I SIMPOSIO NACIONAL de ONCOLOGÍA de PRECISIÓN

Vigo, del **28** de **febrero** al **1** de **marzo** de **2019**

Biopsia líquida: diagnóstico, respuesta y seguimiento

Dra. Atocha Romero

LiquidBiopsyLabPdH (@LiquidBiopsyLab)

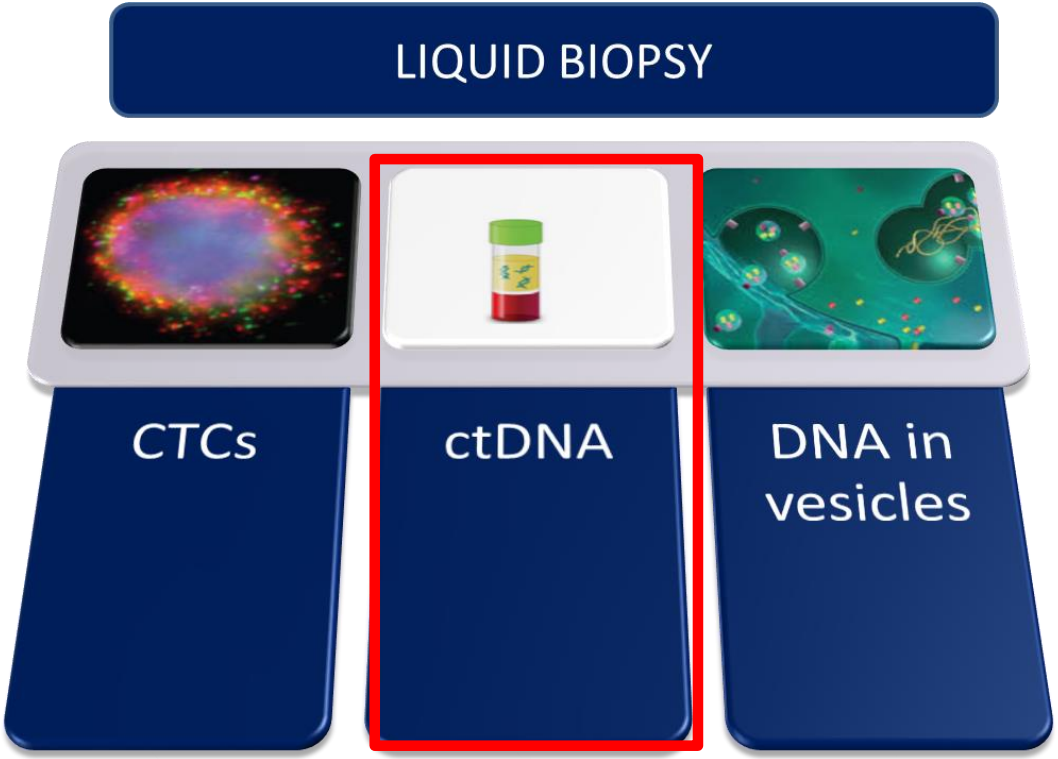
rla red de laboratorios Sistema madrid

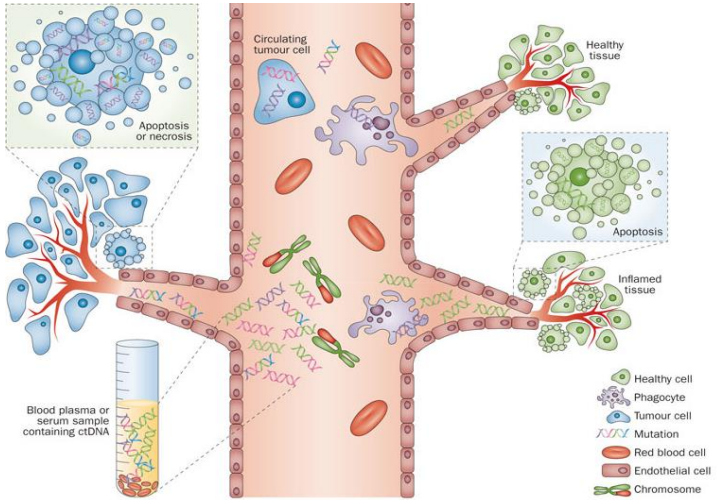
Nº Registro: 419

Hospital Universitario Puerta de Hierro Majadahonda

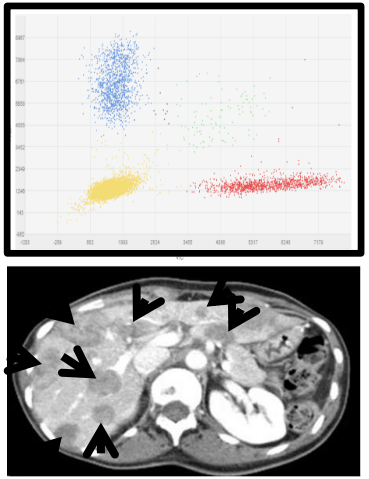
INSTITUTO DE INVESTIGACIÓN SANITARIA Hospital Puerta de Hierro – Segovia de Arana

ENAC ENSAYOS Nº 1296/LE2432

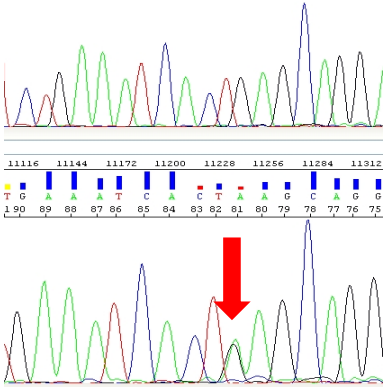

LIQUID BIOPSY
CTCs
ctDNA
DNA in vesicles



Crowley, E. et al. (2013) Nat. Rev. Clin. Oncol.



