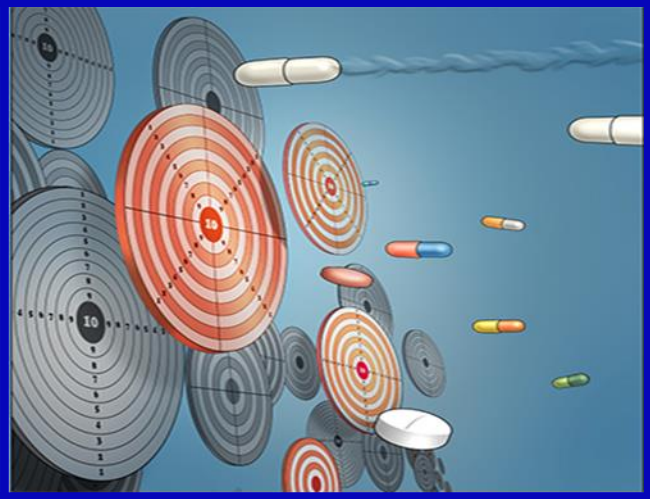
Application of Liquid Biopsy in Lung Cancer





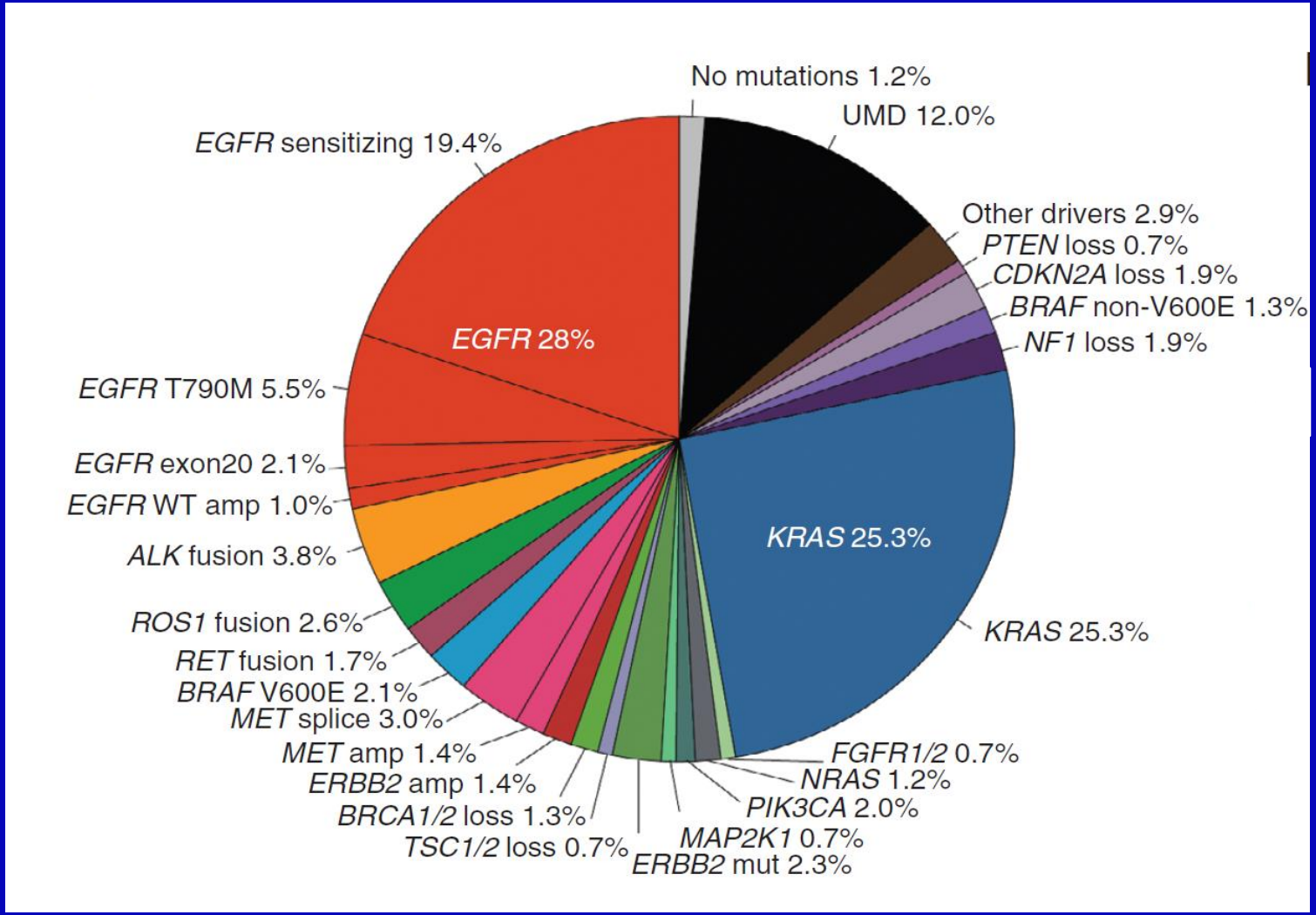
Byoung Chul Cho, M.D., Ph.D.

