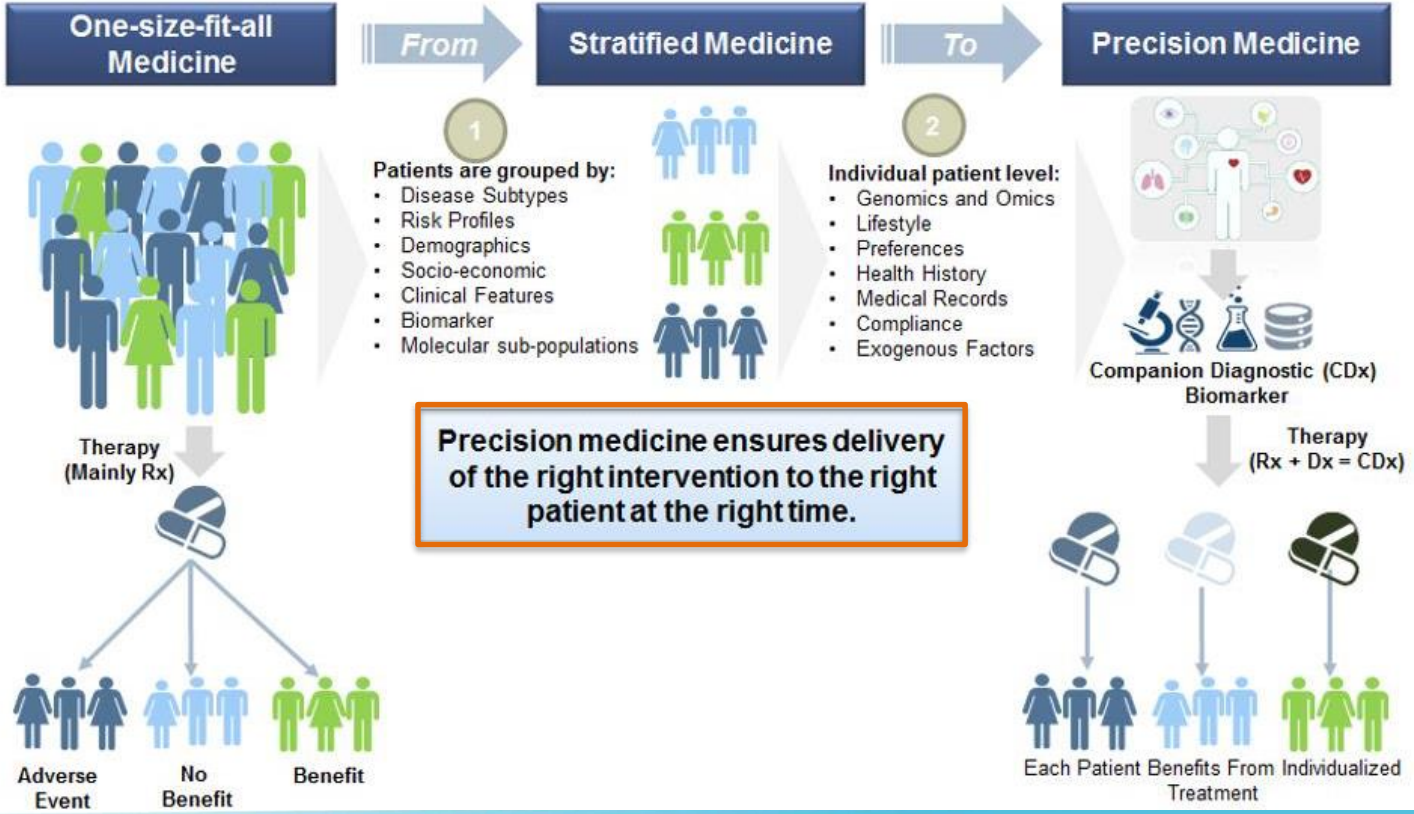
16th ASEICA INTERNATIONAL CONGRESS
Valencia, 6th - 7th - 8th November 2018

Liquid biopsies as important tool for the implementation of precision oncology.

The example of OncoBEAM EGFR mutation assessment in lung cancer.