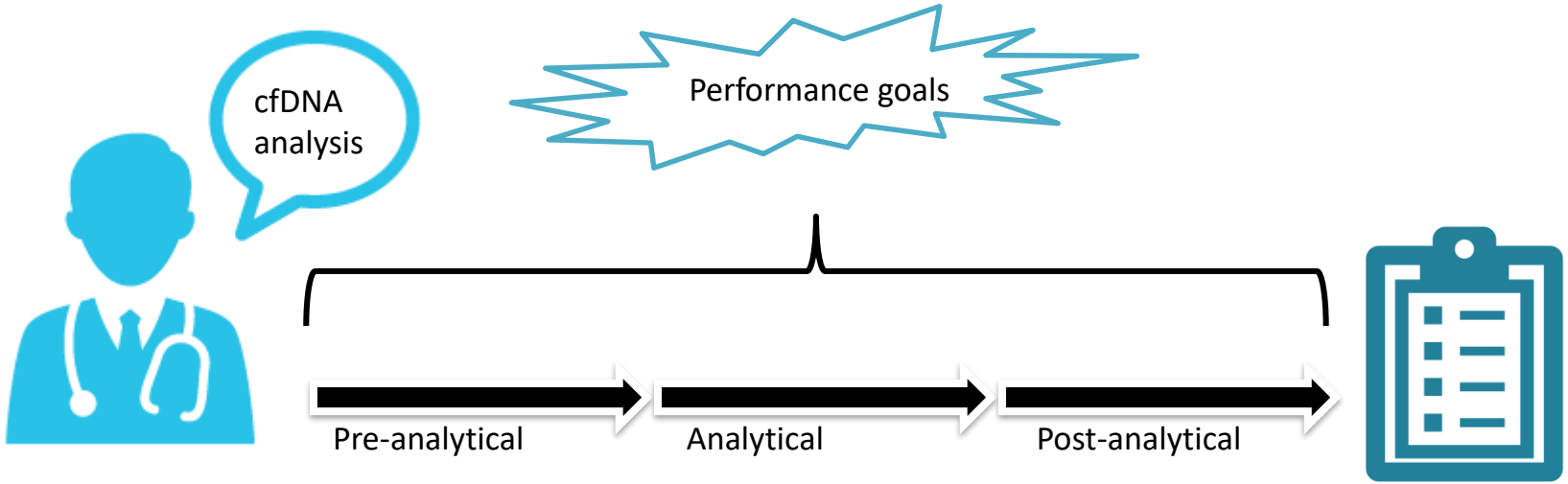
PRIMARY TUMOR



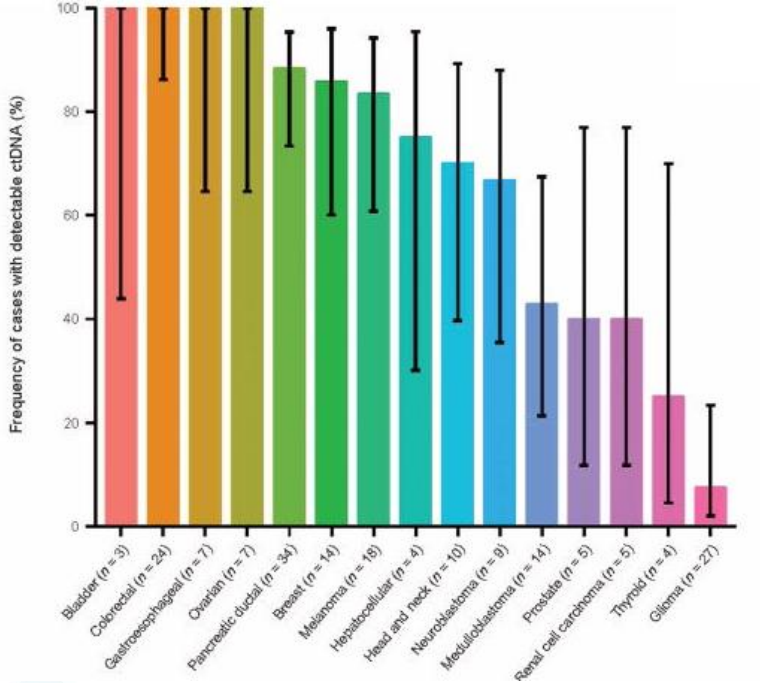
LIVER METASTASIS

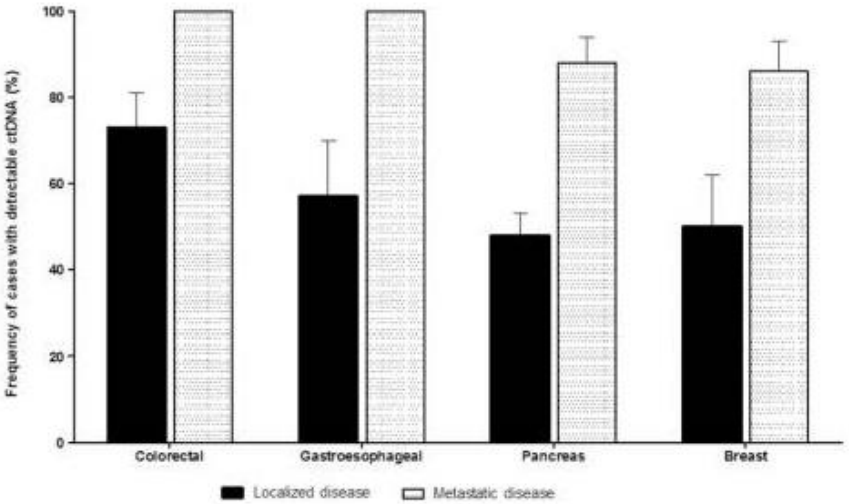
Romero A, et al . Translational Research 2015; 166(6):783-7

Phases of Testing

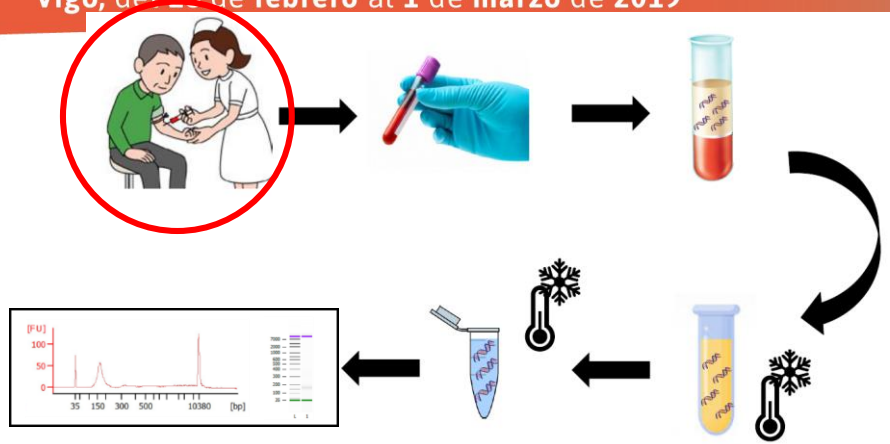


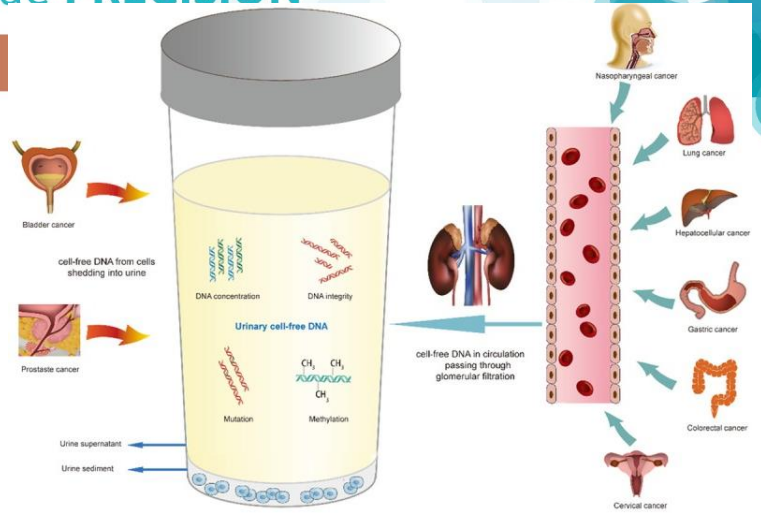
ctDNA detection depends on tumour/patient characteristics