Yonsei Cancer Center

YONSEI CANCER CENTER
SEVERANCE HOSPITAL

세브란스병원
연세암센터

Potentially Actionable Oncogenic Drivers in Lung Adenocarcinoma



Jordan, et al. Cancer Discov 2017

Expanding List of Guideline Recommendations for Genomic Testing in NSCLC

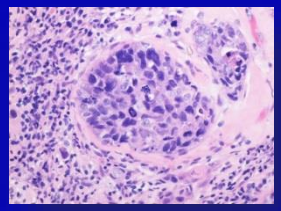
NCCN guideline has advocated broad molecular profiling as a part of the standard diagnostic evaluation for advanced NSCLC with the goal of identifying driver mutations for which effective therapies or clinical trials are available

Genomic alteration	Available targeted agents with activity against driver event in lung cancer
EGFR mutation	Osimertinib, erlotinib, gefitinib, afatinib, dacomitinib
ALK fusion	Alectinib, brigatinib, ceritinib, crizotinib, lorlatinib
ROS1 fusion	Crizotinib, Ceritinib
BRAF V600E mutation	Dabrafenib+ trametinib, vemurafenib
HER2 mutation	Ado-trastuzumab emtansine, afatinib
MET amplification/mutation	Crizotinib
RET fusion	Cabozantinib, vandetanib, LOXO-292
NTRK fusion	Larotrectinib, entrectinib
Tumor mutational burden	Nivoluamb+ ipilimumab, nivolumab

NCCN Clinical Guideline. NSCLC v3 2019

Evolution of Biomarker Test in NSCLC: Past, Current, Tomorrow

Tissue-based

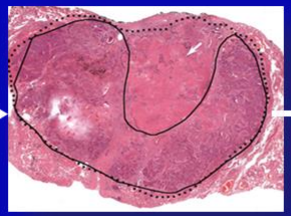


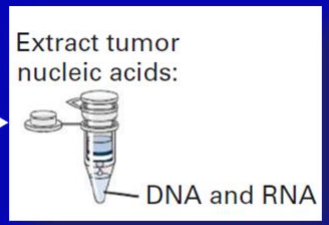
Past

Empirical therapy by clinicopathologic factors to select drugs for individual patients

Current

Target-based therapy by single-gene or multiplexed or NGS for decision-making





Single Biomarker Tests:
- Sanger DNA sequencing or pyrosequencing
- RT-PCR
- FISH

Multiplex, Hotspot Mutation Tests:
- PCR-based SNapShot
- PCR-based Mass Array SNP Sequenom

Next-Generation Sequencing:
- Whole genome or exome capture sequencing (DNA)
- Whole or targeted transcriptome sequencing (RNA)

