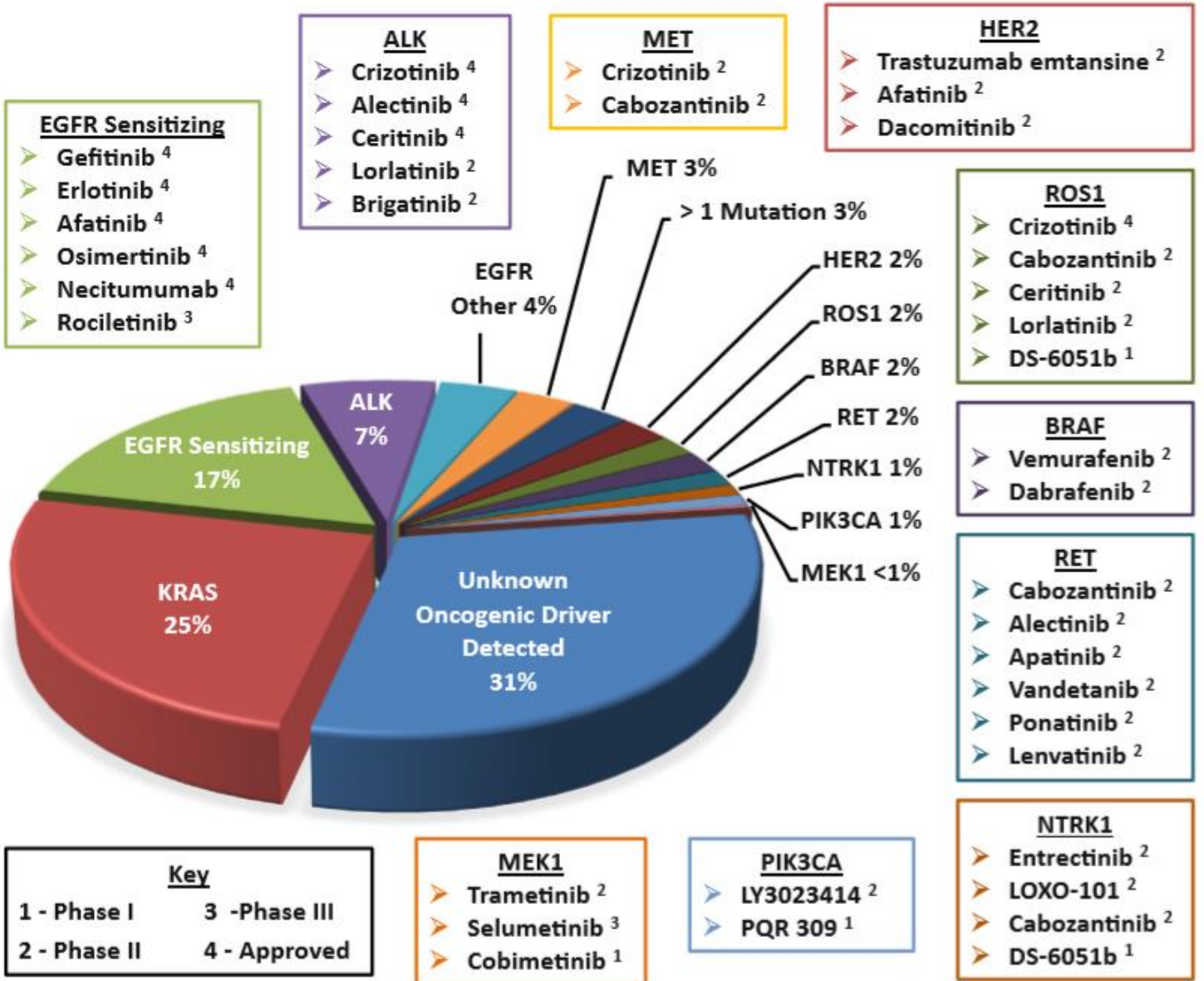
The Role of Liquid biopsy in Treatment of Lung Cancer

2018-04-13 폐암 심포지엄

건국대학교 병원 호흡기내과 김희정

Contents

Driver oncogenes in lung adenocarcinoma

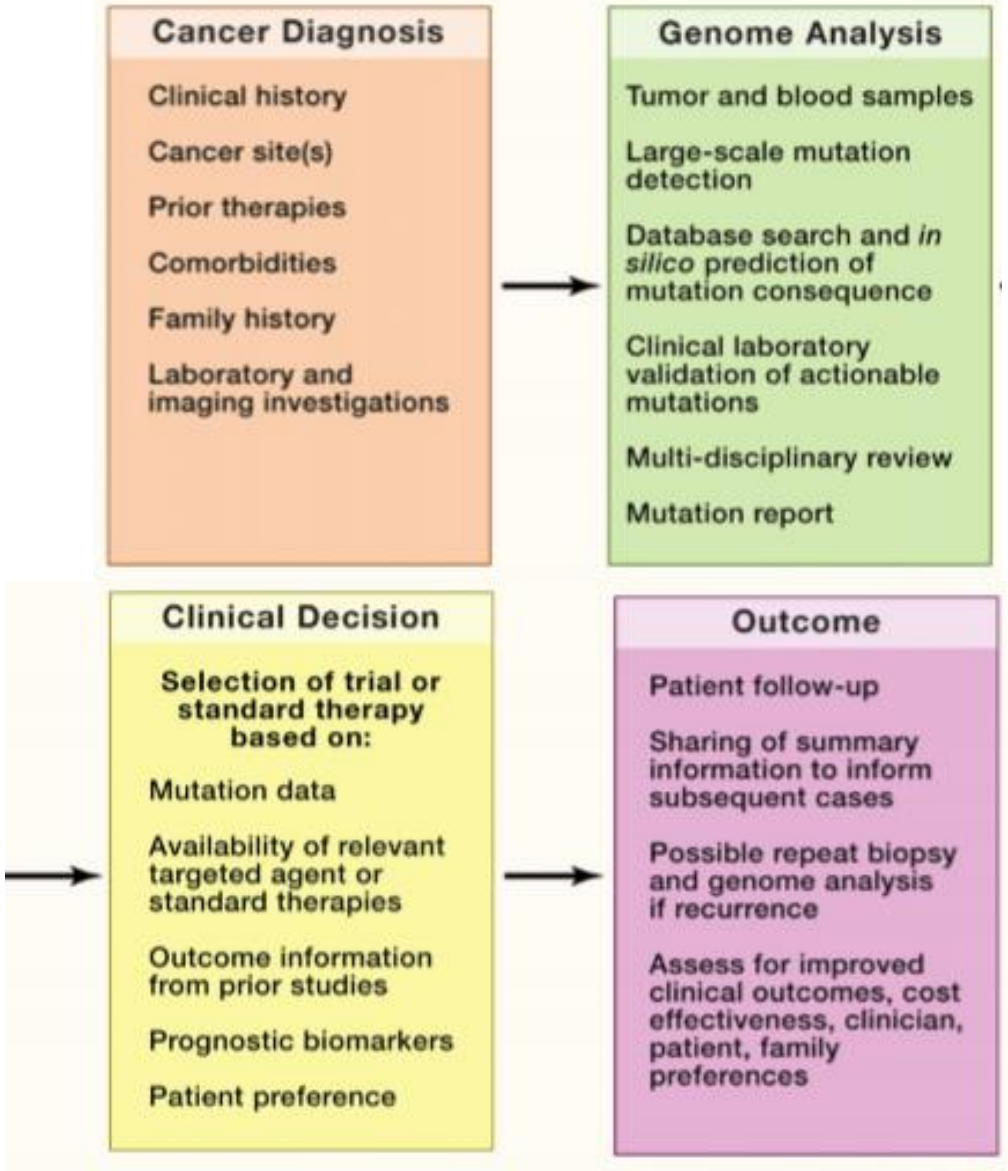


► Addictive driver oncogenes

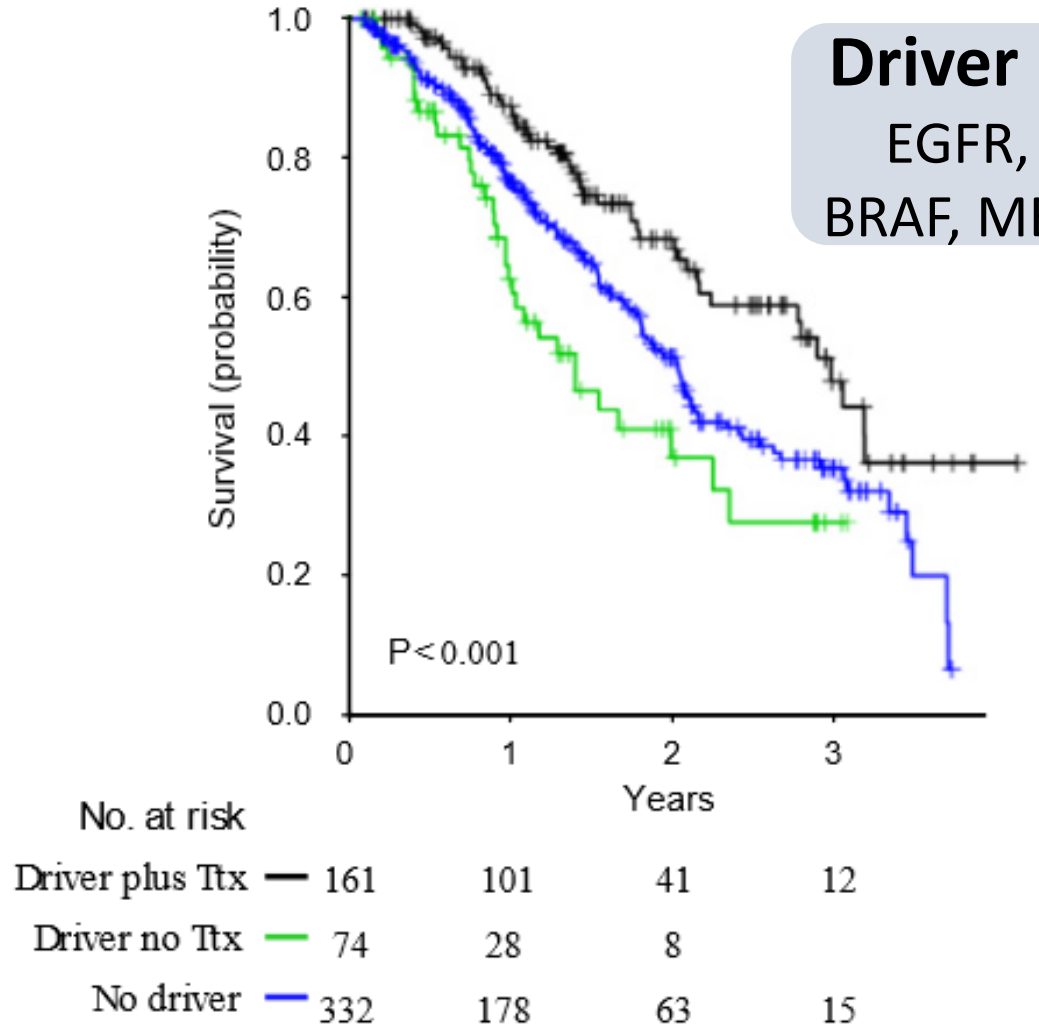
Targets : **Biomarkers**

Tsao et al. J Thorac Oncol. 2016;11(5):613-38.

Genetic basis for lung cancer treatment

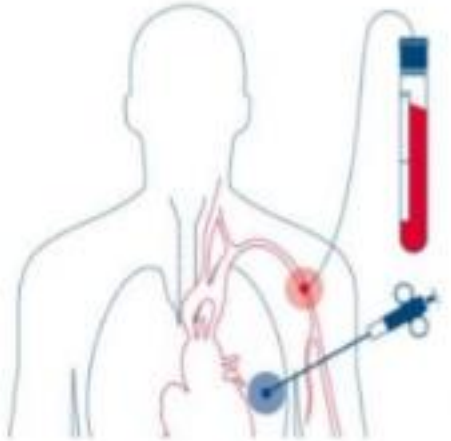


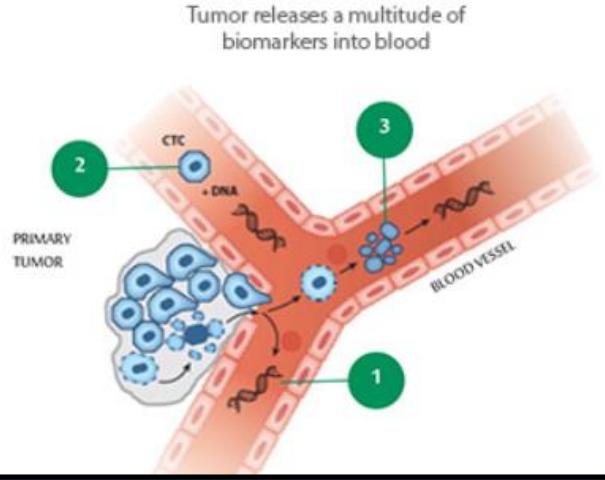
Driver mutations :
EGFR, ALK, ROS-1,
BRAF, MET, ERBB2, RET



Dancey et al. Cell. 2012;148(3):409-20.
Aisner DL et al. Clin Cancer Res. 2018 Mar 1;24(5):1038-1047.

Tumor biopsy vs. Liquid biopsy



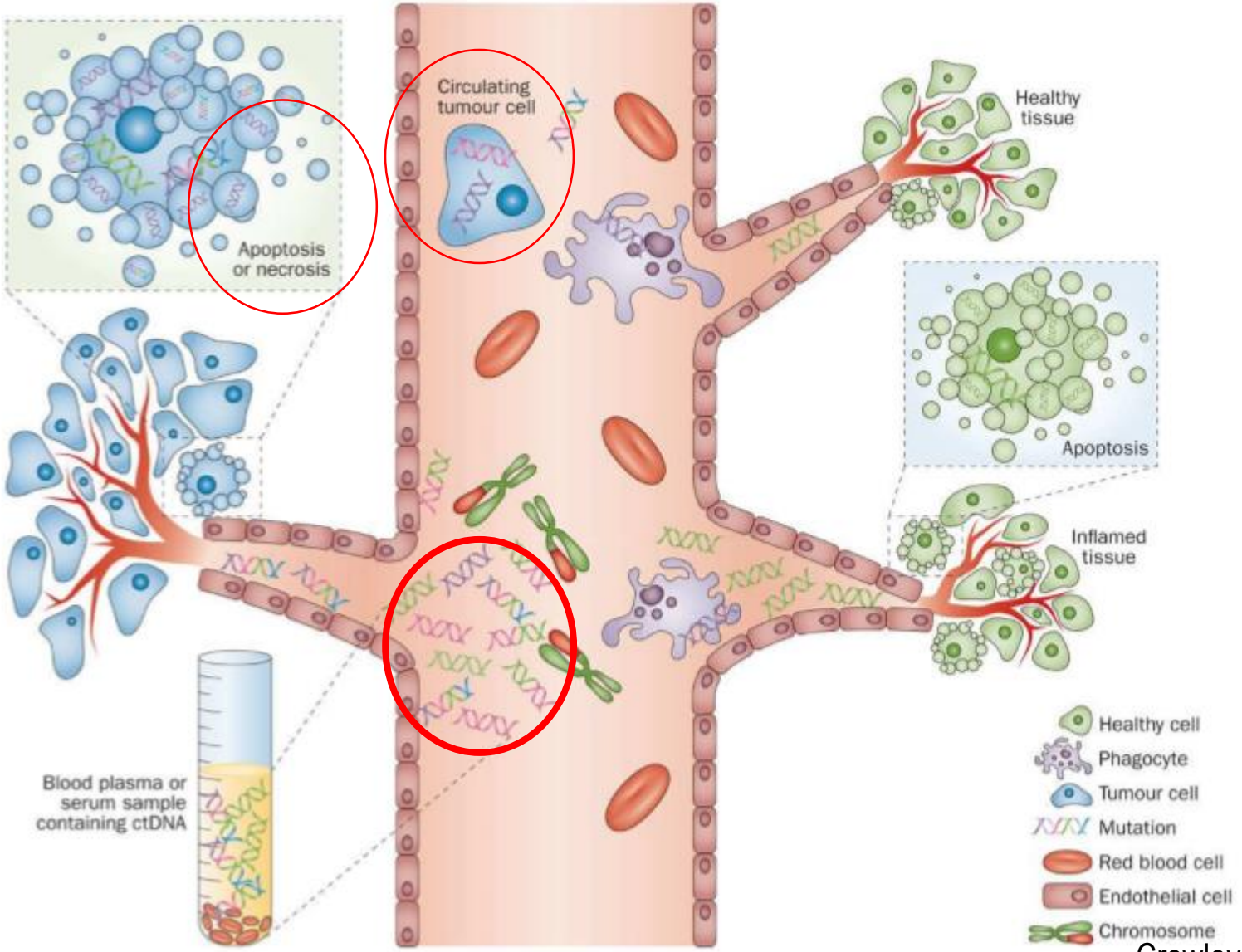


Tumor biomarkers in blood

1. Cell-free DNA (cfDNA)
2. Circulating tumor cells (CTCs)
3. Exosomes & micro vesicles

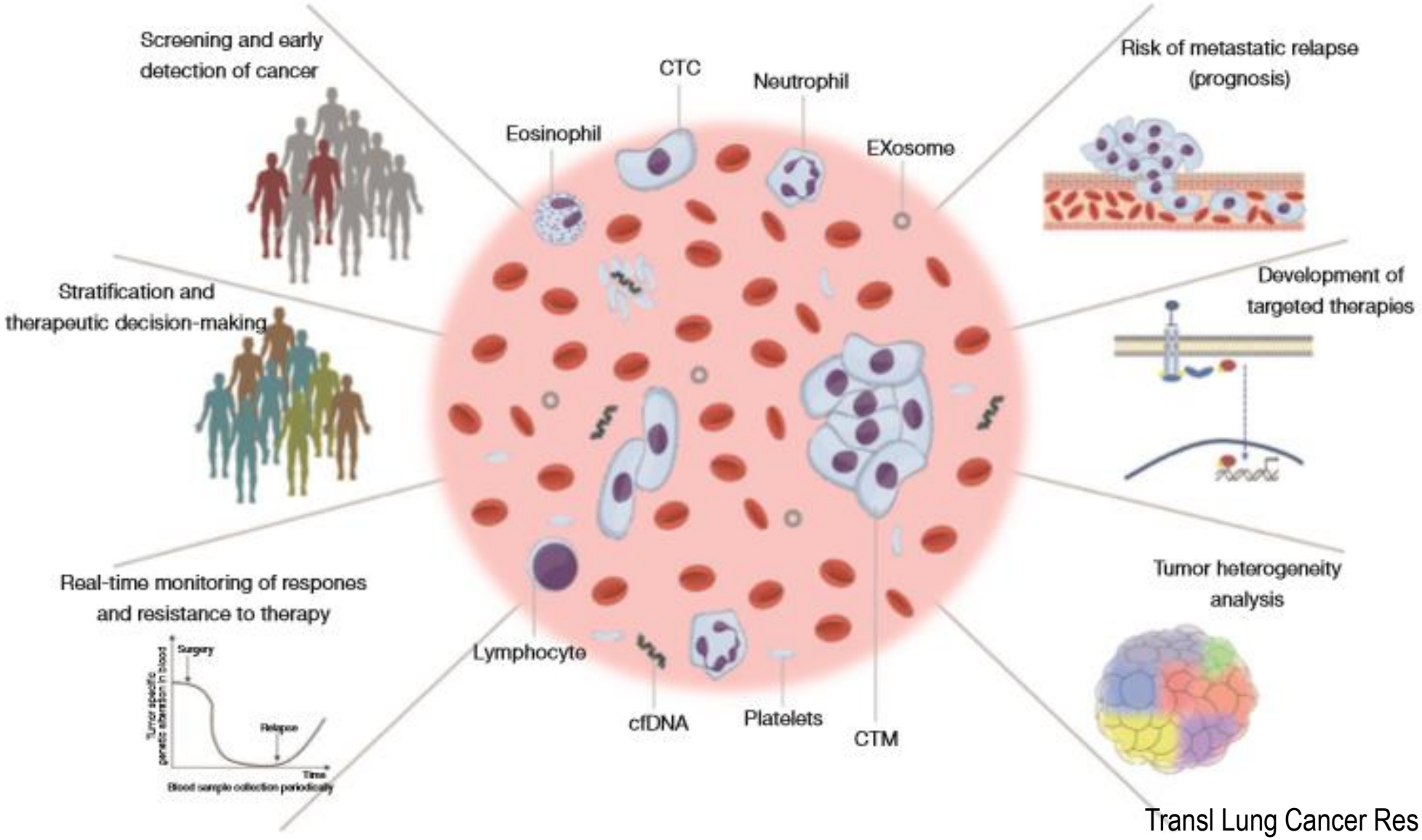
Tumor biopsy	Liquid biopsy
Gold standard for genotyping Diagnostic, prognostic, predictive Histologic diagnosis important FFPE archived tissue Assess tumor content	**Less invasive**, more successible **Serial testing** over time/treatment Avoidance of additional biopsies Minimal processing; turn around time May better capture tumor heterogeneity Potentially quantifiable
Susceptible to preanalytic factors Serial biopsies not practical May not represent tumor heterogeneity Invasive procedure; complications	Susceptible to preanalytic factors (plasma half-life < 2h) Low concentration of ctDNA in background of genomic DNA