Bettegowda C. Sci Transl Med. 2014 Feb 19;6(224):224ra24

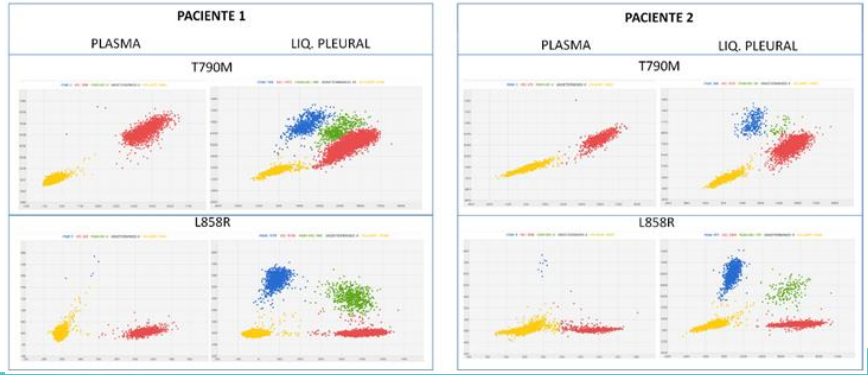


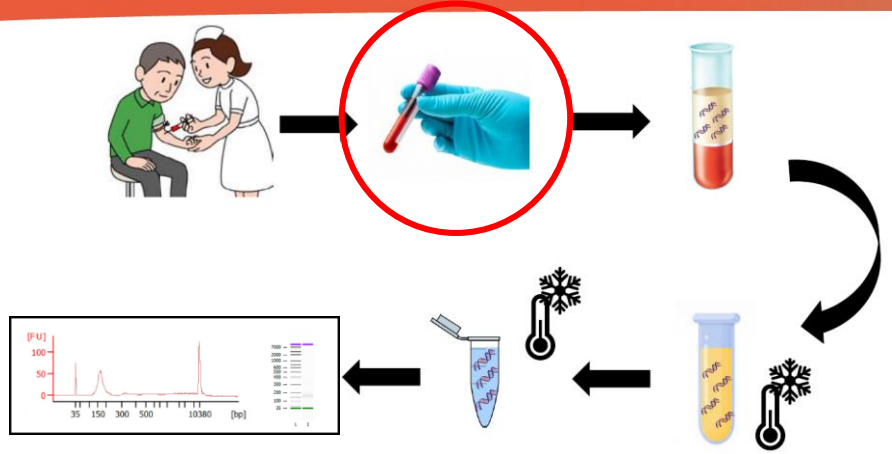


Lu T al. Am J Cancer Res. 2017 Nov 1;7(11):2318-2332

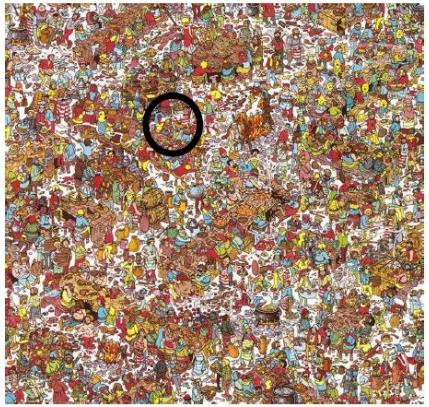
The diagnostic accuracy of pleural effusion and plasma samples versus tumour tissue for detection of EGFR mutation in patients with advanced non-small cell lung cancer: comparison of methodologies

Xiaoqing Liu,[1] Yachao Lu,[2] Guanshan Zhu,[2] Yao Lei,[1] Li Zheng,[2] Haifeng Qin,[1] Chuanhao Tang,[1] Gillian Ellison,[3] Rose McCormack,[3] Qunsheng Ji[2]





1/1000=0,1%

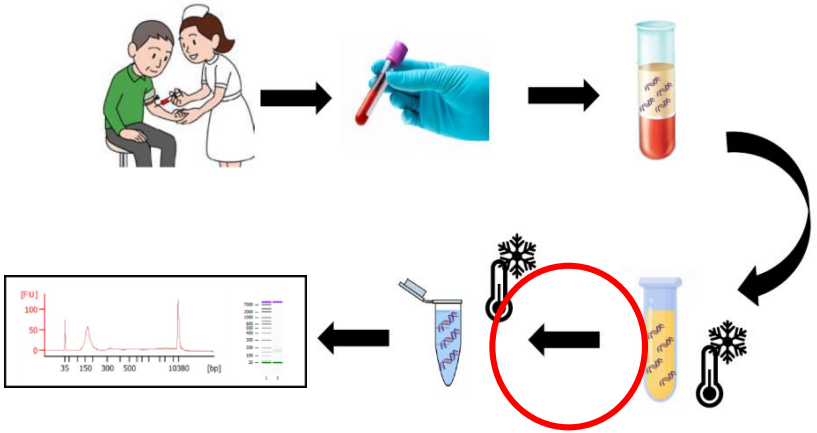


1/35= 2,9%

Table 2. Summary of Key Findings on the Use of ctDNA Analysis in Patients With Cancer

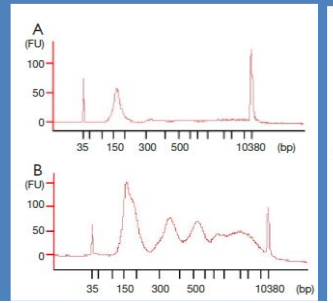
Topic	Key Findings
Preanalytical variables for ctDNA specimens	• Evidence suggests that plasma is the optimal specimen type for ctDNA analysis. • Evidence supports the use of either cell-stabilizing tubes or EDTA anticoagulant tubes. However, EDTA tubes need to be processed as expediently as possible within 6 hours of collection. Leukocyte stabilization tubes allow up to 48 hours from collection to processing, and longer with some tubes. • Further studies are required to address other preanalytical variables that may affect ctDNA testing, including specimen collection, handling variables, storage condition and time, and patient-related biologic factors.

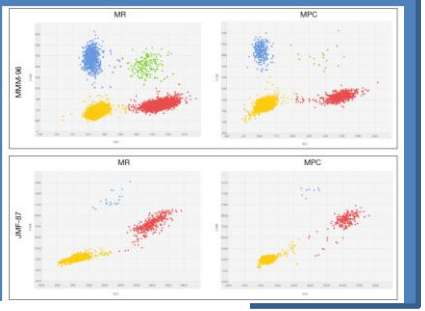
Merker JD, et al. Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint ReviewArch Pathol Lab Med. 2018



Comparison of methods for circulating cell-free DNA isolation using blood from cancer patients: impact on biomarker testing

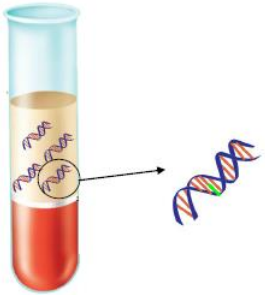
Clara Pérez-Barrios[1*], Irene Nieto-Alcolado[2*], María Torrente[3], Carolina Jiménez-Sánchez[2], Virginia Calvo[3], Lourdes Gutierrez-Sanz[3], Magda Palka[2], Encarnación Donoso-Navarro[1], Mariano Provencio[3], Atocha Romero[2,3]