Silvia Calabuig Fariñas
FIHGUV
UV
CIBERONC

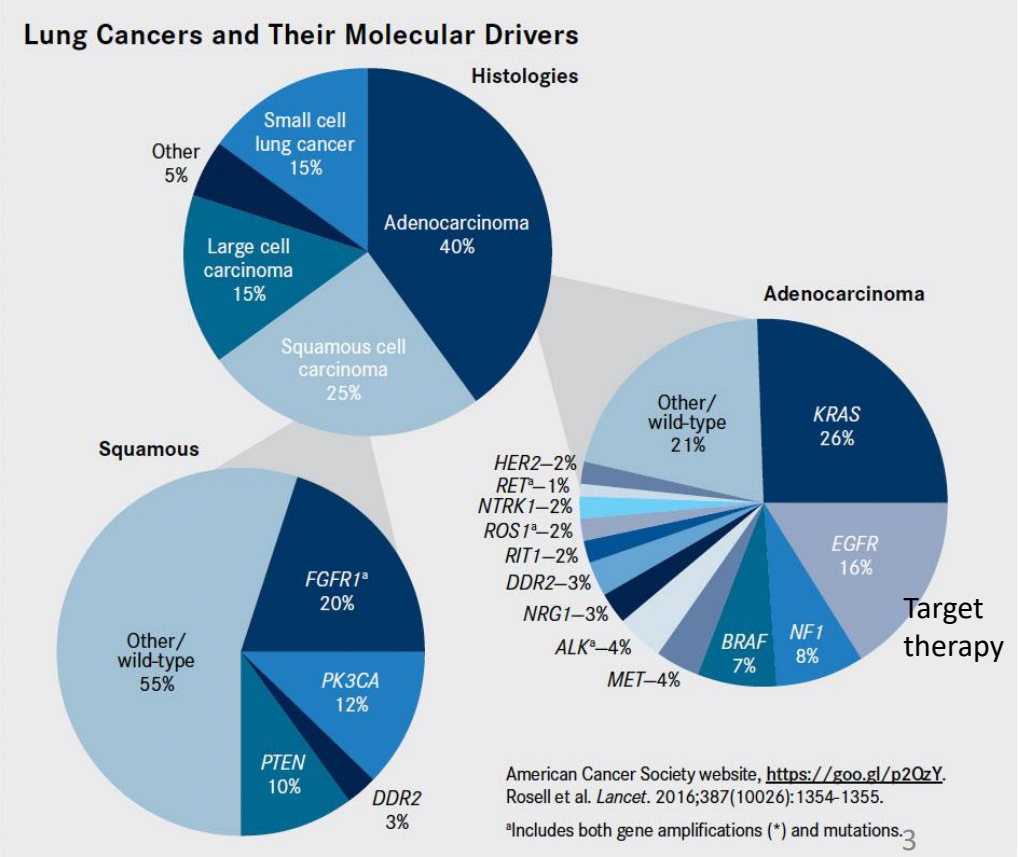
Precision Medicine



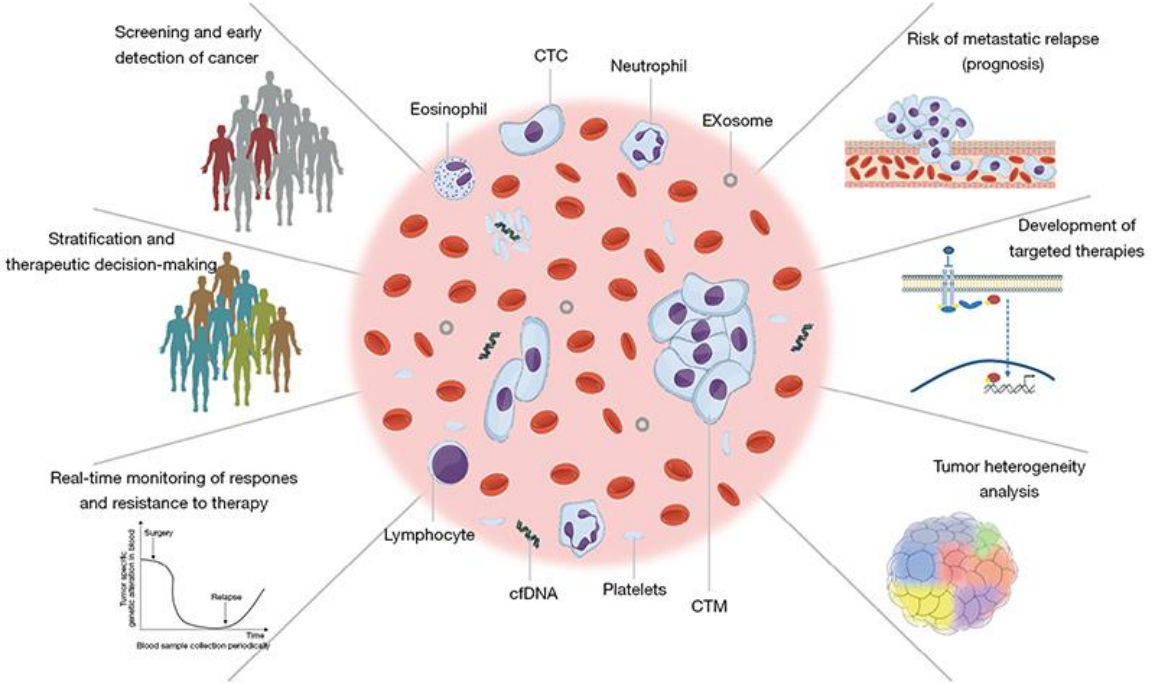
Non-Small Cell Lung Cancer

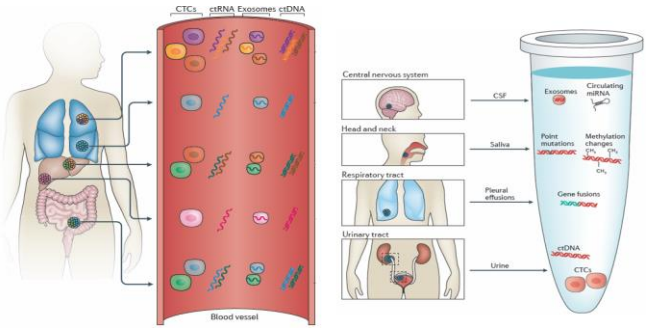
- **Leading cause of cancer deaths worldwide (**1.6M deaths each year);

- In **15-20% of advanced NSCLC** cases, genetic tests are not performed due to: unavailable or insufficient tissue, poor PS, comorbidities.



Liquid biopsies as tool for the implementation of precision oncology.



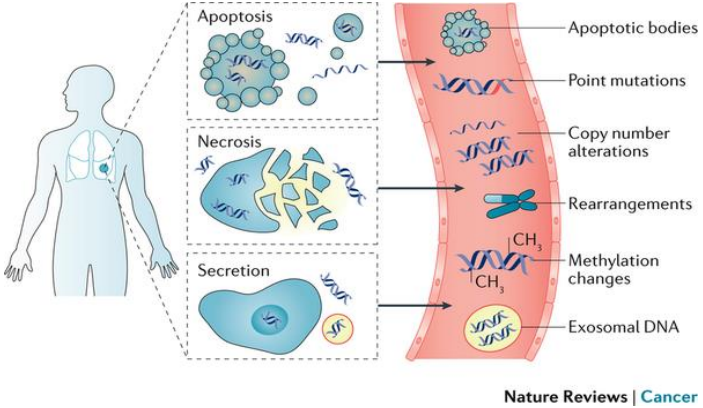


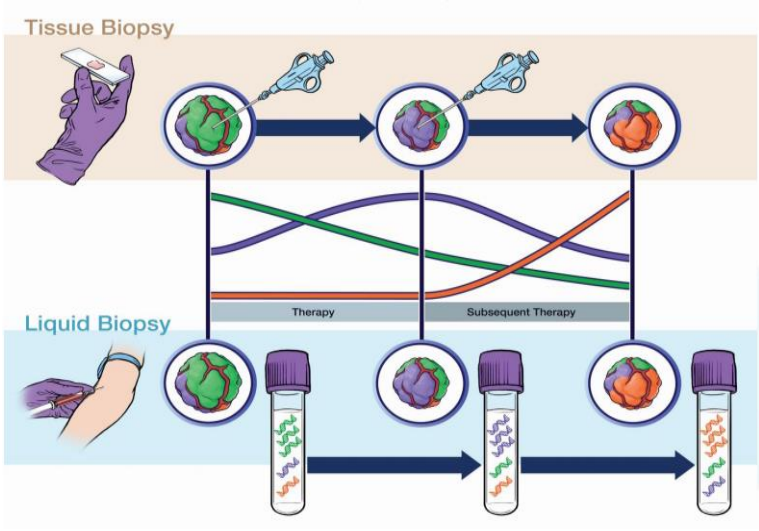
⇨To analyze the clinical utility of plasma using cell-free circulating tumor DNA (ctDNA) for advanced-stage lung ADC patients, as a complement or alternative to tissue-based molecular profiling.

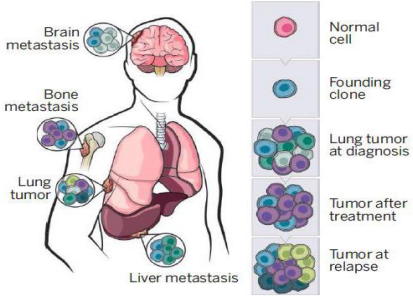
Calabuig-Fariñas et al.Transl Lung Cancer Res 2016

Wan et al. Nature Reviews, 2017

Liquid biopsies as tool for the implementation of precision oncology







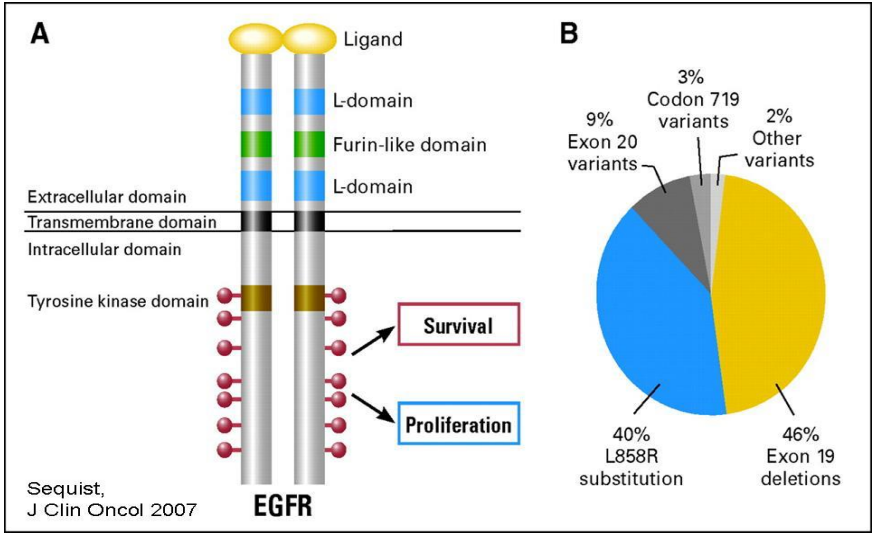
Klein et al. Nature 2013

Siravegna et al. Nature Reviews Clinical Oncology 2017

Govindan et al. Science 2014

EGFR Sensitizing Mutations:

- Found in 10% to 30% of NSCLC pts[1]
- More common in never-smokers, adenocarcinomas, females, Asians[1,2]
- Predominantly located in *EGFR* exons 18-21[2]
  - ~ 85% of *EGFR* mutations are either deletions in exon 19 or a single-point mutation in exon 21 (L858R)[3]
- Specific *EGFR* mutation identified is important
  - There are sensitive mutations, primary resistance mutations (often exon 20), and acquired resistance mutations (T790M)[3]

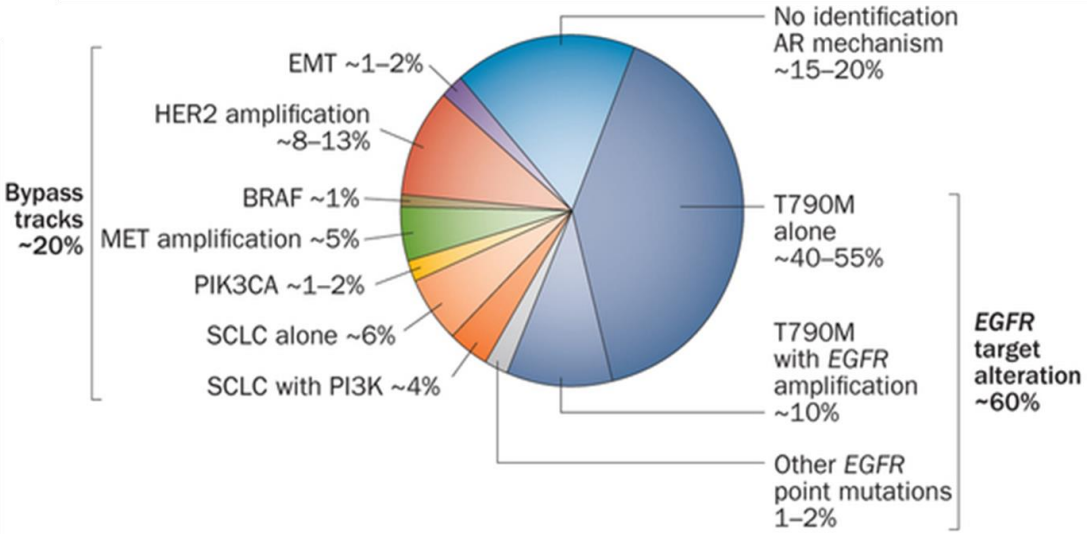


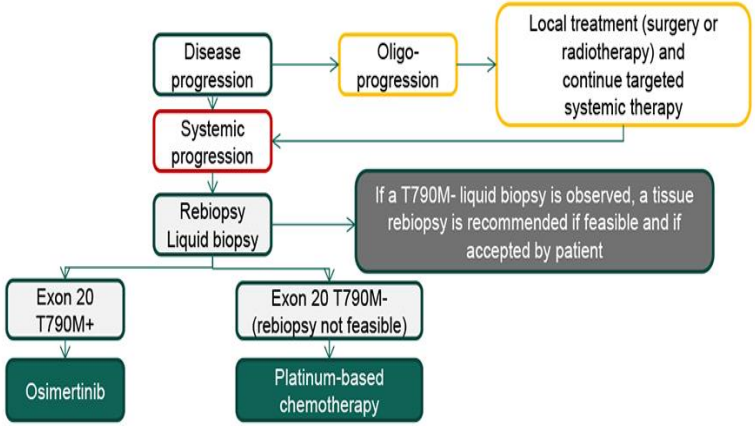
Erlotinib Gefitinib	1st-generation TKI EGFR inhibition	Activity range ▪ Reversible binding to wild-type and mutant EGFR ▪ Inactive on T790M mutant	
Afatinib Dacomitinib	2nd-generation TKI ErbB family blockade	Activity range ▪ Irreversible covalent binding to EGFR, ErbB2 and ErbB4 to inhibit all ErbB family signalling ▪ Broader activity to overcome EGFR TKI-resistant mutations	

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.
Liao BC, et al. Curr Opin Oncol 2015;27:94–101.

Pao W, et al. J Clin Oncol. 2005;23:2556-2568. Wu YL, et al. J Thorac Oncol. 2007;2:430-439. Gazdar AF. Oncogene. 2009;28:S24-S31.

EGFR T790M the most common resistence mechanism to 1st and 2nd EGFR TKIs





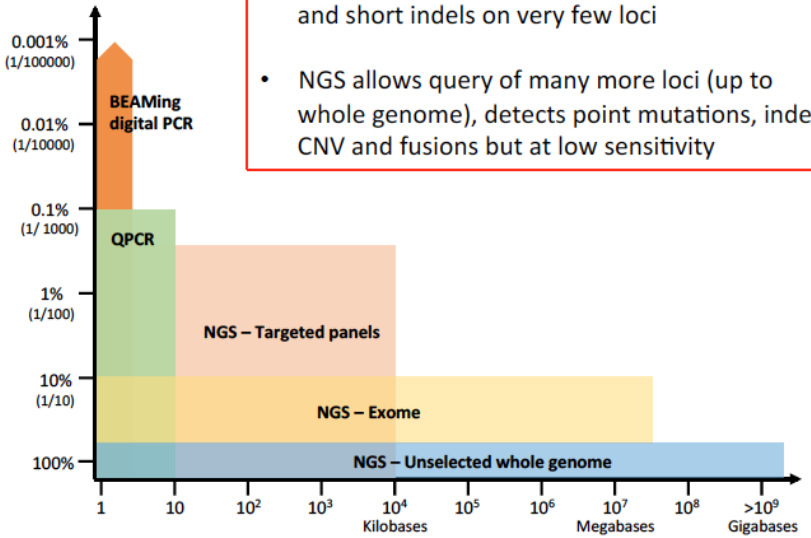
Novello S et al; ESMO Guidelines Committee. *Ann Oncol*. 2016;27(suppl 5):v1-v27.

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor.
Liao BC, et al. Curr Opin Oncol 2015;27:94–101.

Different methodologies

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

- Allele specific- and emulsion-PCR methods are highly sensitive but detect only point mutations and short indels on very few loci
- NGS allows query of many more loci (up to whole genome), detects point mutations, indels, CNV and fusions but at low sensitivity



	Type of mutation	EGFR exon	Mutations
OncoBEAM EGFR Kit v2 (RUO*) 36 EGFR mutations	Sensitising	18	G719A, G719S, G719C
		19	ΔK745, ΔE746, ΔL747
		21	L858R, L861Q
	Resistance	20	T790M, C797S

Diaz and Bardelli.- J Clin Oncol 2014

Digital PCR

Beads

Emulsion

Amplification

Magnetics

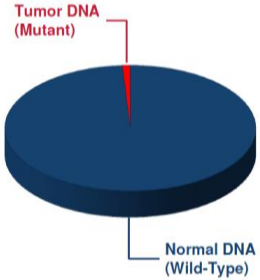
- **High Sensitivity**
- **Suitable for detection of specific point mutations, short indels**
- **No bioinformatic analysis**

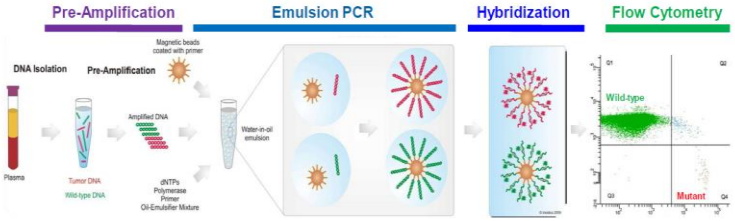
- **One or few gene mutations could be analyzed**
- **Only enables monitoring of known mutations**

