THE ROLE OF LIQUID BIOPSY IN MONITORING THE RESPONSE TO TREATMENT

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ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI-IRCCS-FONDAZIONE G. Pascale – NAPOLI
SC Biologia Cellulare e Bioterapie

DISCLOSURE SLIDE

- **Personal financial interests (speaker's fee and/or advisory boards)**: MSD, Qiagen, Biocartis, Incyte, Roche, BMS, MERCK, Thermofisher, Boehringer Ingelheim, Astrazeneca, Sanofi, Eli Lilly, Bayer, ArcherDX, Illumina

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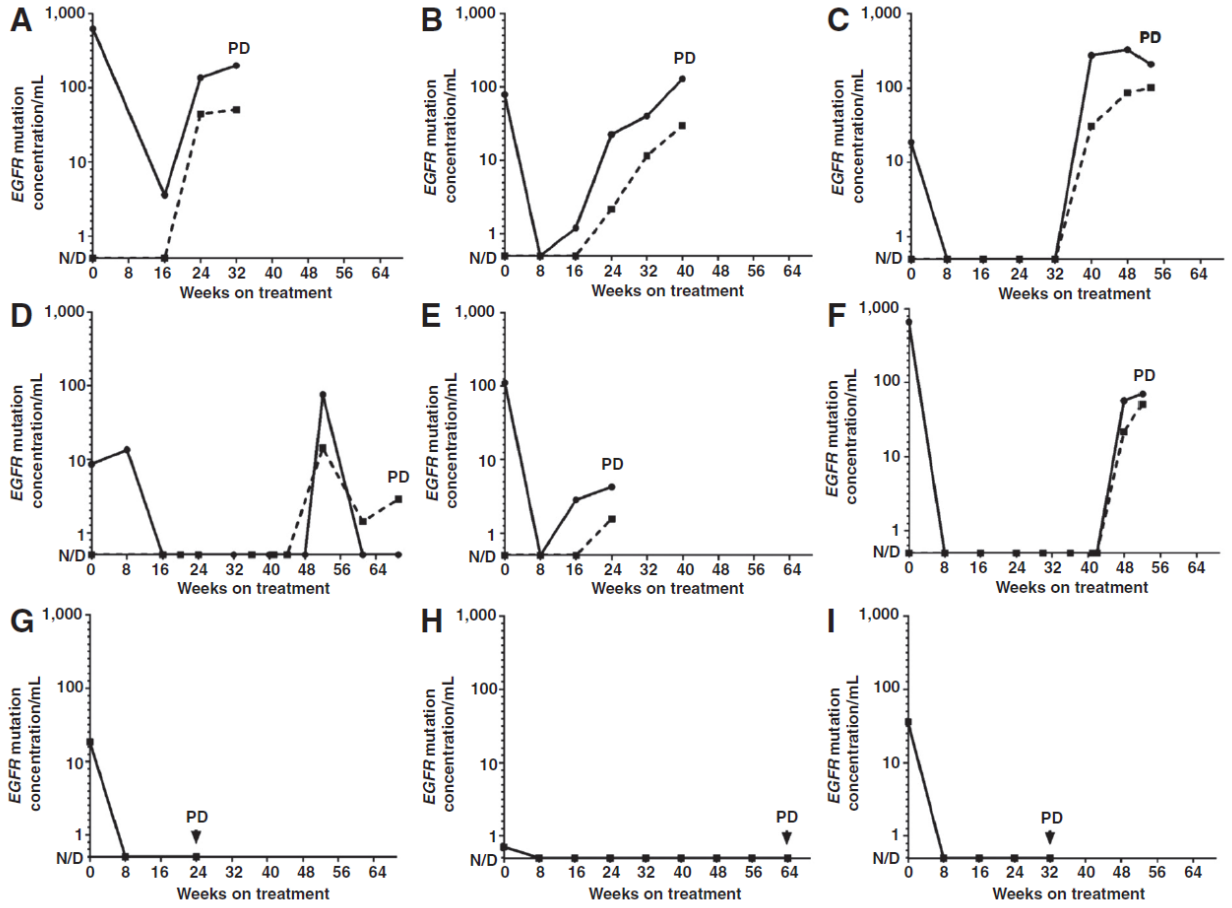
APPLICATIONS OF cfDNA TESTING IN MONITORING

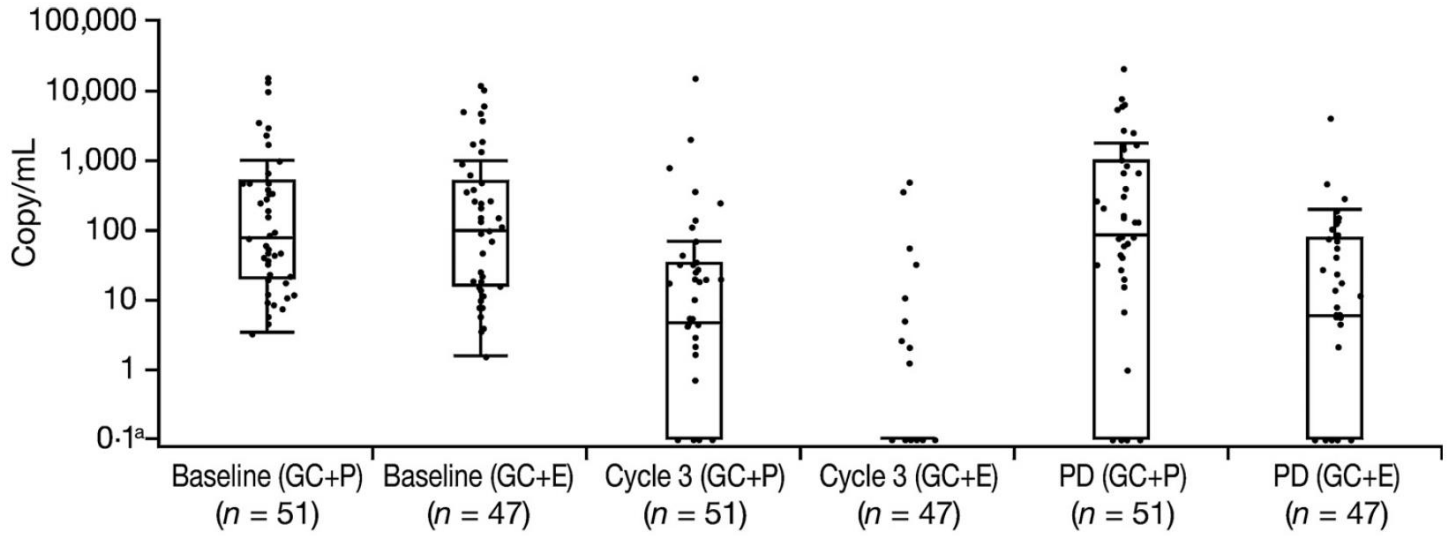
ctDNA as marker of response to therapy

Plasma EGFR mutations during treatment with EGFR TKIs



Oxnard Clin. Cancer. Res. 2014

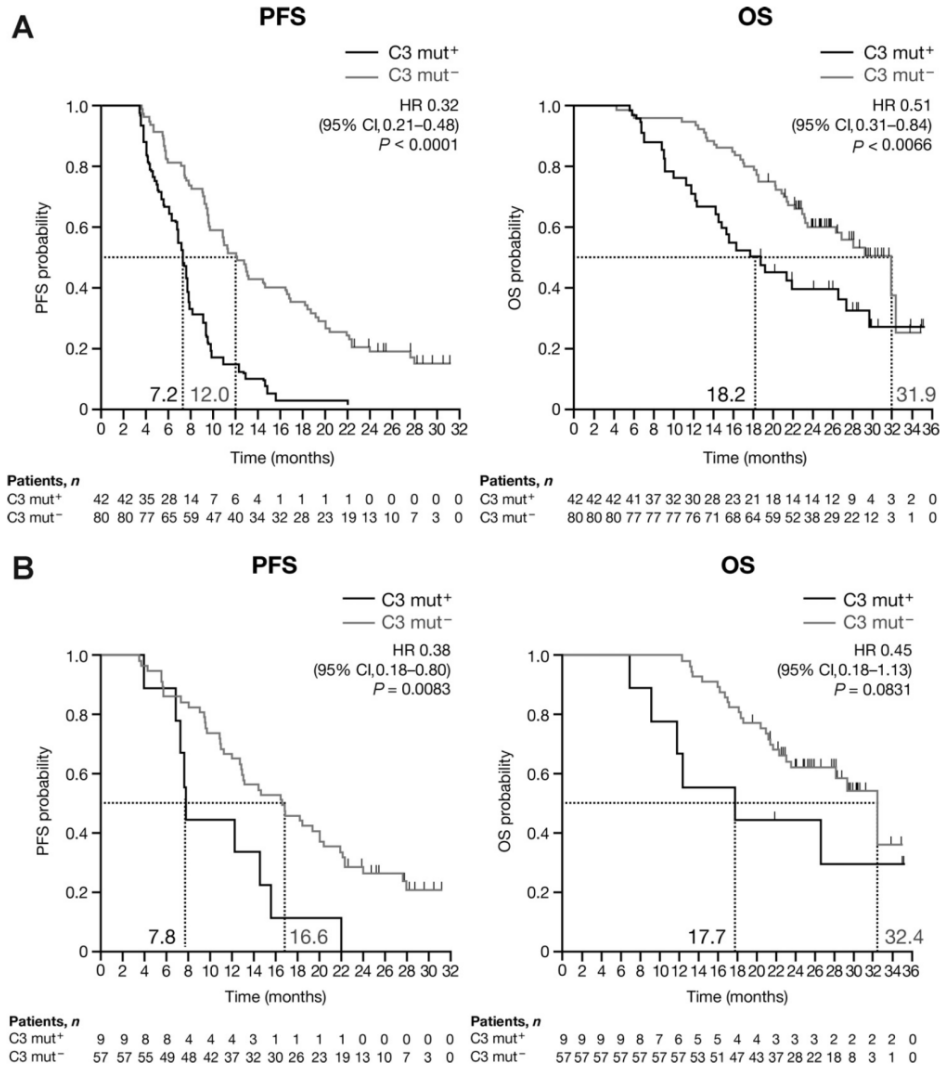
Dynamic quantitative change in EGFR mutant ctDNA in the FASTACT2 trial