Plasma-based

Tomorrow

Comprehensive genomic profiling by NGS of plasma ctDNA for decision-making

Modified from Li T. JCO 2013

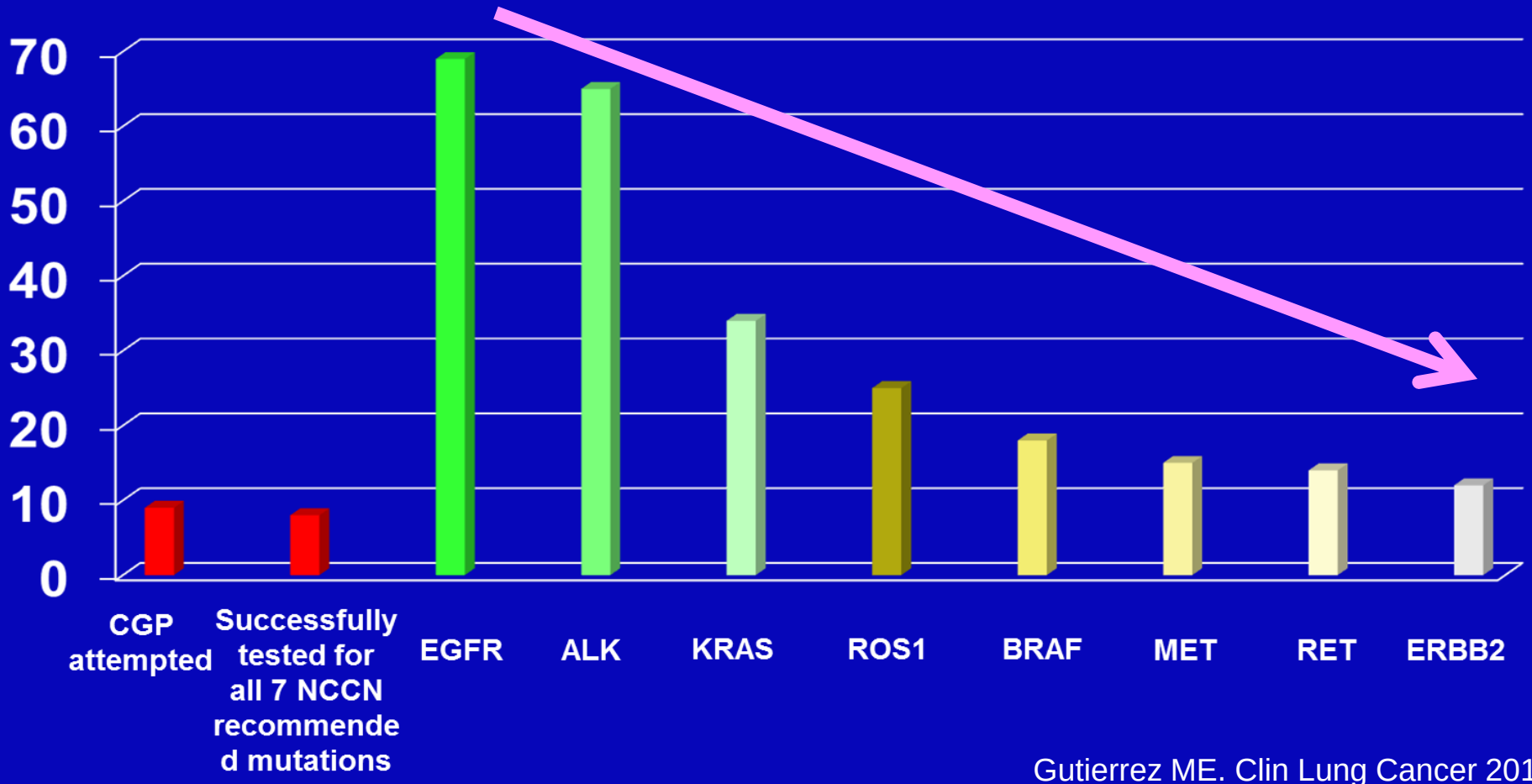
Barriers to Tissue Genomic Testing for Community Oncology Practice