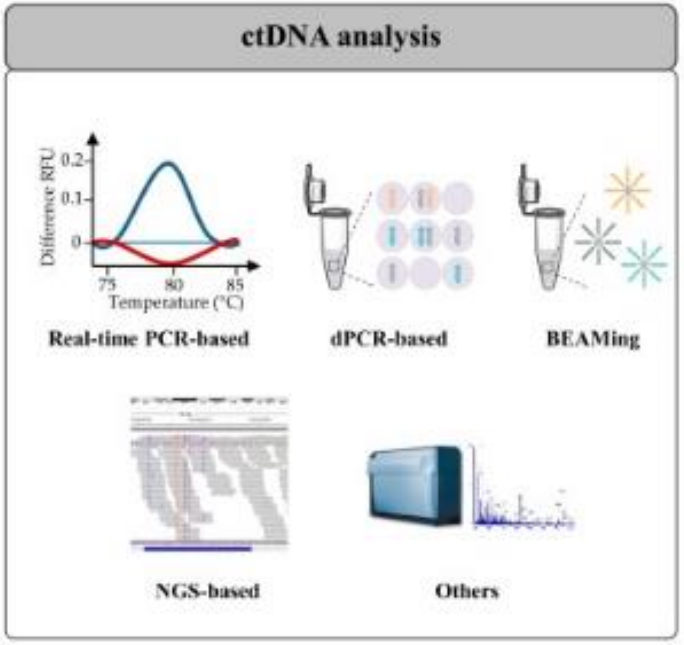


Thermo Fisher	Qiagen		Promega
MagMax cfDNA Kit	CNA Kit	QIAsymphony	Maxwell ccfDNA Plasma Kit
			
Manual, beads	Manual, column	Automated, beads	Automated, beads

Technology



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



Vendrell JA et al.Circulating Cell Free Tumor DNA Detection as a Routine Tool forLung Cancer Patient Management. Int J Mol Sci. 2017;18(2)

Diaz LA . J Clin Oncol. 2014 Feb 20;32(6):579-86.

Technique	Sensitivity	Optimal Application
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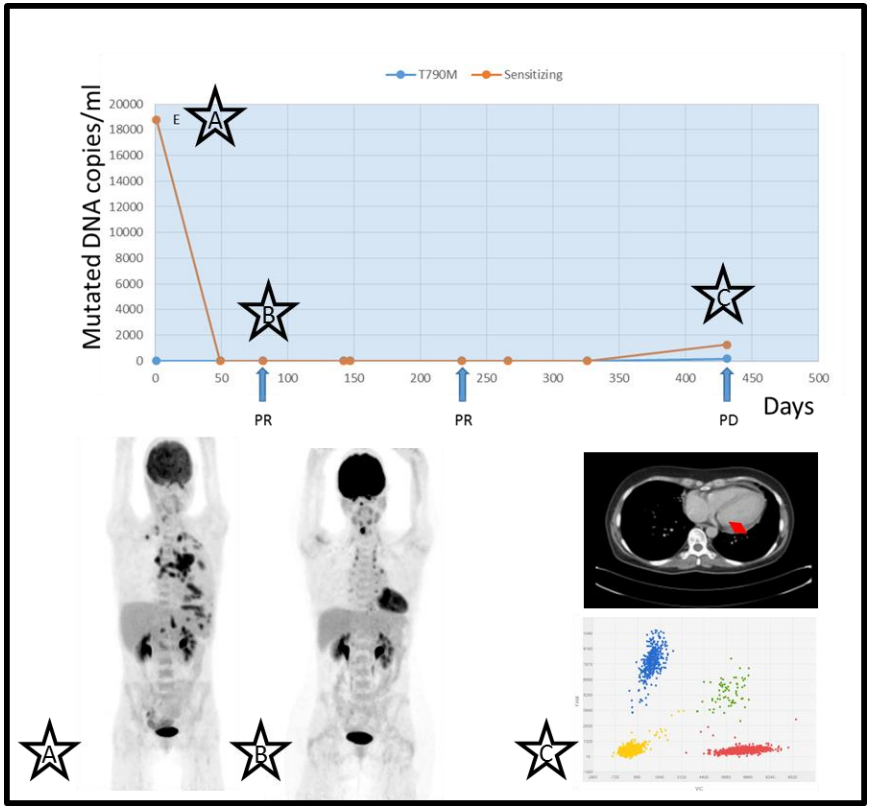
Diaz LA . J Clin Oncol. 2014 Feb 20;32(6):579-86.

PRINCIPLE	*Sensitivity (MAF)*	*Adventage*	*Limitations*
dPCR	1-0.01%	Ease of use Robustness Inexpensive	Only a limited number of mutations can be testedLimited
NGS	1-0.002%	A large number of mutation can be tested at a time	Expensive

Commercial NGS liquid biopsy test

- Guardant 360 (Guardant Health)
- FoundationACT (Foundation Medicine)
- AVENIO ctDNA Targeted Kit, ctDNA Expanded Kit and ctDNA Surveillance Kit (Roche Diagnostics)
- Oncomine Lung cfDNA Assay (Thermo Fisher Scientific
- NEOliquid (New Oncology)
- Reveal ctDNA 28 Kit (ArcherDX)
- OptiSeq™ Pan-Cancer Panel (DiaCarta)
- PlasmaSELECT™-R 64 (PGDx)
- Signatera™ RUO (Natera)

ctDNA to monitor disease



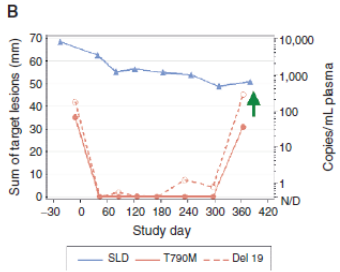
Provencio M , et al . Oncotarget. 2017 Aug 7;8(36):60291-60298.

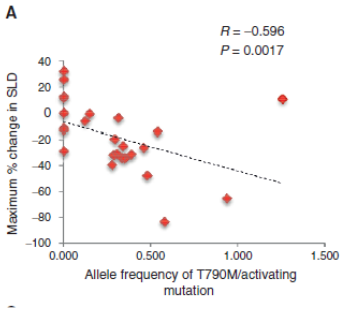
Different line different patterns….

Gene	Number of patients	Baseline alterations	Emergent alterations
EGFR	5	A750P (1)	C797S (1) L798I (1) L692V (1) E709K (1)
PIK3CA	5	E545K (1)	E542K (3) E545K (3) E81K (1)
KRAS	3		G12A (1) Q61H (1) A146T (1)
CDKN2A	3	D74A (1)	D74A (2)
RB1	2	G587* (1)	R787Q (1)
ALK	1		R1061Q(1)
KIT	1		L576P (1)
MET	1	D1304H (1)	

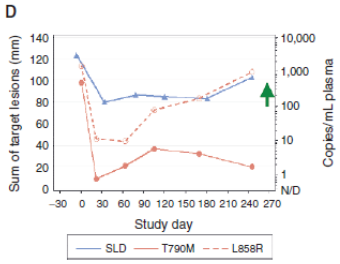
We next sought to explore differences between mechanisms of innate and acquired resistance. Patients with innate resistance were defined as those with PFS shorter than 3 months, while patients with a PFS longer than 3 months were considered as having acquired resistance (Methods). Interestingly, we observed distinct patterns of genomic alterations in patients with innate versus acquired resistance. Specifically, pre-existing copy-number gains in *MET*, *ERBB2* and *EGFR* were significantly more common in patients with innate resistance ($n = 15$), and conversely, emergent or increasing SNVs were more common in patients with acquired resistance ($n = 28$; Fig. 2c, $P < 0.005$, Fisher's exact test). This suggests that detection of copy-number gains leading to EGFR bypass pathway activation before third-generation EGFR TKI therapy may allow identification of patients likely to harbour innate resistance.