ctDNA, cell free DNA

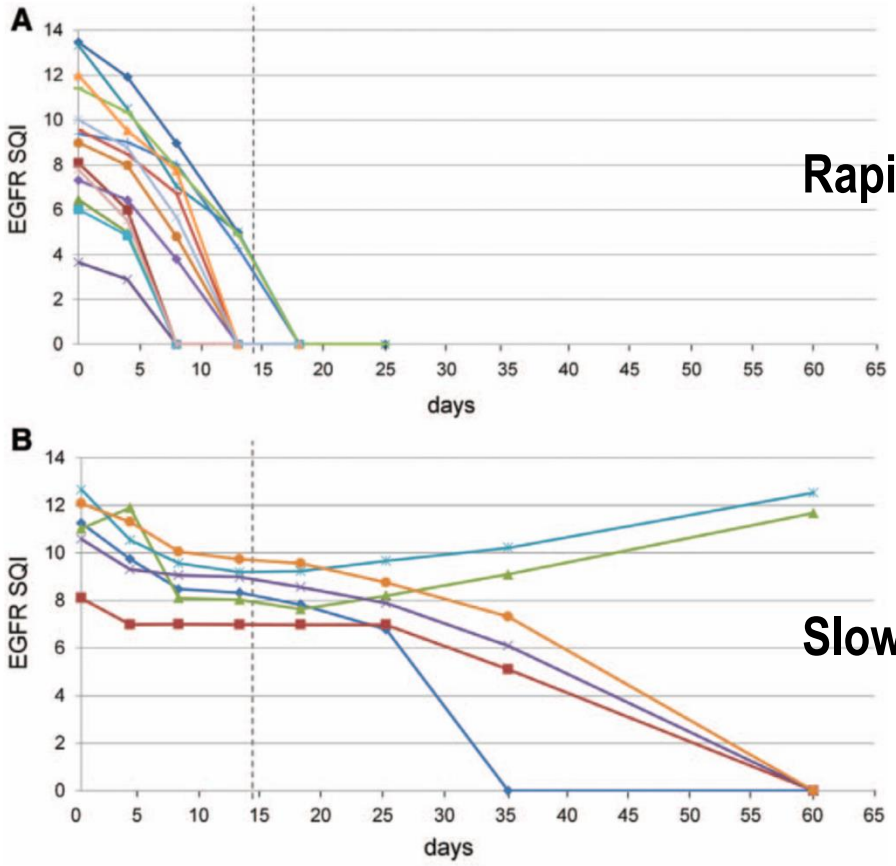
Mok *Clin. Cancer Res.* 2015

PFS and OS for baseline cfDNA mutant patients stratified by C3 ctDNA EGFR status



Mok *Clin. Cancer Res.* 2015

Clearance of EGFR mutations correlates with tumor shrinkage

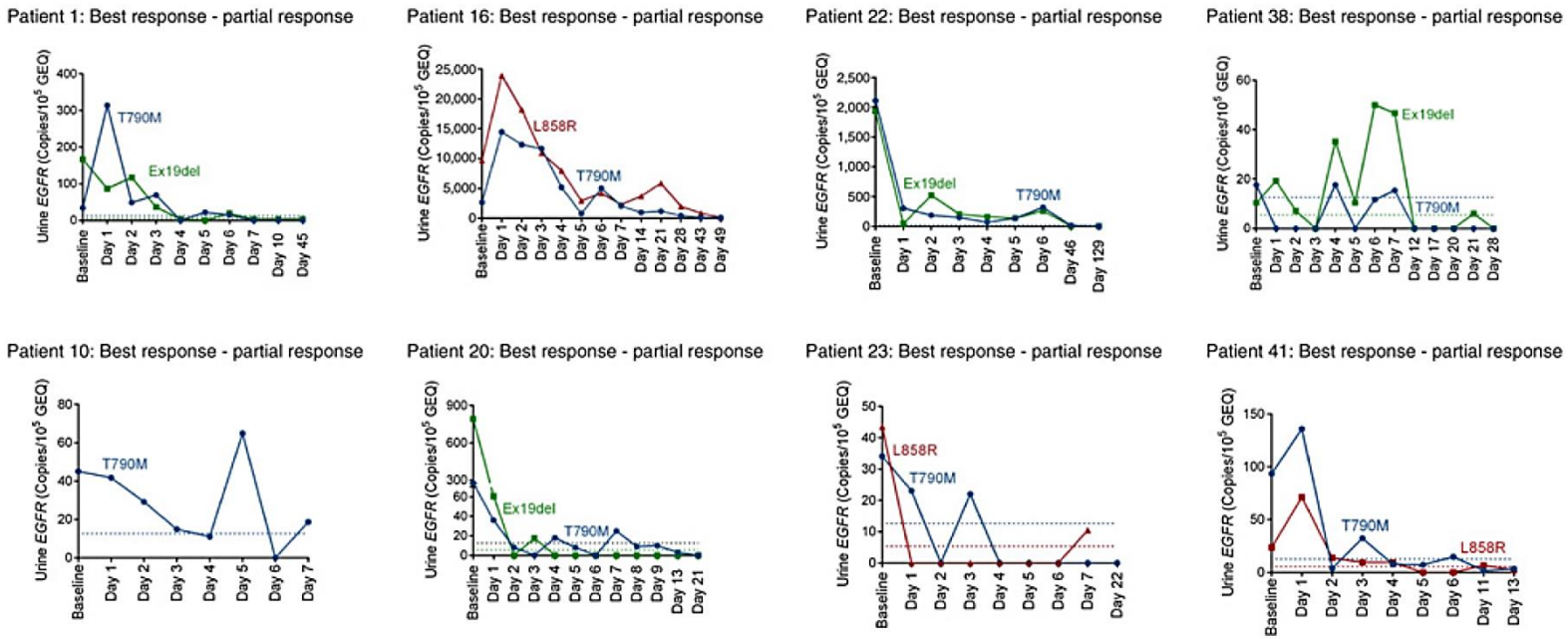


Rapid responders

Slow responders

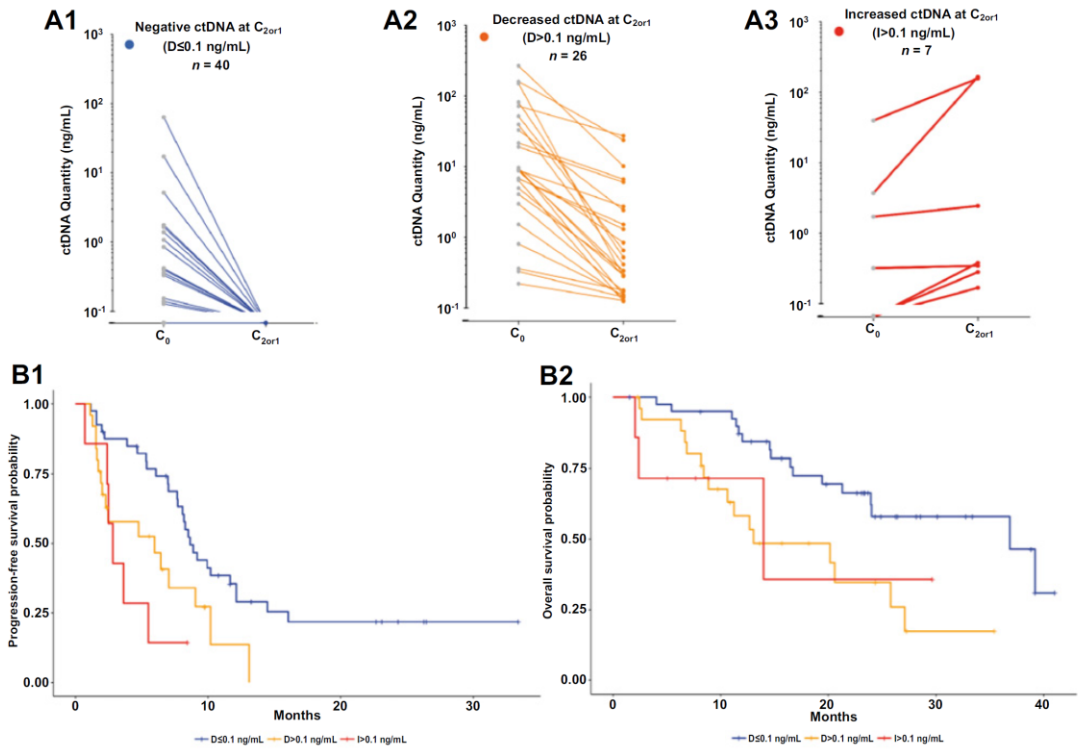
Marchetti JTO 2015

Daily dynamics of ctDNA EGFR mutation levels in urine on second-line osimertinib

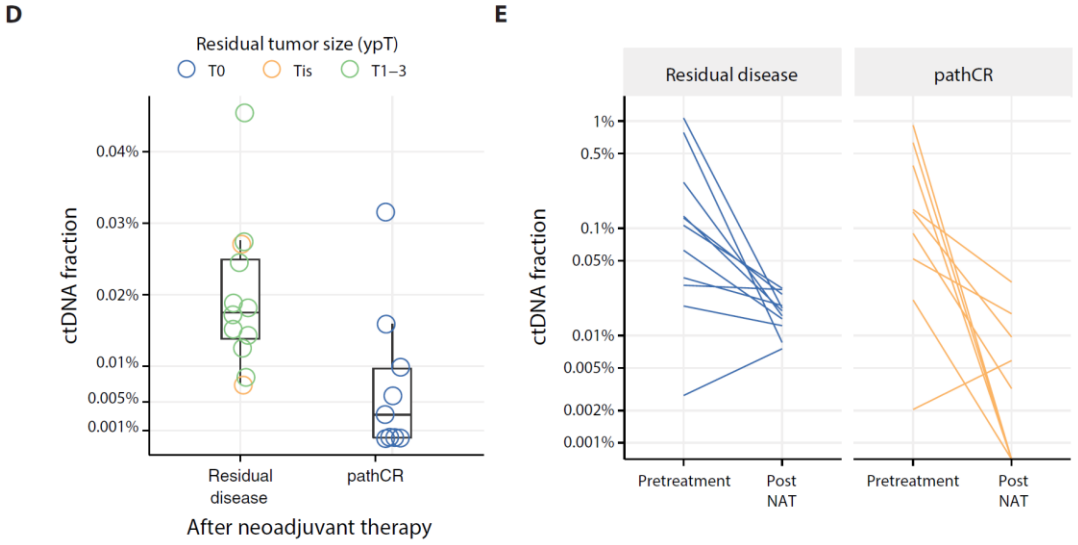


Husain Cancer Res 2017

Correlation between changes in ctDNA and response to chemotherapy

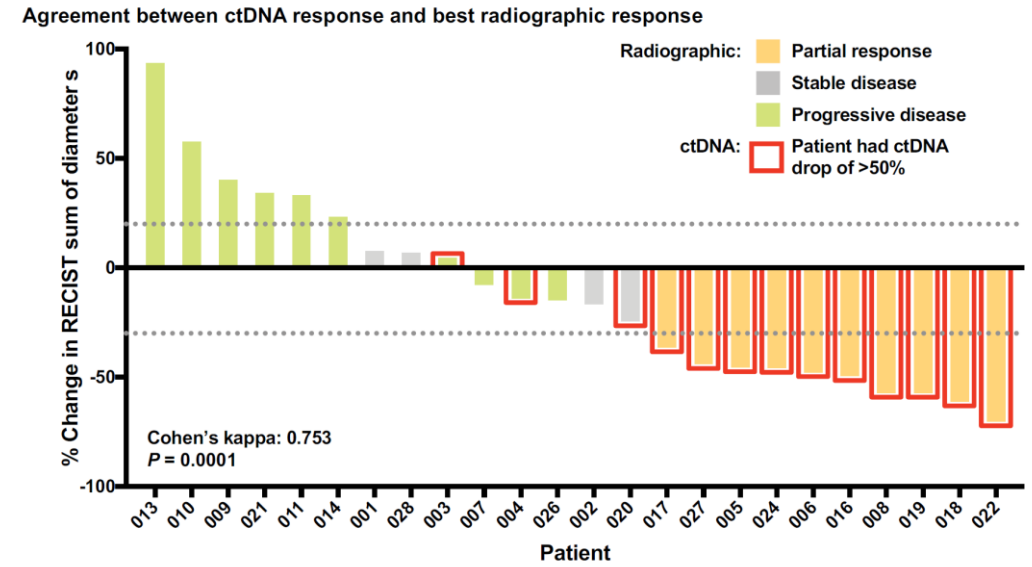


Garlan Clin Cancer Res 2017

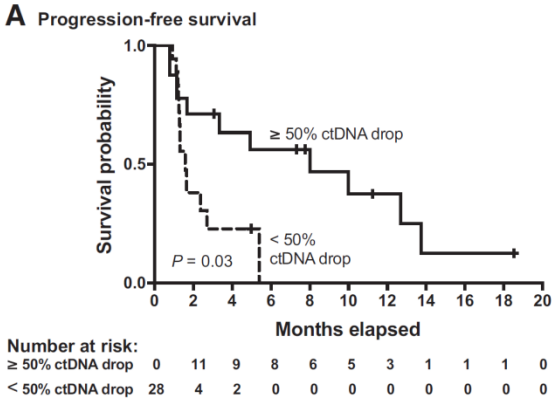


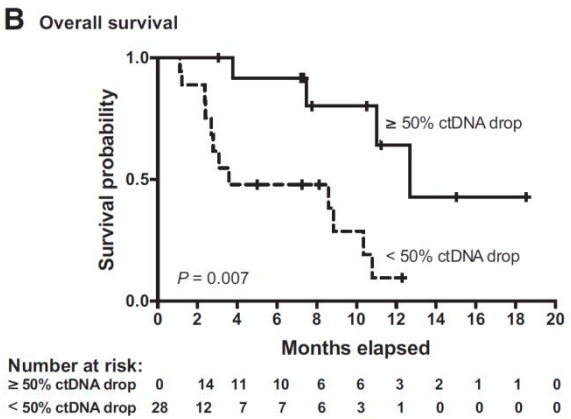
McDonald Sci Transl Med 2019

Assessment of Lung Cancer Immunotherapy Response via ctDNA



Median time to initial response among patients who achieved responses was 24.5 days by ctDNA versus 72.5 days by imaging.

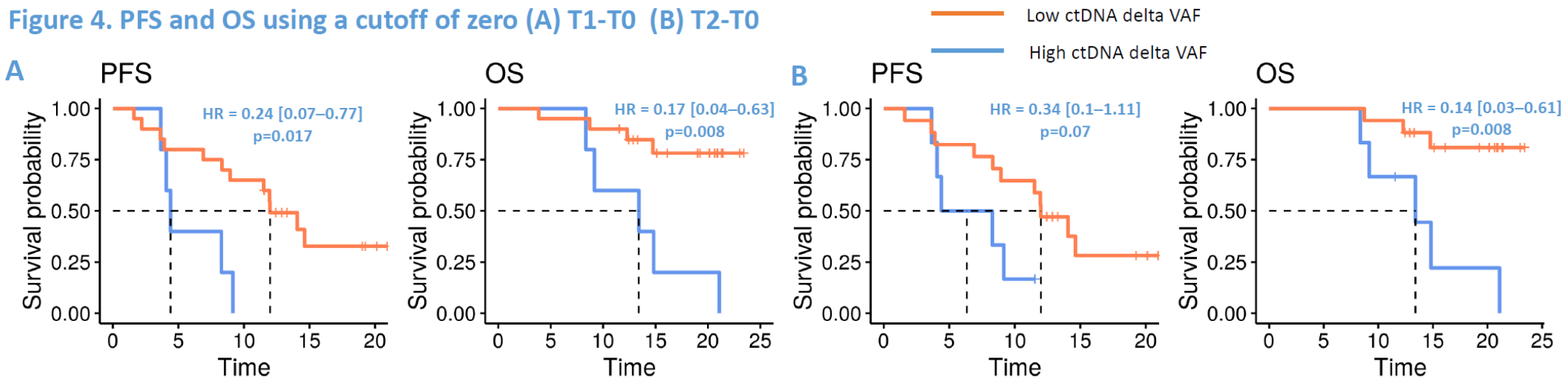




Goldberg CCR 2018

Monitoring cfDNA in NSCLC pts treated with pembrolizumab monotherapy

Figure 4. PFS and OS using a cutoff of zero (A) T1-T0 (B) T2-T0



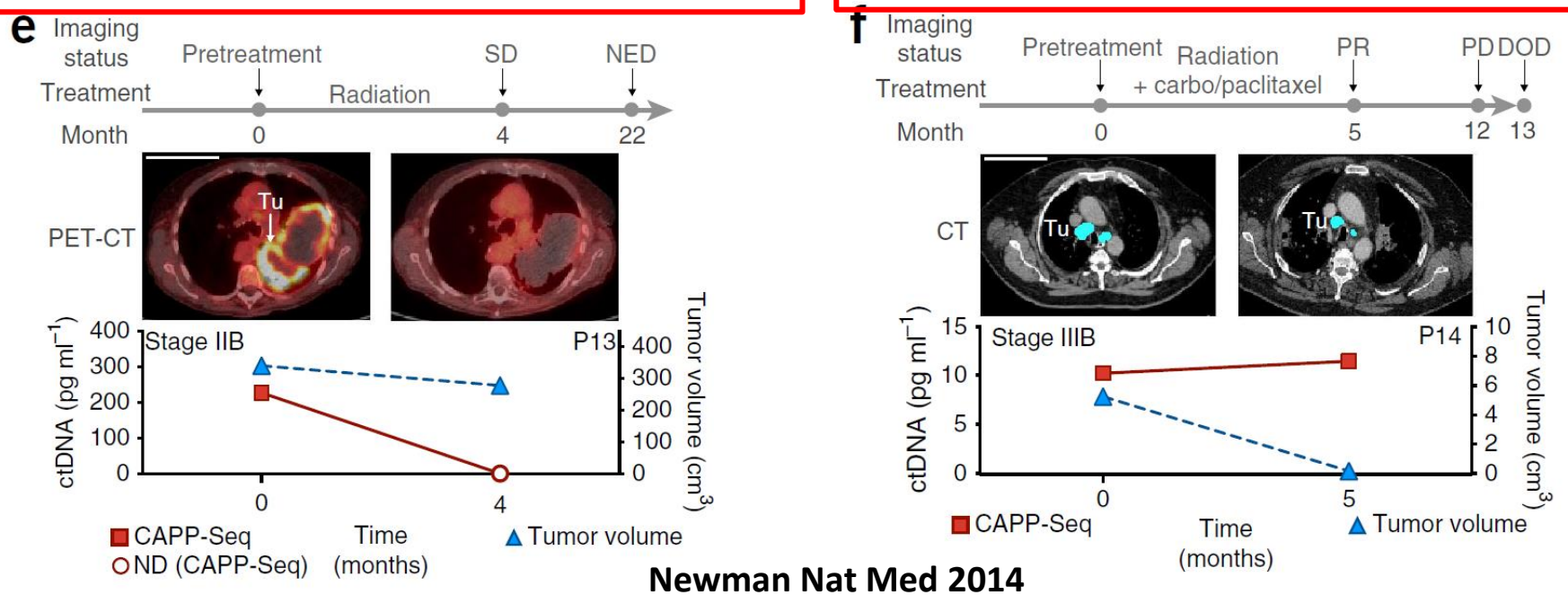
T0: baseline; T1: 9 weeks; T2: 18 weeks

Aggarwal ASCO 2019

ctDNA analysis and outcome in lung cancer patients receiving RT

P13 was treated with RT for stage IIB NSCLC. Follow-up imaging showed a large mass that was interpreted to represent residual disease. However, ctDNA at the same time point was undetectable, and the patient remained disease free 22 months later.