- Insufficient tumor tissue
- Tumor location
- Long turnaround times
- Test reimbursement
- Patient co-morbidities
- Patient harm from the repeat biopsies (bleeding, pneumothorax)

Gutierrez ME. Clin Lung Cancer 2017

Genomic Profiling in advanced NSCLC: In reality…

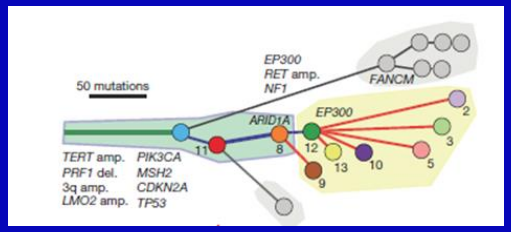
- Diagnostic accuracy is suboptimal ranging from 34-88%
- ~10% of patients are NOT tested because of insufficient tumor tissue
- ~30% of failure rate for NGS in routine pathological samples



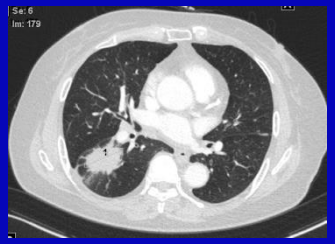
Gutierrez ME. Clin Lung Cancer 2017

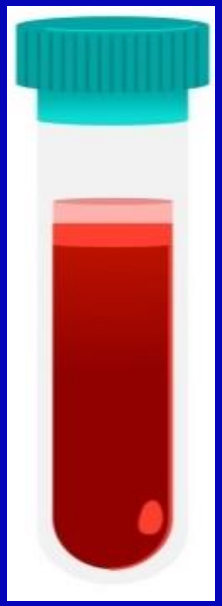
Clinical Application of Liquid Biopsy

Monitoring of tumor evolution

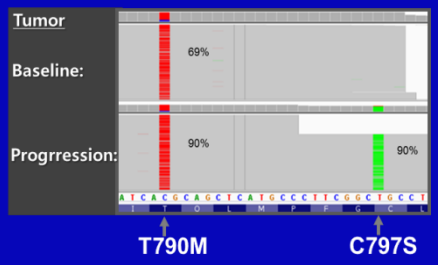


Identification of recurrence

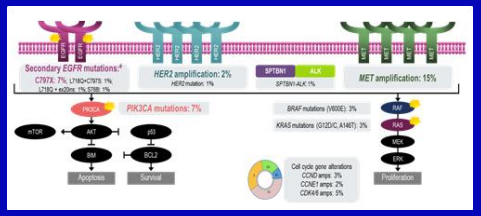




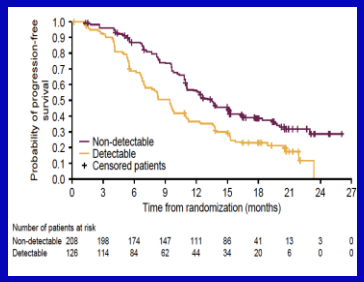
Identification of therapeutic targets



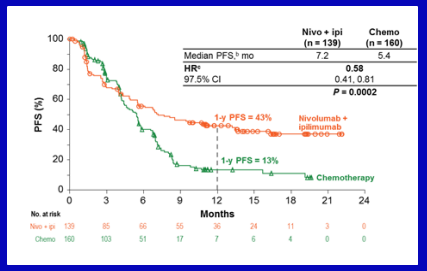
Identification of resistance mechanism



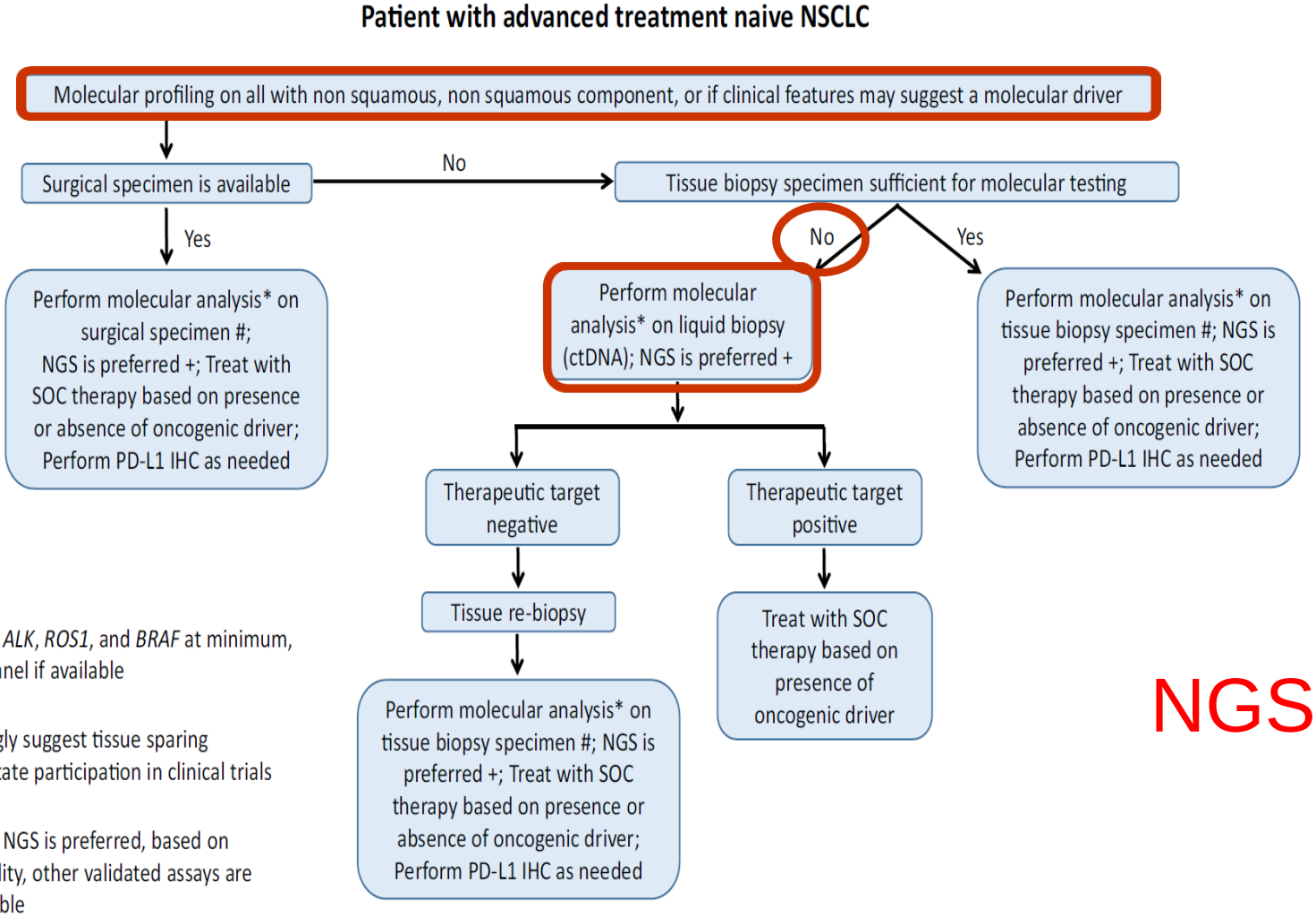
Response monitoring



Tumor mutation burden (Immunotherapy)



Liquid Biopsy for Advanced NSCLC: Consensus Statement from the IASLC (Rolfo C. JTO 2018)



NGS!!