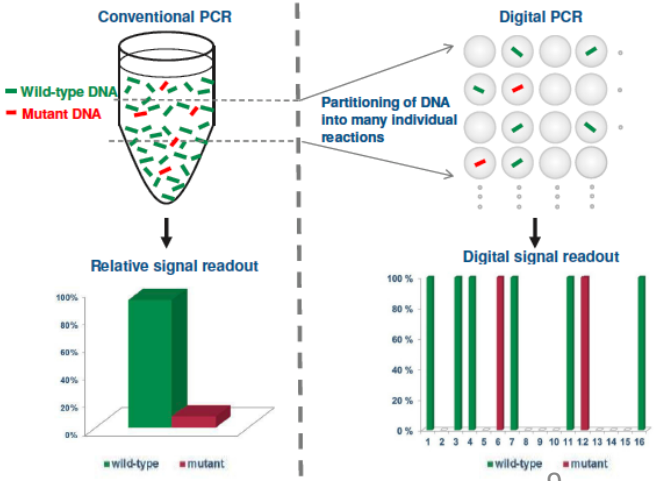
- **We must be SCRUPULOUS in the analytical phase to avoid erroneous results**

- **Clinically irrelevant molecular changes due to the high sensitivity**

Highly sensitive quantitative digital PCR technology that employs bead-based amplification in water-in-oil emulsions and allele-specific hybridization followed by flow cytometry for detecting small amounts of mutated DNA released by tumors into the blood circulation



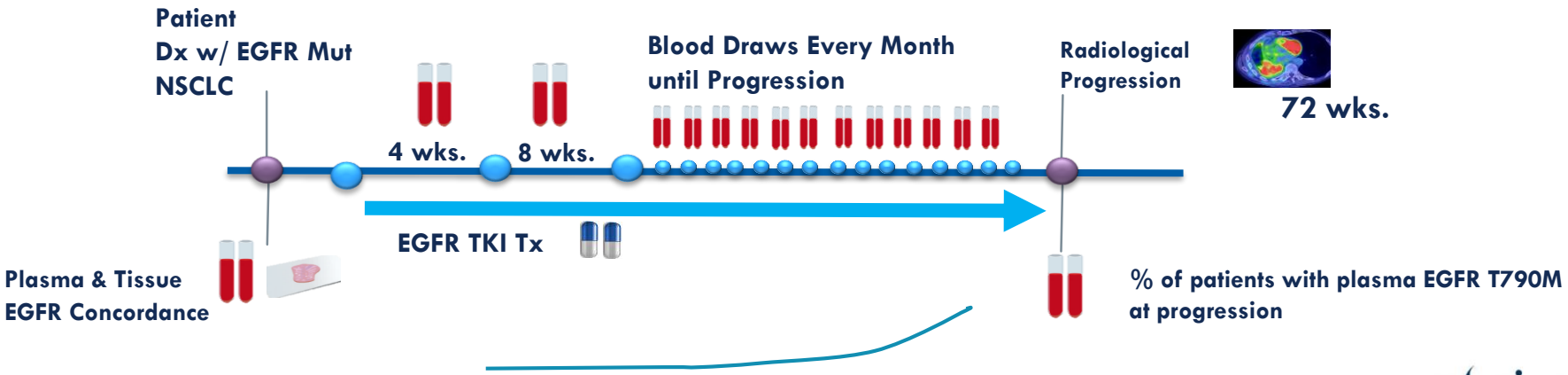




Diehl et al. Curr Opin Oncol.2007

LungBEAM Trial – Study Design & Methods

- Therapy-naïve metastatic NSCLC patients with EGFR-positive sensitizing mutations (del19, L858R) tissue at baseline
- Standard EGFR TKI treatment and follow-up based on local guidelines
- CT every 8 weeks until 6 months and then every 12 weeks. No central review.
- Blood samples throughout standard first-line EGFR TKI treatment until PD or 72 wks.
- BEAMing test performed in a CLIA lab
- Clinicians blinded to blood results.



Goals: Determine concordance at baseline and 'window' of T790M emergence with respect to time of disease progression.

Lead by Garrido P, et al .WCLC 2017& ESMO 2018

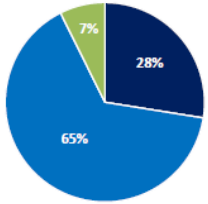
LungBEAM Trial – Study

Patient Characteristics

Patient cases recruited/analyzed for concordance:	109 (Nov 2015-May 2017)
Median Age:	65.5 (35-87)
Gender:	
Female	78 (71,5%)
Male	31 (28.5%)
Smoking status:	
Never Smoker	67 (61.5%)
Former Smoker	33 (30.2%)
Active Smoker	9 (8.3%)
M status:	
M1a	35 (32.1%)
M1b	74 (67.9%)
Metastases:	
brain only	10 (9.2%)
bone only	16 (14.7%)
Stage at diagnosis and tissue acquisition:	
M0	14 (12.8%)
M1	95 (87.2%)
Tissue EGFR mutation status:	
del19+	69 (63.3%)
L858R+	40 (36.7%)

Positive Percent Agreement (PPA) at baseline of EGFR del19 and L858R mutations

109 Total Patients



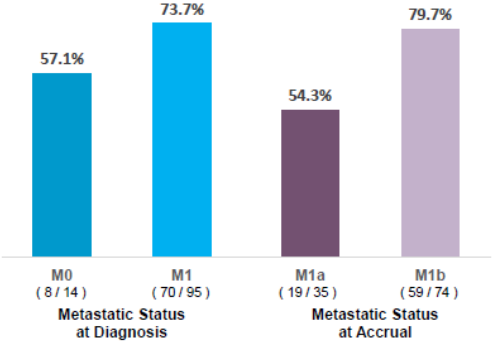
- Patients with NO evidence of progression at 72 weeks (n=30)
- Patients with evidence of progression to date (n=71)
- Patients who have not finished the study (n=8)

PPA at baseline of EGFR del19 and L858R mutational results between plasma and tissue was **71.6% (78/109)**

The **average number of days** between tissue biopsy and blood collection for:

Concordant cases	116 days
Discordant cases	306 days

Significant differences on PPA was observed according to the metastatic status of patients



Progression-free survival indicators

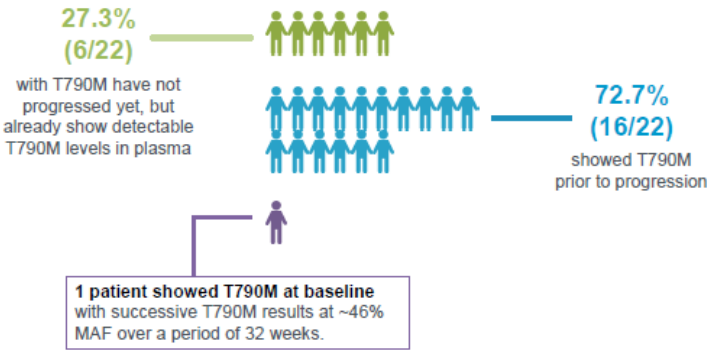
Clearance of EGFR mutations in plasma 8 weeks after initiation of EGFR TKI could be a favorable indicator for PFS:

	Number of Patients	MAF at baseline	Mean PFS
Detectable mutations at 8 weeks	13	13.5%	25.8 weeks
Clearance of mutations at 8 weeks	33	3.8%	40.3 weeks

Garrido P, et al .WCLC 2017& ESMO 2018

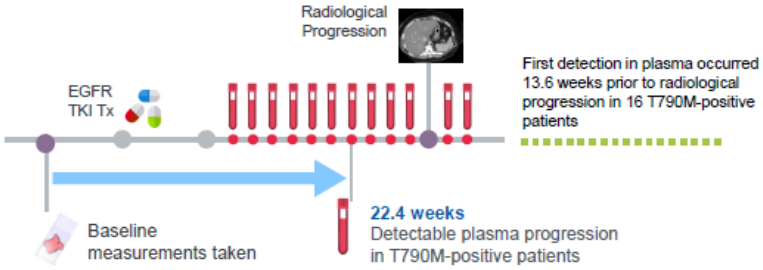
Plasma T790M monitoring during first line EGFR TKI therapy

Of the 23* T790M-positive patients:



*21 of the 23 patients also had an accompanying del19 or L858R mutation

Timing of T790M Detection in Plasma



A total of 23 patients showed T790M-positive (only 16 had radiological progression):

Average time to T790M-positive plasma:	22.4 weeks
Average T790M mutant allelic fraction (MAF):	0.11 %

So far, 71 patients have experienced Radiological Progression. The average time was:

non-T790M patients (n=55):	26.7 weeks
T790M-positive patients (n=16):	35.8 weeks

CONCLUSIONS

- Overall, these initial results show high concordance of plasma and tissue del19 and L858R EGFR mutation status at baseline (~72%).
- Early detection of T790M ctDNA using a highly sensitive method may assist in anticipating T790M resistance to first-line anti-EGFR therapy.
- Clearance of EGFR mutations from plasma 8 weeks after initiation of anti-EGFR therapy could be indicator of a more favorable PFS.