P14 was treated with Chemo RT for stage IIIB NSCLC, and follow-up imaging revealed a near-complete response. However, the ctDNA concentration slightly increased following therapy, suggesting progression of occult microscopic disease. Indeed, clinical progression was detected 7 months later.

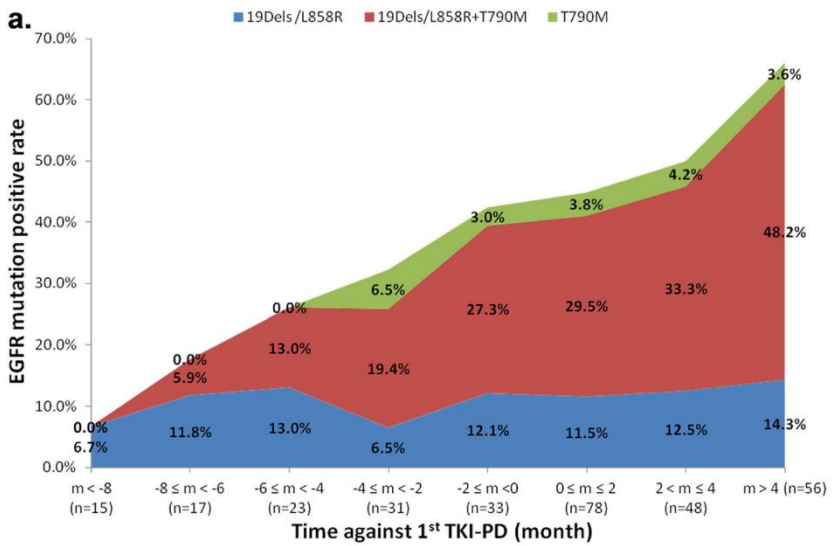


Newman Nat Med 2014

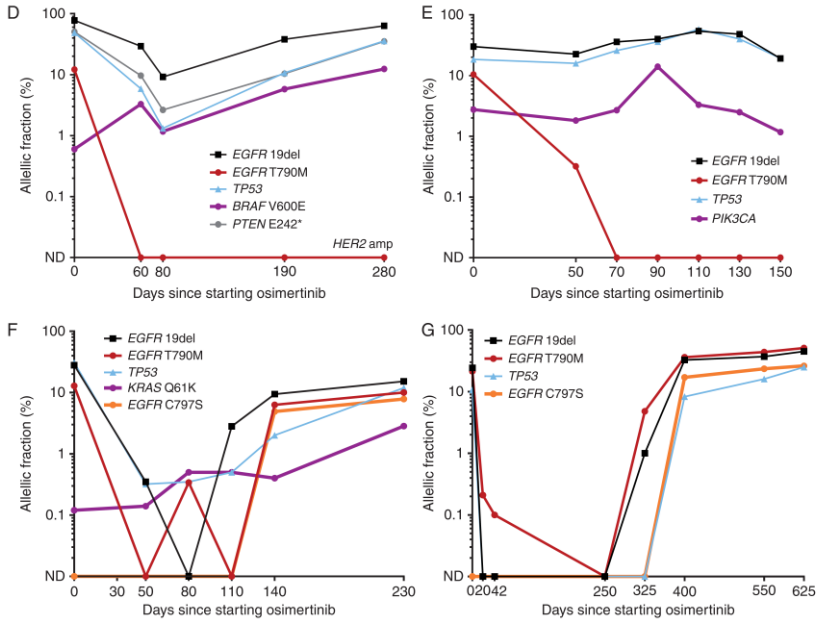
APPLICATIONS OF cfDNA TESTING IN MONITORING

ctDNA as early marker of progession

Dynamic detection of EGFR mutant ctDNA and resistance mechanisms in plasma



Zheng Sci Rep 2016

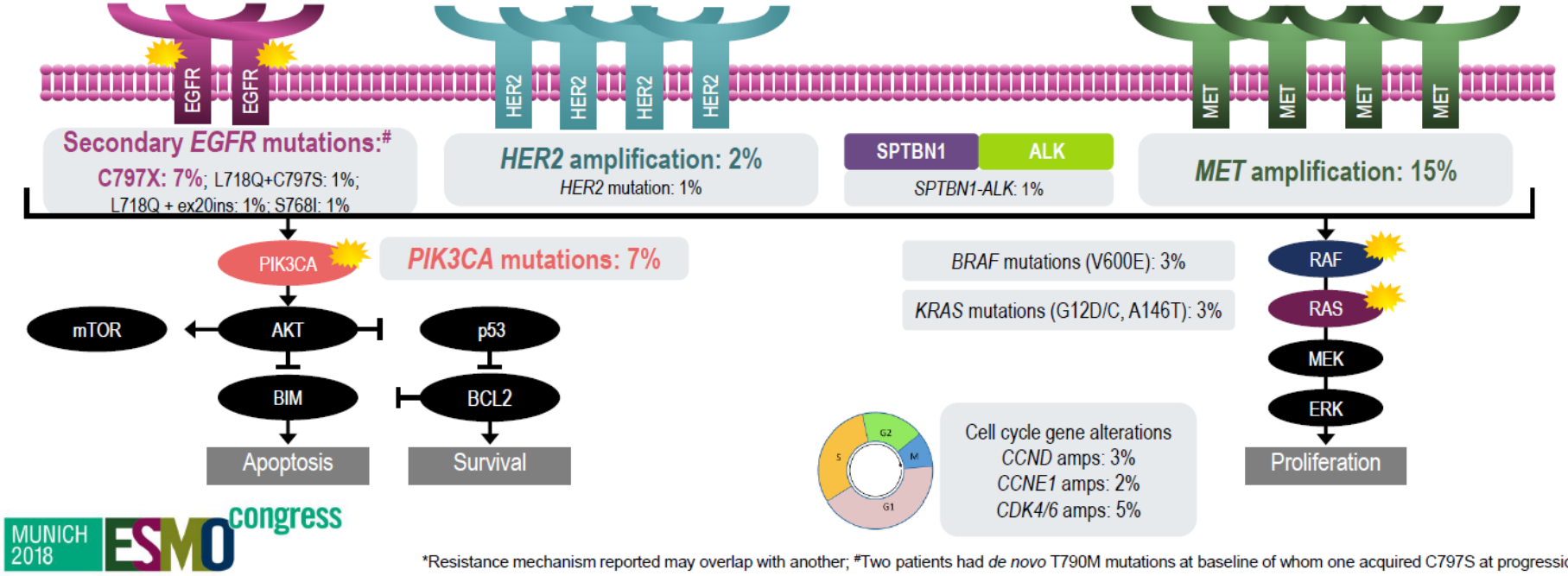


Guibert Ann Oncol 2018

FLAURA trial: first line osimertinib

RESULTS: CANDIDATE ACQUIRED RESISTANCE MECHANISMS WITH OSIMERTINIB (n=91)*

- No evidence of acquired EGFR T790M
- The most common resistance mechanisms were *MET* amplification and EGFR C797S mutation
  - Other mechanisms included *HER2* amplification, *PIK3CA* and *RAS* mutations



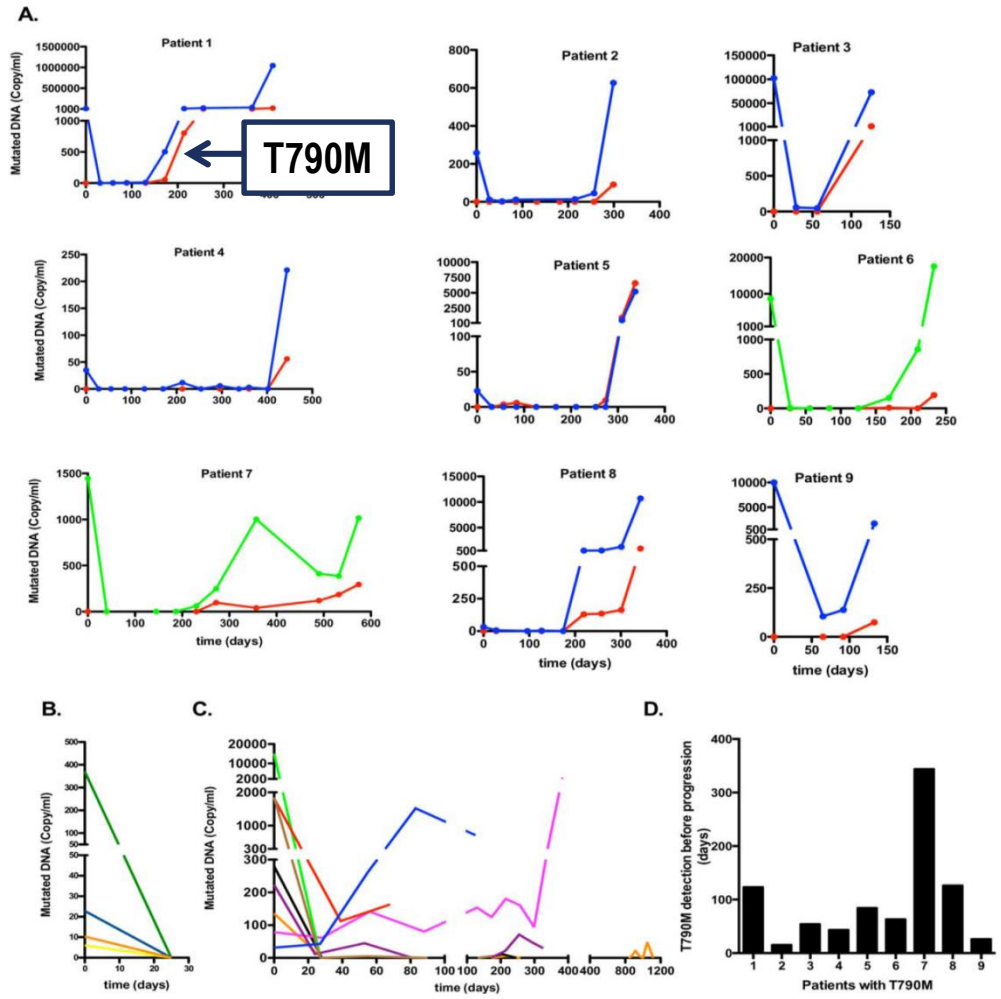
MUNICH 2018 ESMO congress

*Resistance mechanism reported may overlap with another; #Two patients had *de novo* T790M mutations at baseline of whom one acquired C797S at progression

Ramalingam ESMO 2018

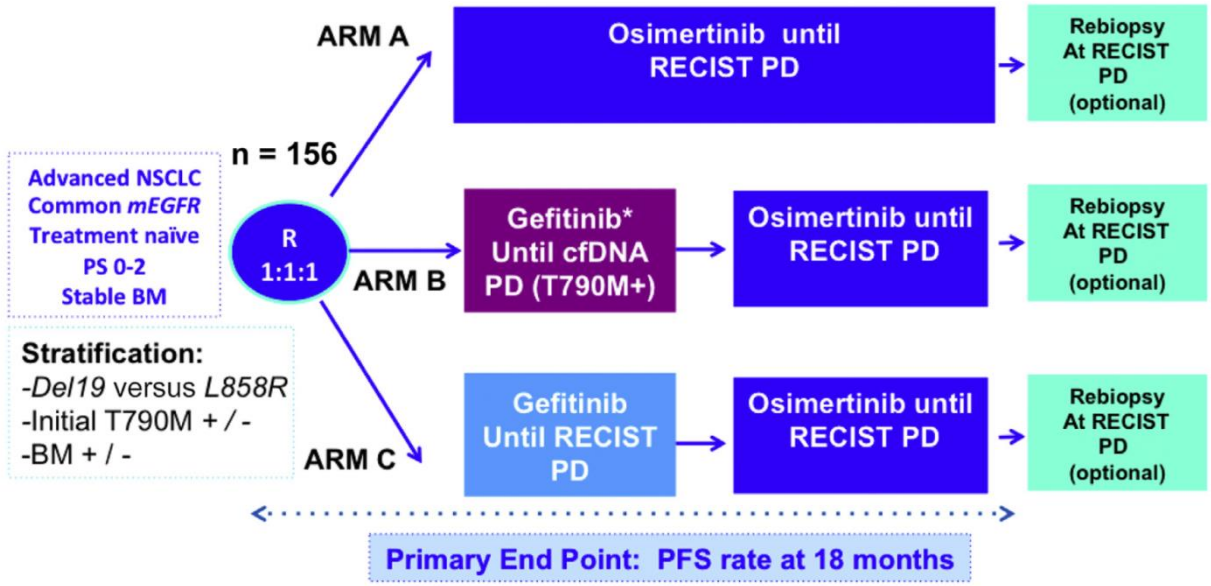
ESMO

Plasma EGFR mutations during treatment with EGFR TKIs



Sorensen *Cancer* 2014

The APPLE Trial: Feasibility and Activity of Osimertinib Treatment on Positive PLasma T790M in EGFR-mutant NSCLC pts