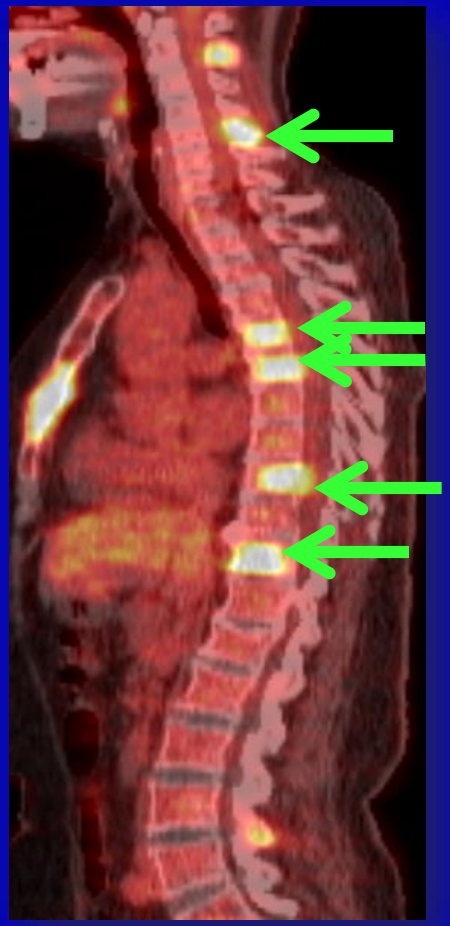
Can Plasma NGS Imporove Detection of Actionable Mutations?

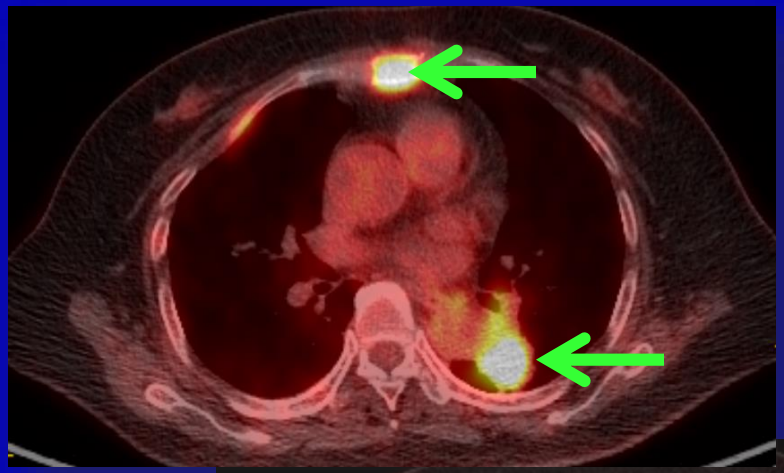
	Number	Success rate of tissue NGS	Plasma NGS	Concordance rate or PPA	Improved detection rate
Aggarwal C, et al. JAMA Oncol 2018	323	62%	Guardant360	81.3%	15.3%
Leighl NB et al. CCR 2019	282	-	Guardant360	>98.2%	48%
Itotani R, et al ESMO 2019	363	67%	Guardant360	75%	~14%

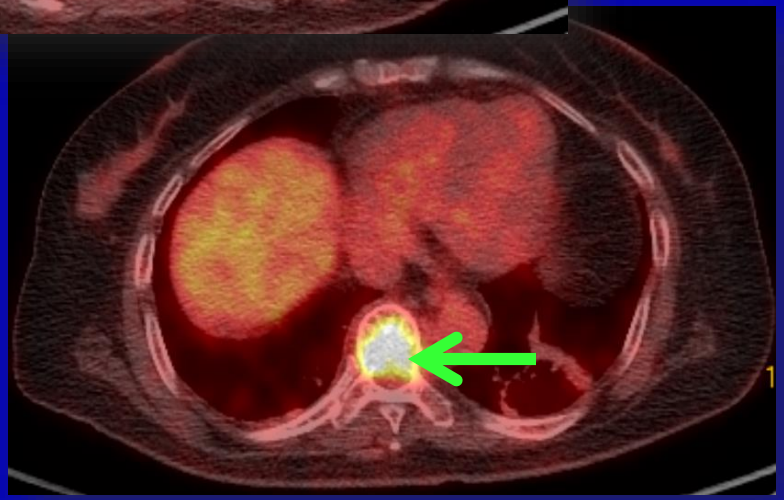
PPA, positive percent agreement

Case #1: 68-year-old lady

- Lifetime never smoker and housewife
- Present with cough and severe chest/back pain







What would you do first?