Liquid biopsy – 3 main areas



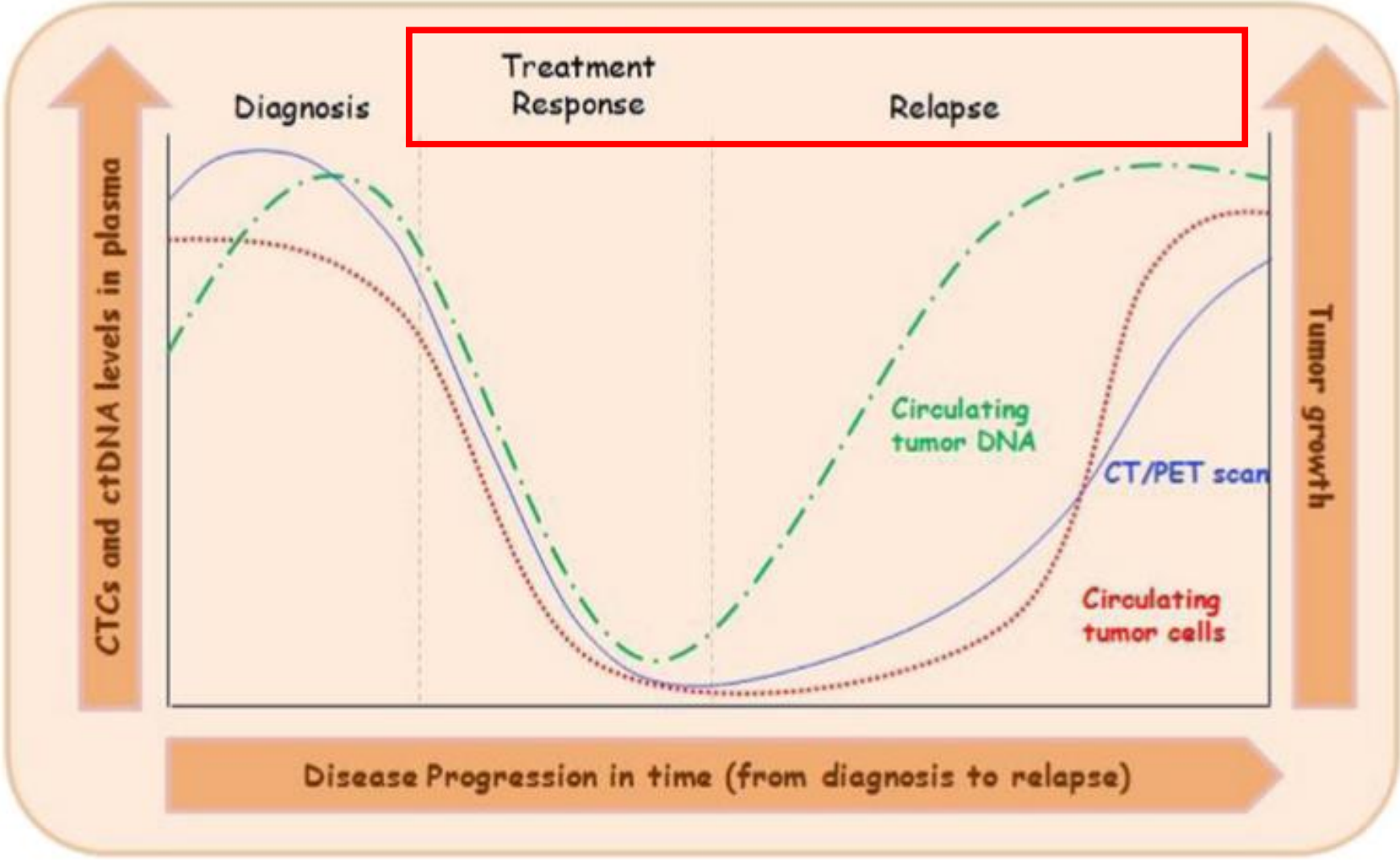
Crowley et al. Nat Rev Clin Oncol 2013;10:472-484.

Clinical applications of Liquid biopsy



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

Dynamic monitoring of CTC and ctDNA



Rolfo et al. Biochimica etBiophysica Acta 1846. 2014;539–546.

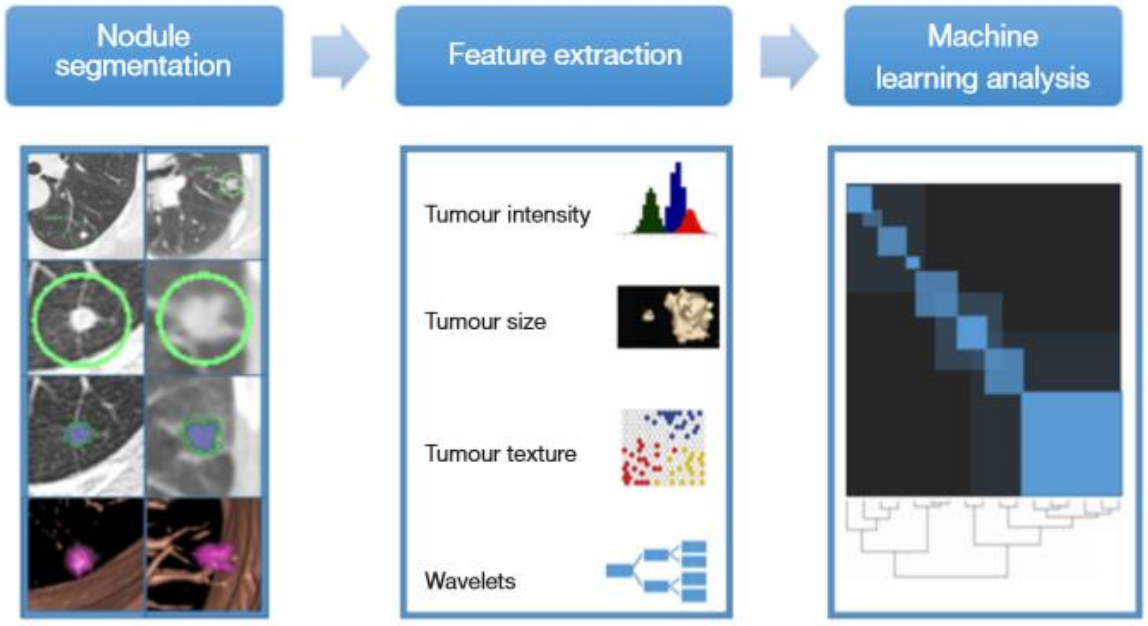
Early stage lung cancer → Resection?

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 4, 2011 VOL. 365 NO. 5

Reduced Lung-Cancer Mortality with Low-Dose Computed Tomographic Screening

The National Lung Screening Trial Research Team*



Clinical Utility of a Plasma Protein Classifier for Indeterminate Lung Nodules

Anil Vachani[1] · Zane Hammoud[2] · Steven Springmeyer[7] · Neri Cohen[3] · Dao Nguyen[4] · Christina Williamson[5] · Sandra Starnes[6] · Stephen Hunsucker[7] · Scott Law[7] · Xiao-Jun Li[7] · Alexander Porter[7] · Paul Kearney[7]

A Blood-Based Proteomic Classifier for the Molecular Characterization of Pulmonary Nodules

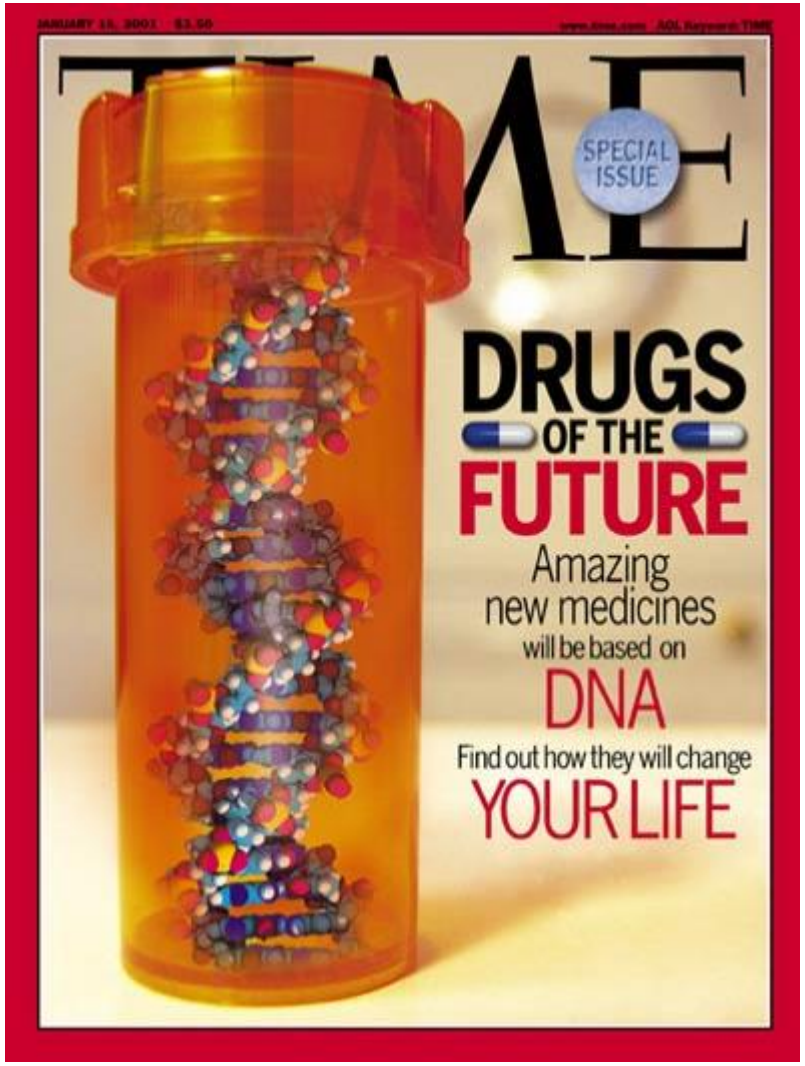
Xiao-jun Li[1,*], Clive Hayward[1], Pui-Yee Fong[1], Michel Dominguez[1,†], Stephen W. Hunsucker[1], Lik Wee Lee[1], Matthew McLean[1,‡], Scott Law[1], Heather Butler[1,§], Michael Schirm[2], Olivier Gingras[2], Julie Lamontagne[2], Rene Allard[2], Daniel Chelsky[2], Nathan D. Price[3], Stephen Lam[4], Pierre P. Massion[5], Harvey Pass[6], William N. Rom[7], Anil Vachani[8], Kenneth C. Fang[1], Leroy Hood[3], and Paul Kearney[1,*]

Assessment of Plasma Proteomics Biomarker's Ability to Distinguish Benign From Malignant Lung Nodules

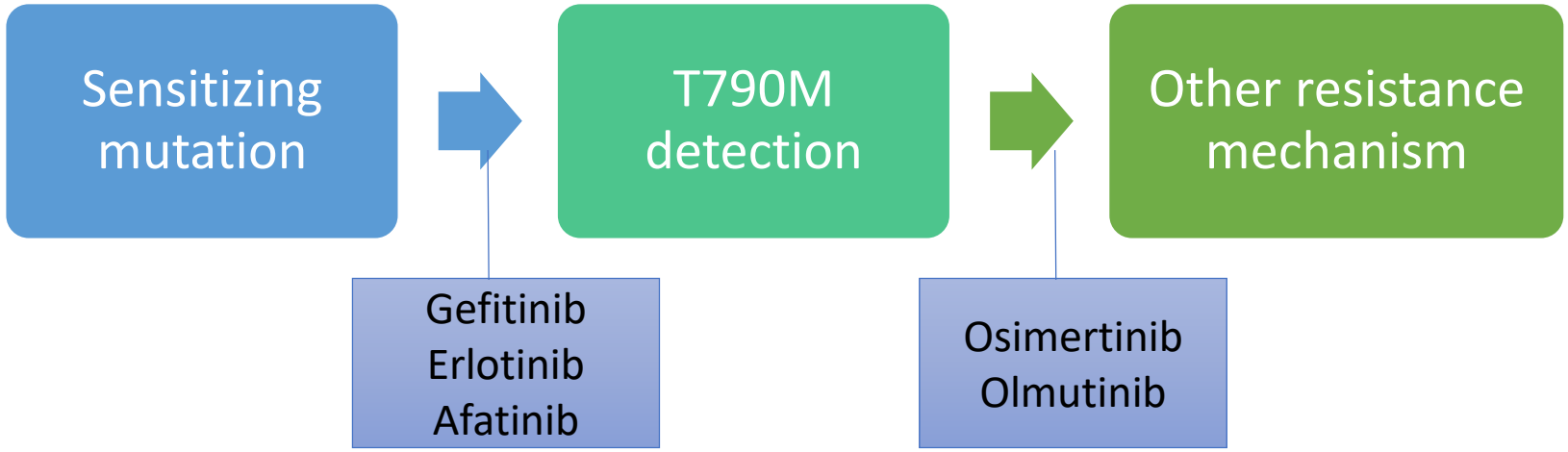
Results of the PANOPTIC (Pulmonary Nodule Plasma Proteomic Classifier) Trial

Vachani A, et al. Lung. 2015;193(6):1023-7.
Li XJ et al. Sci Transl Med. 2013;5(207):207ra142.
Silvestri GA et al. Chest. 2018 Mar 1. pii: S0012-3692(18)30307-6.
Wilson R, et al. Transl Lung Cancer Res. 2017 Feb;6(1):86-91.
National Lung Screening Trial Research Team, et al. N Engl J Med. 2011;365(5):395-409.

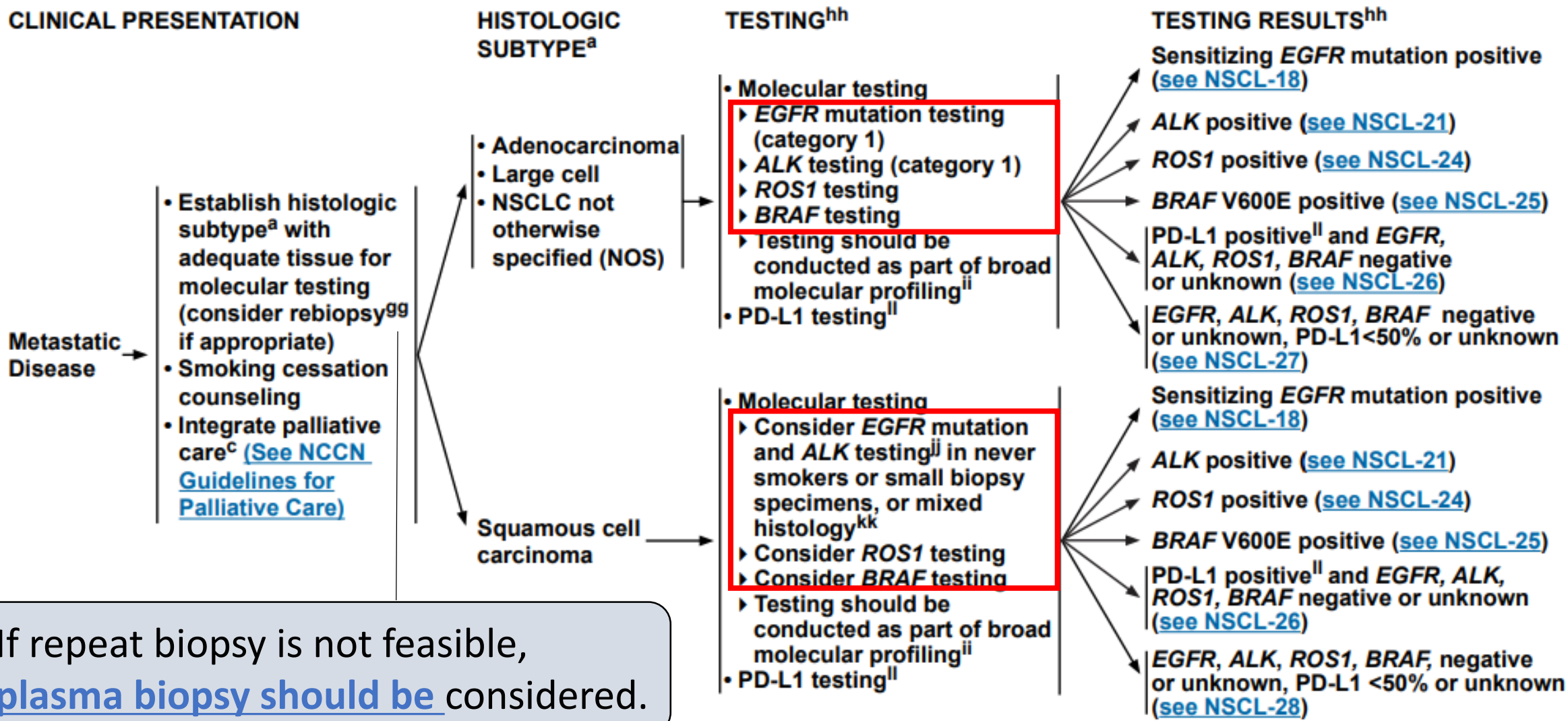
Advanced stage lung cancer



- ▶ Personalized medicine offers
  - The right treatment
  - To the right patient
  - For the right disease
  - At the right time
  - With the right dose
- ▶ Ex.) EGFR mutated tumor



Molecular testing in advanced NSCLC



If repeat biopsy is not feasible, plasma biopsy should be considered.

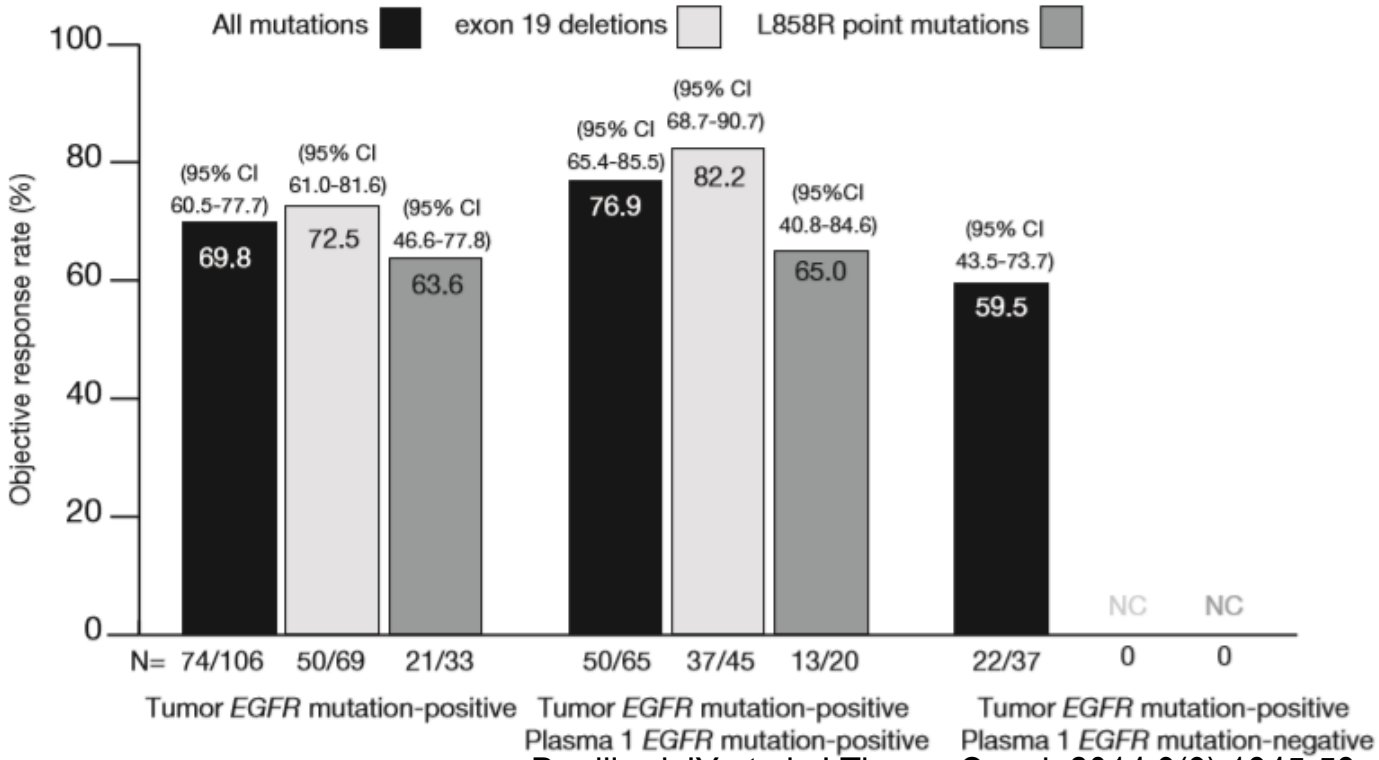
NCCN guidelines version 3. 2018.

ctDNA as a surrogate for determination of EGFR status

- IFUM study; 1st line gefitinib in Caucasian EGFR+ advanced NSCLC patients
- Scorpion ARMS-based EGFR mutation detection kit
  - (Therascreen EGFR RGQ PCR kit; Qiagen, Crawley, UK)

	Plasma 1 *EGFR* Mutation Status, *n*		
	Positive	**Negative**	**Total**
Tumor *EGFR* mutation status, *n*[a]			
Positive	69	36	105
Negative	1	546	547
Total	70	582	652

	n	**Rate, %**	**95% Confidence Interval**
Concordance	652	94.3	92.3–96.0
Sensitivity	105	65.7	55.8–74.7
Specificity	547	99.8	99.0–100.0
Positive-predictive value	70	98.6	92.3–100.0
Negative-predictive value	582	93.8	91.5–95.6



Douillard JY et al. J Thorac Oncol. 2014;9(9):1345-53.