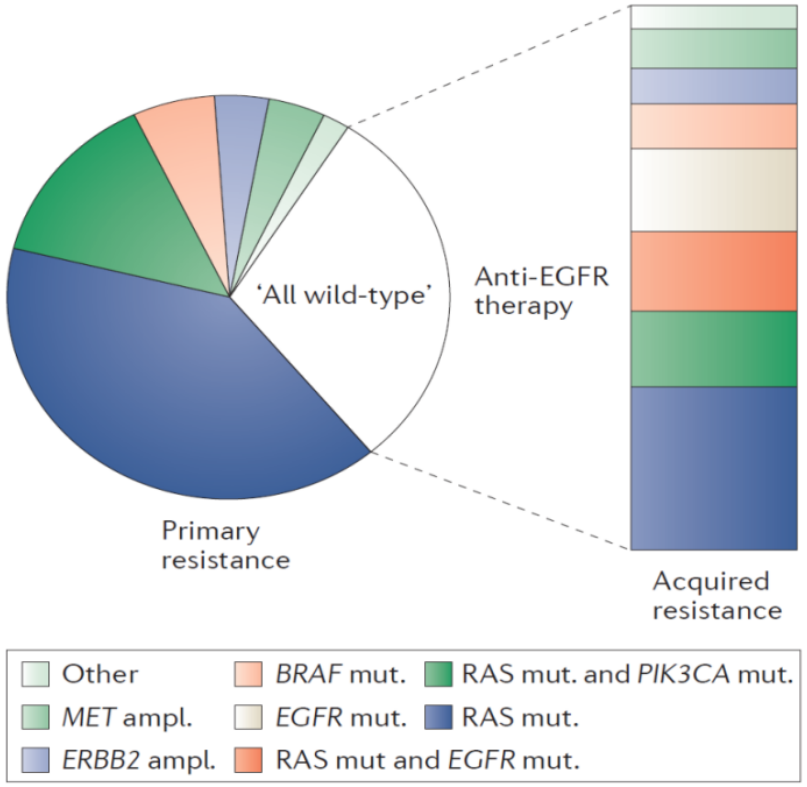


(cfDNA using cobas every 4 weeks and CT scan of the brain-thorax-abdomen every 8 weeks all arms

*In case of RECIST progression without T790M+, patients will be switched

Remon Clin Lung Cancer 2017

Genomic landscape before and after anti-EGFR therapy in mCRC pts



Dienstmann Nat Rev Cancer 2017

Resistance RAS mutations in mCRC according to liquid biopsy

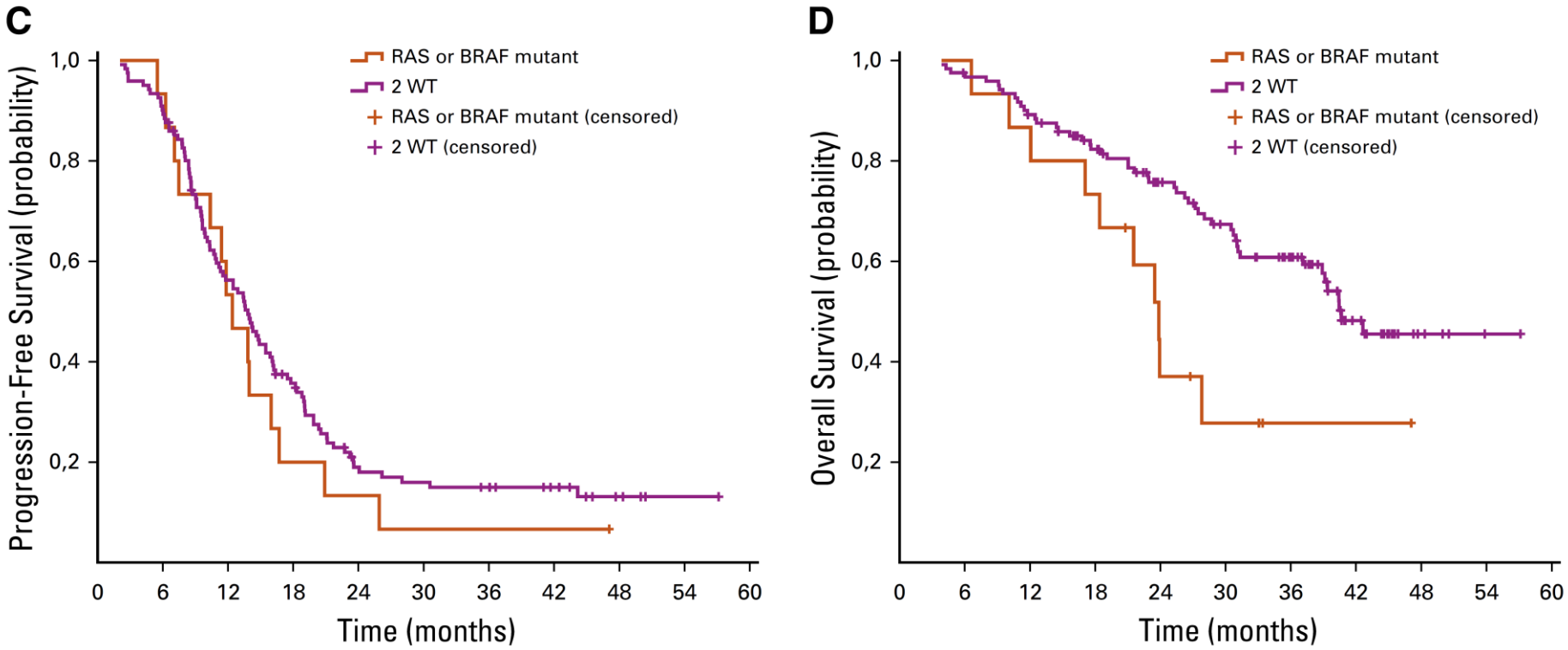
Method	RAS mutant at progression	
	n/N	%
PCR Ligation/BEAMing [103]	9/24*	37.5
NGS/BEAMing [104]	2/3*	66.6
PCR Ligation/BEAMing/SafeSeqS [11]	23/24	95.8
BEAMing [105]	2/4	50.0
ddPCR [106]	11/16	68.8
BEAMing [107]	27/62*	43.5
BEAMing [81]	41/86*	48
NGS (Plasma Select) [108]	53/164	32.3

PCR, polymerase chain reaction; BEAMing, beads, emulsion, amplification and magnetics; NGS, next-generation sequencing; SafeSeqS, Safe-Sequencing System; ddPCR, droplet digital PCR.

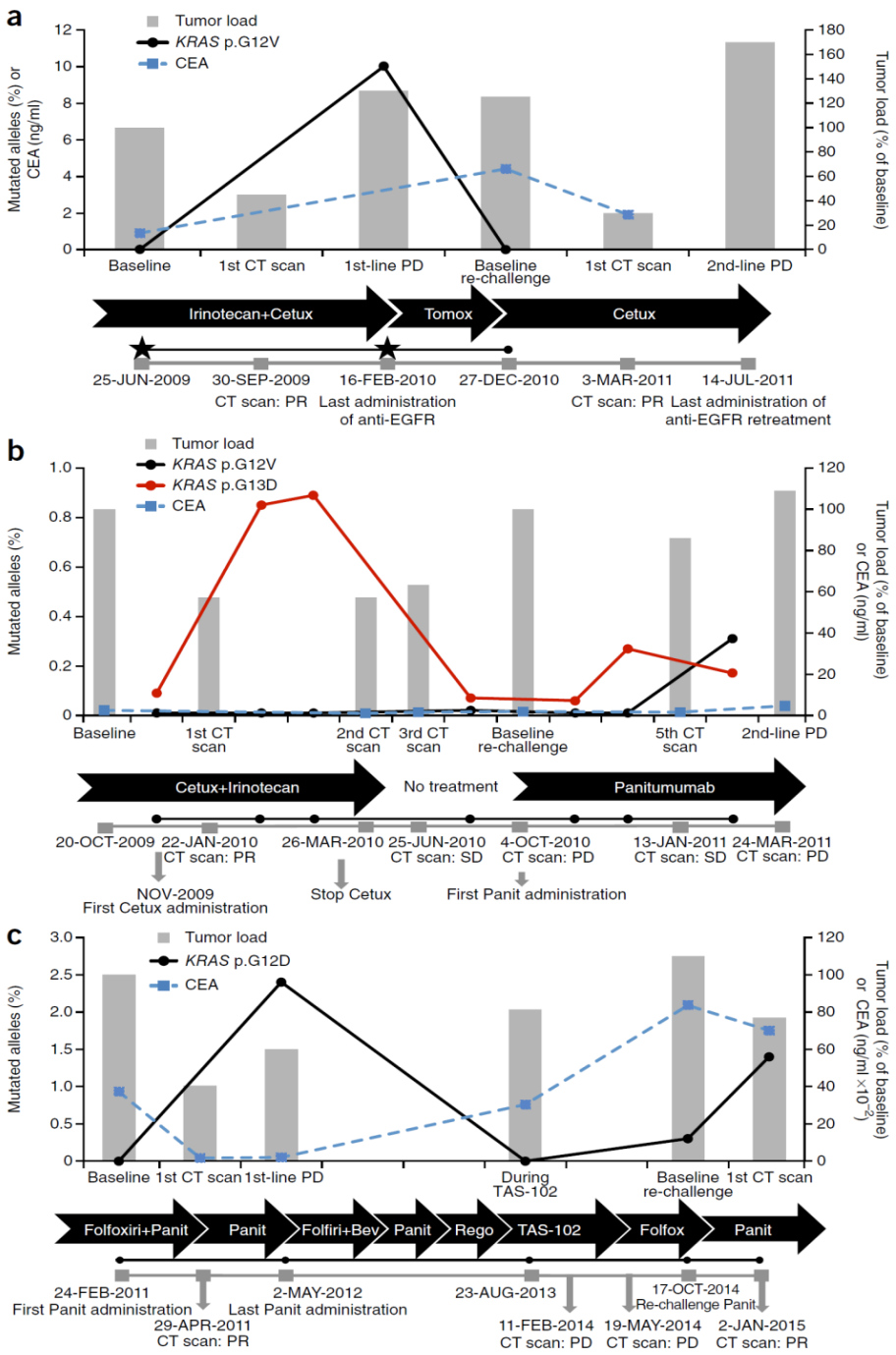
* Only KRAS.

Normanno CTR 2018

PFS and OS of mCRC patients with or without emergence of RAS/BRAF mutations



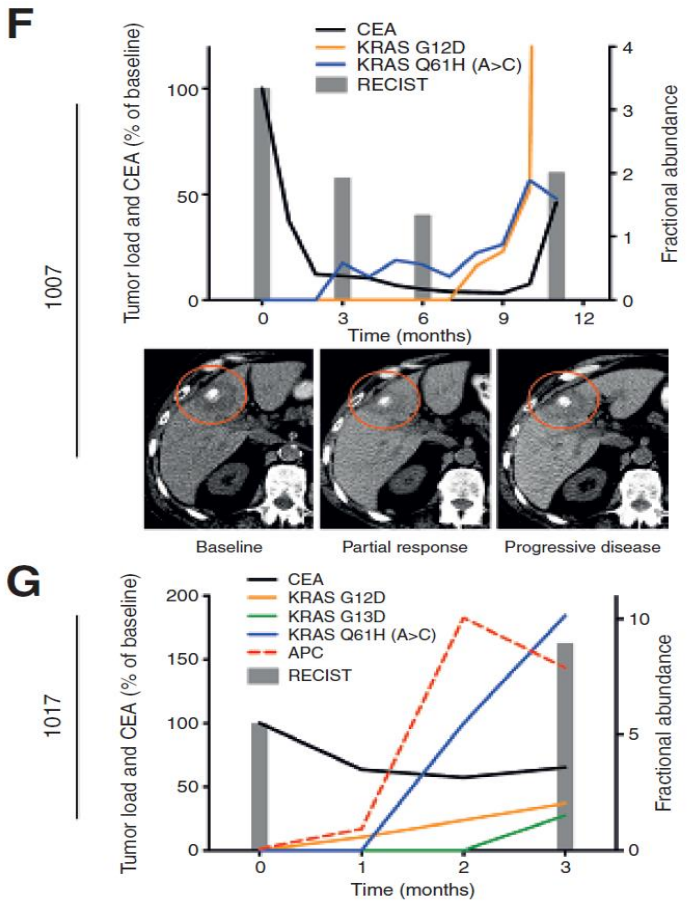
Maurel JCO Precis Oncol 2019



Mutated KRAS mutant clones dynamically evolve in response to pulsatile EGFR-specific antibody therapy