Chabon JJ. Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. Nat Commun. 2016 Jun 10;7:11815





This yielded 25 patients with pretreatment T790M/activating mutation allele frequencies that could be used to estimate the fraction of T790M-positive cells within the biopsy. For each patient, we compared this ratio with the maximum tumor shrinkage observed during rociletinib treatment (Fig. 3A and Supplementary Table S3). This analysis revealed a highly significant relationship showing cancers with a higher baseline fraction of T790M-positive cells had greater tumor shrinkage in response to rociletinib ($P = 0.0017$). Importantly, this result suggests that a more quantitative assessment of T790M burden (as opposed to a binary positive/negative test) may provide additional information regarding the degree to which T790M-positive patients are likely to respond.



Piotrowska Z et al. Heterogeneity Underlies the Emergence of EGFRT790 Wild-Type Clones Following Treatment of T790M-Positive Cancers with a Third-Generation EGFR Inhibitor.
Cancer Discov. 2015 Jul;5(7):713-22.

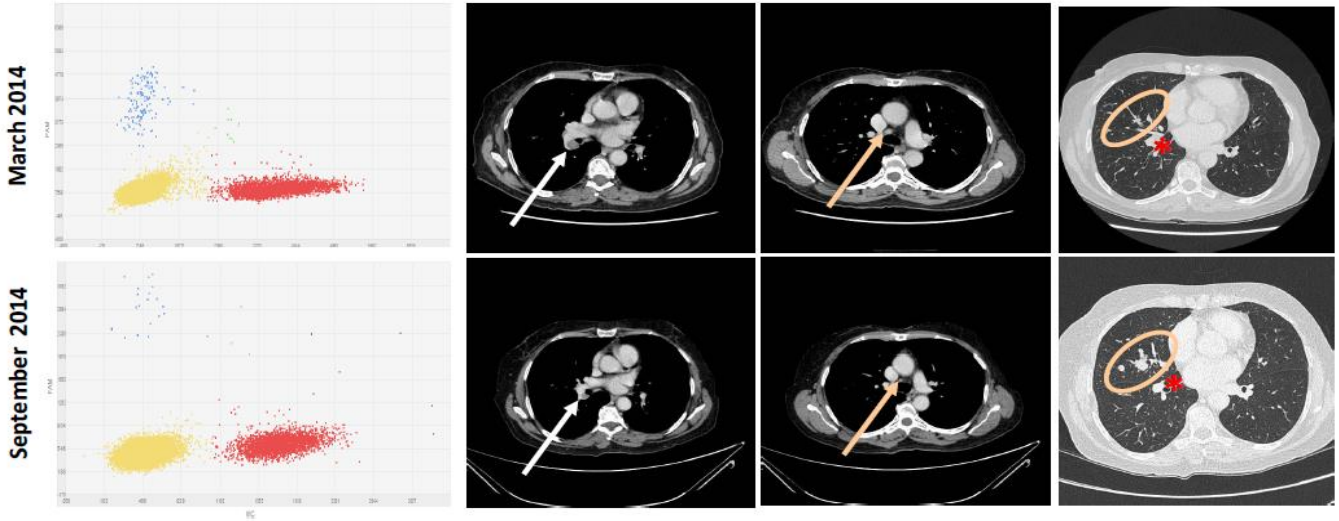
García-Saenz et al. BMC Cancer (2017) 17:210
DOI 10.1186/s12885-017-3185-9

BMC Cancer

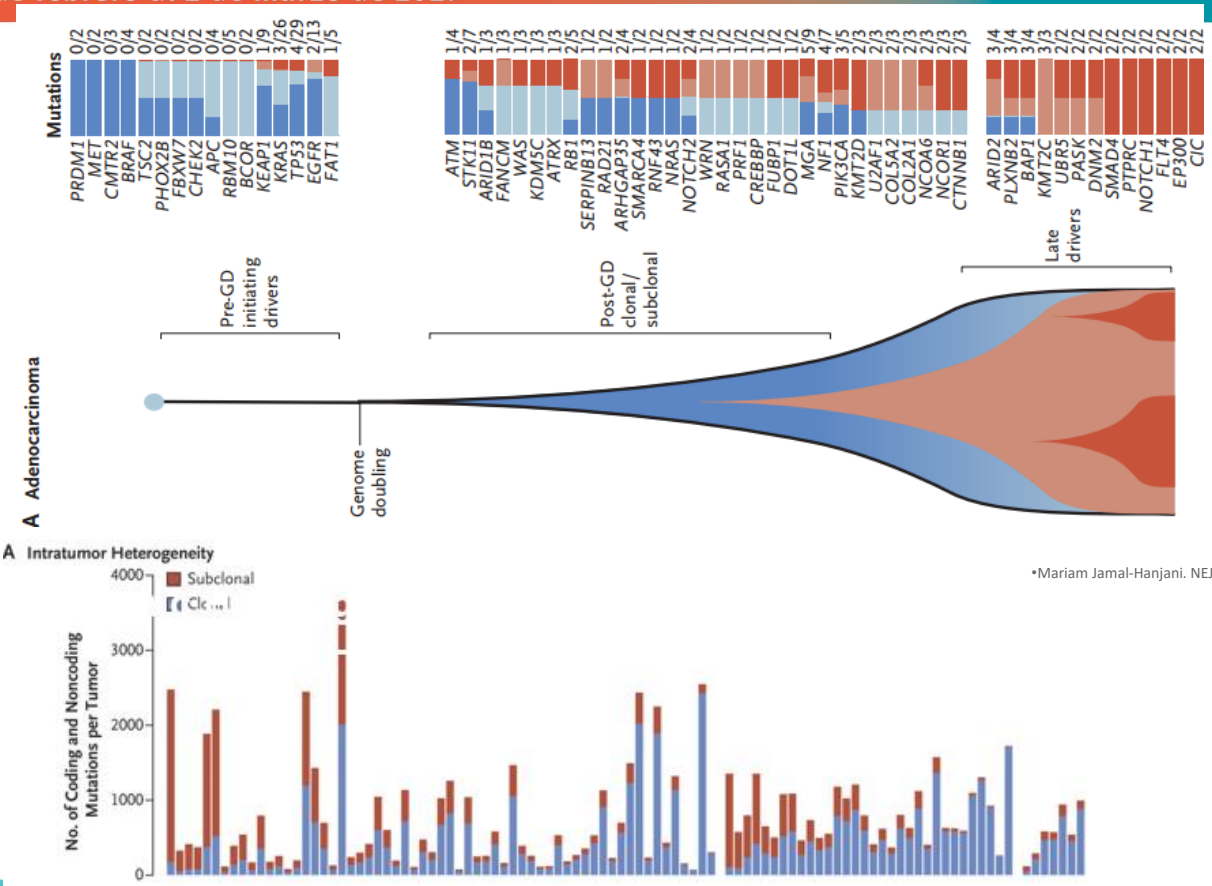
RESEARCH ARTICLE Open Access

Tumor burden monitoring using cell-free tumor DNA could be limited by tumor heterogeneity in advanced breast cancer and should be evaluated together with radiographic imaging