Lead by Garrido P, et al .WCLC 2017& ESMO 2018

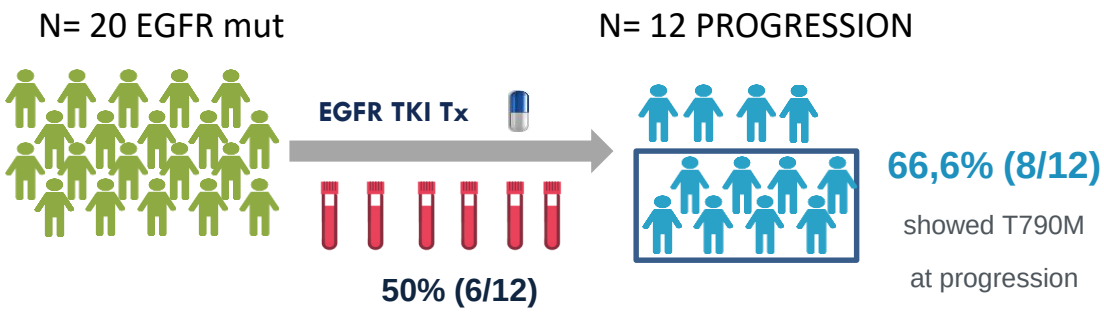
aseica Asociación Española de Investigación sobre el Cáncer

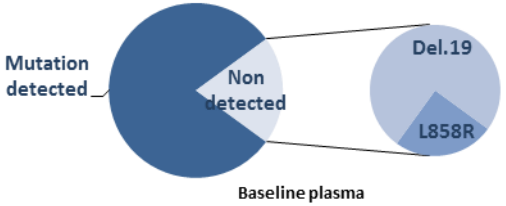
Our experience

Hospital General Universitari de Valencia

Characteristics	N=20
Age at diagnosis (median, range)	70 [47-85]
Gender	
Male	5
Female	15
Smoking status	
Never smoker	12
Former smoker	4
Active smoker	4
EGFR mutation status	
Del.19	8
L858R	12
Progression	
No	8
Yes	12
Dead	
No	15
Yes	5

- Patients recruited between 2014-2018.
- The Positive Percent Agreement (PPA) at baseline of EGFR del19 and L858R mutational results between plasma and tissue was **16/20 = 75%**