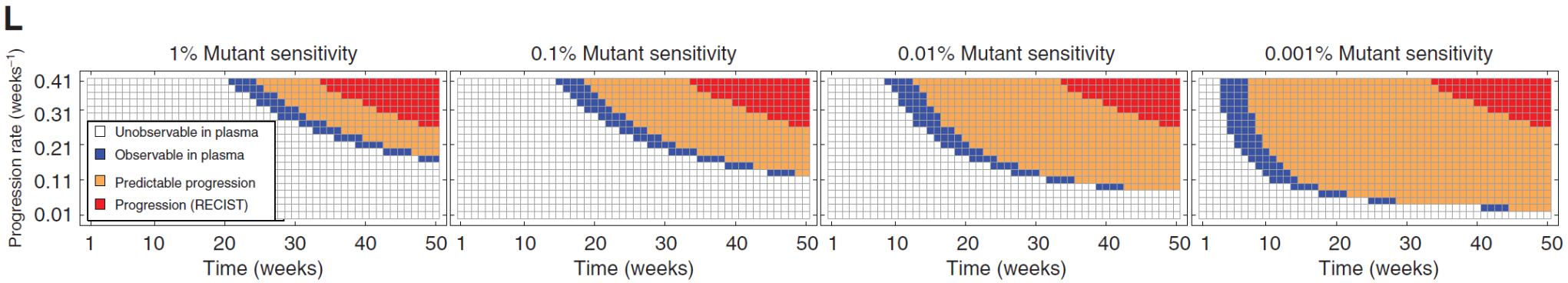
Siravegna Nat Med 2015

Tracking of plasma mutations in pts treated with anti-EGFR MoAbs



Khan Cancer Discov 2018

Predictive power of a mathematical framework applied to cfDNA



Khan Cancer Discov 2018

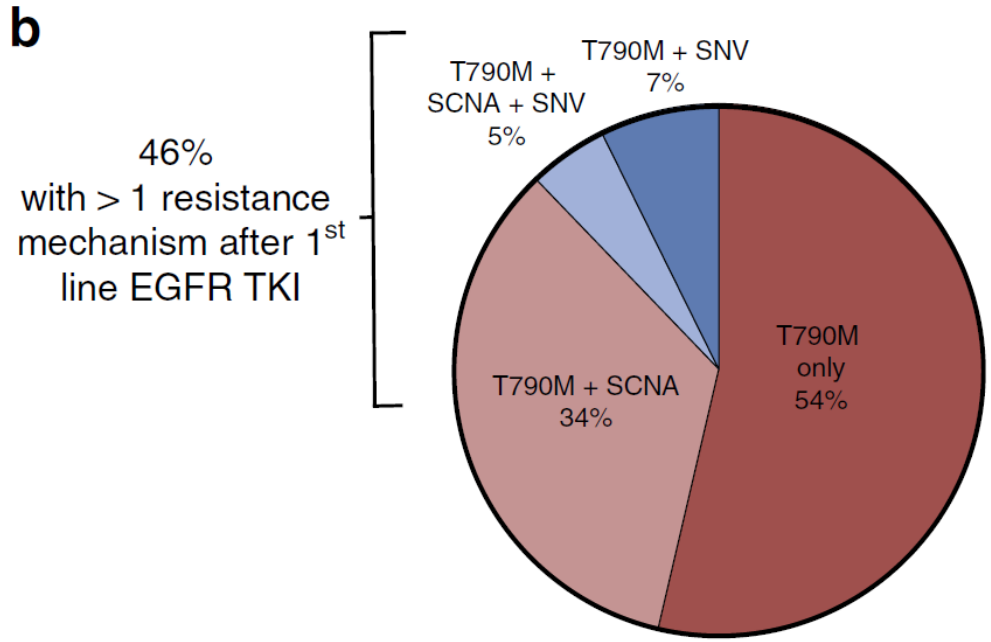
Ongoing ctDNA Interventional Trials in mCRC

Trial (trial identifier) by Disease Type	Study Type	Estimated No. of Patients	Study Population	Criteria for Patient Selection	Study Intervention	Primary Endpoint	Study Location
Metastatic disease							
PANIRINOX (NCT02980510)[g]	II, randomized	209	Stage IV first-line therapy	*RAS/BRAF* wild type	mFOLFOX6 plus panitumumab *vs.* FOLFIRINOX plus panitumumab	CR rate in FOLFIRINOX plus panitumumab arm	France
CHRONOS (NCT03227926)[h]	II	129	Stage IV first-line therapy[i]	*RAS*-extended mutational load between basal and rechallenge mutation load checkpoints	Rechallenge with panitumumab	ORR	Italy
NCT03087071[j]	R-II	84	Stage IV cetuximab-refractory disease	Treatment allocation according to *RAS*, *BRAF*, and *EGFR* mutational status	Panitumumab *v* panitumumab and trametinib	ORR	USA
TRIUMPH (UMIN000027887)[k]	II	25	Stage IV refractory disease	*ERBB2* amplification	Trastuzumab plus pertuzumab	ORR	Japan

gClinicalTrials.gov: PANIRINOX trial. https://clinicaltrials.gov/ct2/show/NCT03259009. hClinicalTrials.gov: CHRONOS trial. https://clinicaltrials.gov/ct2/show/NCT03227926.
iMain eligibility criteria: (1) imaging documented complete or partial response (according to RECIST 1.1 criteria) to first-line anti-EGFR–based therapy and progression while on therapy or maintenance regimen, including anti-EGFR agent; (2) planned second-line treatment of any type with the exclusion of additional anti-EGFRs; (3) *RAS*-extended mutational load with more than 3% fractional abundance, measured on plasma ctDNA at baseline mutational load (maximum within 2 weeks of last anti-EGFR administration); (4) a more than 50% decrease in *RAS*-extended mutational load between baseline mutational load and rechallenge mutational load.
jClinicalTrials.gov: Panitumumab in combination with trametinib in cetuximab-refractory stage IV colorectal cancer. https://clinicaltrials.gov/ct2/show/NCT03087071.
kUMIN Clinical Trials Registry: TRIUMPH trial. https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000031949