José Angel García-Saenz[1], Patricia Ayllón[1], Marion Laig[2], Daniel Acosta-Eyzaguirre[3], Marta García-Esquinas[4,5], Myriam Montes[4], Julián Sanz[6], Miguel Barquín[7], Fernando Moreno[1], Vanesa García-Barberan[1], Eduardo Díaz-Rubio[1], Trinidad Caldes[1*] and Atocha Romero[1,7*]



García-Sáenz JA et al. BMC Cancer 2017;17(1):210



•Mariam Jamal-Hanjani. NEJM 2017

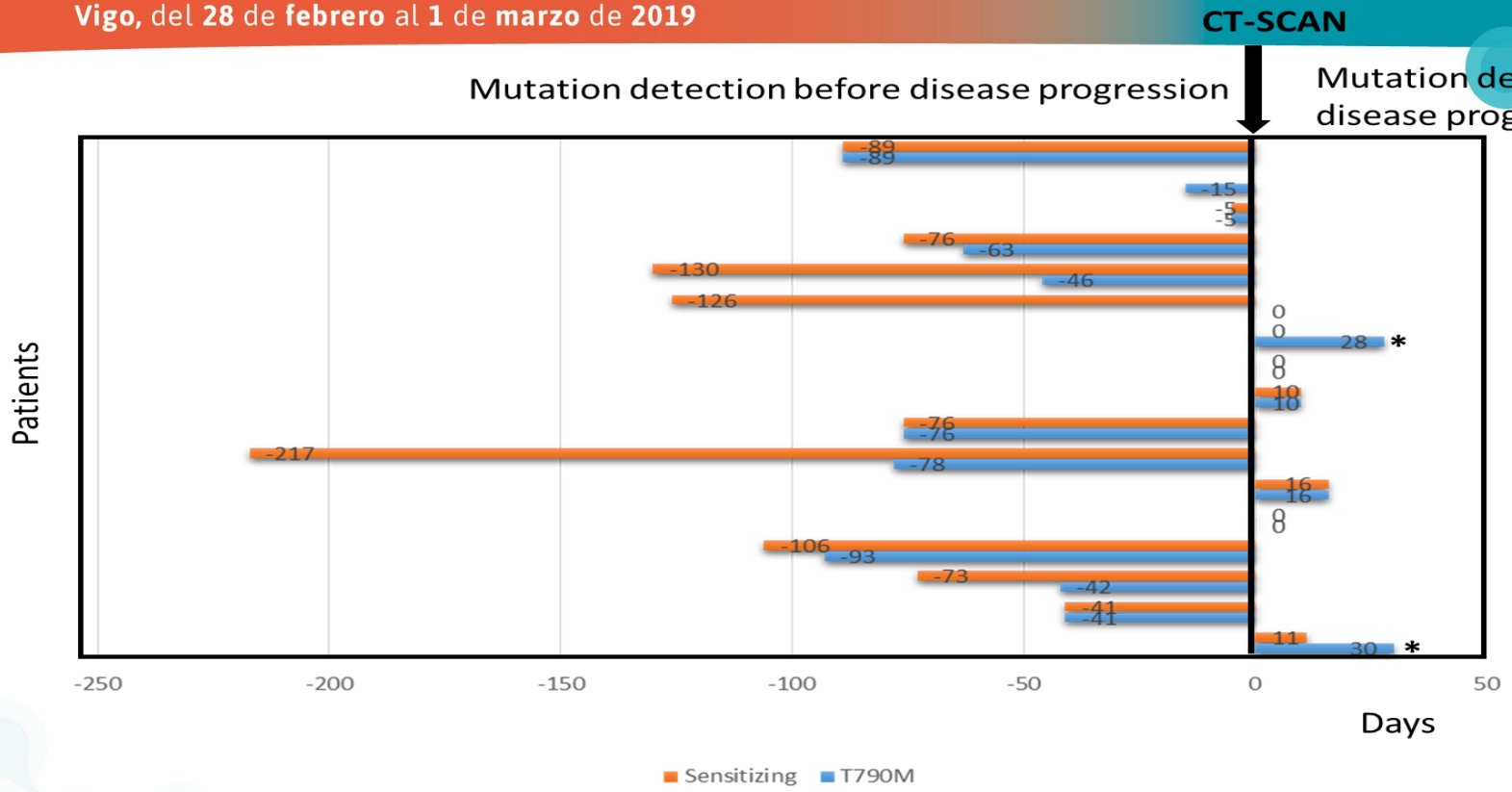
Original Article

Monitoring of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor-Sensitizing and Resistance Mutations in the Plasma DNA of Patients With Advanced Non–Small Cell Lung Cancer During Treatment With Erlotinib

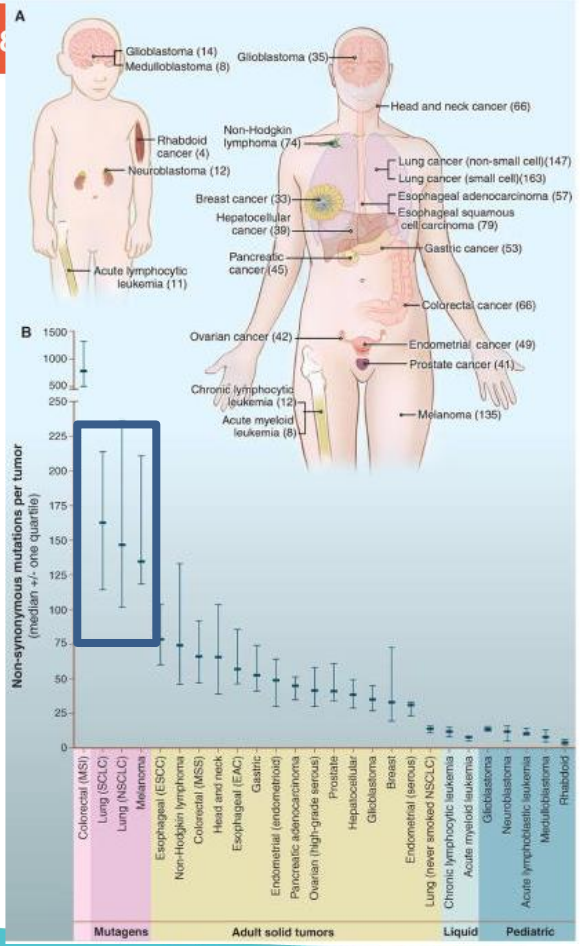
Boe S. Sorensen, MS, PhD[1]; Lin Wu, MS, PhD[2]; Wen Wei, MS, PhD[2]; Julie Tsai, BS[2]; Britta Weber, MD, PhD[1,3]; Ebba Nexo, MD, PhD[1]; and Peter Meldgaard, MS, PhD[3]