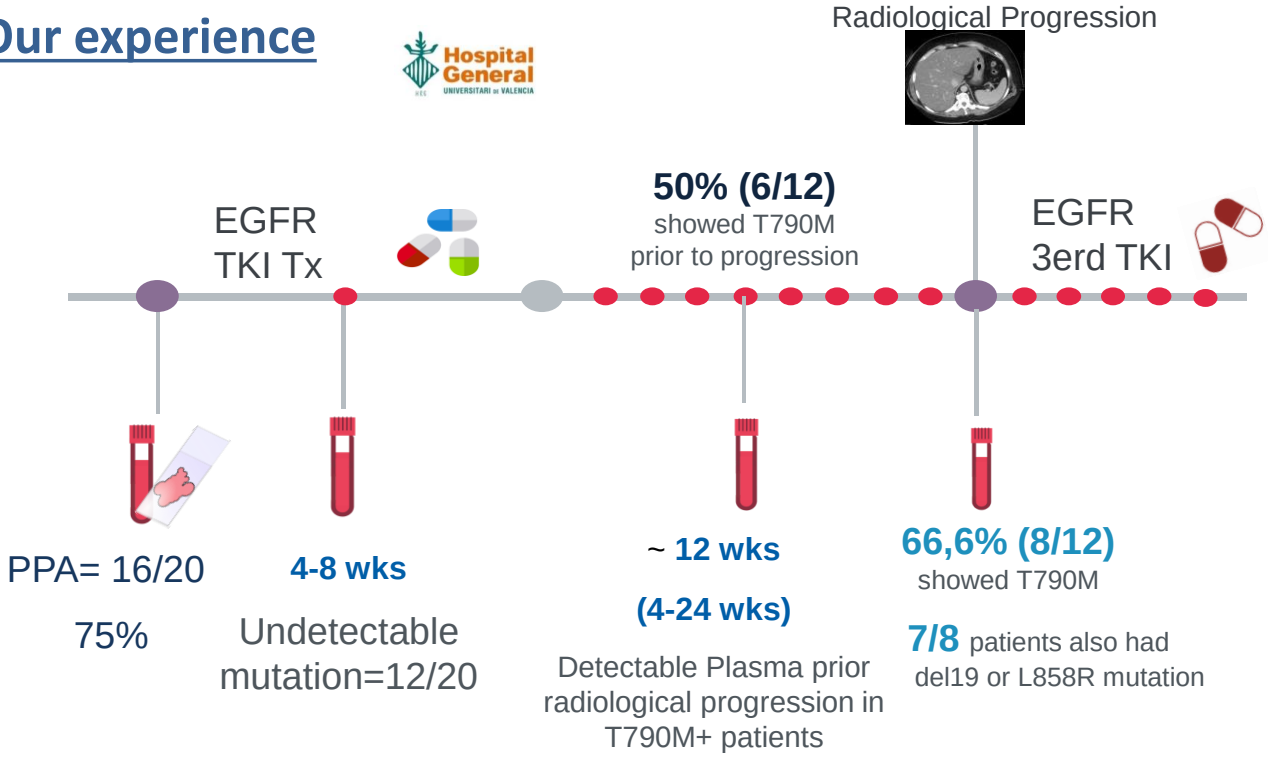




aseica Asociación Española de Investigación sobre el Cáncer

Jantus & Camps Lab, 2018

Our experience

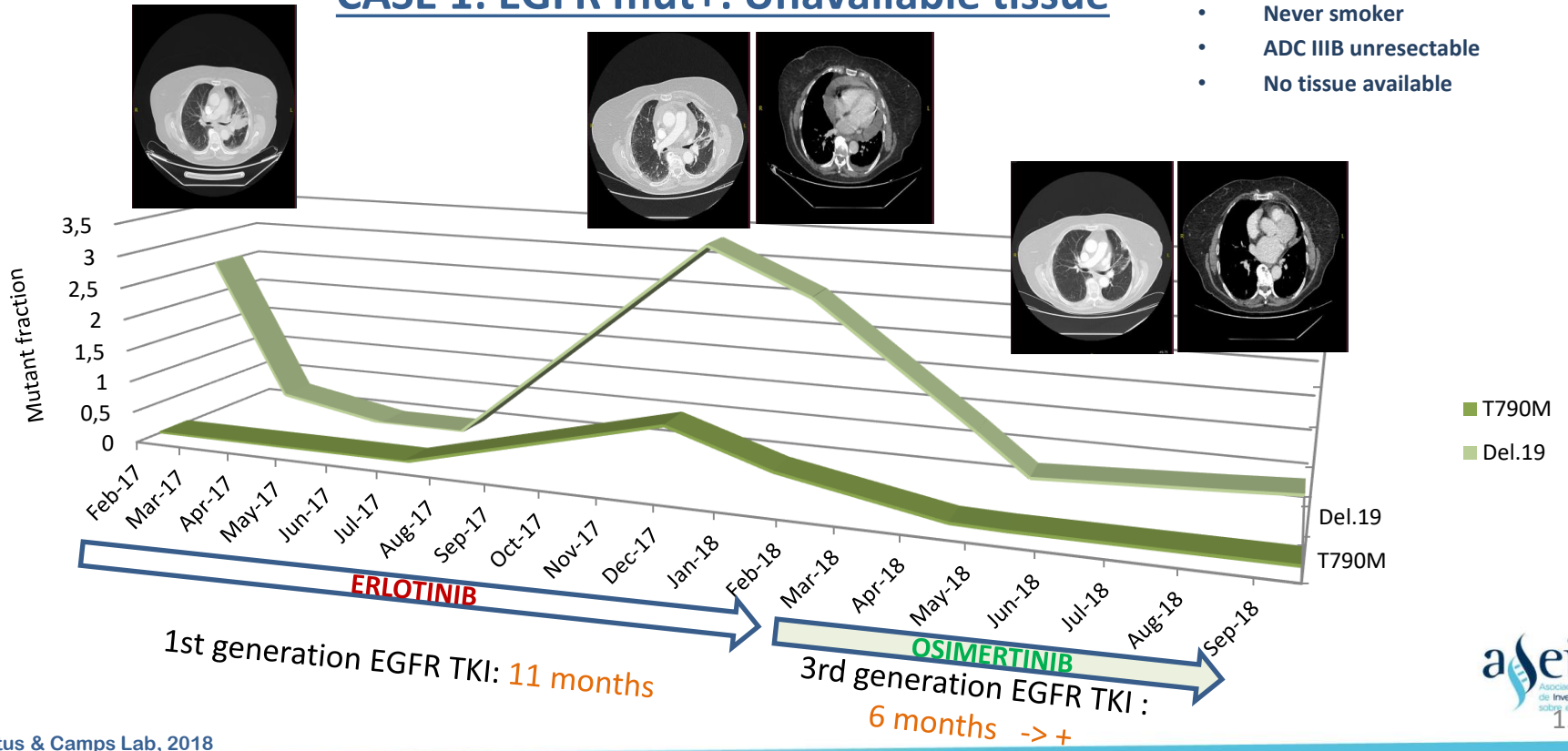


- High **concordance** of plasma and tissue at baseline.
- **Clearance** of mutations from plasma after initiation of targeted therapy could be **indicator of a more favorable PFS.**
- **Early detection** of resistance mutations -> highly sensitive method may assist in anticipating resistance to anti-targeted therapy.

Jantus & Camps Lab, 2018

CASE 1: EGFR mut+: Unavailable tissue

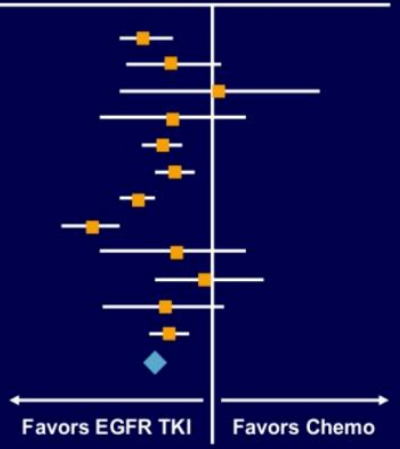
- 85 years old woman
- Never smoker
- ADC IIIB unresectable
- No tissue available



Jantus & Camps Lab, 2018

First-line Treatment With EGFR TKIs vs. Chemotherapy in EGFR-Mutated NSCLC

Study	HR (95% CI)
EGFRmut (first-line therapy)	
EURTAC	0.37 (0.25-0.54)
First-SIGNAL	0.54 (0.27-1.10)
GTOWG	1.08 (0.24-4.90)
INTACT1-2	0.55 (0.19-1.60)
IPASS	0.48 (0.36-0.64)
LUX LUNG3	0.58 (0.43-0.78)
NEJ002	0.32 (0.24-0.44)
OPTIMAL	0.16 (0.11-0.26)
TALENT	0.59 (0.21-1.67)
TOPICAL	0.90 (0.39-2.06)
TRIBUTE	0.49 (0.20-1.20)
WJTOG3405	0.52 (0.38-0.72)
Subtotal	0.43 (0.38-0.49)



"If repeat biopsy is not feasible, plasma biopsy should be considered"
"Testing should be conducted as part of broad molecular profiling"

NCCN 2017 NSCLC Practice Guidelines

"Key new recommendations include the inclusion of additional genes (*ERBB2*, *MET*, *BRAF*, *KRAS*, and *RET*)…and the use of cell-free DNA to "rule in" targetable mutations when tissue is limited or hard to obtain.

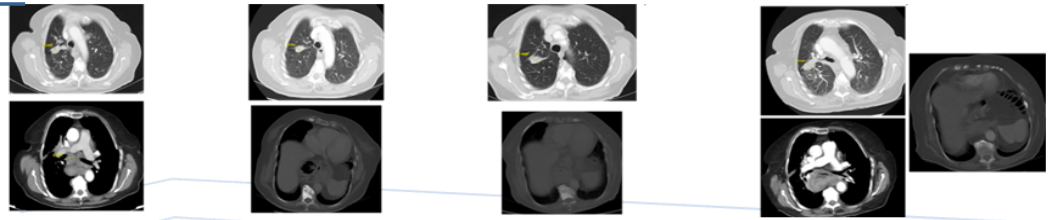
AMP/CAP/IASLC 2018 Molecular Testing Guidelines for Lung Cancer

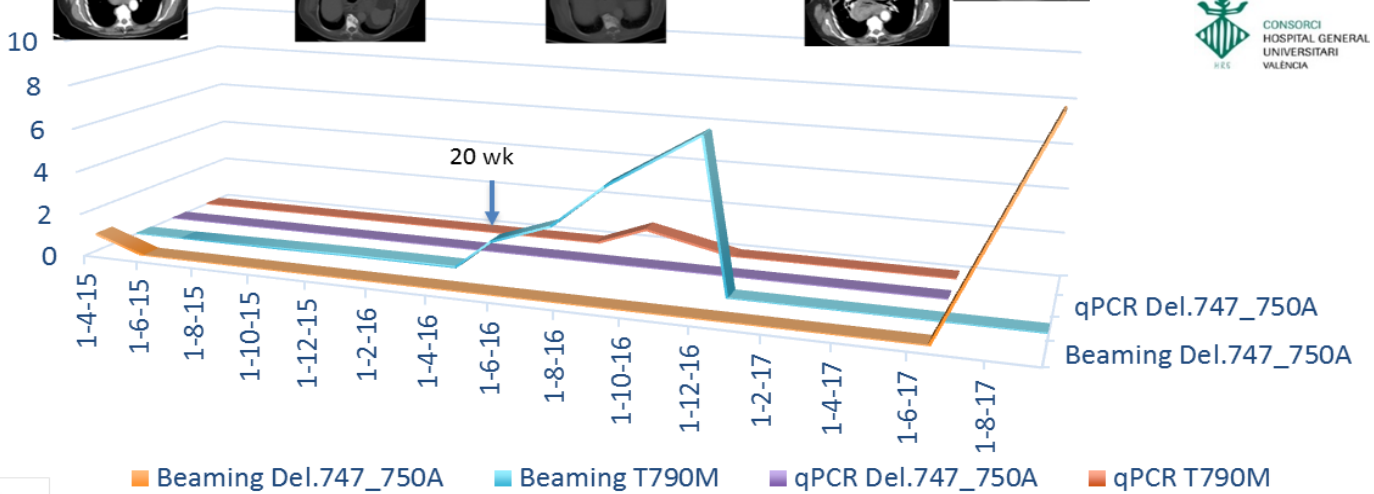
"Even for patients who are able to undergo a traditional tissue biopsy, a liquid biopsy may be safer, quicker, and more convenient—and perhaps even more informative."