Douillard JY et al. Br J Cancer 2014;110:55–62.

Concordance of EGFR mutation status between matched tissue/cytologic and plasma samples

- ▶ Noninterventional diagnostic study, 56 centers (Europe, Japan)
- ▶ Newly diagnosed locally advanced/ metastatic NSCLC patients
- ▶ 1162 matched tissue/cytologic and blood samples

	Concordance Rate		Sensitivity		Specificity	
Characteristic	n/n (%)	95% CI	n/n (%)	95% CI	n/n (%)	95% CI
Overall (n = 1162)	1035 of 1162 (89)	87.1-90.8	87 of 189 (46)	38.8-53.4	948 of 973 (97)	96.2-98.3
Japan (n = 281)	227 of 281 (81)	75.7-85.2	34 of 86 (40)	29.2-50.7	193 of 195 (99)	96.3-99.9
Europe (n = 881)	808 of 881 (92)	89.7-93.4	53 of 103 (51)	41.4-61.4	755 of 778 (97)	95.6-98.1
Qiagen therascreen EGFR RGQ PCR Kit (n = 138)	131 of 138 (95)	89.8-97.9	16 of 22 (73)	49.8-89.3	115 of 116 (99)	95.3-100
Roche cobas EGFR Mutation Test (n = 23)	22 of 23 (96)	78.1-99.9	3 of 4 (75)	19.4-99.4	19 of 19 (100)	82.4-100
Cycleave (n = 190)	161 of 190 (85)	78.8-89.5	29 of 57 (51)	37.3-64.4	132 of 133 (99)	95.9-100
PNA-LNA PCR Clamp[a] (n = 91)	76 of 91 (84)	74.3-90.5	15 of 29 (52)	32.5-70.6	61 of 62 (98)	91.3-100
Other[b]	20 of 191 (10)		7 of 119 (6)			

Reck M et al. J Thorac Oncol. 2016;11(10):1682-9.

ctDNAfor the detection of EGFR mutation in NSCLC

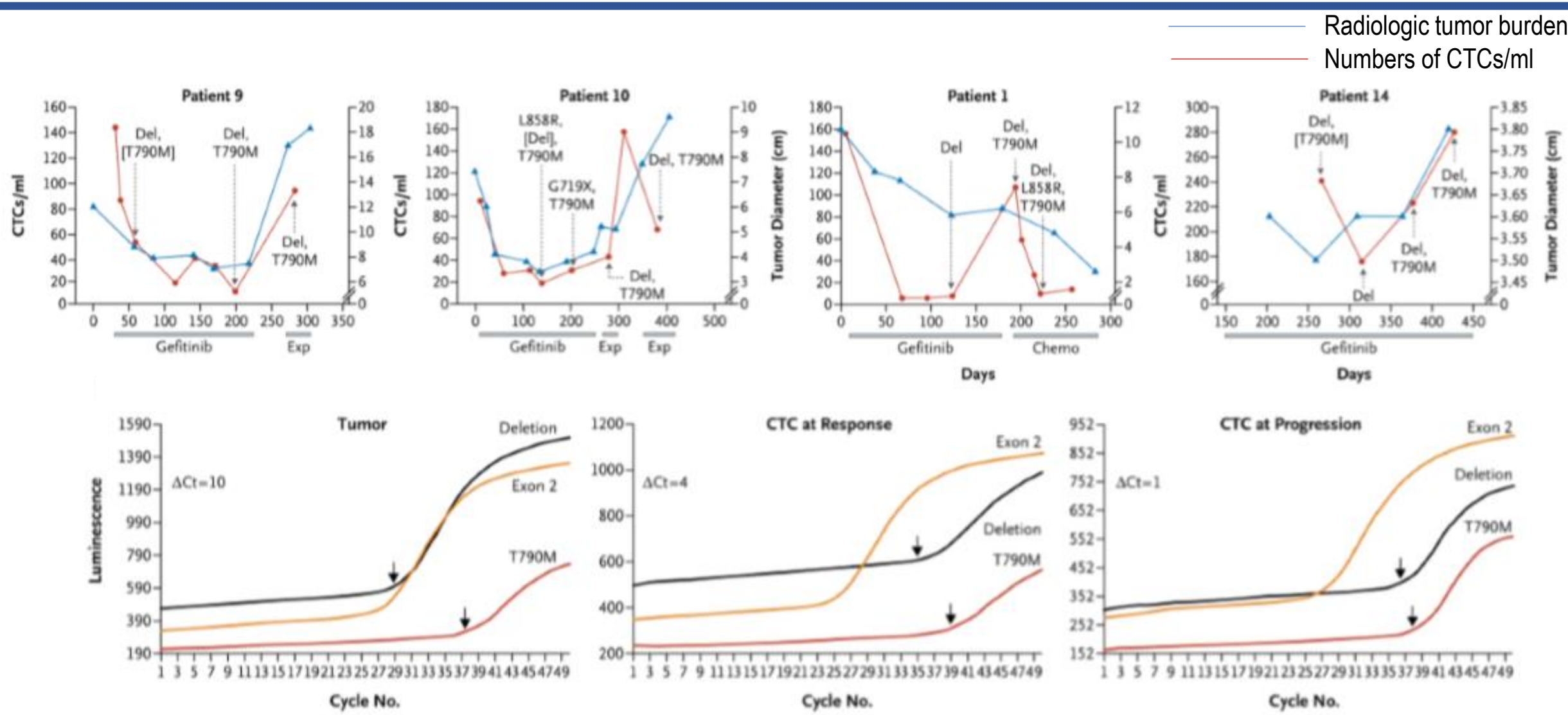
	Studies	AUSROC	Sensitivity	Specificity
Overall	27	0.91 (0.89–0.94)	0.620 (0.513–0.716)	0.959 (0.929–0.977)
Format of blood sample				
Plasma	18	0.92 (0.89–0.94)	0.599 (0.468–0.717)	0.960 (0.925–0.979)
Serum	9	0.90 (0.87–0.92)	0.658 (0.463–0.811)	0.954 (0.864–0.986)
TNM stage				
I-IV	6	0.94 (0.91–0.95)	0.786 (0.420–0.949)	0.921 (0.751–0.978)
Advanced	14	0.96 (0.94–0.97)	0.521 (0.399–0.641)	0.962 (0.940–0.977)
Storage method of tumor tissues				
FFPE	14	0.93 (0.90–0.95)	0.607 (0.484–0.718)	0.957 (0.925–0.975)
Frozen	4	0.84 (0.81–0.87)	0.627 (0.253–0.893)	0.908 (0.479–0.991)
Detection methods				
ARMS	9	0.88 (0.85–0.91)	0.549 (0.419–0.672)	0.975 (0.937–0.991)
AS-APEX	2	0.96 (0.94–0.98)	0.859 (0.189–0.994)	0.935 (0.527–0.995)
DHPLC	2	0.82 (0.78–0.85)	0.628 (0.572–0.681)	0.846 (0.813–0.874)
HRM	2	0.91 (0.88–0.93)	0.887 (0.402–0.989)	0.736 (0.042–0.994)
ME-PCR	3	0.97 (0.95–0.98)	0.556 (0.290–0.794)	0.975 (0.906–0.994)
Collection time of blood sample				
BC	6	0.89 (0.86–0.91)	0.647 (0.375–0.848)	0.967 (0.773–0.996)
AC	2	0.81 (0.78–0.85)	0.307 (0.149–0.528)	0.961 (0.732–0.995)

Abbreviations: BC, before chemotherapy; AC, after chemotherapy.

- Meta-analysis
- 3,110 subjects (27 studies)
- Gold standard; tissue EGFR

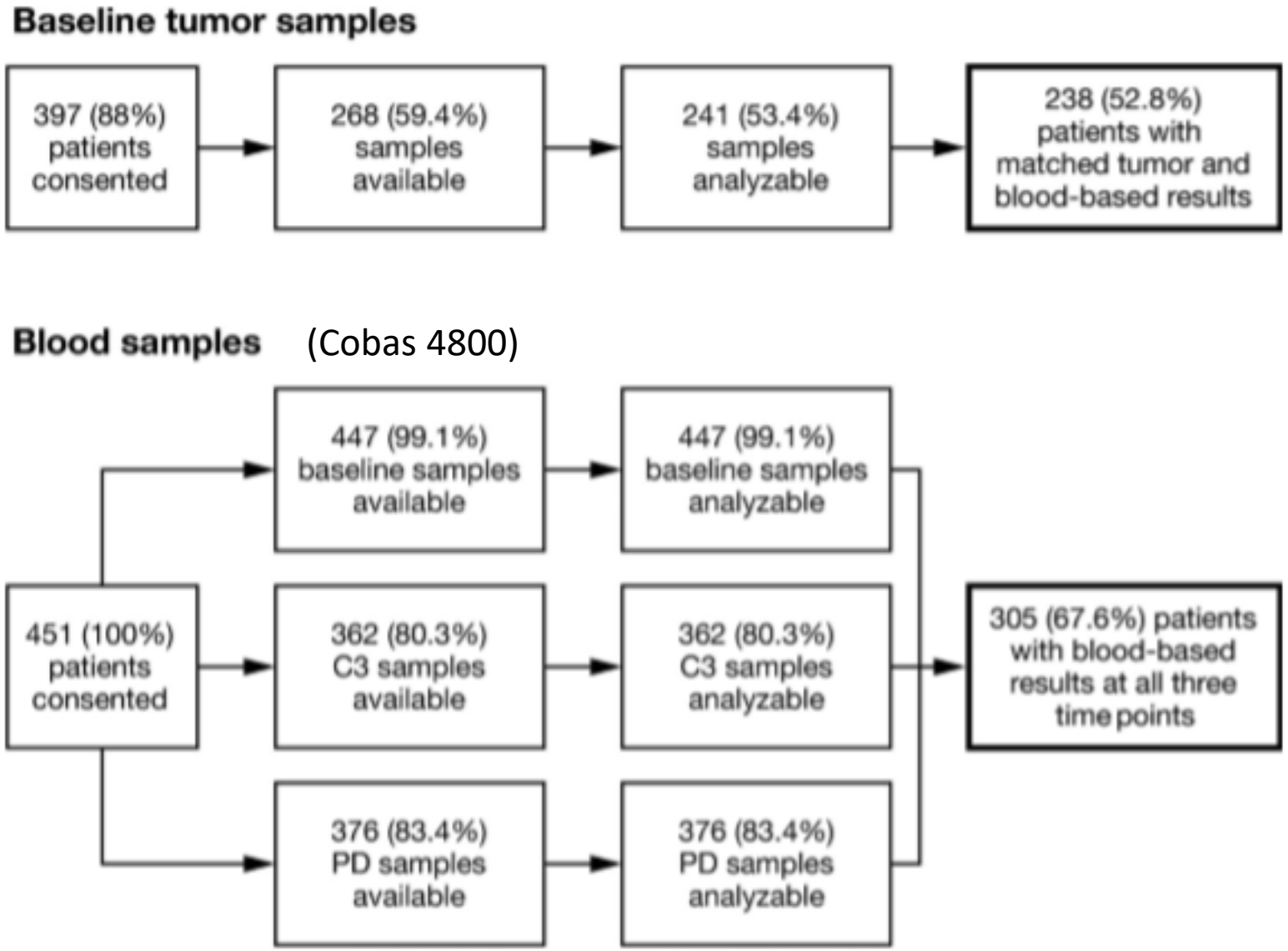
Qiu M, et al. Cancer Epidemiol Biomarkers Prev. 2015;24(1):206-12.

Disease monitoring; CTCs



Maheswaran S et al. N Engl J Med. 2008;359(4):366-77.

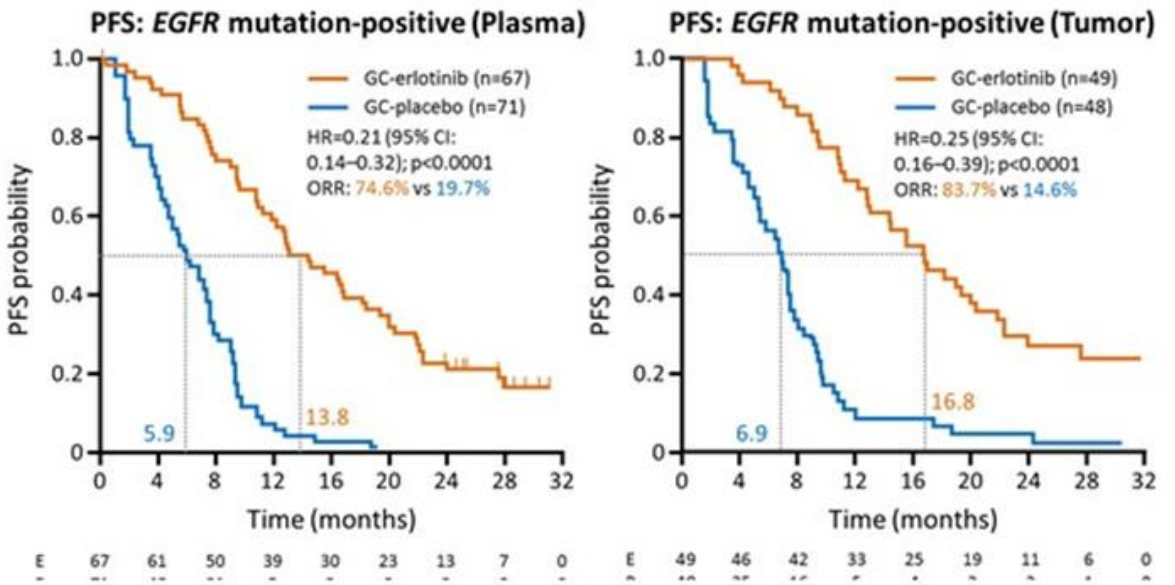
Serial EGFR mutation analysis

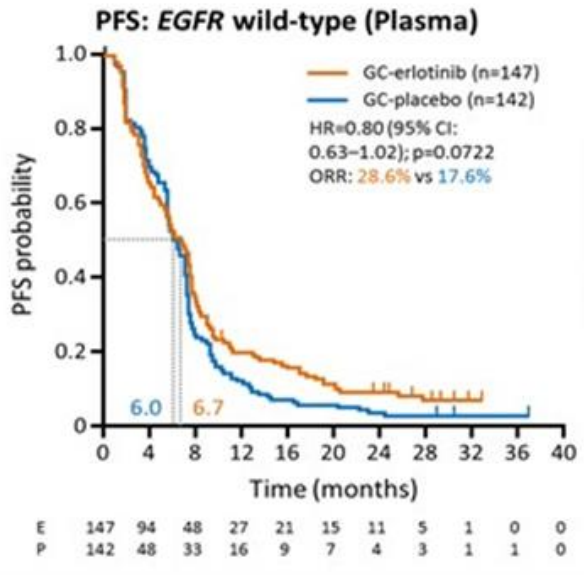


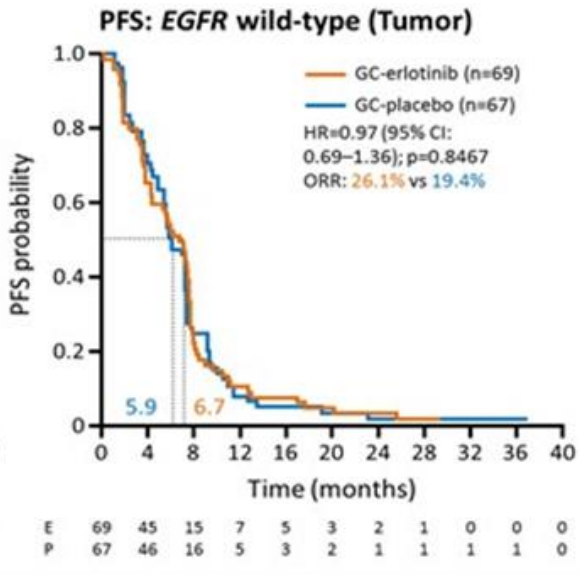
- FASTACT-2 study
  - Untreated advanced NSCLC
  - Gemcitabine+Platinum# 6
  - Sequential erlotinib vs. placebo

Mok T et al. Clin Cancer Res. 2015;21(14):3196-203

Plasma EGFR mutation as a predictive marker







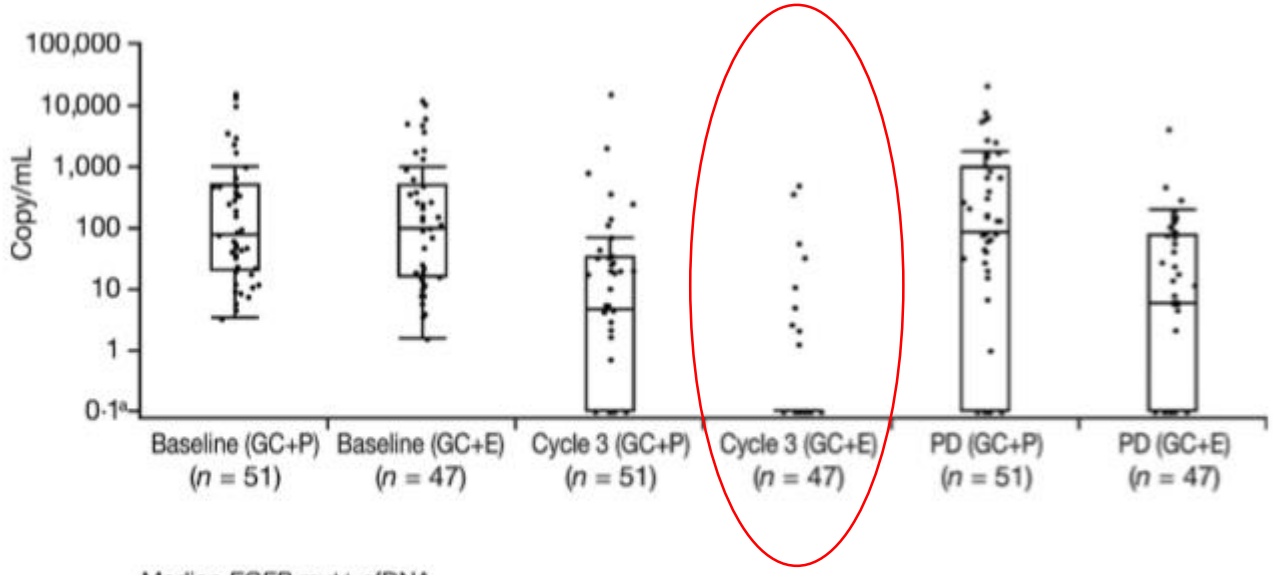
Mok T et al. Clin Cancer Res. 2015;21(14):3196-203

Serial EGFR mutation analysis

Table 2. Concordance between tumor and cfDNA mutation results at baseline

EGFR TKI-sensitive mutations	cfDNA *EGFR* mut$^+$	cfDNA *EGFR* mut$^-$	Total
Tumor tissue *EGFR* mut$^+$	72	24	96
Tumor tissue *EGFR* mut$^-$	5	137	142
Total	77	161	238

NOTE: For concordance calculations only, single resistant mutations found in the tumor were counted as mutation negative.