BACKGROUND: The feasibility of monitoring epidermal growth factor receptor (EGFR) mutations in plasma DNA from patients with advanced non–small cell lung cancer (NSCLC) during treatment with erlotinib and its relation to disease progression was investigated. **METHODS:** The amount of EGFR-mutant DNA was tested in plasma DNA from patients with advanced NSCLC with allele-specific polymerase chain reaction assays. Blood samples from 23 patients with adenocarcinoma of NSCLC that carried tyrosine kinase inhibitor-sensitizing EGFR mutations were taken immediately before treatment with erlotinib. Additional blood samples were taken at timed intervals until erlotinib treatment was withdrawn. **RESULTS:** The amount of plasma DNA with sensitizing EGFR mutations was found to be reduced after the first cycle of erlotinib treatment in 22 of 23 patients (96%). No patients presented with the resistant T790M mutation in the pretreatment sample, but at the time of disease progression the mutation was detected in plasma from 9 patients (39%). The quantitative data from the current study demonstrated that when a T790M mutation emerged in the blood it was accompanied by an increase in the original sensitizing EGFR mutation. When T790M was detected, it was found to be present in all subsequent blood samples from that patient. Most interestingly, the results of the current study demonstrated that monitoring the EGFR mutations in the blood allows for the detection of the T790M mutation up to 344 days before disease progression is clinically evident (range, 15-344 days). **CONCLUSIONS:** The results of the current study demonstrated that serial monitoring of EGFR mutations in plasma DNA is feasible and may allow for the early detection of resistance mutations. These results warrant further studies to explore the clinical usefulness of such analysis. ***Cancer* 2014;120:3896-901.** © 2014 The Authors. *Cancer published by American Cancer Society.* This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

KEYWORDS: epidermal growth factor receptor (EGFR) mutations, plasma DNA, erlotinib, lung cancer, resistance.

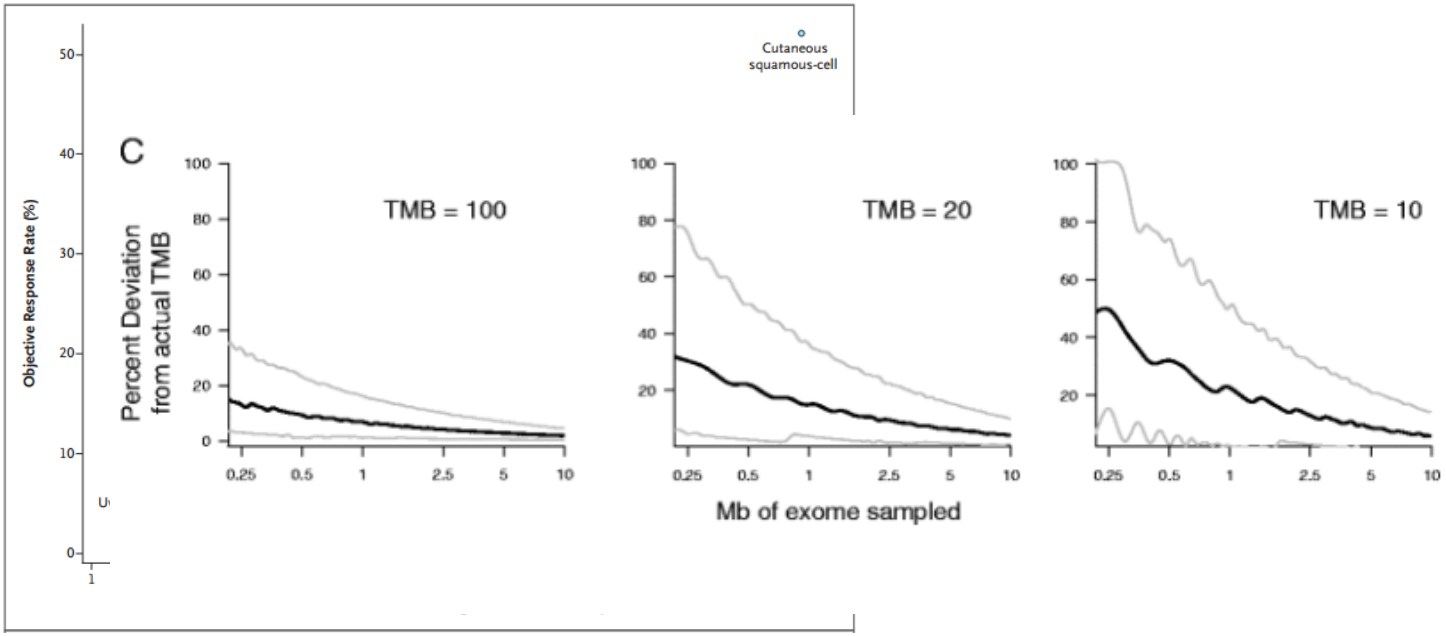


Provencio M , et al . Oncotarget. 2017 Nov 16;9(1):488-494



Vogelstein B et al. Cancer genome landscapes Science. 2013 Mar 29;339(6127):1546-58.

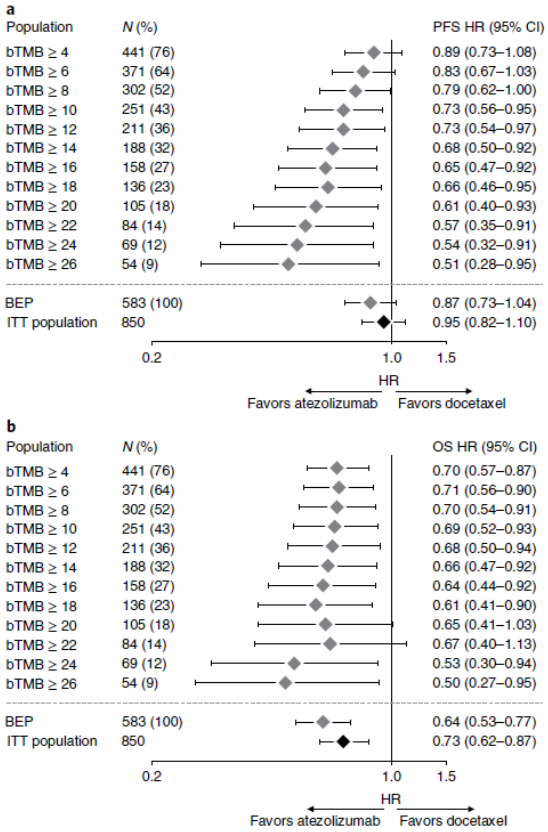
Chalmers ZR et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden Genome Med. 2017;9:34..