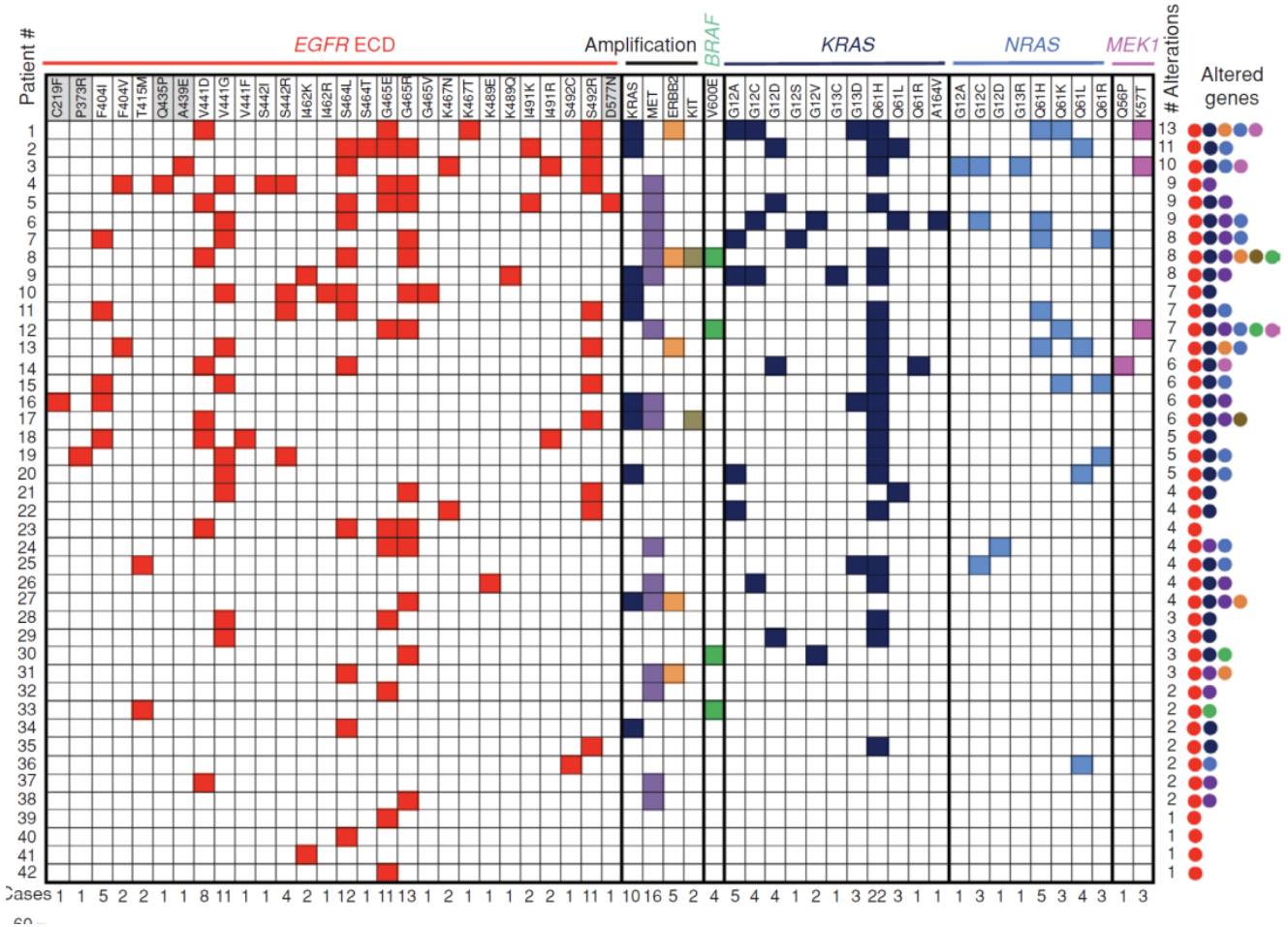
Yoshino ESMO 2019

Heterogeneity of anti-EGFR resistance alterations in aNSCLC

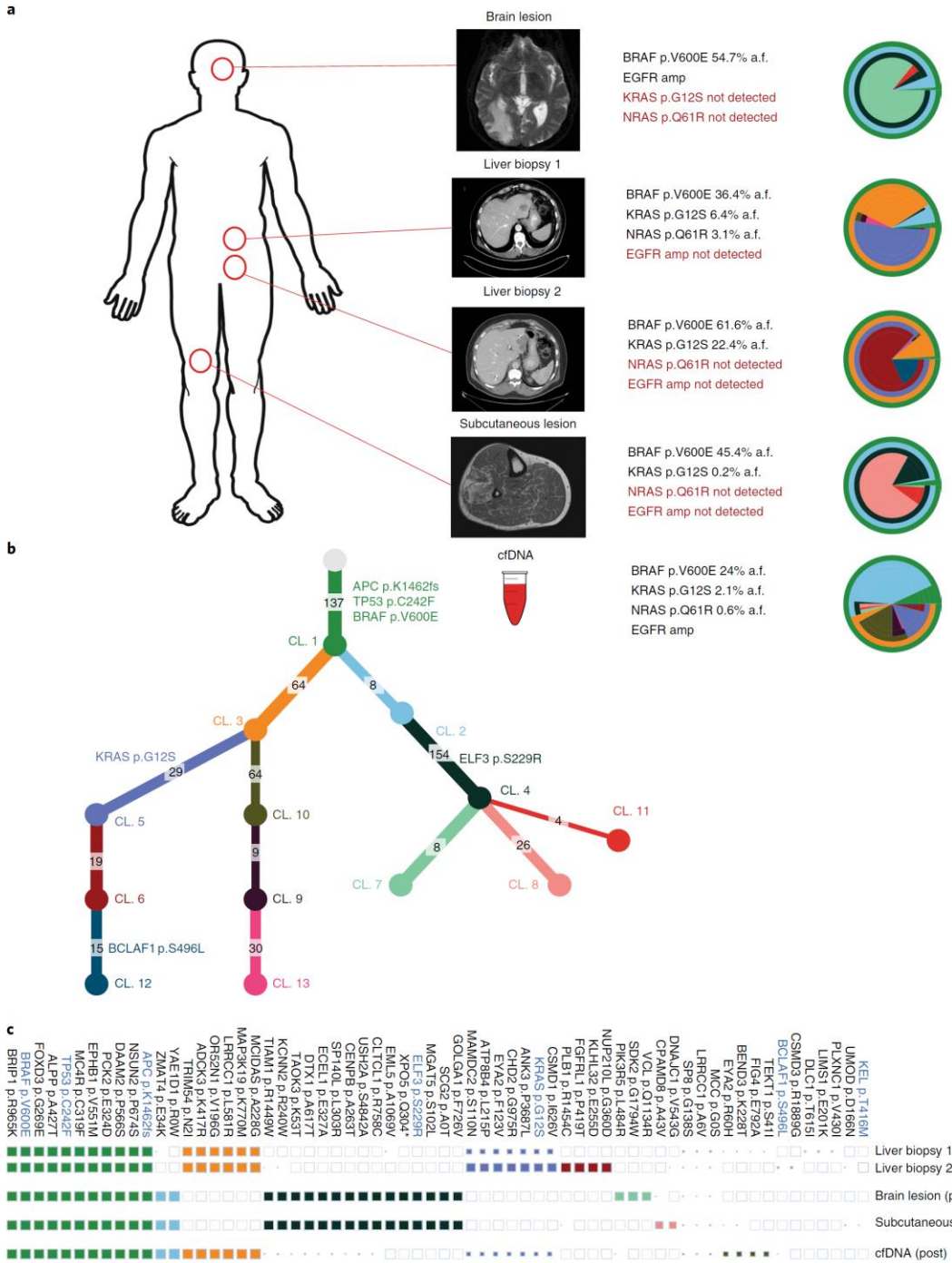


Chabon Nat Comm 2016

Heterogeneity of anti-EGFR resistance alterations in mCRC patients



Strickler Cancer Discov 2018

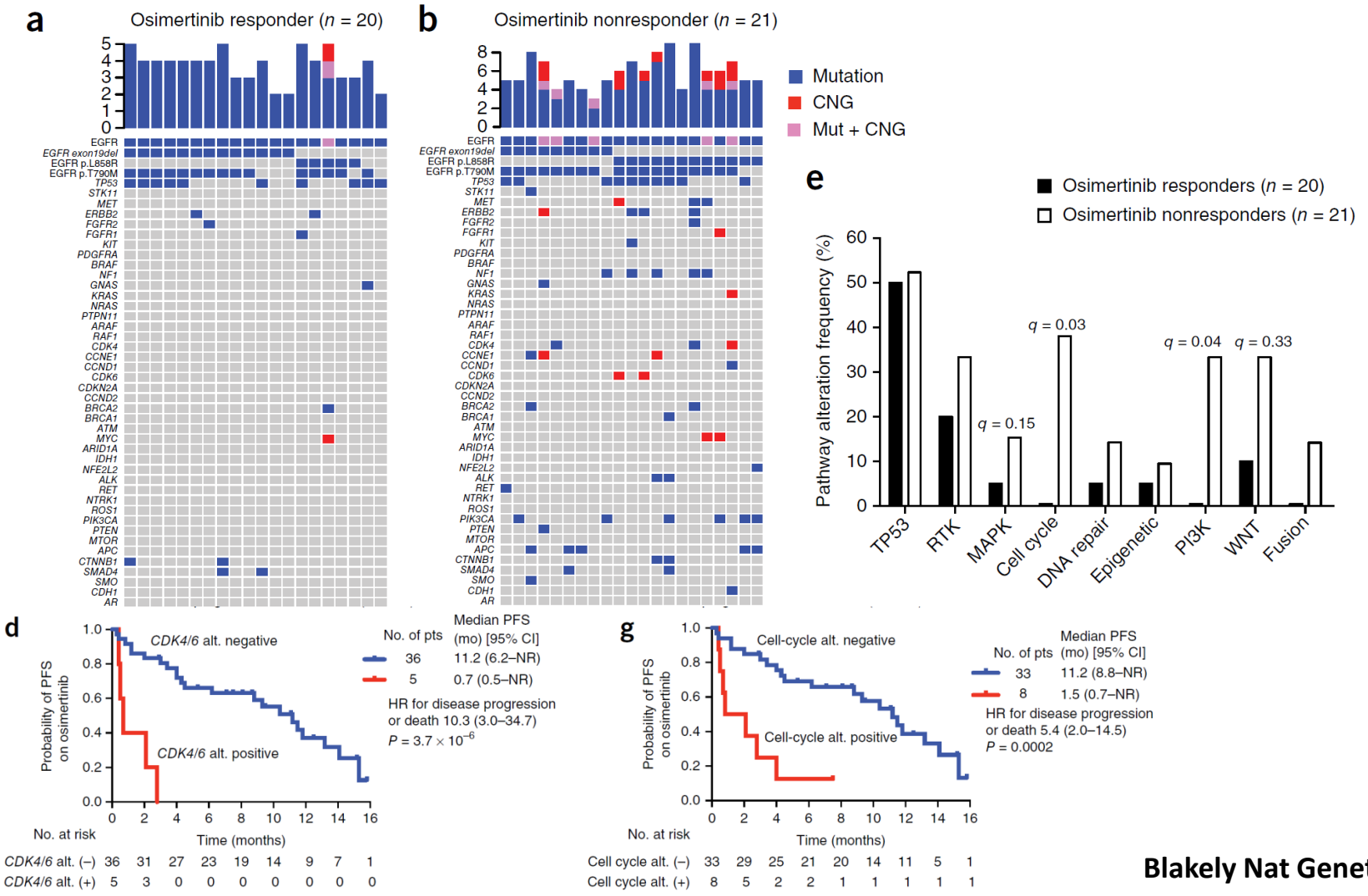


Comparison of multiple tumor biopsies versus liquid biopsy in a BRAF-mutant CRC patient

TPS037—a 53-year-old male with metastatic BRAFV600E CRC—was treated with a combination of EGFR, BRAF and phosphoinositide 3-kinase inhibitor-α inhibitors (cetuximab, encorafenib and alpelisib; NCT01719380) for 16 months before tumor progression

Parikh Nat Med 2019

Effects of cfDNA detectable co-occurring genetic alterations on osimertinib response

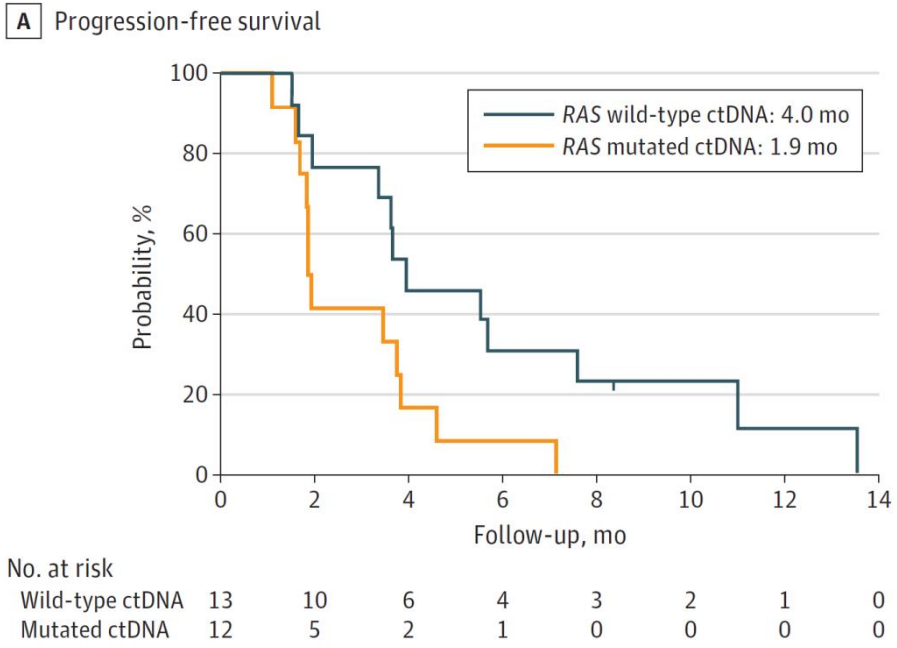


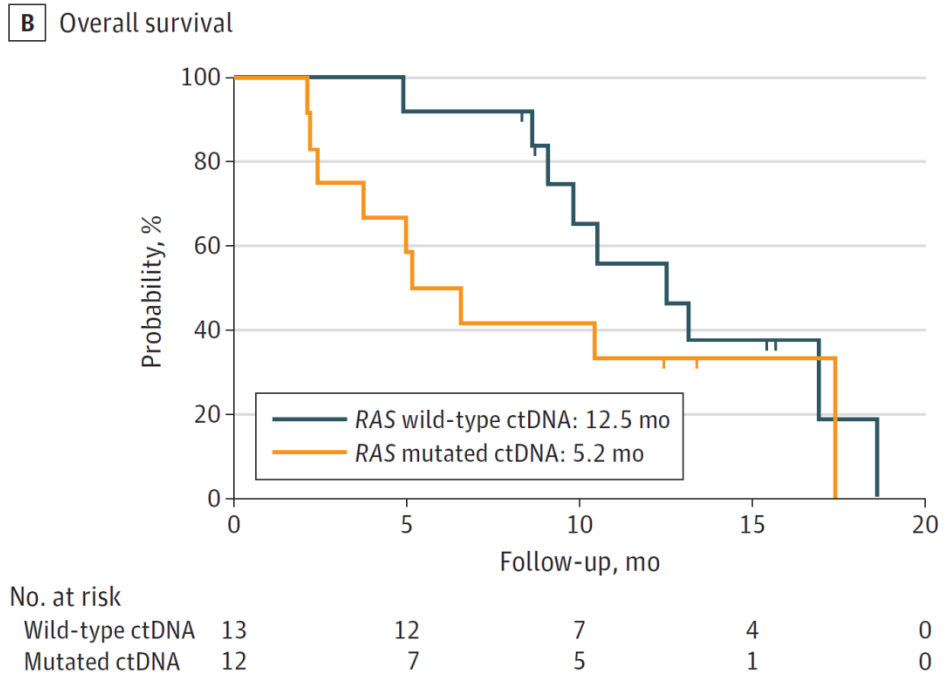
Blakely Nat Genetics 2017

APPLICATIONS OF cfDNA TESTING IN MONITORING

ctDNA and rechallenge with targeted therapies

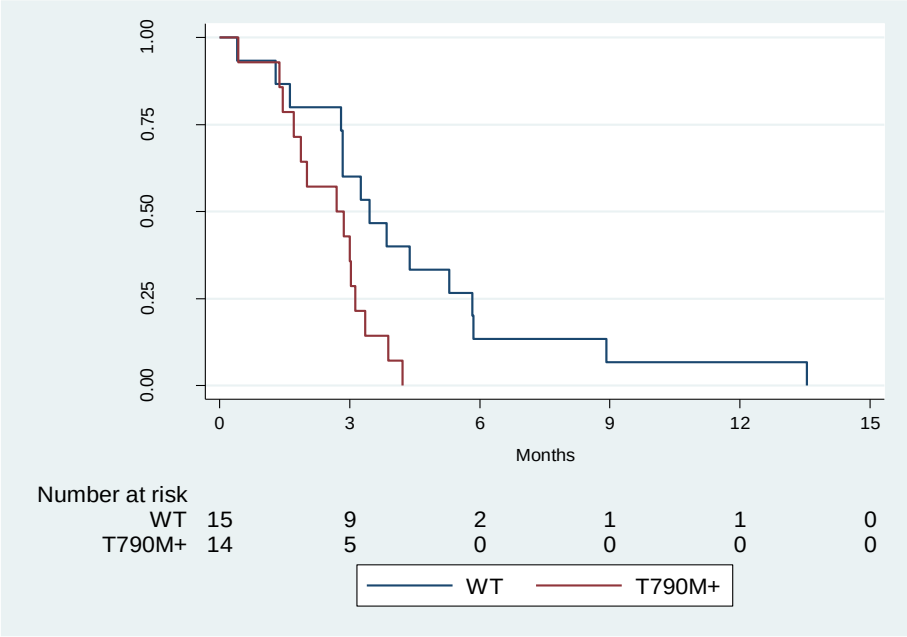
PFS and OS according to RAS and BRAF ctDNA Status in mCRC patients rechallenged with anti-EGFR MoAbs

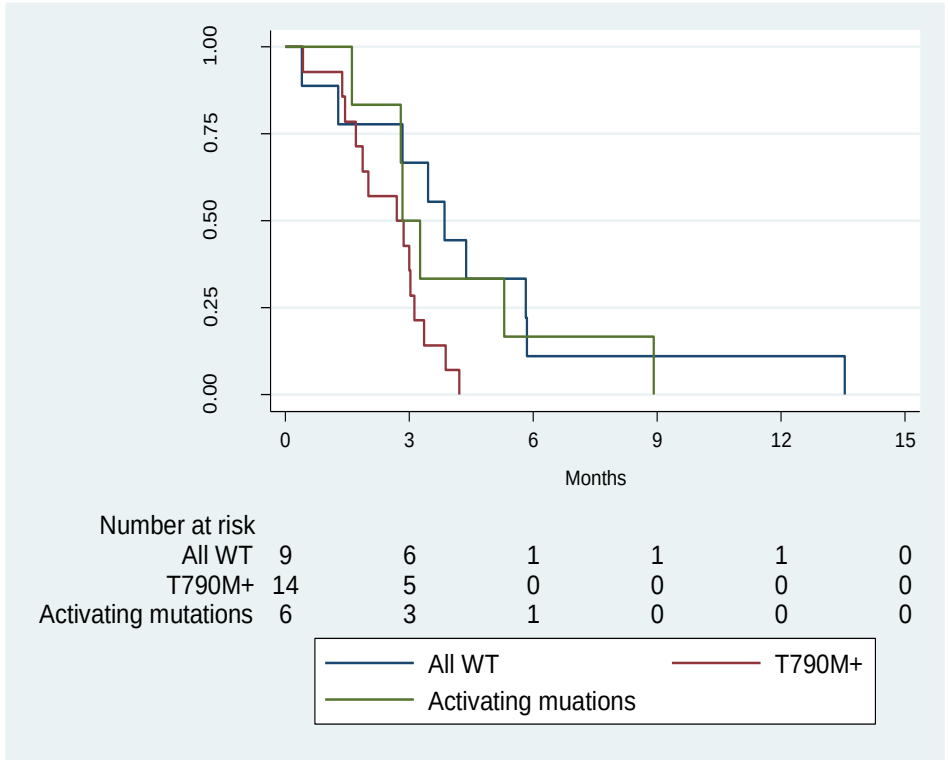




Cremolini JAMA Oncol 2018

Gefitinib rechallenge in EGFR mutant NSCLC pts



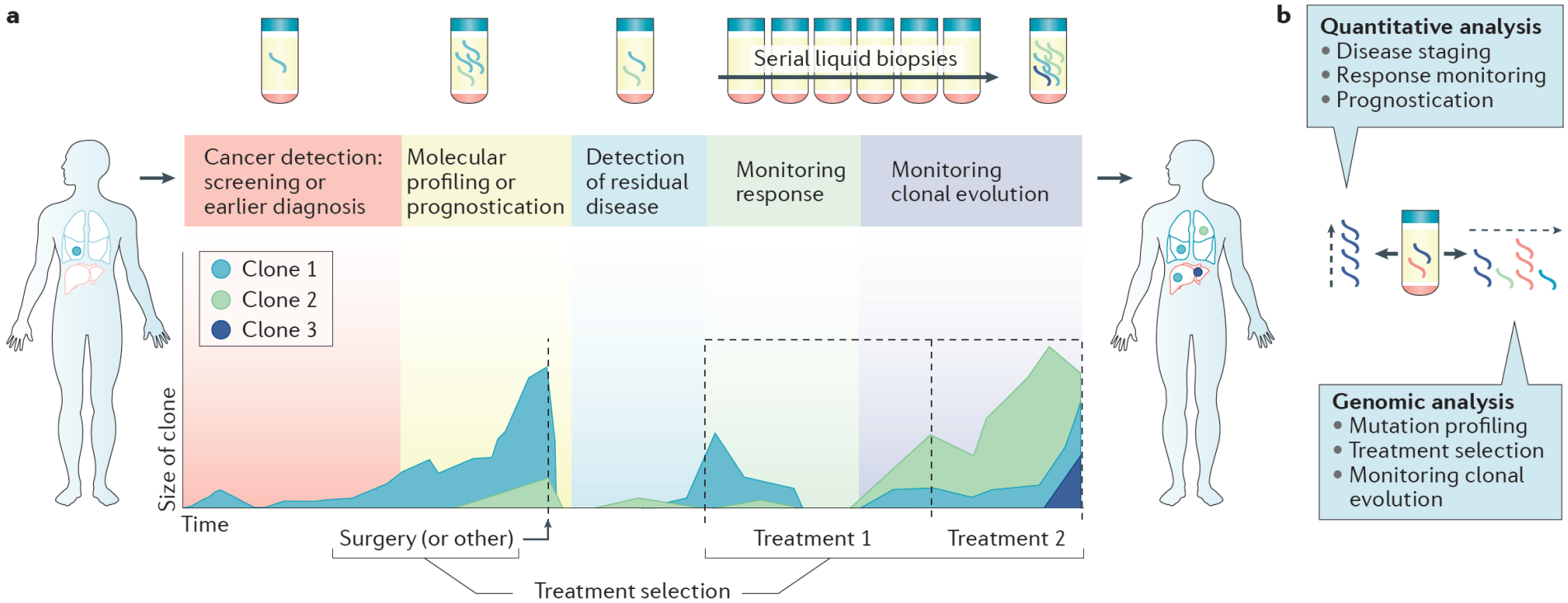


Esposito Abate Cancers 2019

APPLICATIONS OF cfDNA TESTING IN MONITORING

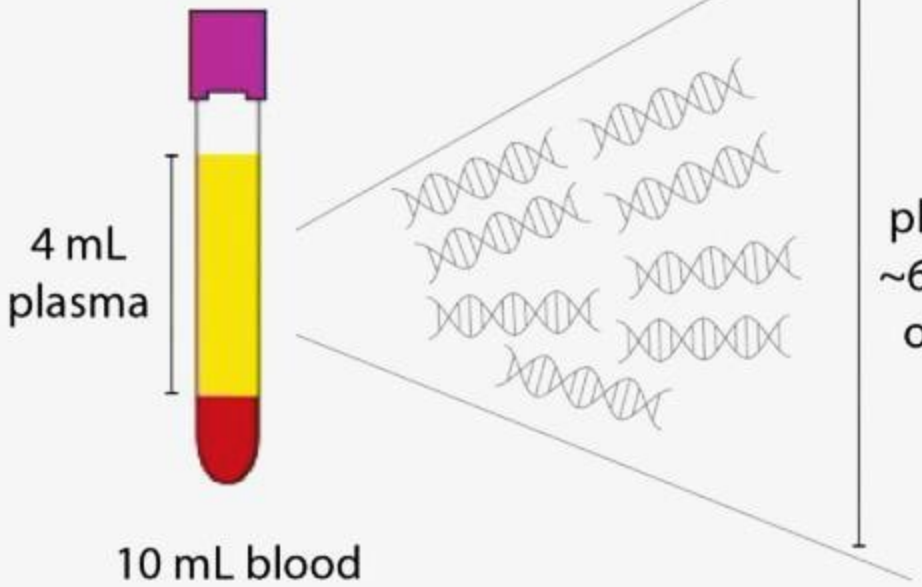
ctDNA as marker of MRD in early cancer patients