Median *EGFR* mut$^+$ cfDNA (copy/mL of blood)	GC+P	GC+E
Baseline	78	94
C3	5	0
PD	83	6

- ▶ Sensitivity : 75.0% (72/96)
- ▶ Specificity : 96.5% (137/142)
- ▶ Positive predictive value : 93.5% (72/77)
- ▶ Negative predictive value : 85.1% (137/161)
- ▶ Overall concordance : 87.8% (209/238)

Mok T et al. Clin Cancer Res. 2015;21(14):3196-203

Efficacy outcomes by C3 cfDNA mutation status

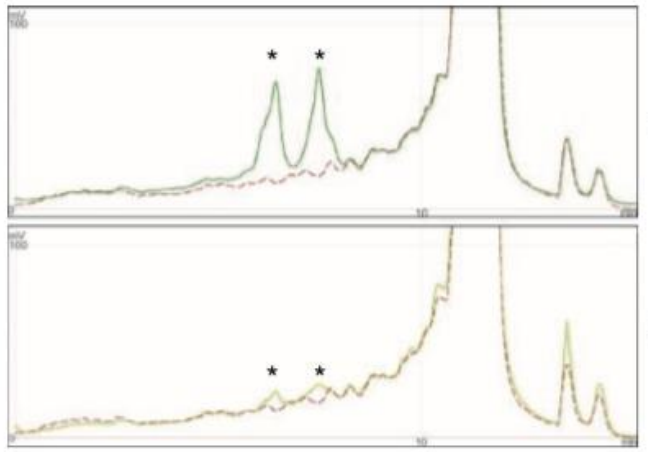
Table 3. Efficacy outcomes for baseline cfDNA mut$^+$ patients by C3 cfDNA mutation status

C3	ORR, %	Median PFS, mo	Median OS, mo
EGFR mut$^+$			
GC+P ($n = 33$)	24.2	6.8	18.8
GC+E ($n = 9$)	66.7	7.8	17.7
	OR, 6.25 (95% CI, 1.26–30.90)	HR, 0.38 (95% CI, 0.17–0.90)	HR, 0.98 (95% CI, 0.40–2.42)
EGFR mut$^-$			
GC+P ($n = 23$)	26.1	7.8	26.3
GC+E ($n = 57$)	82.5	16.6	32.4
	OR, 13.32 (95% CI, 4.20–42.23)	HR, 0.23 (95% CI, 0.13–0.41)	HR, 0.61 (95% CI, 0.31–1.21)

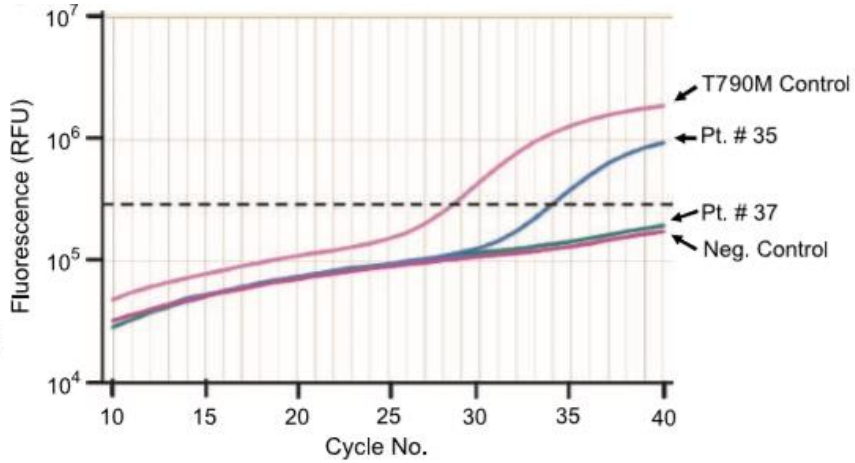
Mok T et al. Clin Cancer Res. 2015;21(14):3196-203

Noninvasive detection of EGFR T790M

No. of patients	*n* = 54
Gender	
Male	10 (18.5%)
Female	44 (81.5%)
EGFR TKI treatment	
Gefitinib	17 (31.4%)
Erlotinib	33 (61.1%)
None	4 (7.5%)
Response to prior EGFR TKI treatment	
Partial response	28 (56%)
Stable disease	14 (28%)
Progressive disease	8 (16%)
Not treated	4 (7.5%)
Tumor EGFR mutation	
Exon 19 deletion	20 (37.0%)
L858R	7 (12.9%)
L861Q	1 (1.9%)
Exon 20 insertion	2 (3.7%)
Wild type	13 (24.1%)
Unknown	11 (20.4%)



WAVE-HS system/ Surveyor



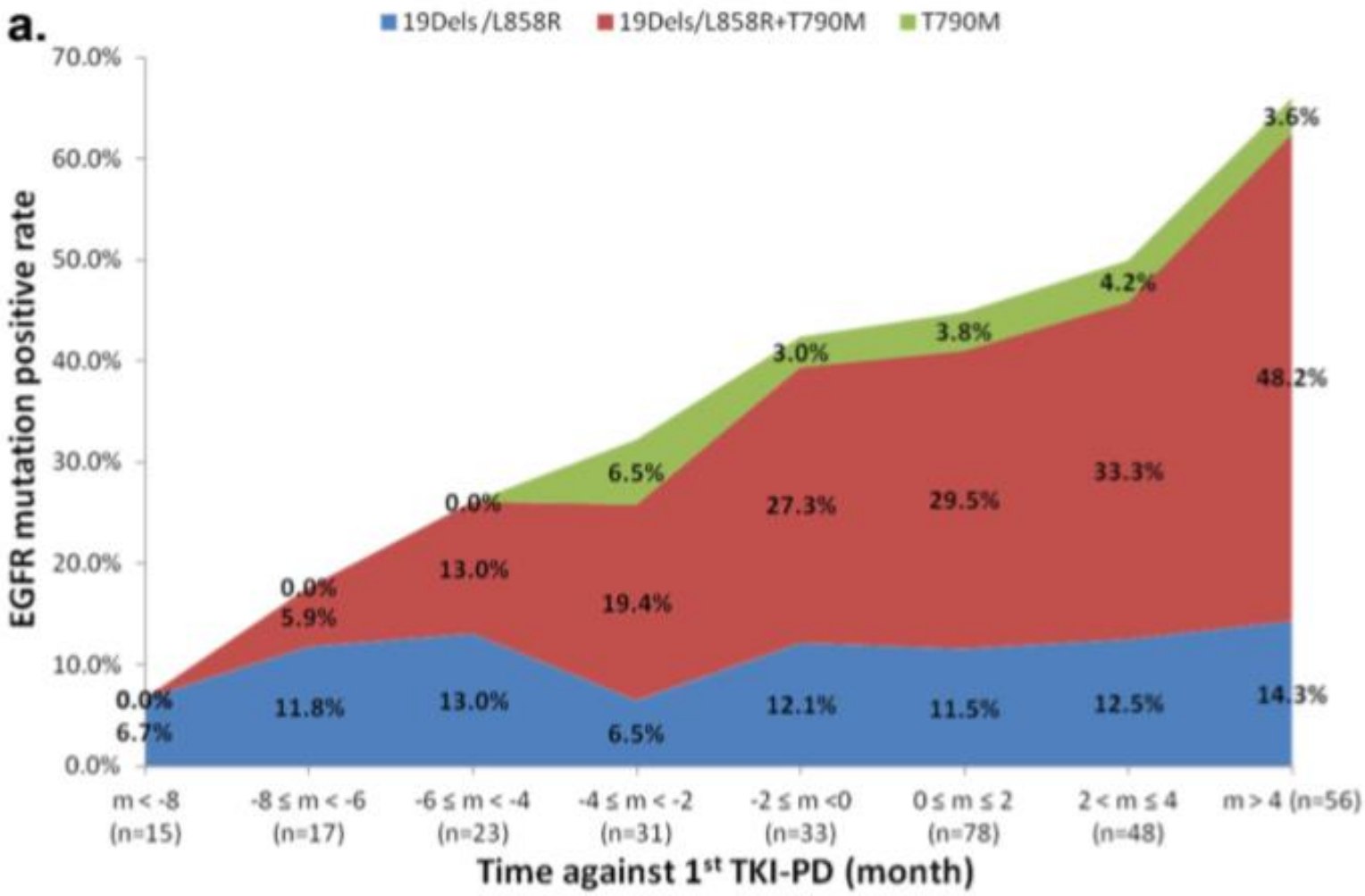
SARMS

EGFR mutation	Del E746_A750	L858R	T790M
Plasma DNA alone			
Total positive patients			
SARMS-positive			
WAVE/Surveyor-positive			
Concordance			
Plasma DNA and whole genome a			
Total positive patients			
SARMS-positive			
WAVE/Surveyor-positive			
Concordance			

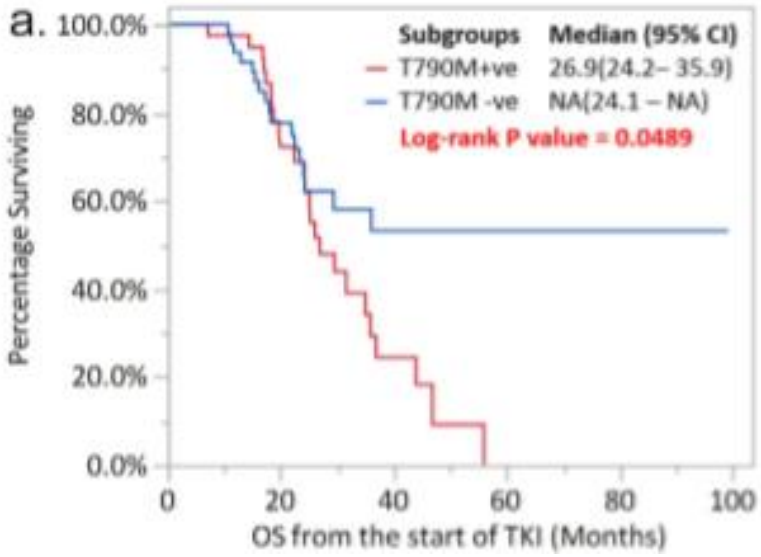
		Plasma *EGFR* T790M	
		Yes	No
Response to prior EGFR TKI Therapy			
Partial response	28	15	13
Stable disease	14	4	10
Progressive disease	8	0	8
Untreated	4	0	4
Tumor *EGFR* T790M	7	5	2

Kuang Y et al. Clin Cancer Res. 2009;15(8):2630-6.

Dynamic monitoring of EGFR mutant ctDNA

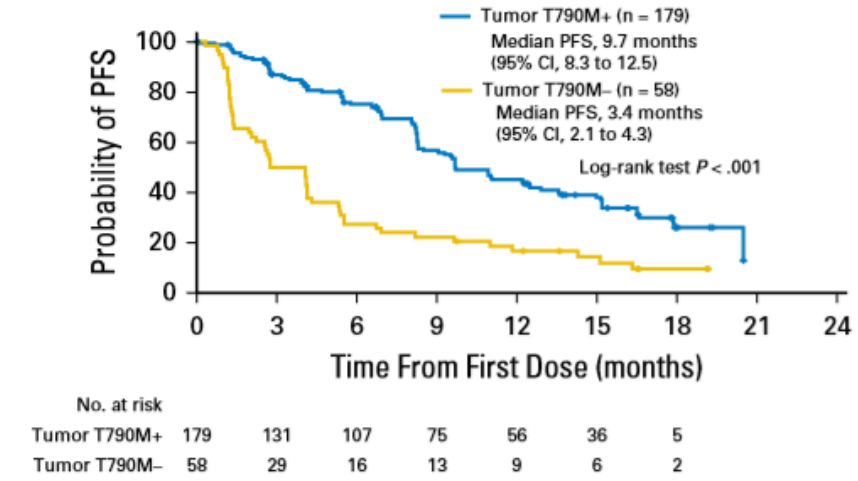


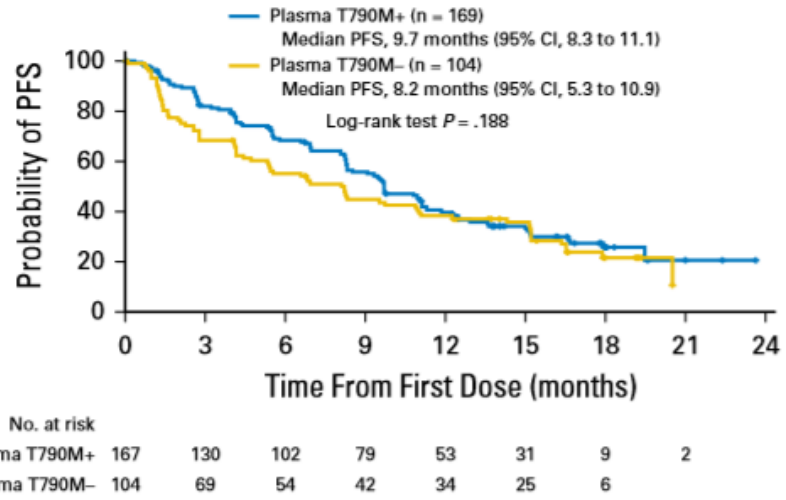
- ▶ 117 patients
- ▶ Before and after EGFR-TKI progression
- ▶ Every 2 months



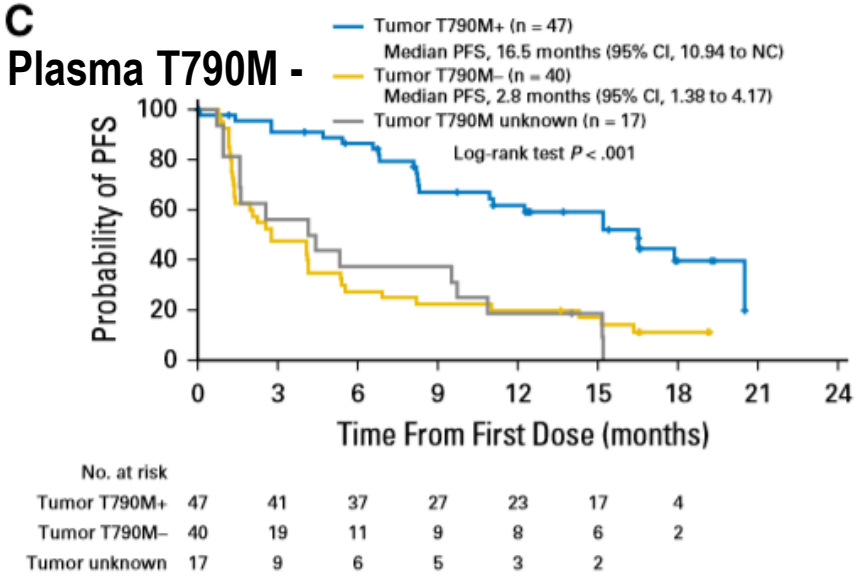
Zheng D et al. Sci Rep. 2016;6:20913.

Response according to tumor/plasma T790M

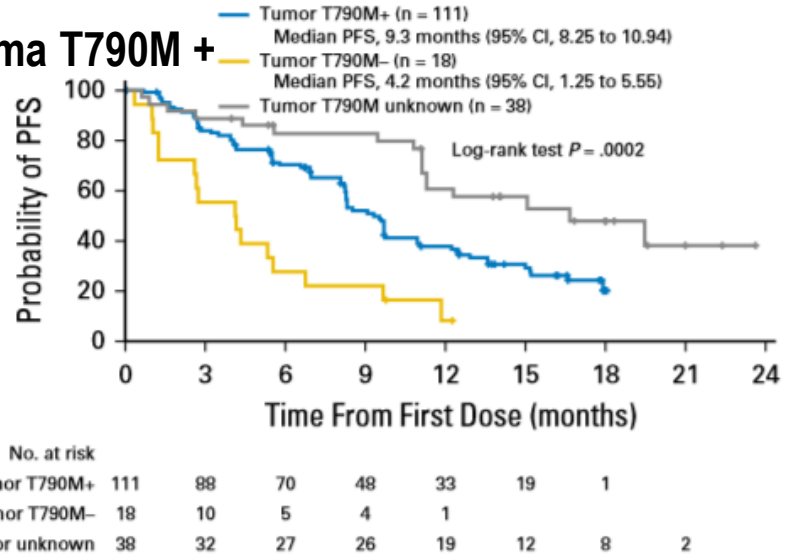




C
Plasma T790M -



D
Plasma T790M +



- Retrospective
- 216 patients
- BEAMing

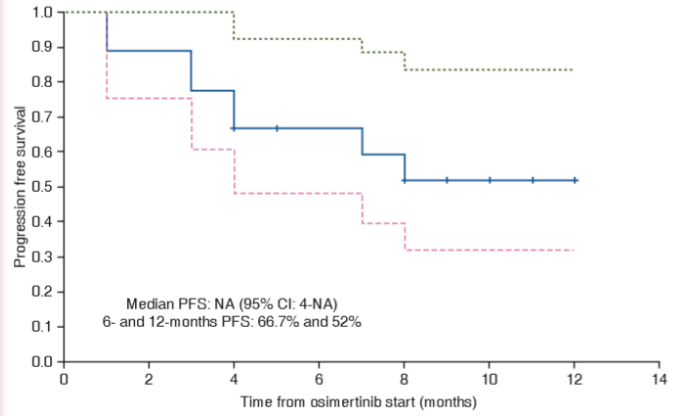
- Limitation of plasma T790M
  - Low sensitivity (false negative)

Oxnard GR et al. J Clin Oncol. 2016 Oct 1;34(28):3375-82.

Response to osimertinib in ctDNA T790M+ patients

Patient	Gender	Age (years)	Pack-years	EGFR mutation	*T790M* AF (%)	Previous systemic treatments	Previous EGFR TKI	Other mutations[a]	Last treatment before osimertinib	RECIST osimertinib
1	M	51	0	Del19	0.41	3	1	TP53 (P151X, R273H)	Erlotinib	NE
2	F	56	0	Del19	15.96	3	2	TP53 (Q331*, V225A)	Erlotinib	SD (−10%)
3	M	54	6	Del19	0.86	3	1	TP53 (R337C) STK11 (P179L)	Erlotinib -BVZ	PR (−50%)
4	F	37	0	Del19	1.06	3	2	TP53 (Q165*)	Erlotinib	PR (−84%)
5	M	67	6	Del19	1.60	4	2	CTNBB1 (S37S)	Pem/Cis	PR (−50%)
6	F	83	10	Del19	6.96	2	2	CTNNB1 (S33C)	Erlotinib	SD (0%)
7	F	67	0	Del19	19.60	1	1	CDKN2A (frameshift) TP53 (frameshift)	Erlotinib	PR (−50%)
8	F	70	0	L858R	0.25	2	1	NRAS (A59G)	Pem	SD (−26%)
9	F	66	5	L858R	0.07	10	3[b]	–	Erlotinib	PR (−65%)
10	F	81	0	L858R	5.38	4	3	TP53 (P60X, splice) PIK3CA (E545K)	Pem	PR (−33%)
11	F	70	0	Del19	0.31	3	1	TP53 (R282W)	Pem	NE
12	F	58	0	Del19	0.24	6	2	–	Erlotinib	PR (−68%)
13	F	54	0	Del19	2.24	3	2	–	Pem/Cb	SD (9%)
14	F	59	10	Del19	0.14	2	1	TP53 (I232S)	Gefitinib	PR (−50%)
15	F	67	2	L858R	0.30	3	1	EGFR (K860I)	Erlotinib	SD (−20%)
16	M	61	20	Del19	0.70	5	3[c]	TP53 (E343*, C238Y, C135X)	Afatinib	SD (−18%)
17	F	54	3	Del19	3.95	2	1	TP53 (R249S)	Gefitinib	PR (−32%)
18	F	65	0	Del19	0.68	1	1	CTNNB1 (S37C)	Gefitinib	PR (−32%)

- ctDNA
- Inivata InVision™ (eTAm-Seq™) assay



Remon J et al. Annals of Oncology 2017; 28: 784–790.