2017 ASCO Clinical Cancer Advances

Mok TS, et al. *N Engl J Med*. 2009;361(10):947-957. Han J-Y, et al. *J Clin Oncol*. 2012; 30:1122-1128. Mitsudomi T, et al. *Lancet Oncol*. 2010;11(2):121-128. Maemondo M, et al. *N Engl J Med*. 2010;362(25):2380-2388. Zhou C, et al. *Lancet Oncol*. 2011;12(8):735-742. Zhou C, et al. *J Clin Oncol*. 2012;30(suppl): abstract 7520. Rosell R, et al. *Lancet Oncol*. 2012;13: 239-246.

CASE 2: T790M mut.





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

Erlotinib → Osimertinib

Jantus & Camps Lab, 2018

Methods	Sample, n	T790M, %	PPA, %	NPA, %	OPA, %	Therapy Response Rate, %	
						Tissue T790M	Plasma T790M
cobas EGFR Mutation Test	72	41	73	67	70	61	59
cobas EGFR Mutation Test	110	21	64	98	86	52	44
BEAMing Digital PCR	77	66	73	NA	NA	-	-
BEAMing Digital PCR	216	60	70	69	70	62	63
Droplet Digital PCR	60	58	77	63	72	NA	NA
NGS	63	41	93	94	78	NA	NA

NPA: negative percentage agreement; OPA: overall percentage agreement; PPA: positive percentage agreement.

Passaro A et al. *Pharmacol Res*. 2017;117:406-415.

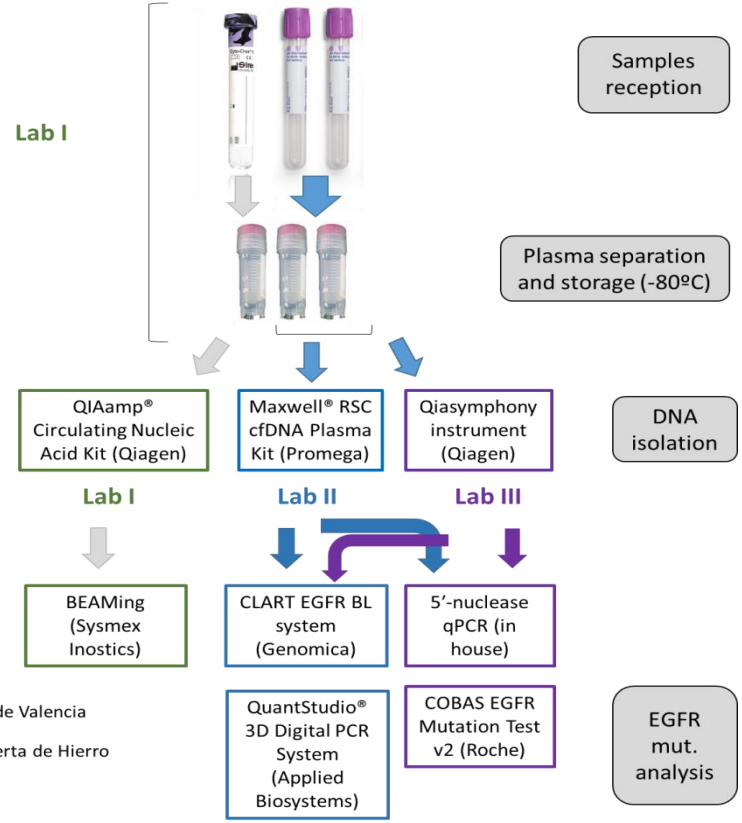
Using the Cobas tissue test as a reference, sensitivity for the detection of T790M was increased for ddPCR and NGS compared with AS-PCR. In AURA I, ctDNA (BEAMing) "**rescue" ~30% of tissue *T790M*-negative tumors**

Is test approved by FDA the best?

Ahn- WCLC 2017; Jenkins – JTO 2017
Oxnard – JCO 2016; Remón - ELCC2018

In Liquid biopsies, sensitivity is an important issue to consider

RING Study



- Lab Oncología Molecular, Fundación Htal Gral Univ de Valencia
- Lab de Biopsia Líquida, Fundación del Htal. Univ. Puerta de Hierro
- Pangaea Oncology

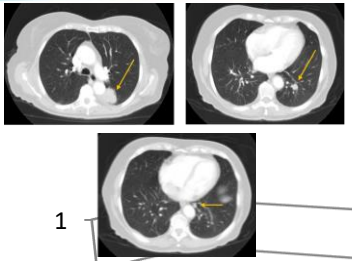
"T790M mutation testing in blood by different methodologies"

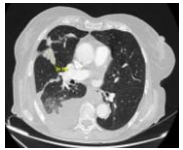
A study to compare 3 purification methods and 8 techniques to determine p.T790M (including NGS)

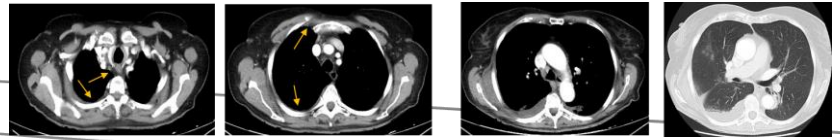
Protocol code: GECP 17/03; ESR-17-12988
PI: Mariano Provencio

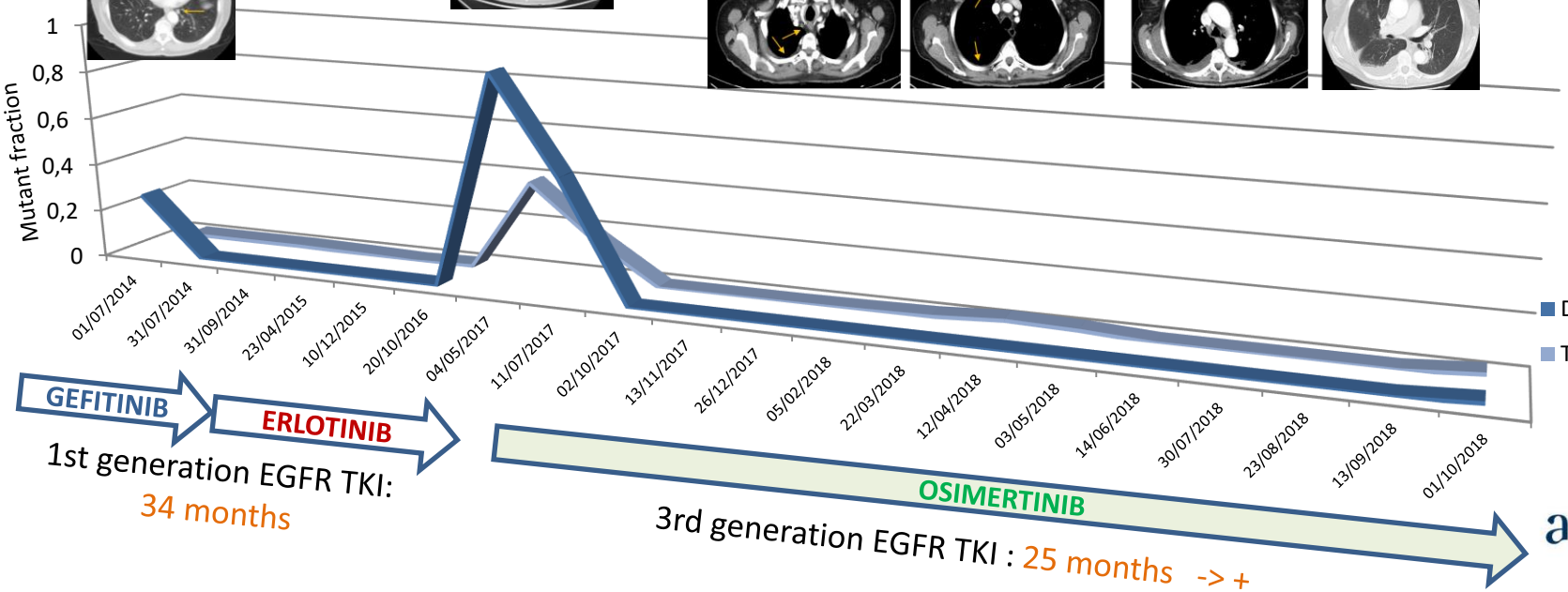
CASE 3: Monitoring of EGFR mut+

- 76 years old woman
- Never smoker
- Poor differentiated lung carcinoma E-IV
- Del. 19 (tissue sample)