Applications of circulating tumour DNA analysis during the course of disease management



Wan NRC 2017

The challenge of ctDNA detection and quantification stems from sampling noise



~20ng
plasma DNA
~6000 copies
of genome

tumor fraction		tumor genome copies
1%	=	60
0.1%	=	6
0.01%	=	0.6
0.001%	=	0.06

Median Tumor Fraction in ctDNA at Presentation
Metastatic Breast Cancer: 2.4%-12.5%
Localized Breast Cancer: 0.11%

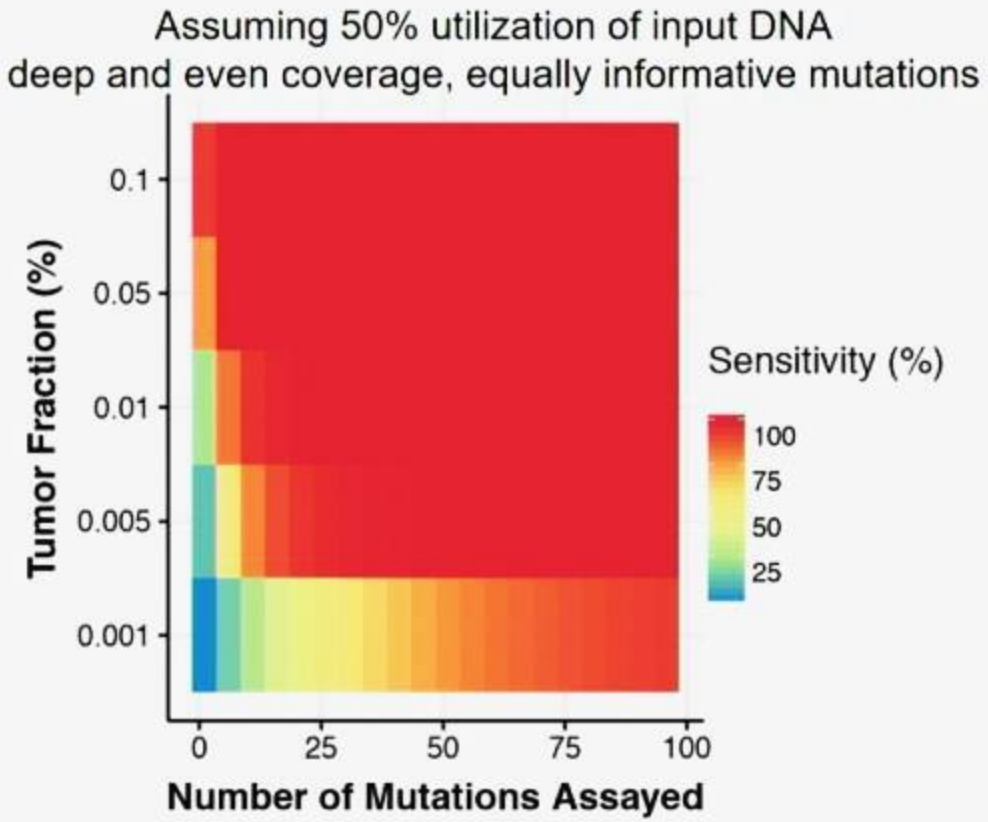
Murtaza AACR 2019

The challenge of ctDNA detection and quantification stems from sampling noise

Number of Mutations Assayed

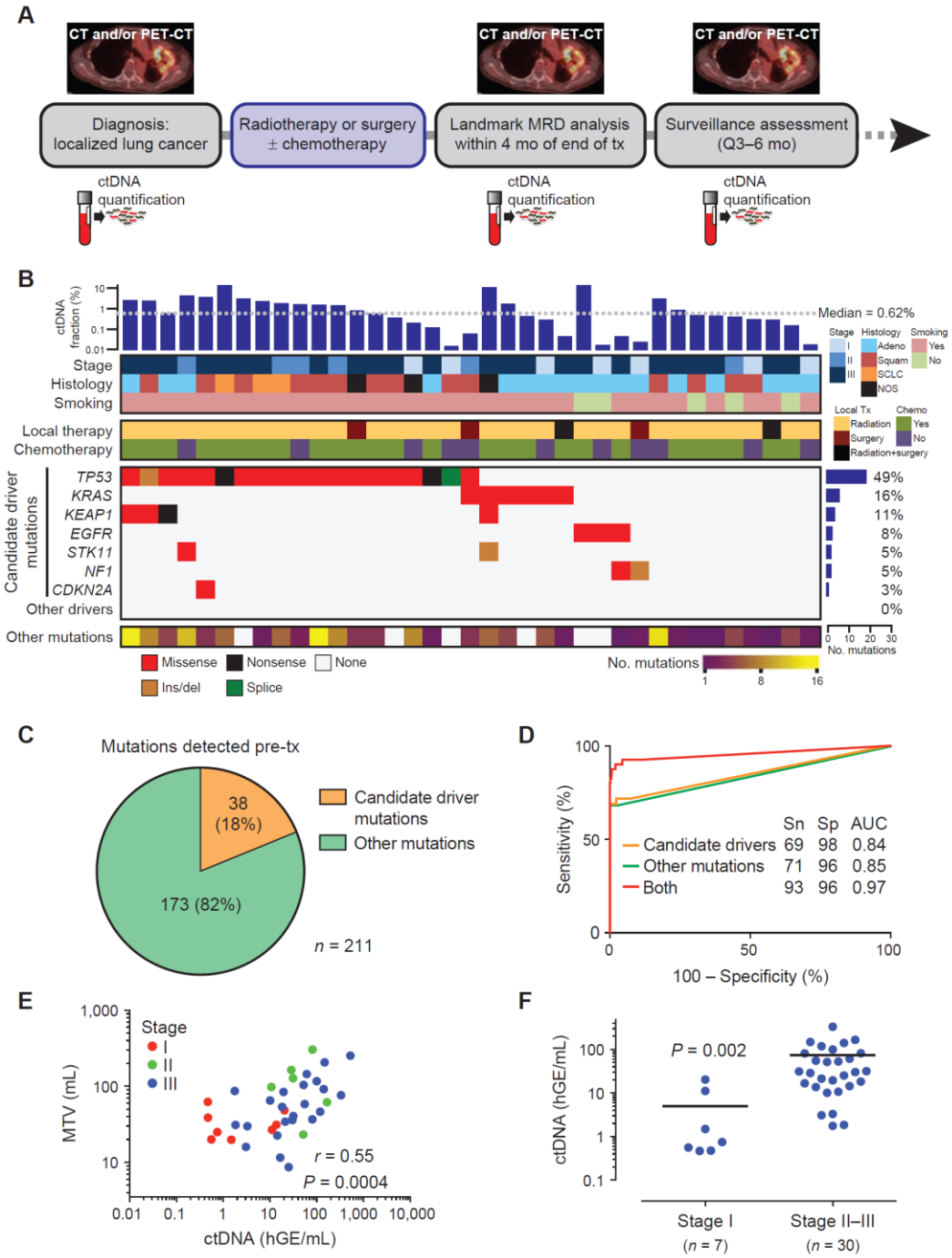
Tumor Fraction	1	2	5	10	50
0.01%	**0.6**	**1.2**	**3**	**6**	**30**
0.001%	**0.06**	**0.12**	**0.3**	**0.6**	**3**