Clinical outcomes of third generation EGFR TKIs

Study (author, Year)	Patients' (or Tumor) characteristics	Treatment	Sample (plasma or tissue), volume, if possible	Testing platform	Clinical outcomes	
					T790M +	T790M-
Janne et al., 2015 [19]	Adenocarcinoma and Squamous cell carcinoma patients	Osimertinib (AZD9291)	Tissue	Cobas (Roche)	ORR: 61% (95% CI, 52–70) DCR: 95% (95% CI, 90–98) Median PFS: 9.6 months (95% CI, 8.3 to not reached)	ORR: 21% (95% CI, 12–34) DCR: 61% (95% CI, 47–73) Median PFS: 6 months (95% CI, 2.1–4.3)
Oxnard GR et al., 2016 [35]	Adenocarcinoma and Squamous cell carcinoma patients	Osimertinib (AZD9291)	Tissue	Cobas (Roche)	ORR: 62% (95% CI, 54–70) Median PFS: 9.7 months (95% CI, 8.3–12.5)	ORR: 26% (95% CI, 15–39) Median PFS: 3.4 months (95% CI, 2.1–4.3)
			Plasma (10–20 ml blood)	BEAMing	ORR: 63% (95% CI, 55–70) Median PFS: 9.7 months (95% CI, 8.3–11.1)	ORR: 46% (95% CI, 36–56) Median PFS: 8.2 months (95% CI, 5.3 to 10.9)
Yang J et al., 2016 [95]	Advanced NSCLC	Osimertinib (AZD9291)	Tissue	Cobas (Roche)	ORR: 71% (95% CI 57–82) DoR: 9.6 months (95% CI 7.7–15.6) Median PFS: 9.7 months (95% CI 8.3–13.6)	NR
Wu YL et al., 2016 [94]	Advanced NSCLC	Avitinib (AC0010)	Tissue	Cobas (Roche)	ORR: 52% DCR: 94%	NA
Mok et al., 2017 [18]	Advanced NSCLC	Osimertinib (AZD9291)	Plasma	Cobas (Roche)	ORR: 71% Median PFS: 10.1 months	NA

Liang Z et al. Cancer Lett. 2017;403:186-194.

Concordance of EGFR T790M mutation

Study group	Sample	Method		Parameters		
		Plasma detection	Tissue detection	Sensitivity/Positive percent agreement (PPA)	Specificity/Negative percent agreement (NPA)	Concordance with tissue
Thress et al. [44]	Plasma N = 38	Cobas (Roche)	Cobas (Roche)	41%	100%	57%
		ddPCR (Bio-rad)		71%	83%	74%
		BEAMing		71%	67%	70%
		ARMS (Qiagen)		29%	100%	48%
Zheng et al. [50]	Plasma N = 117	ddPCR (Bio-rad)	ARMS (AmoyDx)	81%	100%	88%
Karlovich C et al. [41]	Plasma N = 153	Cobas (Roche)	Cobas (Roche)	64%[a]	98%[b]	86%
		BEAMing		73%[a]	50%	67%
Ishii H et al. [77]	Plasma N = 18	ddPCR (Bio-rad)	ddPCR (Bio-rad)	81.8%	85.7%	83.3%
Reckamp KL et al. [62]	Plasma N = 60	NGS (Illumina, MiSeq)	Cobas (Roche) and ARMS (Qiagen)	93%	94%	Good, but percentage NR
Takahama T et al. [72]	Plasma N = 41	ddPCR (Bio-Rad)	ddPCR (Bio-Rad)	64.5%	70.0%	65.9%
Han J Y et al. [63]	Plasma N = 39	PANAMutyper R EGFR kit	Ion Torrent NGS	58%	68%	63%
Chai X et al. [78]	Plasma N = 61	cSMART	ARMS (AmoyDx)	100%	NR	98.4%
Wu YL et al. [79]	Plasma N = 399	Cobas (Roche)	Cobas (Roche)	51%	77%	NR

Liang Z et al. Cancer Lett. 2017;403:186-194.

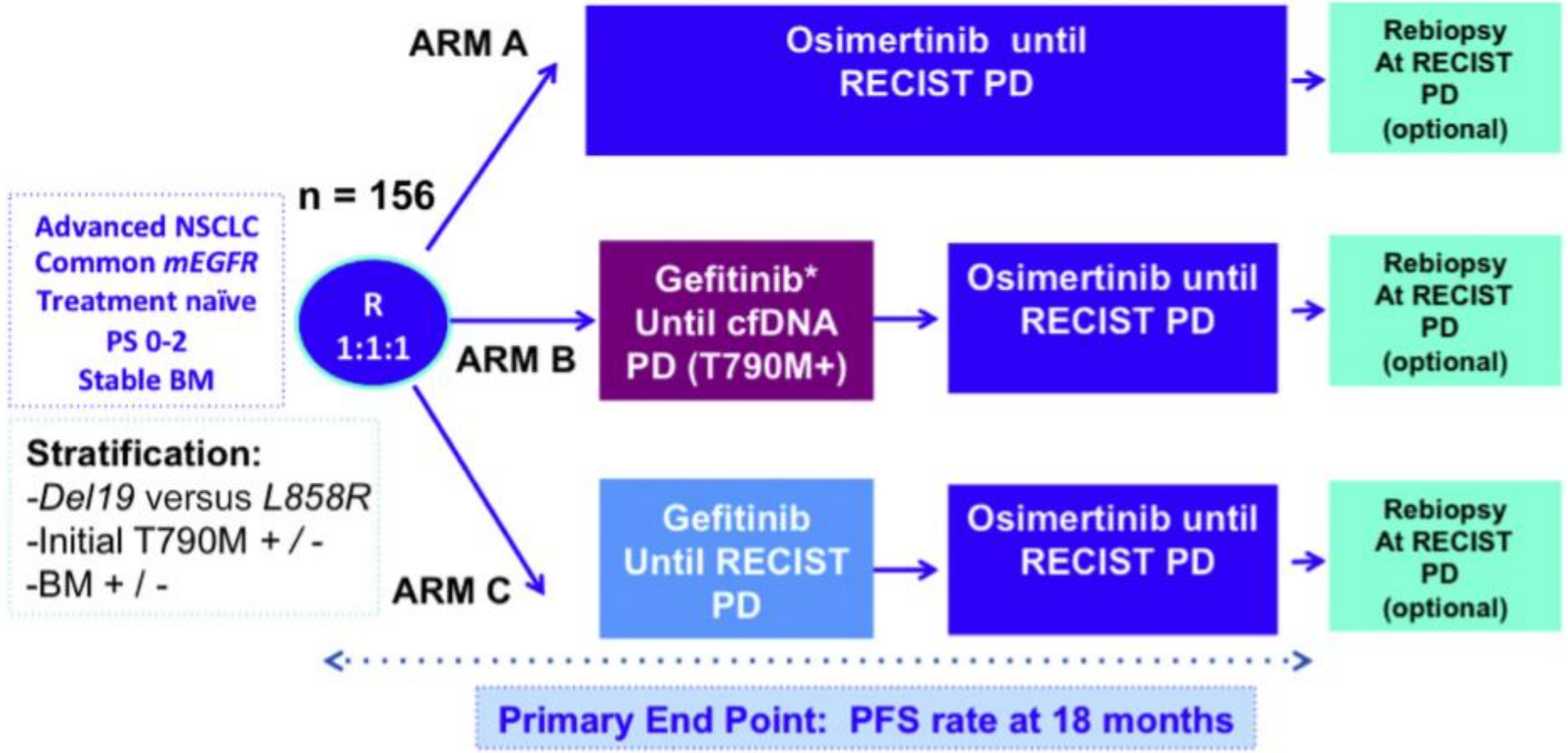
FDA approval of cobas® EGFR MUTATION TEST V2

► Date of approval : September 28, 2016

Drug	FFPET	Plasma
TARCEVA® (erlotinib)	Exon 19 deletions and L858R	Exon 19 deletions and L858R
TAGRISSO™ (osimertinib)	T790M	T790M*

Drug	FFPET	Plasma
TARCEVA® (erlotinib)	G719X, exon 20 insertions, T790M, S768I and L861Q	G719X, exon 20 insertions, T790M, S768I and L861Q
TAGRISSO™ (osimertinib)	G719X, exon 19 deletions, L858R, exon 20 insertions, S768I, and L861Q	G719X, exon 19 deletions, L858R, exon 20 insertions, S768I, and L861Q

Feasibility and Activity of Osimertinib on Positive PLasma T790M in EGFR-mutant NSCLC Patients



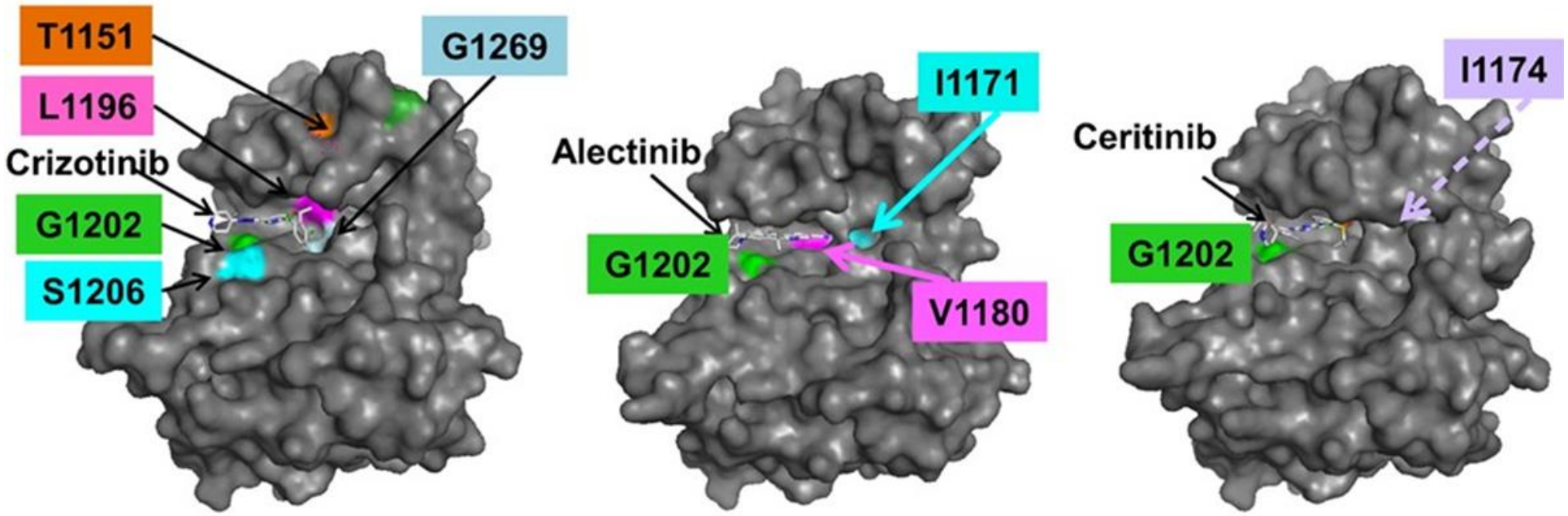
(cfDNA using cobas every 4 weeks and CT scan of the brain-thorax-abdomen every 8 weeks all arms

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

Remon J et al. Clin Lung Cancer. 2017;18(5):583-588.

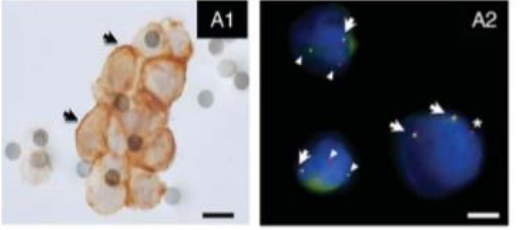
ALK gene rearrangement : Target agents

Various mutations in ALK confer resistance to Crizotinib, Alectinib or Ceritinib



Crizotinib resistance mutations	**Alectinib** resistance mutations	**Ceritinib** resistant mutations
L1196M, G1269A, C1156Y, L1152R, I1171T, F1174V, G1202R, E1210K	I1171T/N/S, G1202R, V1180L	F1174C/V, G1202R, G1202del

Detection of ALK rearrangement; CTCs

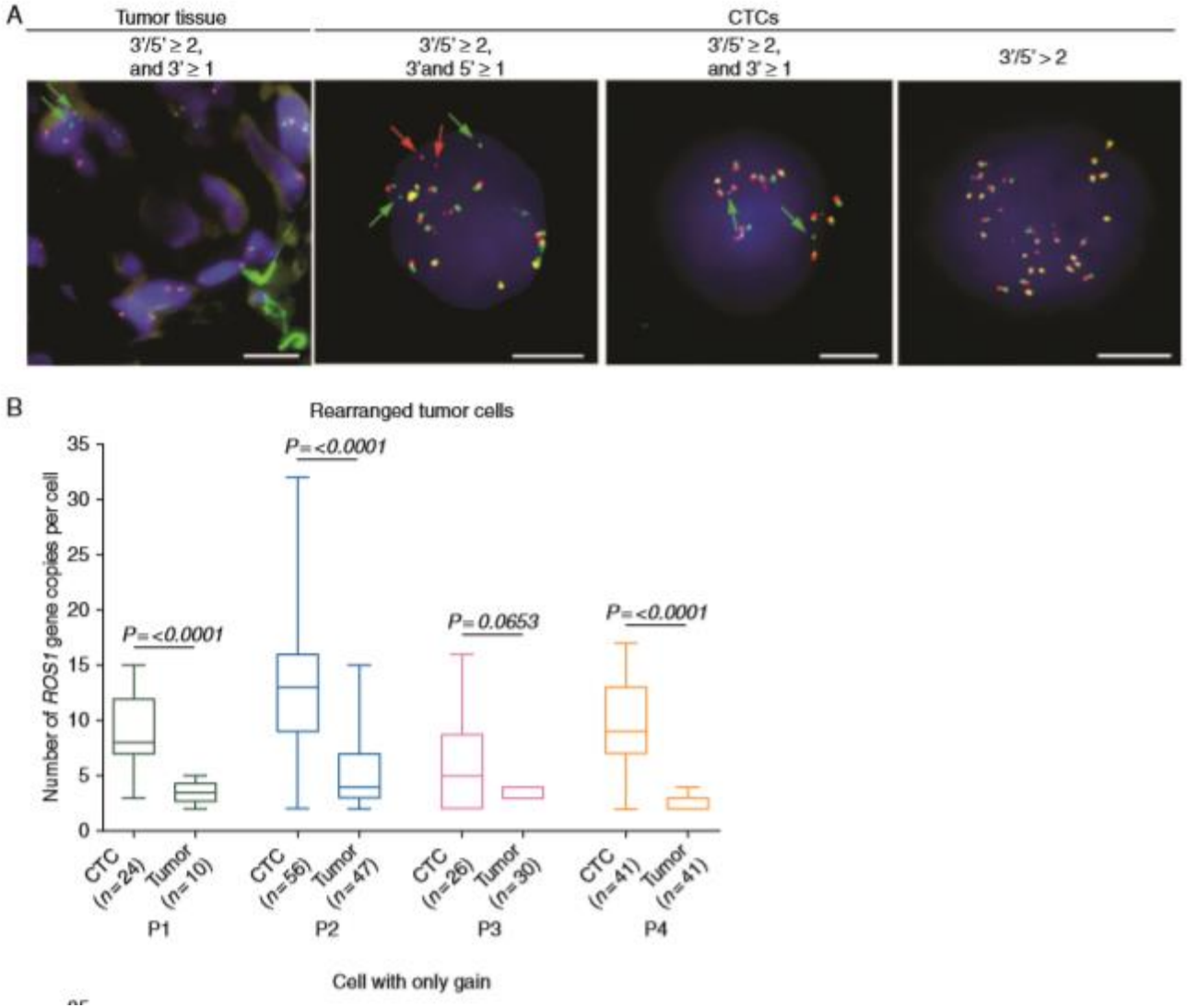


Case number	1	2	3	4	5
Sex	Male	Male	Female	Male	Female
Age (years)	45	48	47	52	43
Smoking status	Never smoked	Never smoked	Never smoked	Never smoked	Never smoked
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian
pTNM stage	IIA	IIA	IIIB	IV	IV
Histology	Adenocarcinoma with solid architecture	Adenocarcinoma with solid architecture	Adenocarcinoma with solid architecture	Adenocarcinoma with solid architecture	Adenocarcinoma with solid architecture
Status for *EGFR*, *KRAS*, *BRAF* mutations	Wild-type	Wild-type	Wild-type	Wild-type	Wild-type
ALK FISH (tumour)	Positive (40% of cells)	Positive (50% of cells)	Positive (60% of cells)	Positive (40% of cells)	Positive (50% of cells)
ALK IHC (tumour)	Positive (100% of cells)	Positive (100% of cells)	Positive (100% of cells)	Positive (100% of cells)	Positive (100% of cells)
Number of CNHC-MF	≥50 cells (70–90 cells)	≥50 cells (60–150 cells)	≥50 cells (70–100 cells)	≥50 cells (60–100 cells)	≥50 cells (80–120 cells)
ALK FISH (CTCs)	Positive (100% of cells)	Positive (100% of cells)	Positive (100% of cells)	Positive (100% of cells)	Positive (100% of cells)
ALK ICC (CTCs)	Positive (100% of cells)	Positive (100% of cells)	Positive (100% of cells)	Positive (100% of cells)	Positive (100% of cells)
Follow-up (5 years)	Alive (no relapse)	Alive (no relapse)	Deceased	Deceased	Deceased

Ilie M et al. Ann Oncol. 2012;23(11):2907-13.

ROS1 rearrangement; CTCs

Patients	Sex	Age	Smoking status	BOR	PFS	Tumor		CTCs		
						Biopsy origin[a]	% of rearranged cells	Sample	Rearranged CTCs detected by ISET (/3 ml)	CTCs counts by Cellsearch (/7.5 ml)
P1	F	74	0	SD	3.3	Lung (MT)	20%	Day 1	24	NA
								Day 75[b]	39	0
P2	M	66	0	PR	6.8+	Pleura (MT)	44%	Day 1	55	0
								Day 40	32	0
P3	F	37	16	PD	1.5	Node (MT)	60%	Day 1	28	2
								Day 45	29	6
P4	M	63	2	PR	7.1+	Node (MT)	82%	Day 1	41	2
								Day 80	22	0
PN1	M	35	0	–	–	Pleura (MS)	–	–	7	0
PN2	M	55	0	–	–	Lung (PT)	–	–	8	6
PN3	F	43	10	–	–	Lung (PT)	–	–	7	0
PN4	F	68	40	–	–	Node (MS)	–	–	11	1



Pailler E et al. J Clin Oncol 2013;31(18):2273–81.

Deep sequencing of circulating free DNA

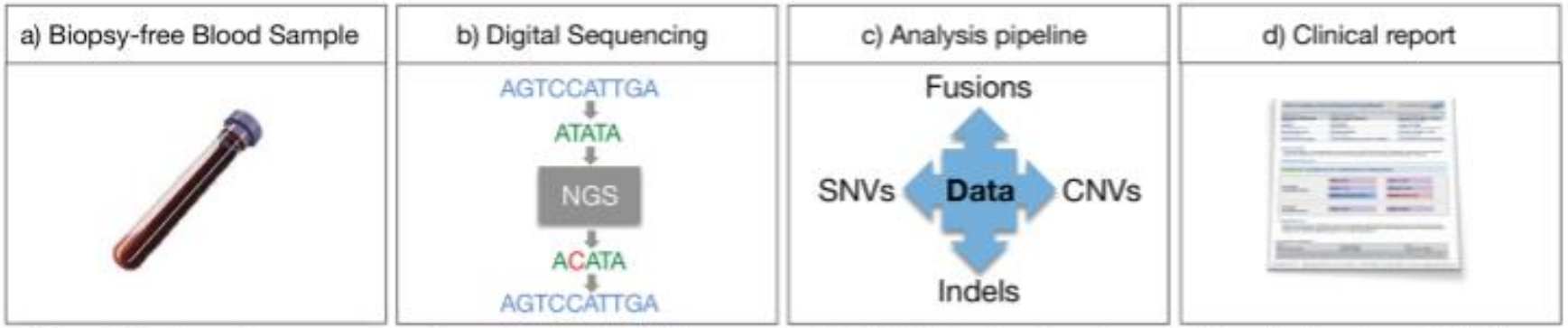
		cfDNA+		cfDNA−			
	n	tDNA+	tDNA−	tDNA−	tDNA+	Mutation detection rate (sensitivity)	Concordance rate
PI3KCA exon 9	61		1	59	1	–	97%
PI3KCA exon 20	60			60		–	100%
EGFR exon 18	59	3		56		100%	100%
EGFR exon 19	59	11	2	37	9	55%	81%
EGFR exon 20	60			58	2	–	97%
EGFR exon 21	61	6		53	2	75%	97%
BRAF exon 11	60			60		–	100%
BRAF exon 15	61			58	3	–	95%
KRAS exon 2	59	3		55	1	75%	98%
KRAS exon 3	58			58		–	100%
ERBB2 exon 19	59			59		–	100%
ERBB2 exon 20	63	3		58	2	60%	97%
Overall (95%CI; all patients, at least one amplicon)	68	26	3	20	19	58% (43%; 71%)	68% (56%; 78%)
All 12 amplicons together (95% CI)	56	21	3	16	16	57% (41%; 71%)	66% (53%; 77%)

- ▶107 plasma samples
- ▶Matched tumor DNA; 68 cases
- ▶Multiplex PCR-based assays vs. NGS IonTorrent Personal Genome Machine Platform

		cfDNA+		cfDNA−		
	n	tDNA+	tDNA−	tDNA−	tDNA+	Specificity
PI3KCA exon 9	7		1	6		86%
PI3KCA exon 20	6			6		100%
EGFR exon 18	8	2		6		100%
EGFR exon 19	33	7	2	15	9	88%
EGFR exon 20	13			11	2	100%
EGFR exon 21	18	3		13	1	100%
BRAF exon 11	8			8		100%
BRAF exon 15	31			29	2	100%
KRAS exon 2	9	2		6	1	100%
KRAS exon 3	6			6		100%
ERBB2 exon 19	6			6		100%
ERBB2 exon 20	28	3		23	2	100%
Overall (95%CI; all patients, at least one amplicon)	51	17	2	13	19	87% (62%; 96%)

Couraud S et al. Clin Cancer Res. 2014;20(17):4613-24.