1) Only tumor biopsy with reflex single-gene assays of EGFR/ALK/ROS1 (turnaround time 1 weeks)
2) Only tumor biopsy with NGS on tissue for broader molecular profiling (turnaround time 5 weeks)
3) Tumor and liquid biopsy simultaneously
4) Liquid biopsy first

Tissue is an issue...?

Day 1
Came to clinic
Order for bronchoscopy

Day 4
Bronchoscopy done

Day 8
No evidence of malignancy reported

Day 8
Plasma ctDNA orderd

Day 15
Order for CT-guided NAB

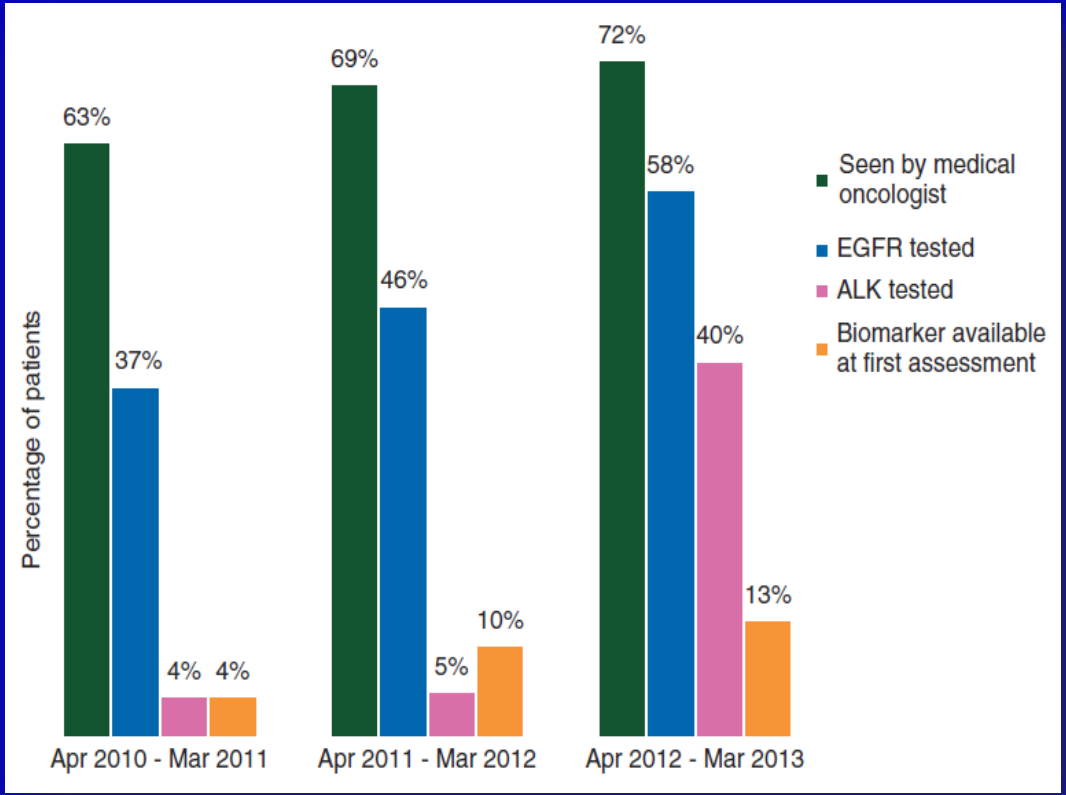
Day 18
CT-guided NAB done

Day 18
Plasma EGFR mutation reported

Day 23
Adenocarcinoma *EGFR* mutation reported

Day 38
Started osimertinib

Concurrent tissue and liquid biopsy should have been ordered….