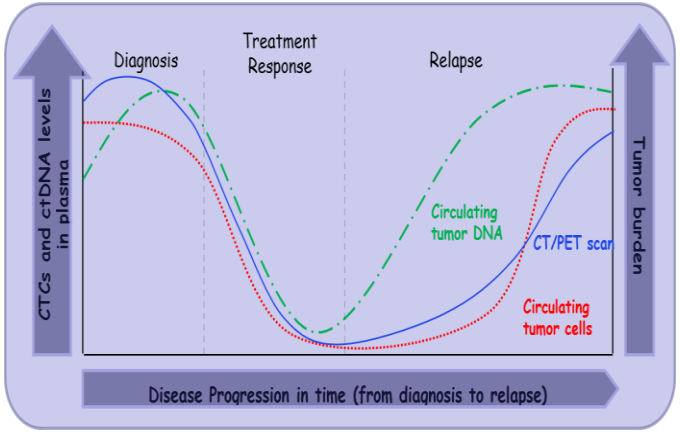


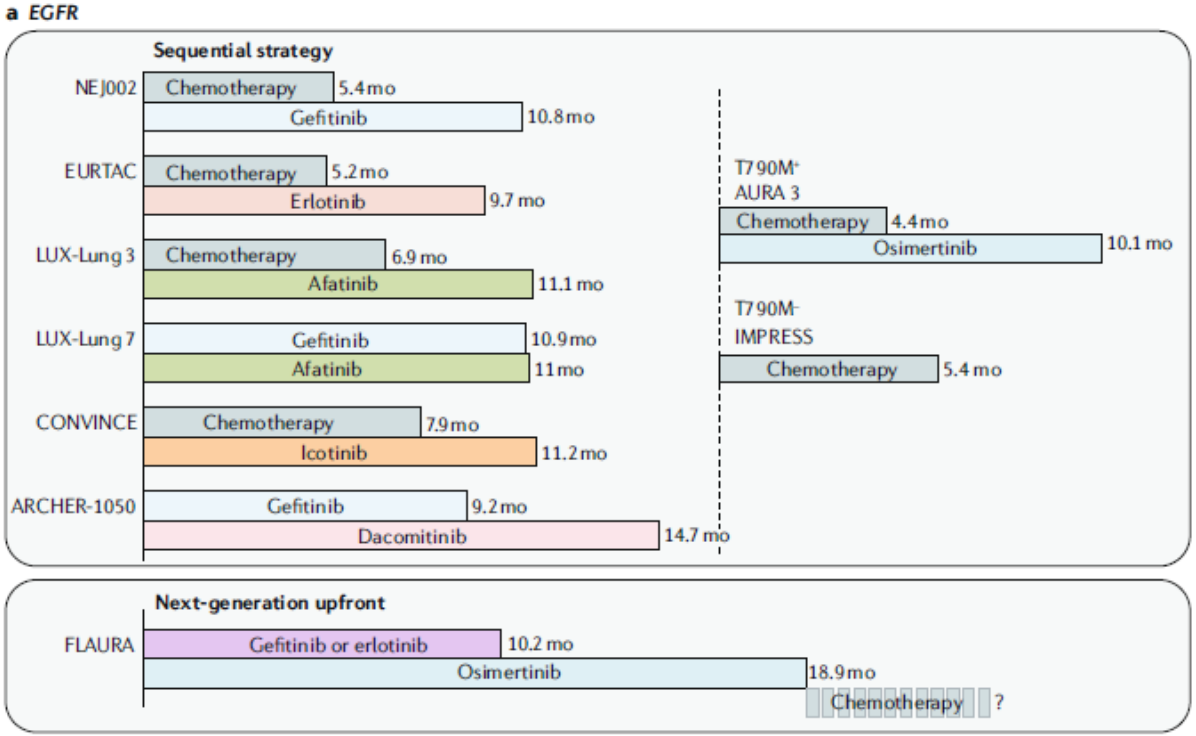
1st generation EGFR TKI: 34 months

3rd generation EGFR TKI : 25 months -> +

Jantus & Camps Lab, 2018

Optimal sequencing treatment



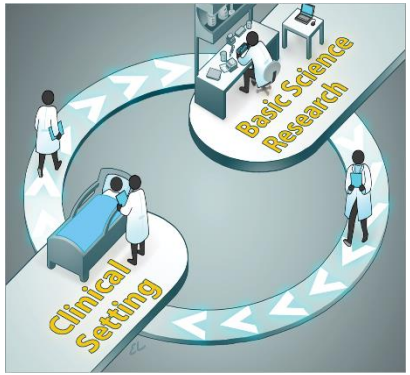


Rolfo, Castiglia, Russo et al. Biochim Biophys Acta. 2014 Dec;1846(2):539-46

Recondo et al. Nature Reviews, Clinical Oncology 2018

Take home message

Precision Medicine



From bench to bedside

GENOMIC analysis

Complementary biopsies

Next Generation Sequencing

Digital PCR

RTqPCR

BEAMing

Different methodologies

Multidisciplinary team

Quality controls

Translational Res.

FUNDACIÓ INVESTIGACIÓ HOSPITAL GENERAL UNIVERSITARI VALÈNCIA

MOLECULAR ONCOLOGY LAB.

Carlos Camps, Eloisa Jantus, Silvia Calabuig, Rafael Sirera, Sandra Gallach, Eva Escorihuela, Marais Mosqueda, Alejandro Herreros, Elena Duréndez, Héctor Amado, Clara(Ning Dong), Bruno (Feiyu Zhang), Susana Torres, Andrea Moreno.

CLINICAL INVEST.

Vicente Castellano, Paula Matoses, Ana Saval, Lola Serra

Clinical Res.

CONSORCI HOSPITAL GENERAL UNIVERSITARI VALÈNCIA

MEDICAL ONCOLOGY

Carlos Camps, Alfonso Berrocal, Ana Blasco, Mª José Safont, Cristina Caballero, Mª José Godes, Vega Iranzo, Francisco Aparisi, Miriam Lobo, Mireia Gil, Alberto Cunquero.

THORACIC SURGERY: Ricardo Guijarro, Eva del Olmo

PATHOLOGY: Miguel Martorell, Atilio Navarro, Lara Navarro

21:00 **YOUNG ONCOLOGISTS NETWORKING EVENING**
(Only for assistents under 45 years old)

Venue: ADEIT | Fundación Universidad-Empresa
de la Universidad de Valencia
Plaza Virgen de la Paz, 3, 46001 Valencia

16th ASEICA INTERNATIONAL CONGRESS
Valencia, 6th - 7th - 8th November 2018

Liquid biopsies as important tool for the implementation of precision oncology.

The example of OncoBEAM EGFR mutation assessment in lung cancer.

Silvia Calabuig Fariñas.
FIHGUV
UV
CIBERONC

aseica
Asociación Española de Investigación sobre el Cáncer