Validation of a digital sequencing panel



Sample Requirements:
> 5 ng cell-free DNA

Custom collection kit with two 10 ml Streck tubes

No local centrifugation, refrigeration or cold bricks

Proprietary, high-yield cfDNA extraction

Fraction of samples with sufficient content for analysis: 99.8%

Conversion of single DNA molecules to robust Digital Sequences

Optimized NGS (paired-end SBS of both 160-170 bp DNA strands to 8,000x average coverage (99% at > 2,000x)

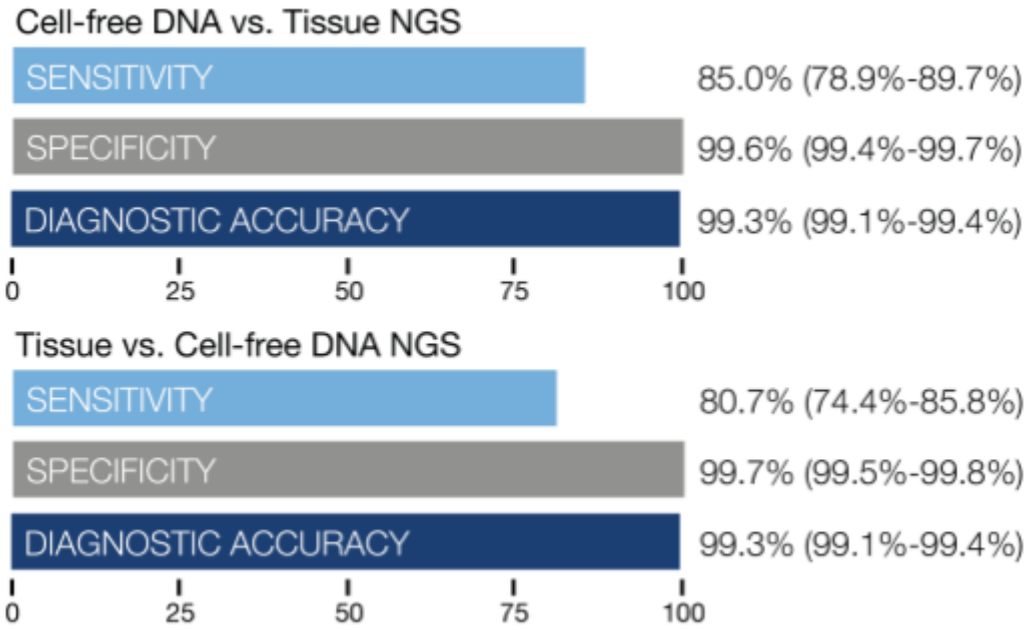
Digital Sequence decoding to genomic sequences

>100X reduction in error rates

Sensitivity to single-molecule alterations with >99.9999% accuracy

Detection of Single Nucleotide Variants (SNVs), Copy Number Variants (CNVs), Indels and Fusions by the same technology

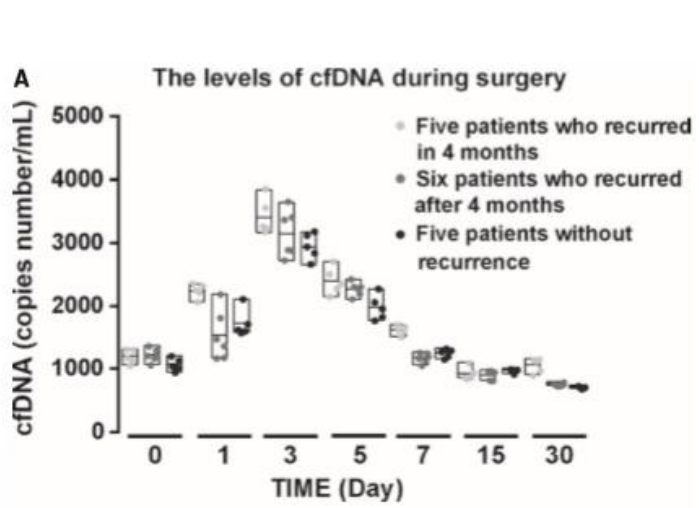
- ▶Guardant360 cfDNA assay
- ▶Illumina Hiseq 2500
- ▶165 paired samples
- ▶5 centers

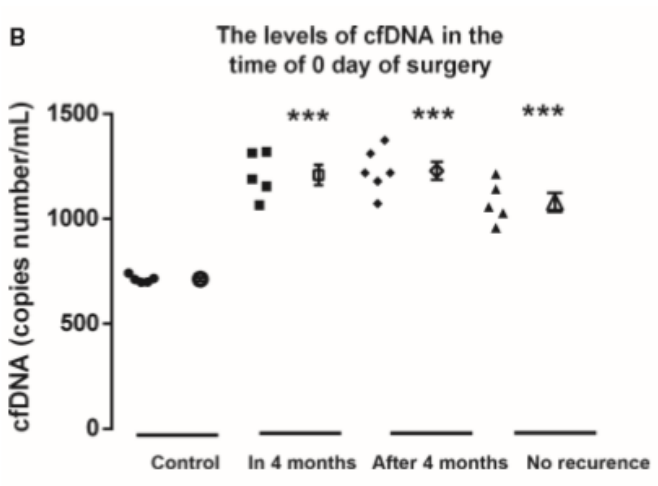


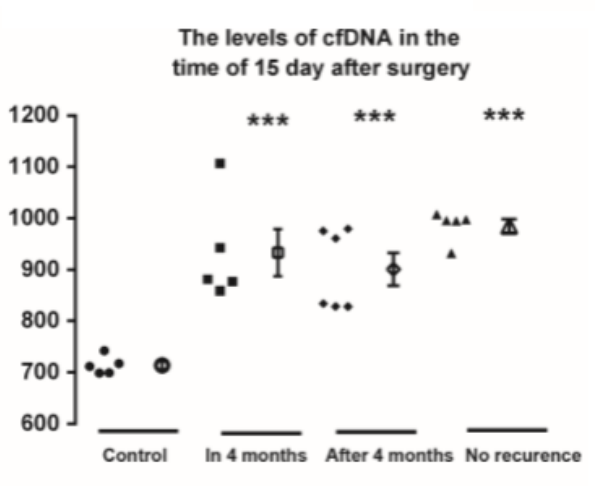
Lanman RB et al. PLoS One. 2015 Oct 16;10(10):e0140712.

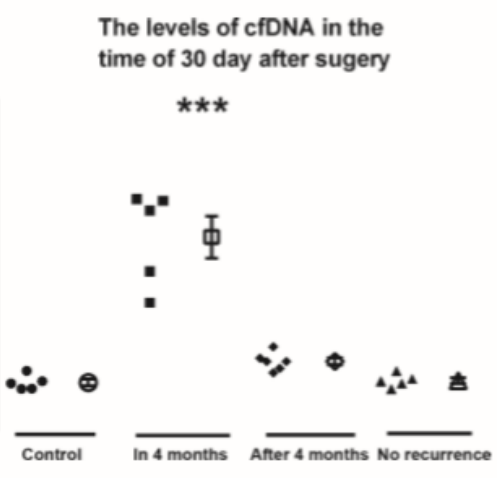
Post surgery ctDNA for relapse of lung cancer

- 168 lung cancer patients
- Copy number of Kras and EGFR : competitive allele- specific TaqMan PCR (CAST- PCR)
- Five patients who recurred in 4 months had significantly higher circulating cell-free DNA ($P < 0.001$)



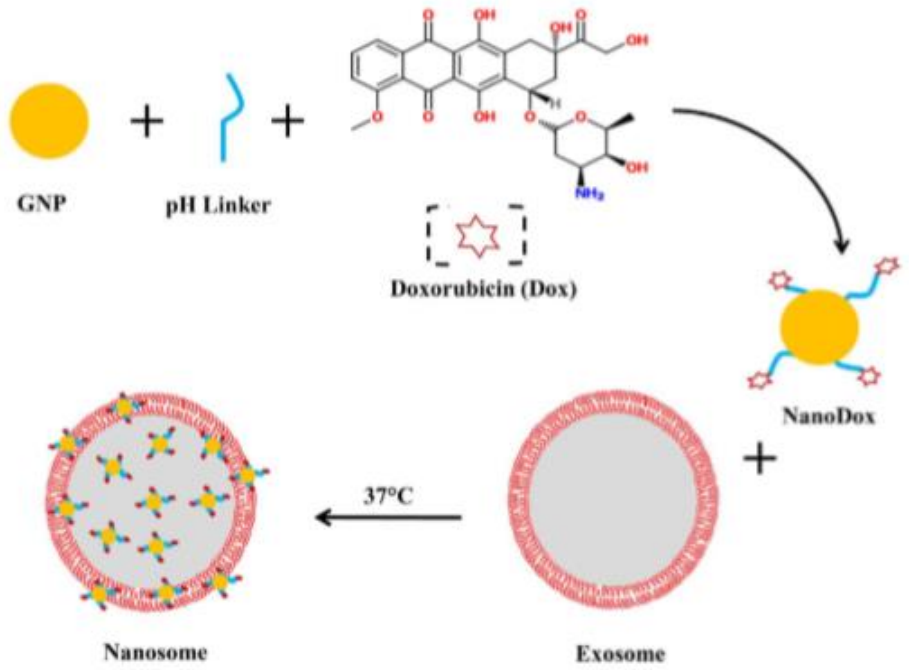


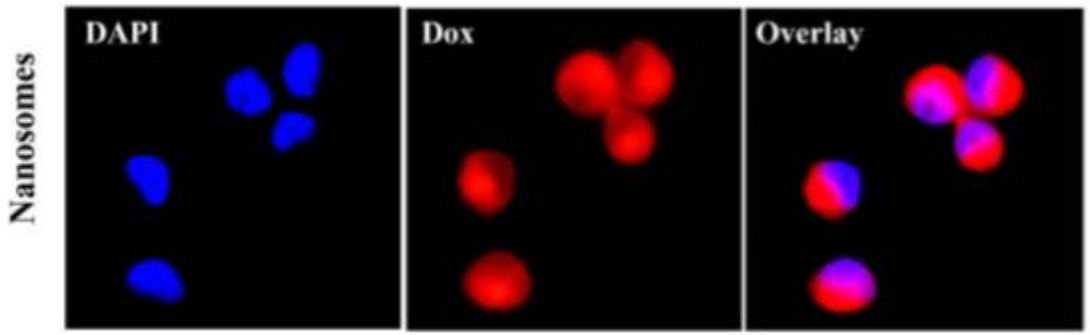


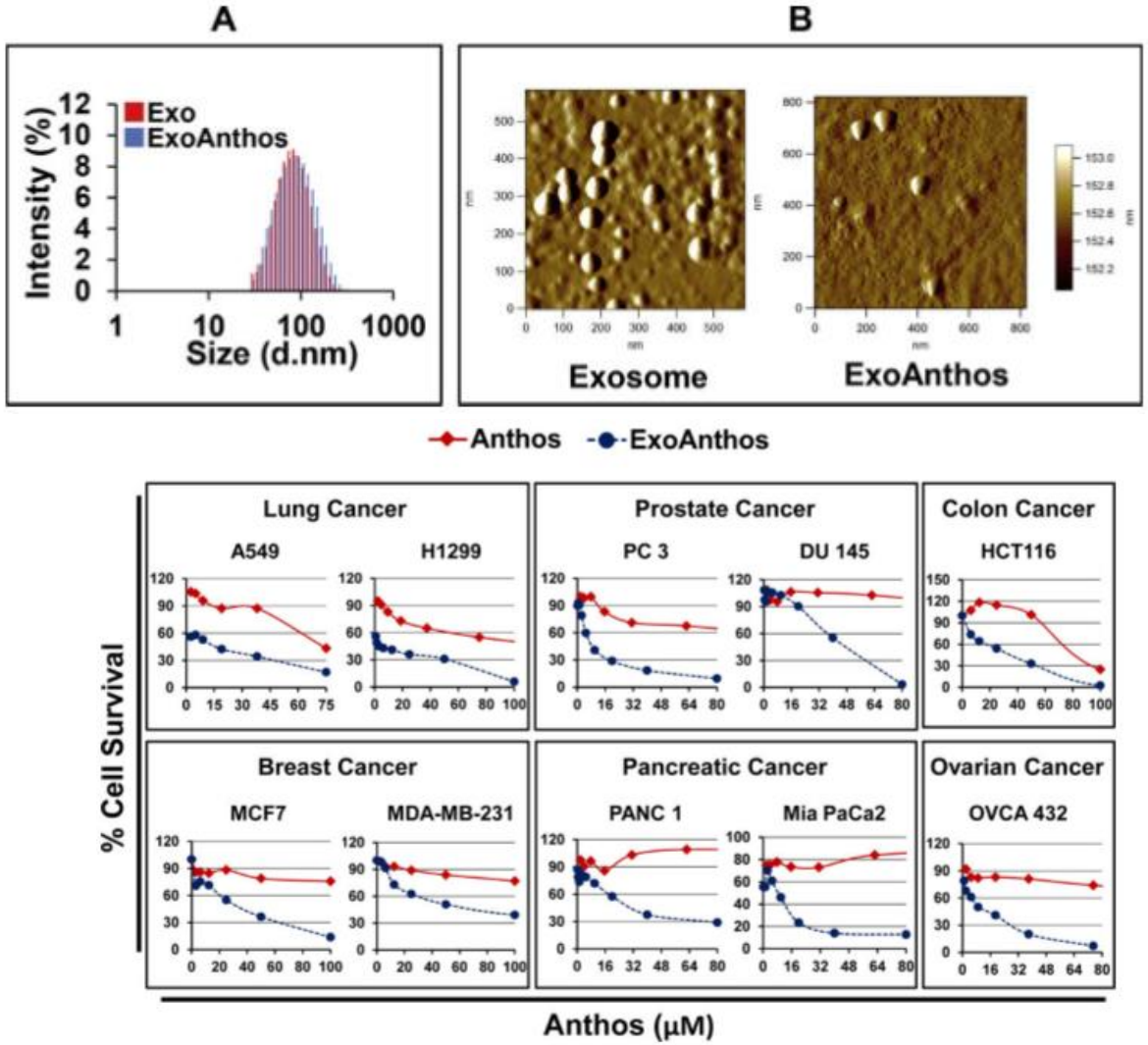


Hu W et al. Cancer Med. 2017 May;6(5):962-974.

Exosomes as a drug delivery system



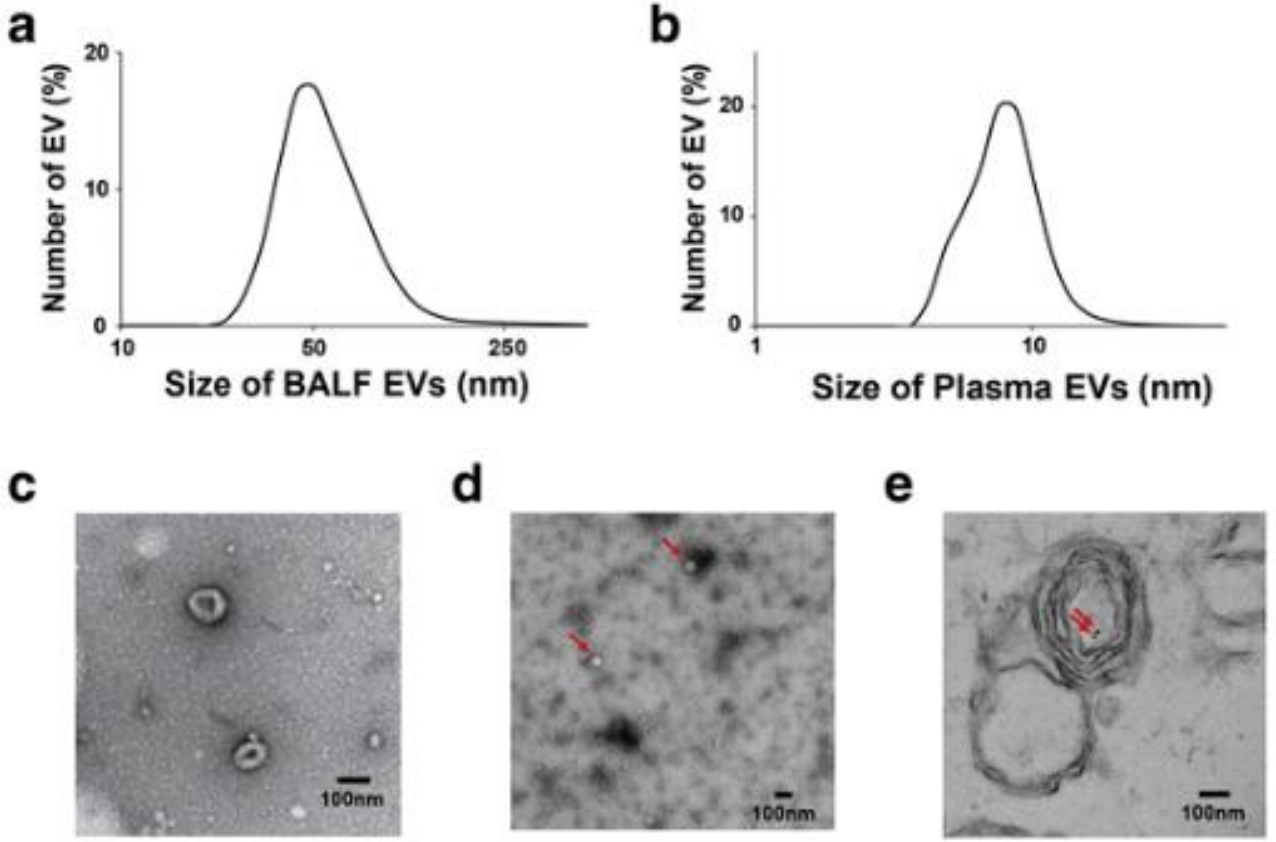


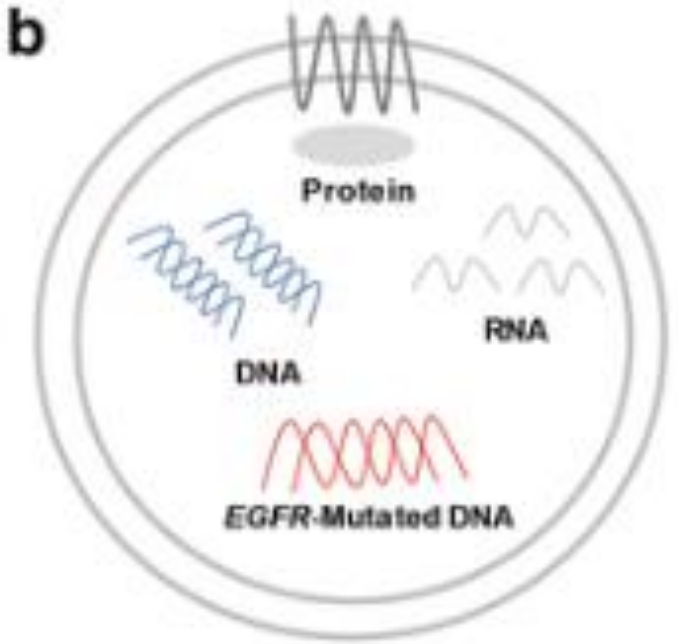


Srivastava A et al. Sci Rep. 2016;6:38541.
Munagala R et al. Cancer Lett. 2017;393:94-102.

Clinical experiences in KUMC

- Bronchoalveolar lavage fluid (BALF), Extracellular vesicles (EVs)





Hur JY et al. Mol Cancer. 2018;17(1):15.