Number of tumor DNA fragments on average

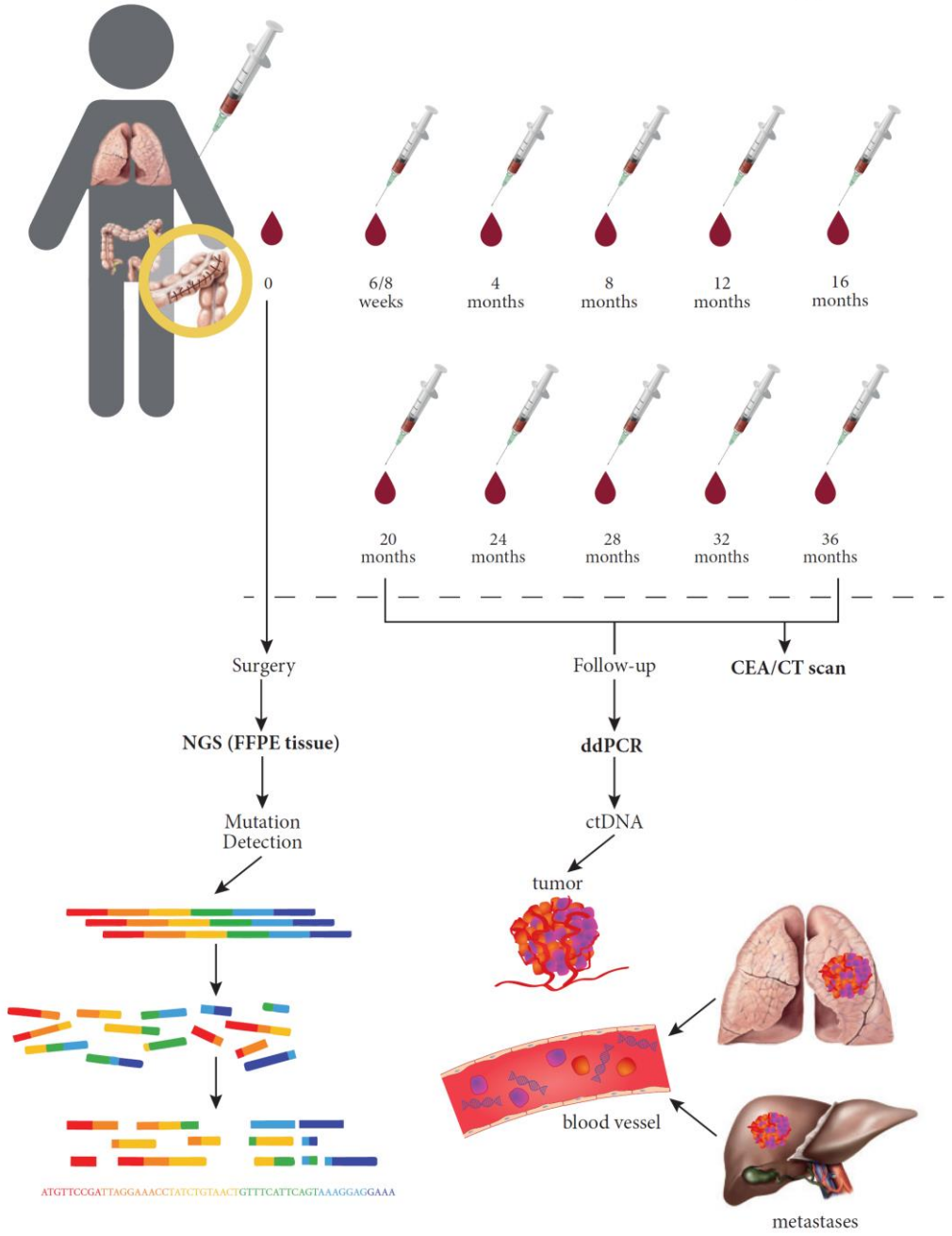


Murtaza AACR 2019

MRD testing: broad NGS panels

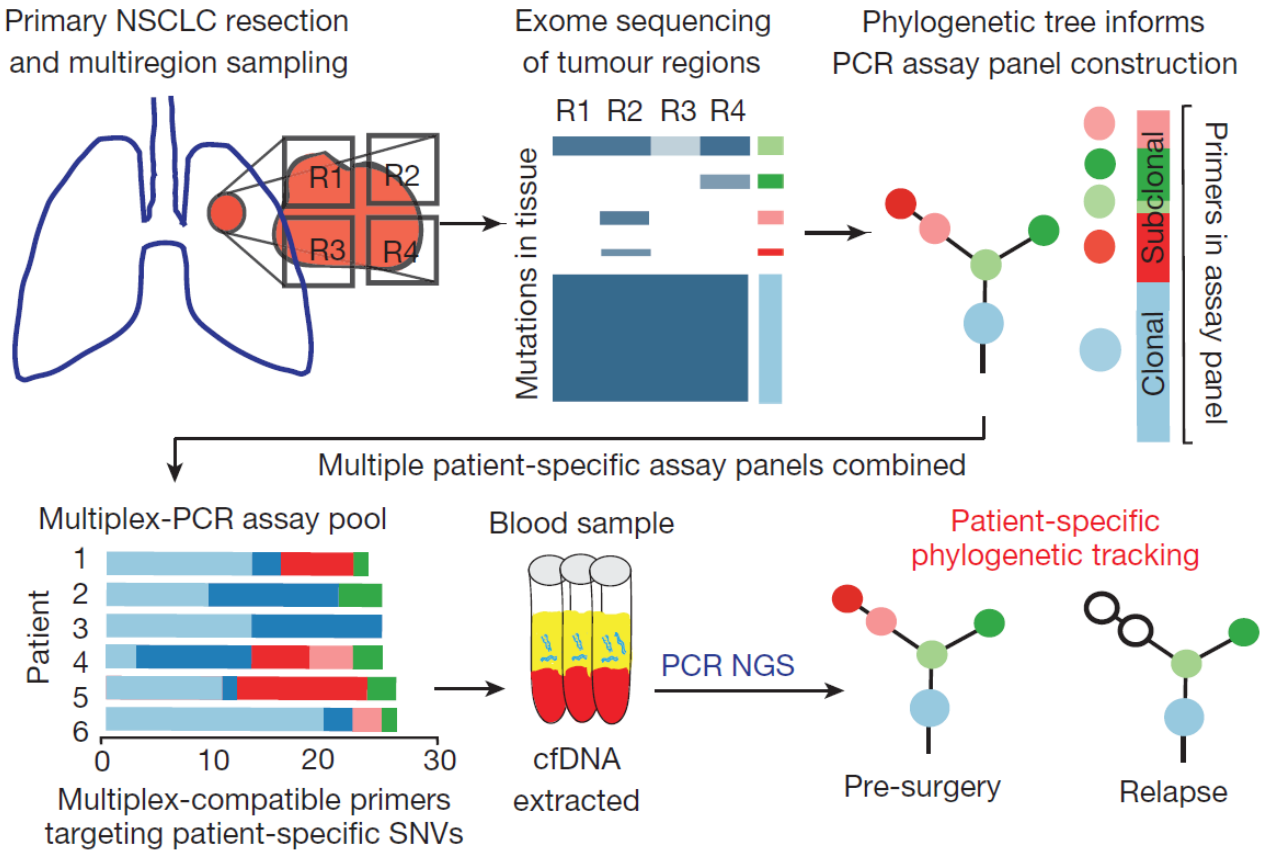


Chaudhuri Cancer Discov 2017



MRD testing: NGS on tumor tissue followed by personalized dPCR

Tarazona Ann Oncol 2019



MRD testing: NGS on tumor tissue followed by multiplex PCR-based NGS

Abbosh Nature 2017

cfDNA tests for MRD detection

Broad NGS panels

Pros:
No need to test tumor tissue
Fast TAT
Track clonal evolution
Relatively low cost

Cons:
Some patients negative at baseline
Relatively low sensitivity

NGS tissue followed by dPCR

Pros:
dPCR robust technique
dPCR available in many labs
Relatively low cost

Cons:
Long TAT to develop dPCR assays
No track clonal evolution
Relatively low sensitivity

NGS tissue followed by NGS

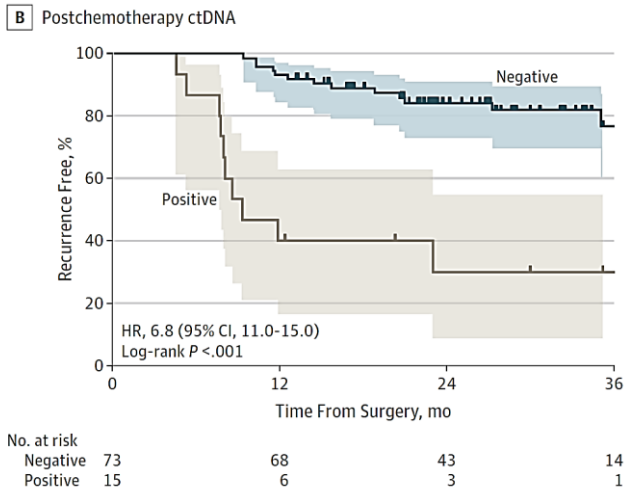
Pros:
High sensitivity (high n target mutations)
Track clonal evolution

Cons:
Long TAT to develop NGS assays
Relatively high cost

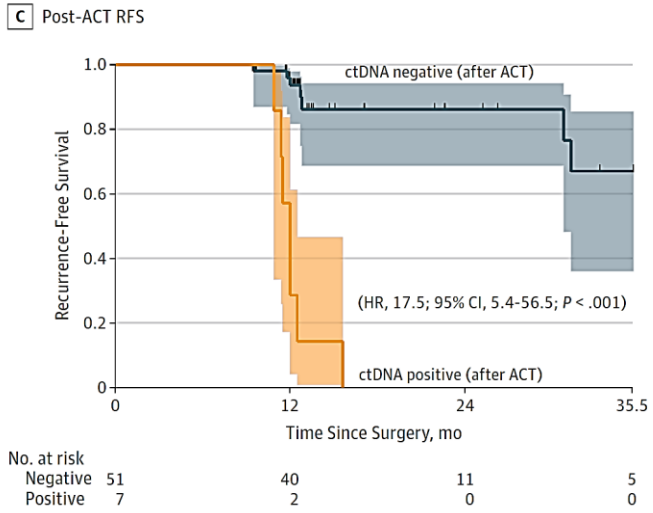
MRD detection by cfDNA testing

Tumor type	Pts (screened)	Stage	Time point	Method	HR RFS	Author, year
NSCLC/SCLC	37 (40)	IB, II, III	1 mo post-surgery	CAPP-seq	43.4	Chaudhuri, 2017
NSCLC	26 (175)	I-III	3 d post-surgery	cSMART	7.5	Chem, 2019
Colon ca.	230 (231)	II	4-10 w post-surgery	Safe-SeqS	18	Tie, 2016
Colon ca.	96	III	32-52 d post-surgery	Safe-SeqS	3.8	Tie, 2019
Colon ca.	130	I-III	30 d post-surgery	Natera	7.2	Reinert, 2019
Colon ca.	132 (155)	I-III	6-8 w post-surgery	dPCR	11.6	Tarazona, 2019
Breast ca.	55	early	2-4 w post-surgery	dPCR	25.1	Garcia-Murillas, 2015
Breast ca.	49	IA-IIIC	variable	Natera	11.8	Coombes, 2019
Bladder ca.	68	MIBC	after cystectomy	Natera	129.6	Christensen, 2019

Predictive value of ctDNA detection after adjuvant therapy in CRC pts



Tie JAMA Oncol 2019



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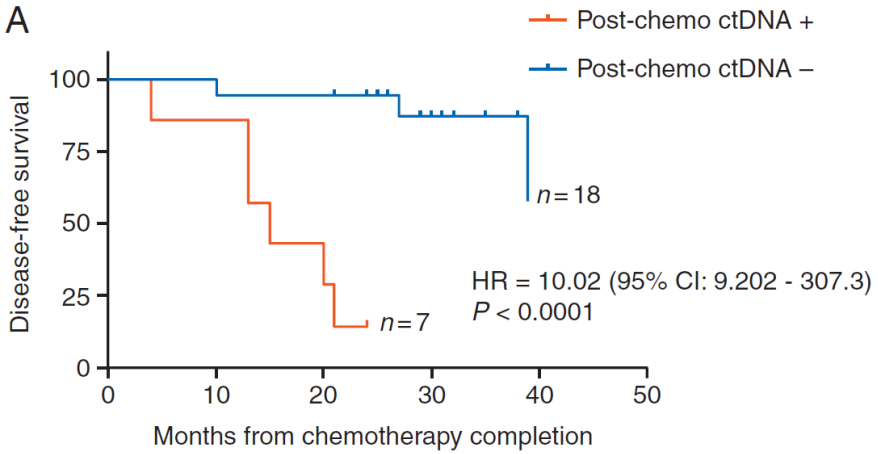

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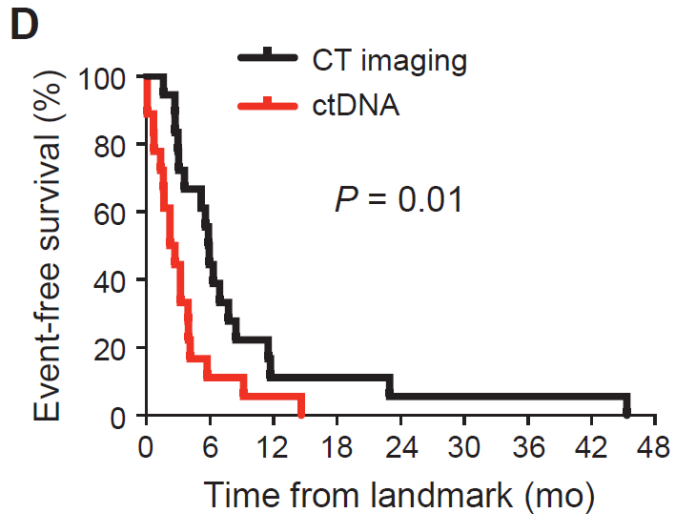
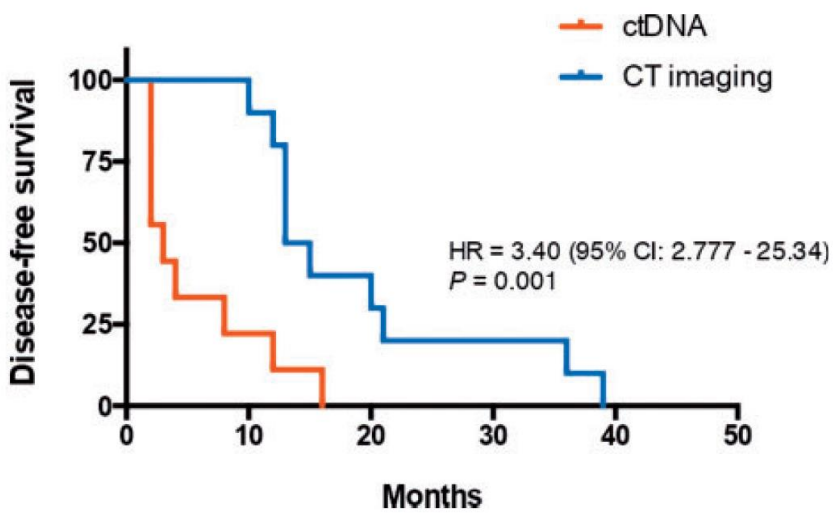
Time to ctDNA detection and time to imaging progression

NSCLC



Chauduri Cancer Discov 2017

CRC



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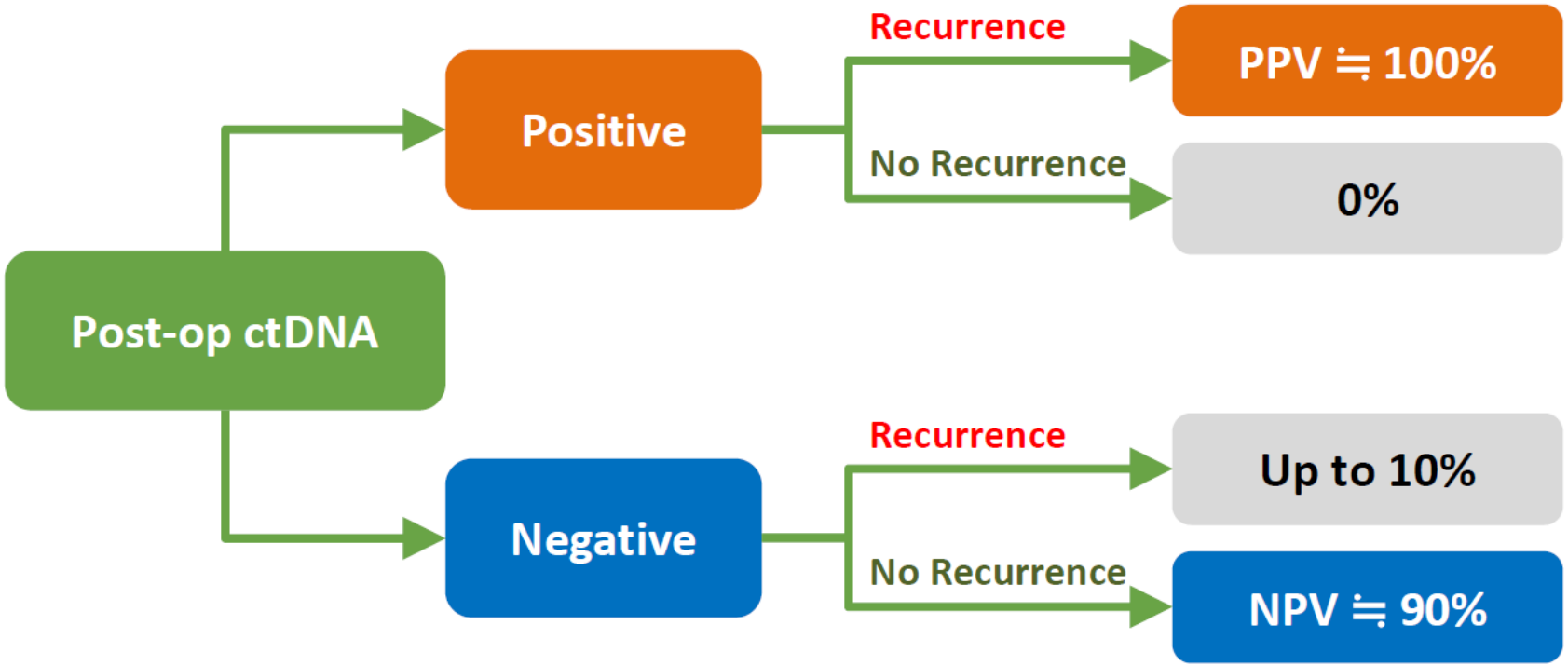
ctDNA and 3-year Recurrence Prediction



Yoshino ESMO 2019

Take home messages

- cfDNA testing allows monitoring response to treatment (target therapy, chemotherapy, immunotherapy, radiotherapy)
- Reduction of ctDNA levels is an earlier marker of response to therapy as compared with imaging
- Increase in ctDNA levels predict progression before clinical and/or radiological evidence
- cfDNA testing can be used to identify resistance mechanisms in patients treated with targeted agents
- cfDNA testing provides relevant clinical information in patients candidate to re-challenge with targeted agents
- In patients with early cancer, ctDNA is emerging as a sensitive marker of MRD and strongly correlates with patients' outcome