1. Yarchoan M et al. *N Engl J Med*. 2017;377:2500-2501.

Blood-based tumor mutational burden as a predictor of clinical benefit in non-small-cell lung cancer patients treated with atezolizumab

nature medicine



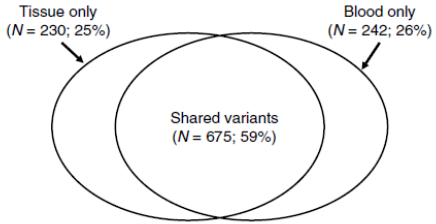
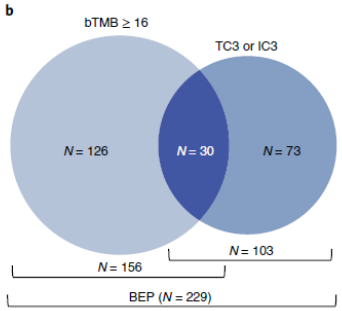


Table 1 | OS and PFS HRs in the OAK BEP with valid bTMB and PD-L1 IHC results

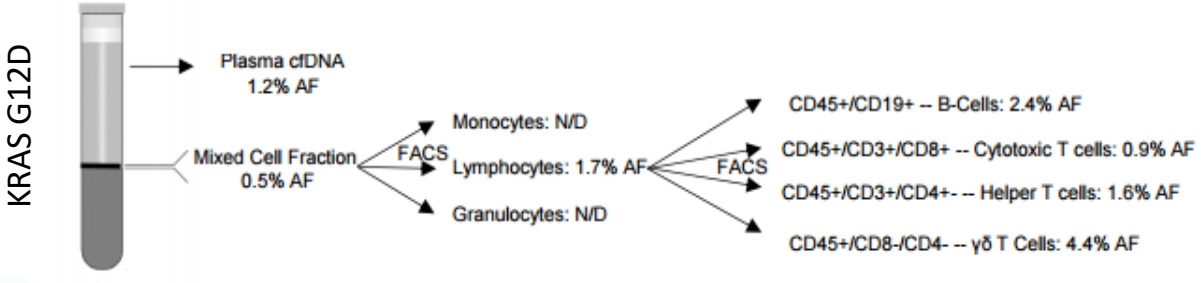
	N	PFS HR (95% CI)	OS HR (95% CI)
bTMB ≥ 16	156	0.64 (0.46–0.91)	0.64 (0.44–0.93)
TC3 or IC3	103	0.62 (0.41–0.93)	0.44 (0.27–0.71)
bTMB ≥ 16 and TC3 or IC3	30	0.38 (0.17–0.85)	0.23 (0.09–0.58)

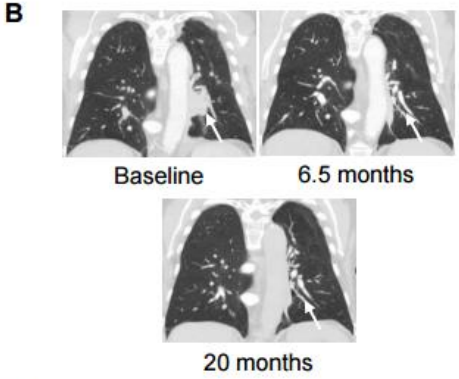
N represents the number of patients in each subgroup. TC3 or IC3, ≥50% of tumor cells or ≥10% of tumor-infiltrating immune cells expressing PD-L1.



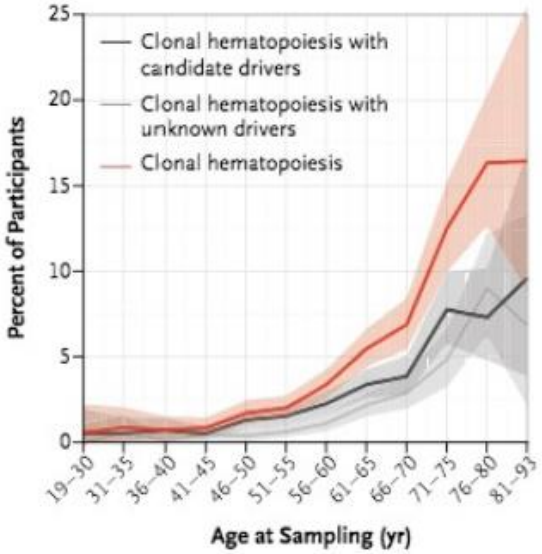
Source of bias. Clonal haematopoiesis

- CHIP is defined by the presence of somatic mutation in blood or BM b without other diagnostic criteria for a haematological malignancy.
- More frequent in aged patients, patients with solid tumours
- More likely to be detected with deeper sequencing approaches.
- May account for false positive ctDNA sequencing results.



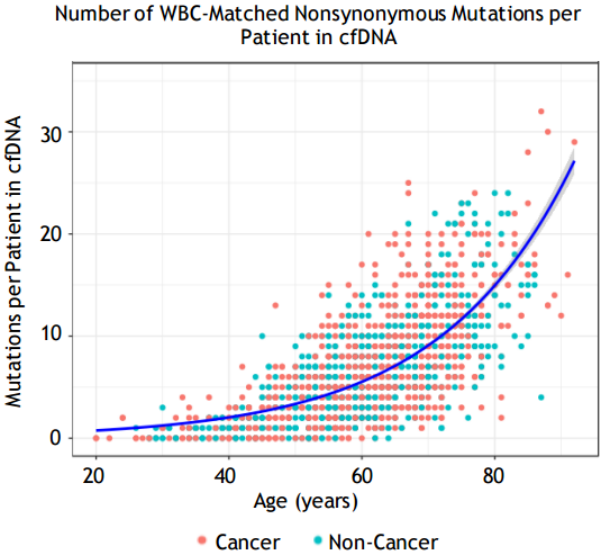


Hu Y et al. False-Positive Plasma Genotyping Due to Clonal Hematopoiesis.Clin Cancer Res. 2018 Mar 22.

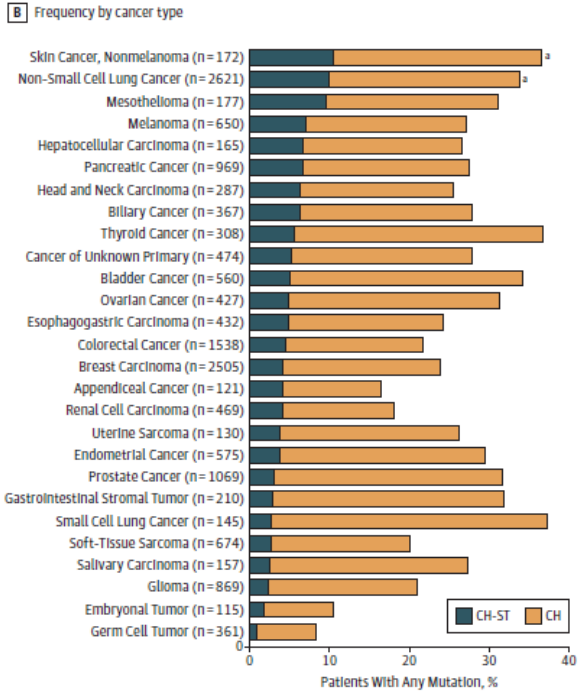


Genovese G., *et al.* 2014 NEJM

The Circulating Cell-free Genome Atlas Study (CCGA)



Charles Swanton et al. Journal of Clinical Oncology 36, no. 15_suppl (May 20 2018) 12003-12003.



Ptashkin RN et al JAMA Oncol. 2018 Jun 5.

Thank you!!

LiquidBiopsyLabPdH (@LiquidBiopsyLab)

Unión Europea
Fondo Europeo de Desarrollo Regional

AstraZeneca

Fundación JL Castaño SEQC

Plataforma Diagnóstica
1DENTIFY EGFR M+
de AstraZeneca Oncology España

Boehringer Ingelheim

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

isc Instituto de Salud Carlos III

BIG Medilytics

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Big Data for Precision Medicine