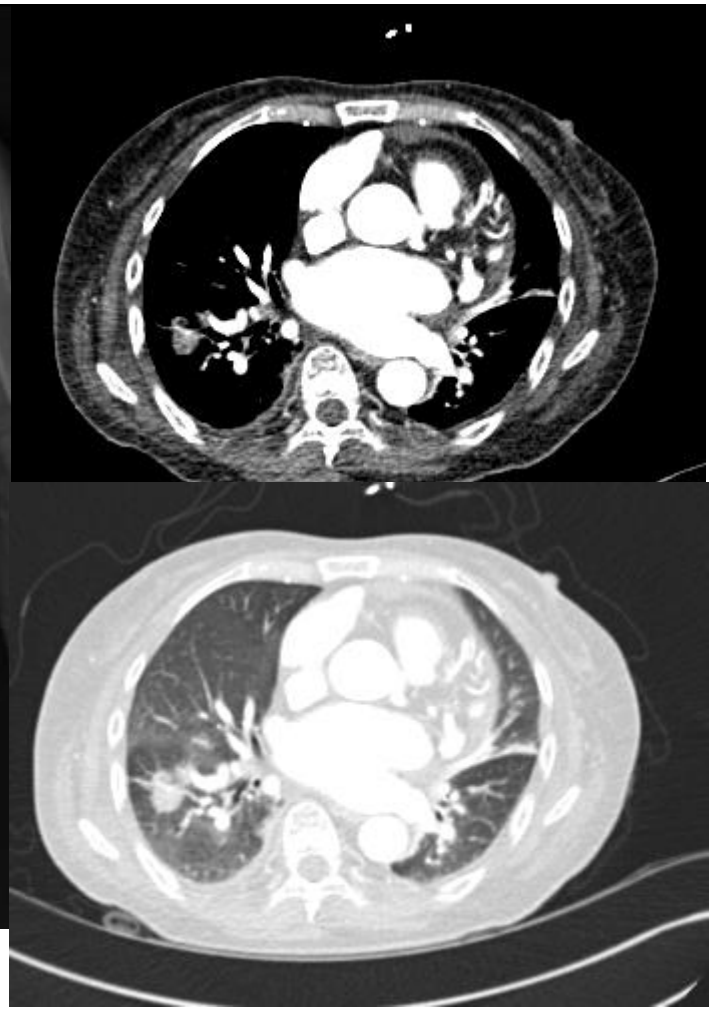
Case #1 F/65

- 2018.2.1. dizziness 주소로 신경과 입원
- cerebral infarction 진단 하에 원인 질환 감별 위한 검사 진행
- coronary CT에서 RML nodule 발견되어 의뢰됨.

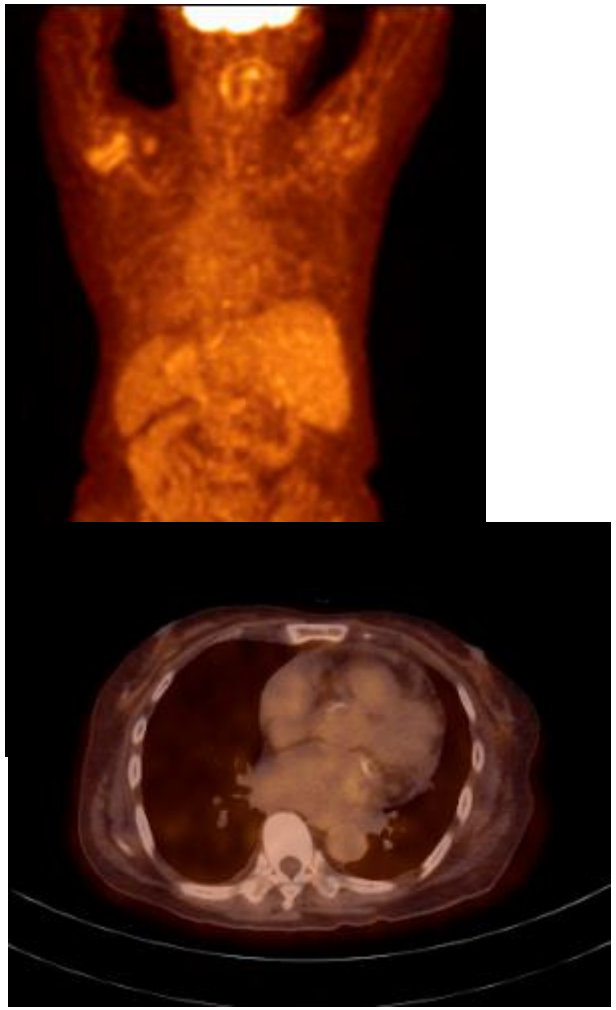
- 기저 질환 : hypertension, thyroid cancer, ESRD on HD

Case #1 F/65

2018-02-02



2018-02-02



2018-02-07

►2.5 cm RLL nodule, SUVmax 2.0

Case #1

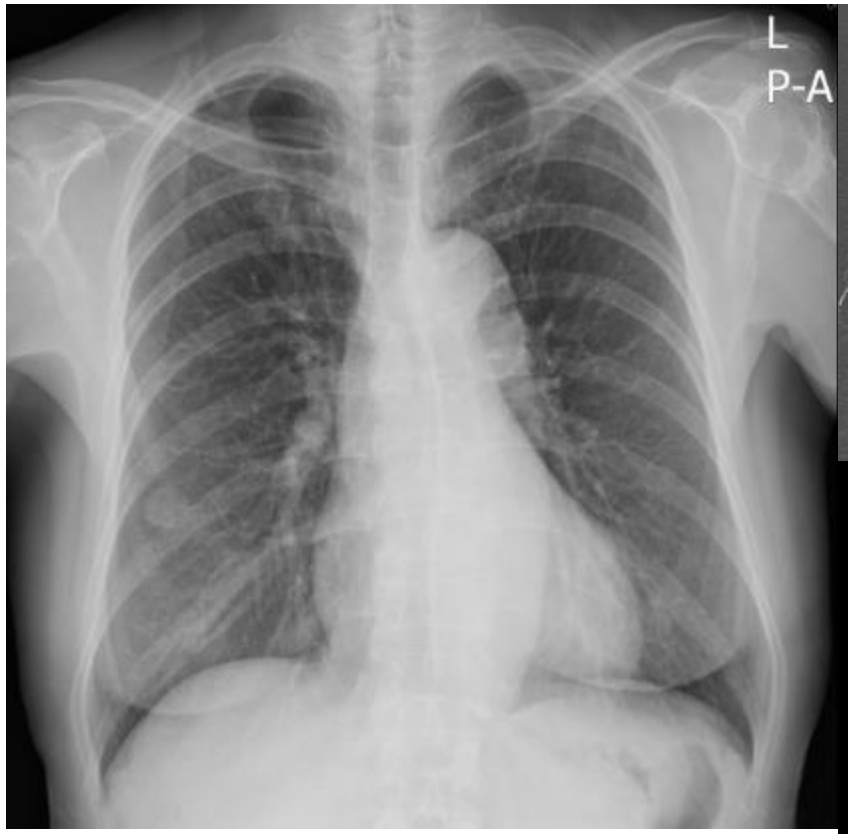
- ►2018-02-12 Bronchoscopy
- ►BAL fluid Extracellular vesicles (EV) EGFR mutation : 19 del +

- ►2018-03-28 VATS biopsy : adenocarcinoma
  Lobectomy
- ►EGFR mutation : 19 del +

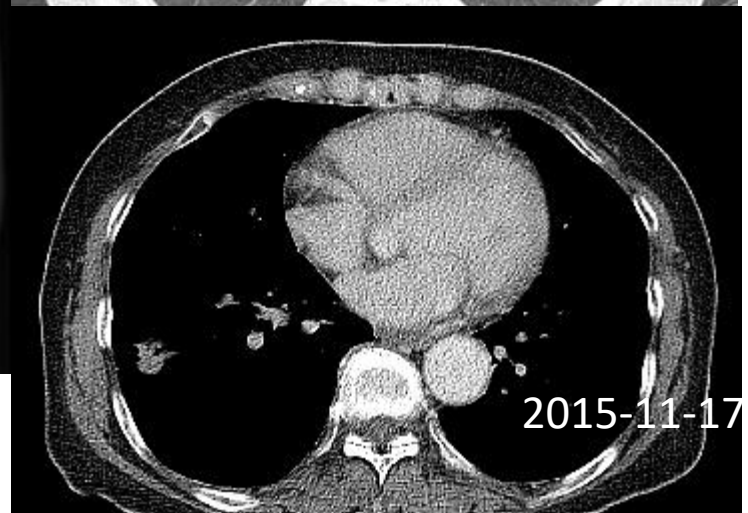
Case #2 F/73

►2015.10. Lt. shoulder mass with pain 주소로 내원

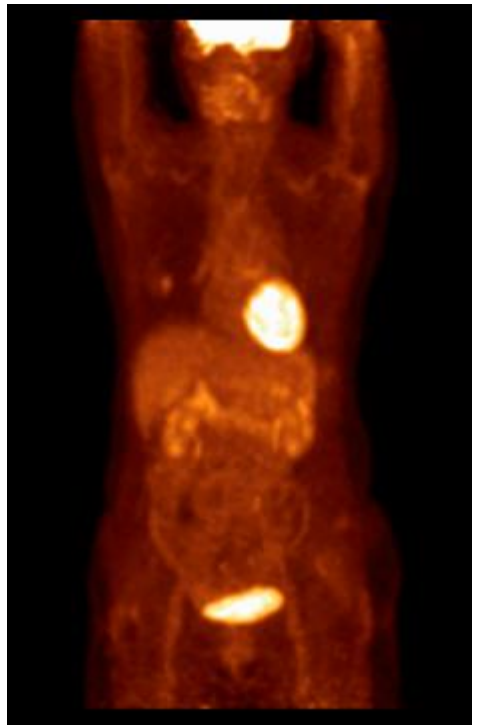
►수술 전 평가에서 RLL nodule 발견



2015-11-05



2015-11-17



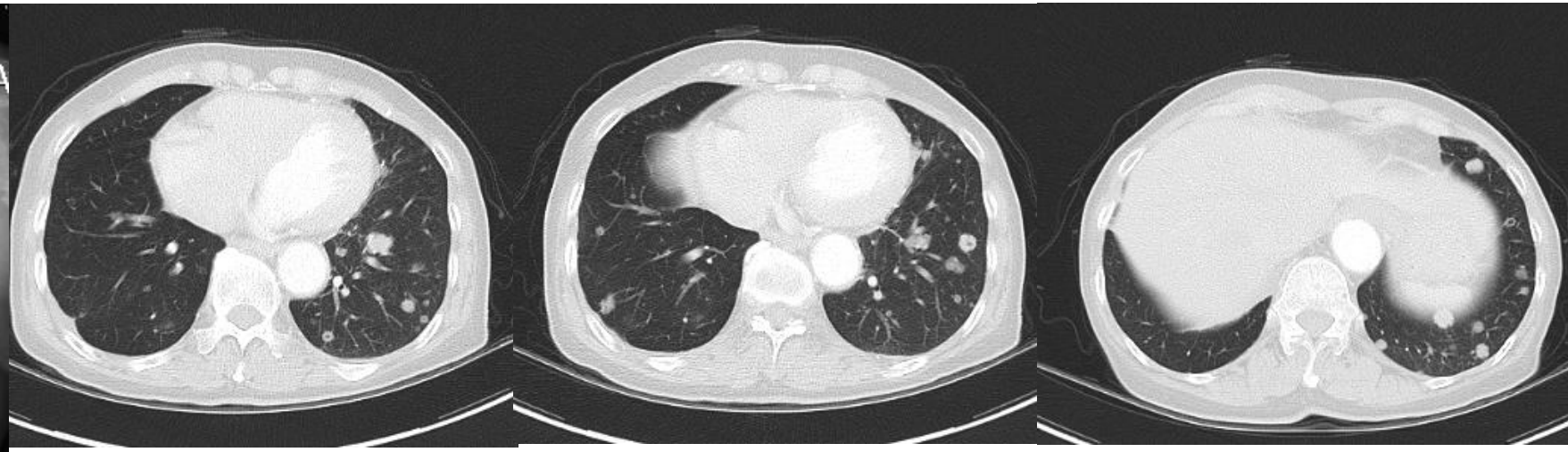
2015-11-20

Case #2

- ►2015-11-27 RLLobectomy

  adenocarcinoma, T1aN0M0, stage IA

- ►Relapse after 22 months



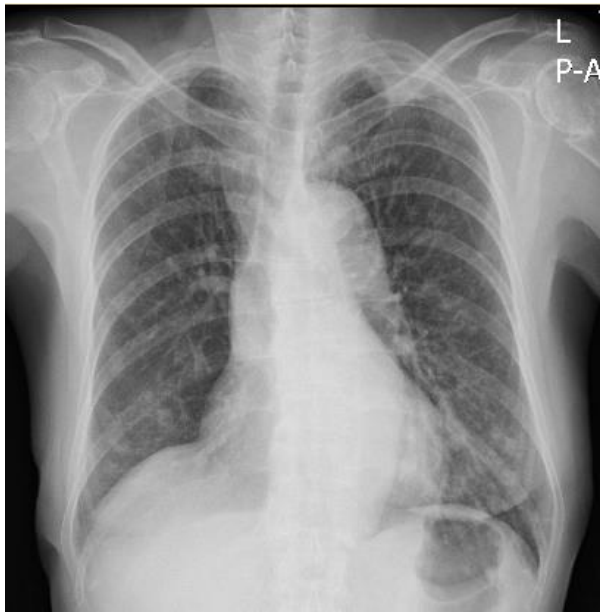
2017-09-12

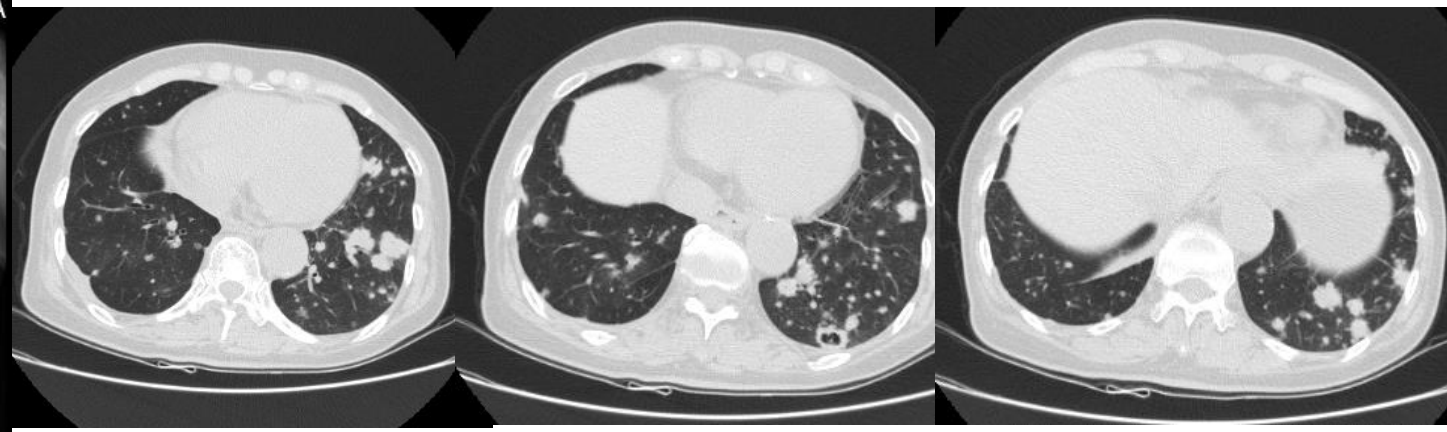
Case #2

►BAF fluid EV EGFR mutation : exon 21 L861Q

►수술 조직 EGFR mutation : wild type

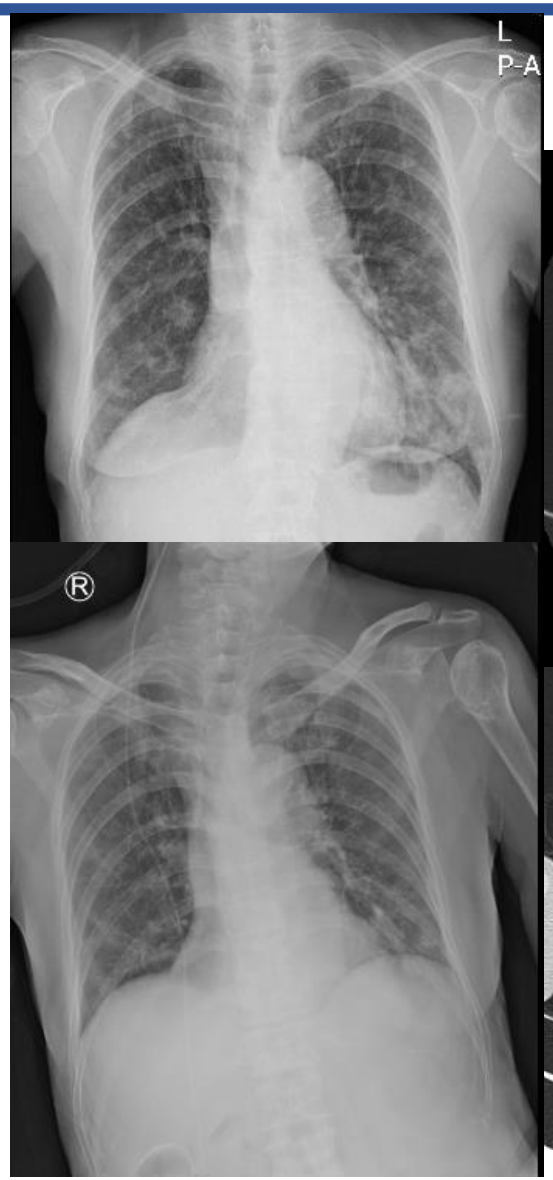
►Palliative chemotherapy : pemetrexed #6 (+ cisplatin #4), SD for 5 mon



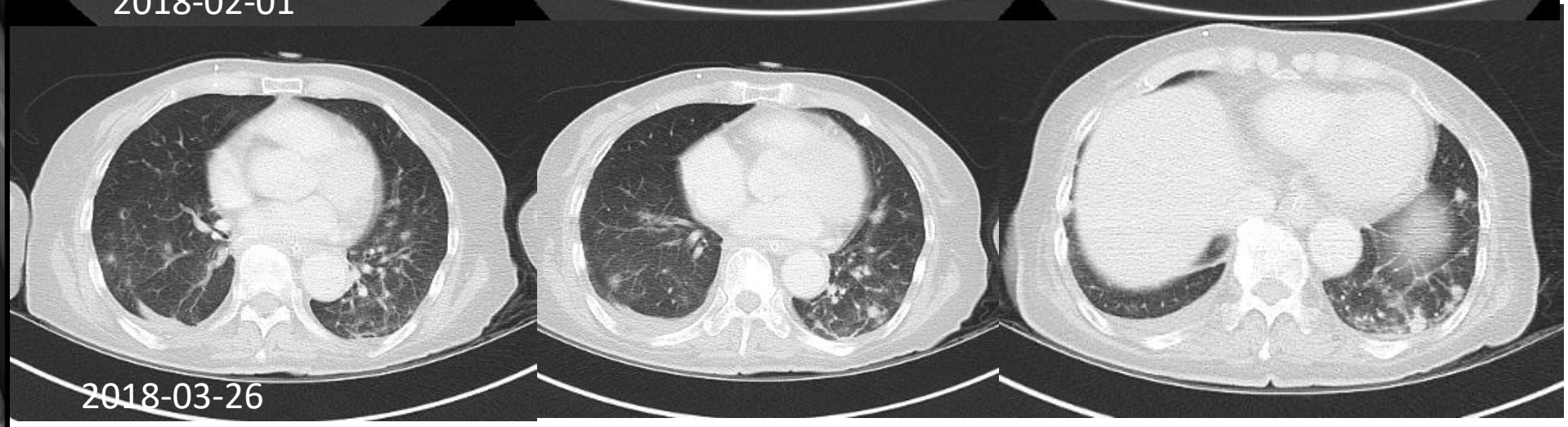


2018-02-01

Case #2

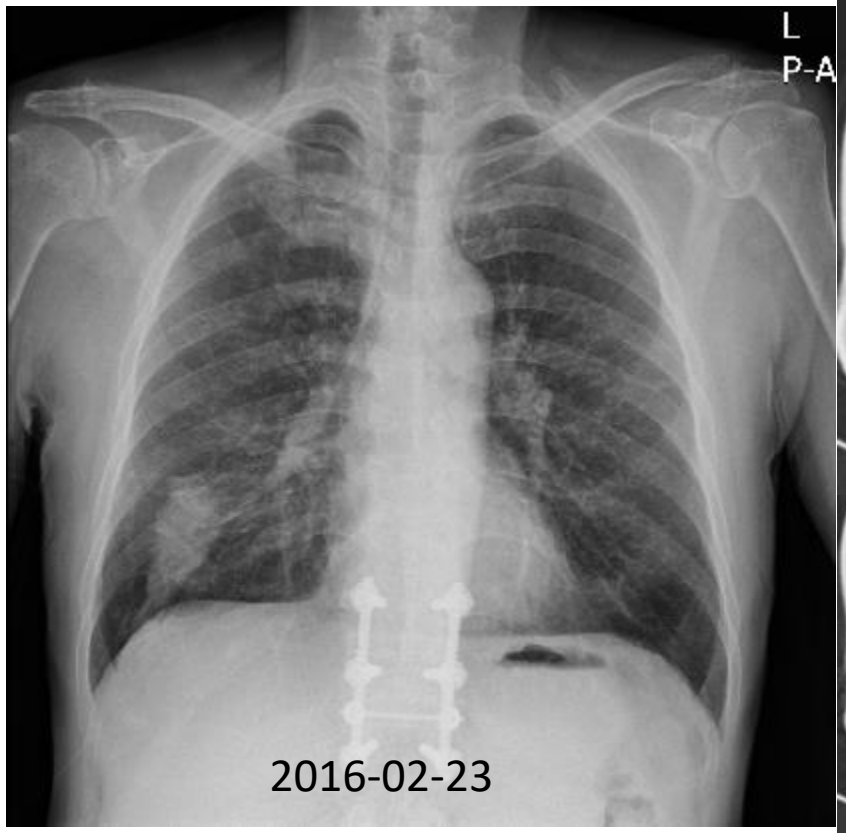


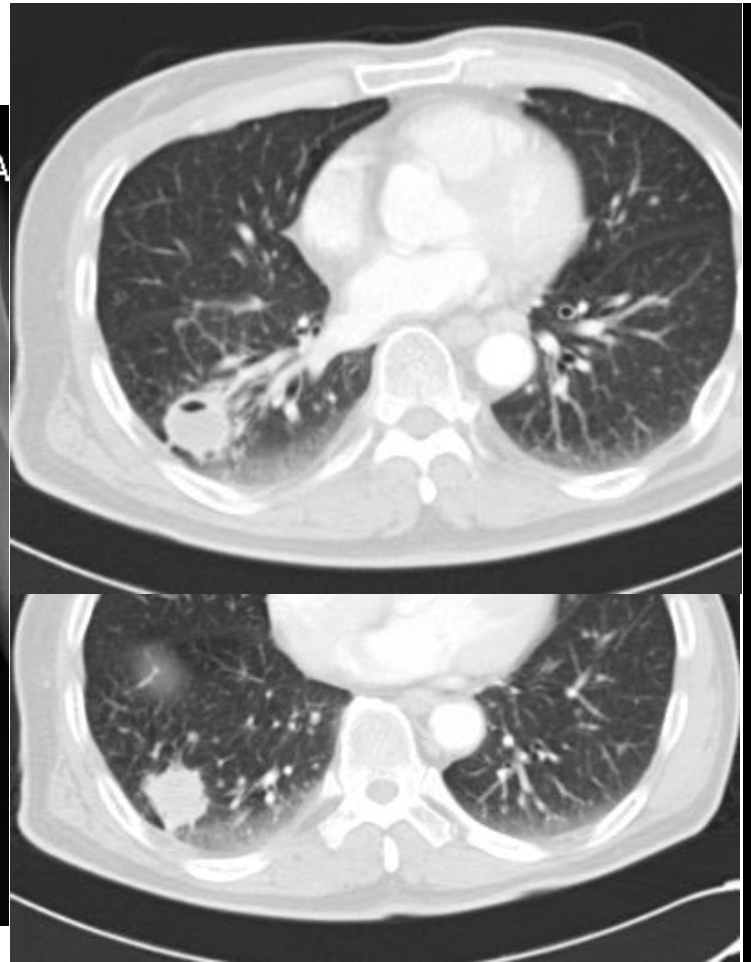
► PR to erlotinib

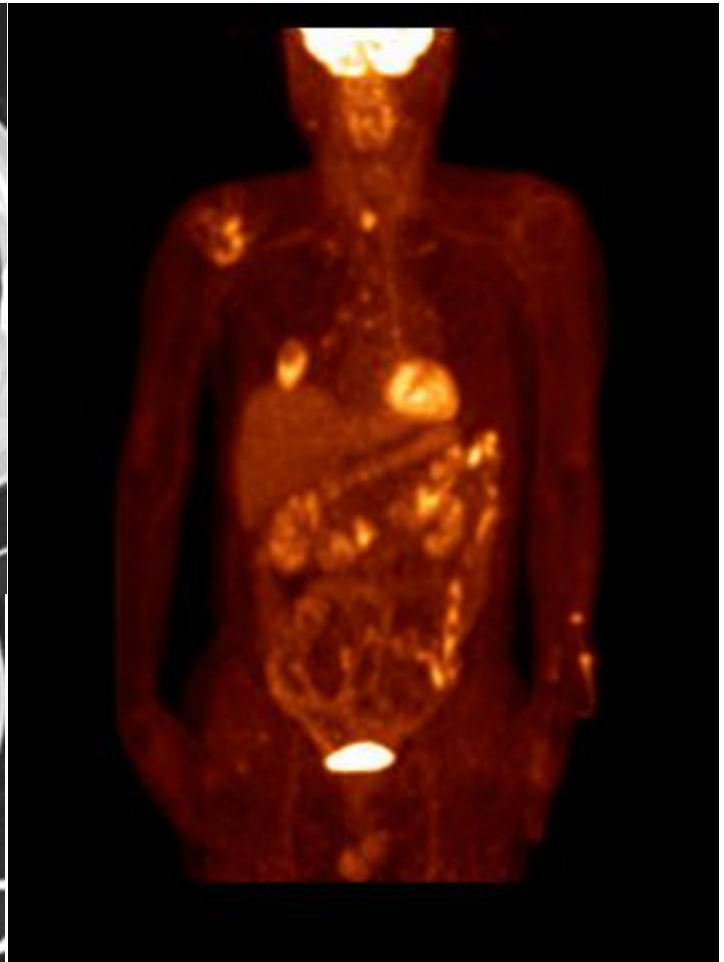


Case #3 – M/67

- ▶Spinal stenosis 수술 시행 전 평가에서 이상 소견 보여 내원
- ▶호흡기계 증상 호소 없음.
- ▶흡연 : 40 갑년







Case #3 Diagnosis

► PCNBx. : Non-neoplastic lung parenchyme
with (1) interstitial wall thickness
(2) lymphoplasmacytic infiltration
"**Failed**"

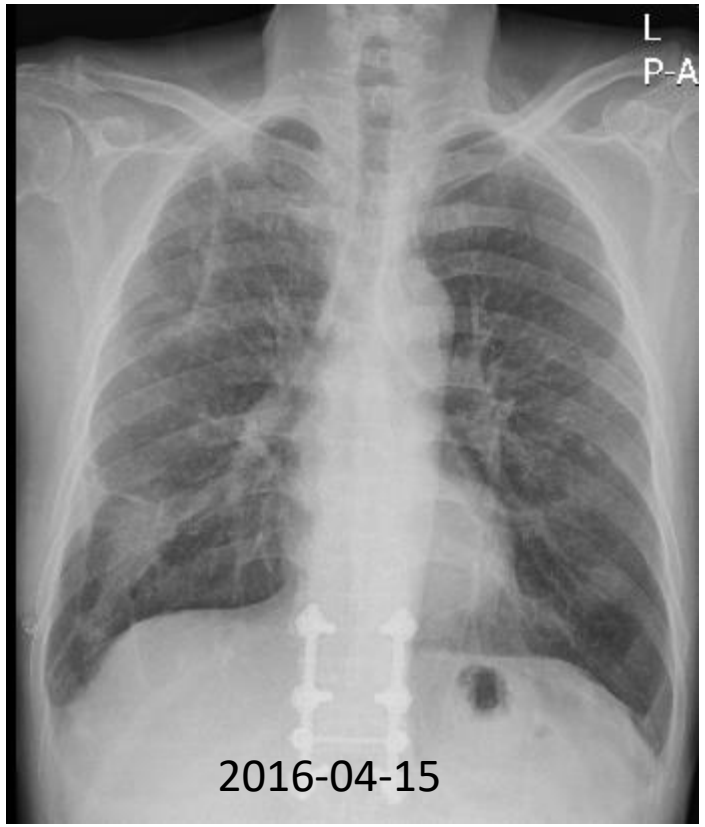
► 2016-03-17 Lumbar and lumbosacral fusion

► 2016-03-31 Wedge resection
- **ADENOCARCINOMA**
- Acinar (100%),) Invasion to visceral pleura: PRESENT

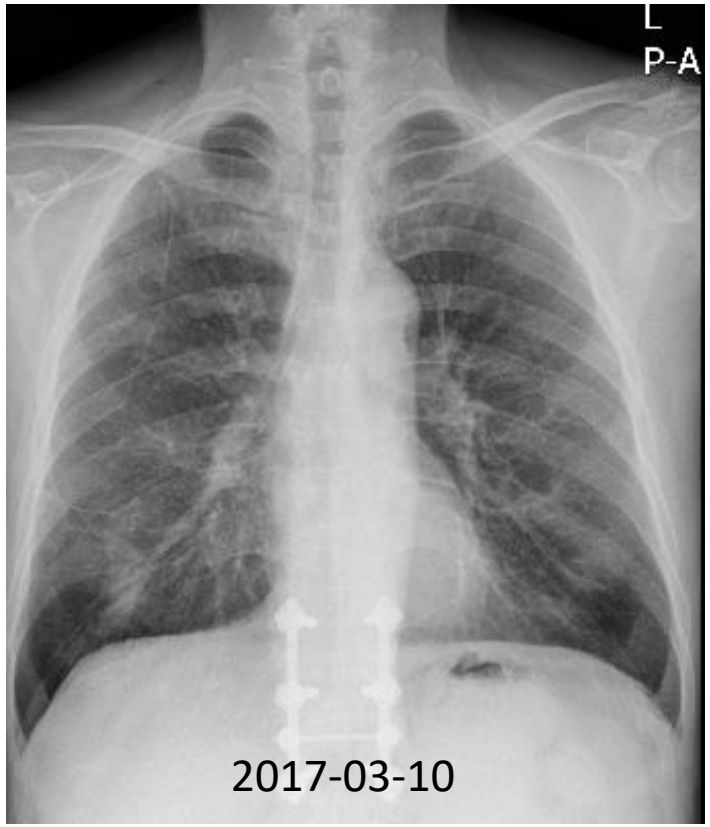
► Exon 19 : Deletion : c.2240_ 2257 del., p.del L747_P753 ins

Case #3 Treatment

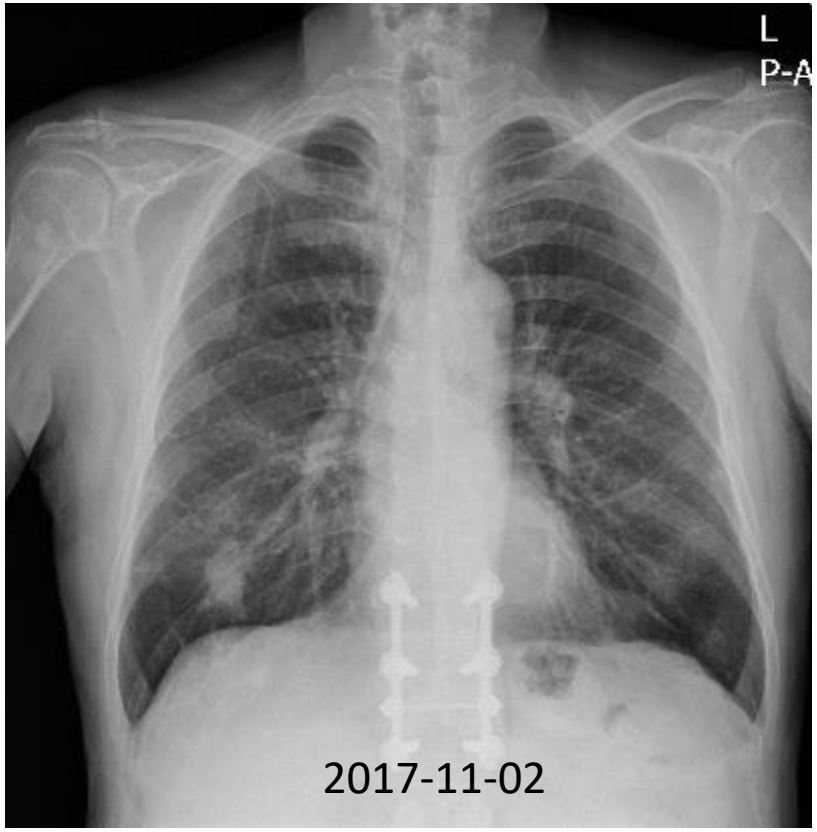
- Adenocarcinoma, 19 del, Stage IV (lung to lung metastasis)
- 2016-04-15 - 2017-11-13 : Gefitinib (PR), 19 months



2016-04-15



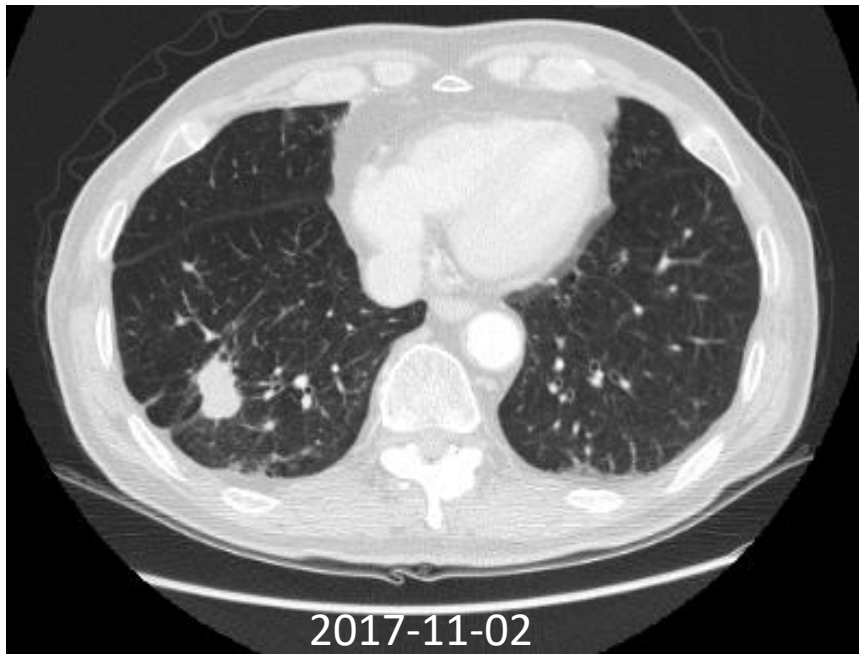
2017-03-10

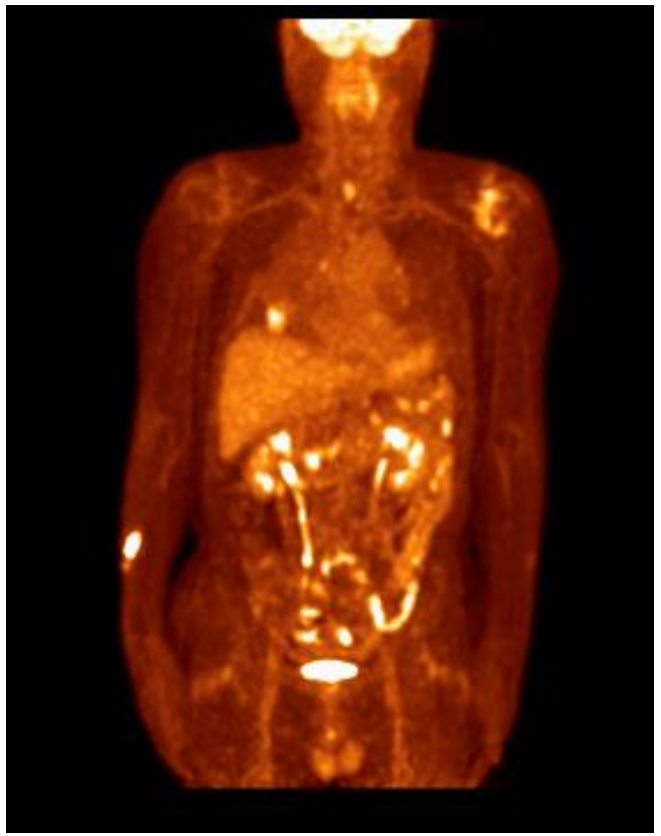


2017-11-02

Case #3 Rebiopsy

- ▶ Rebiopsy
- ▶ PCNBx : adenocarcinoma → EGFR mutation : 19 del
- ▶ BAL EV DNA : 19 del + **T790M**

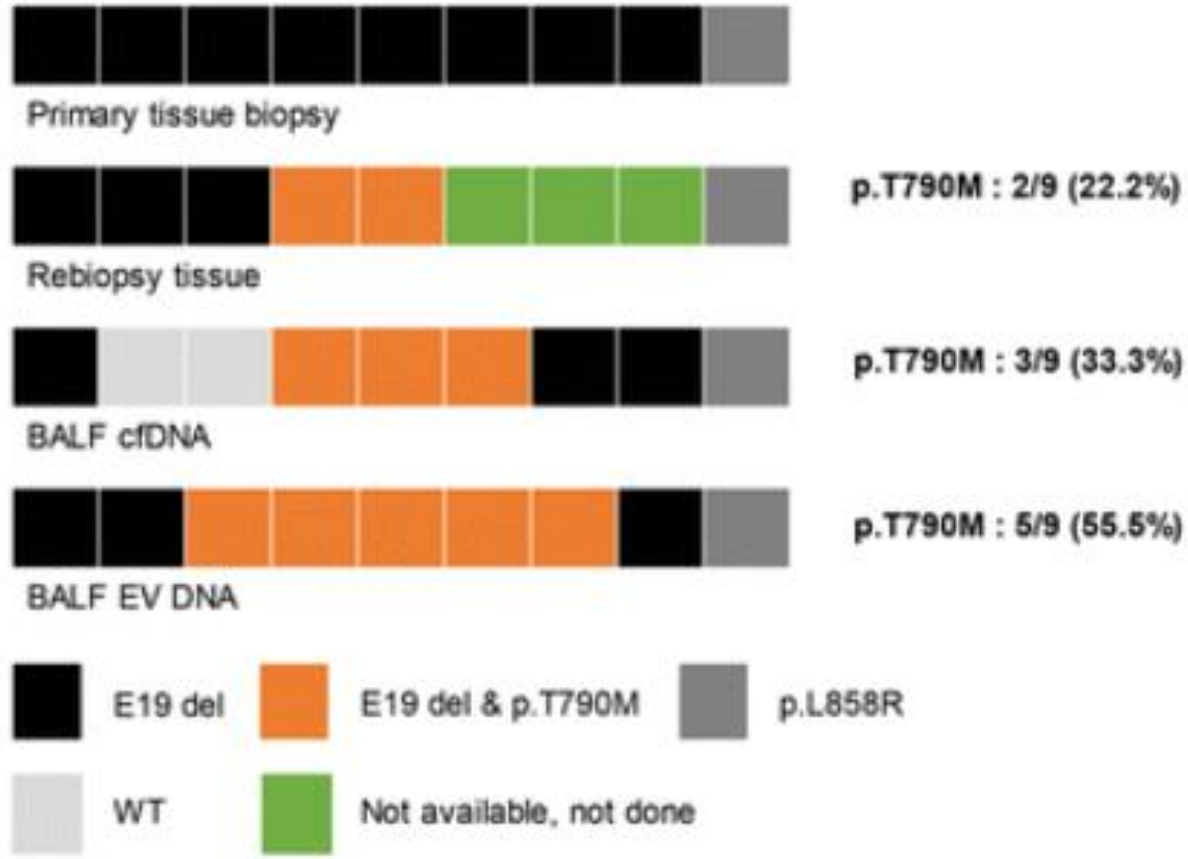




Case #3 Response evaluation

► 2017-11-28 : Olmutinib 800mg QD

BAL fluid extracellular vesicles



Hur JY et al. Mol Cancer. 2018;17(1):15.

Can liquid biopsy replace tissue biopsy?

▶ Strengths

- No tissue biopsy needed
- No FFPE fixation
- Profiling the overall genotype of cancer
  - primary cancer
  - circulating cells
  - metastases
- Better evaluation of :
  - reaction to therapy
  - development of resistance

▶ Weaknesses

- **Sensitivity** : sometimes extremely low levels of ctDNA (1.0%) are present
- **DNA extraction** : the pre-analytical phases of cfDNA need to be better defined
- **Assay standardization** : procedures need to be standardized
- **Quantification** : accurate quantification is needed
- **Variability of assay platform** : different platform assays have different assay sensitivity and specificity and analytical approach

Summary

- Liquid biopsy
  - Non invasive, rapid, serial testing
- Detection of genetic alteration
  - High concordance rate, good predictive marker
- Disease monitoring
  - Mutation clearance – Good predictive marker
- Resistance mechanism
  - T790M: sensitivity about 60-70%
  - Early detection of T790M; insufficient data for making a decision
- Rare oncogenes: NGS using ctDNA


감사합니다
Thank you~!