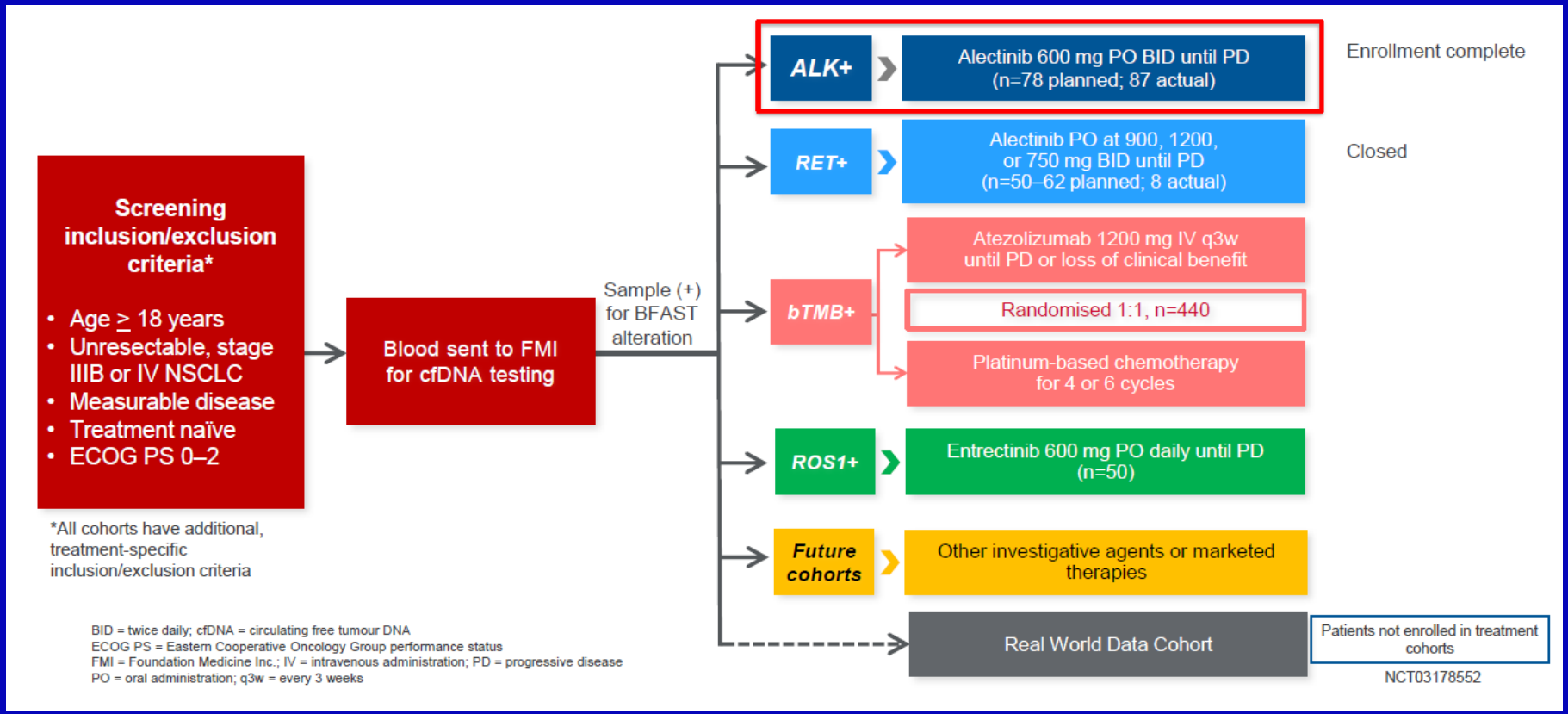


- Only 21% of patients with biomarker testing had results available at their initial oncology consultation
- 13% underwent repeat biopsy for molecular testing
- Delay treatment decision/initiation for advanced NSCLC

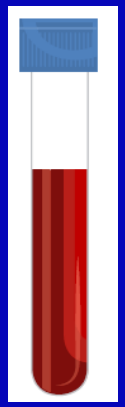
Lim C. Ann Oncol 2015

Can we start targeted therapy based on plasma result?

BFAST: blood-first screening trial in treatment-naive NSCLC

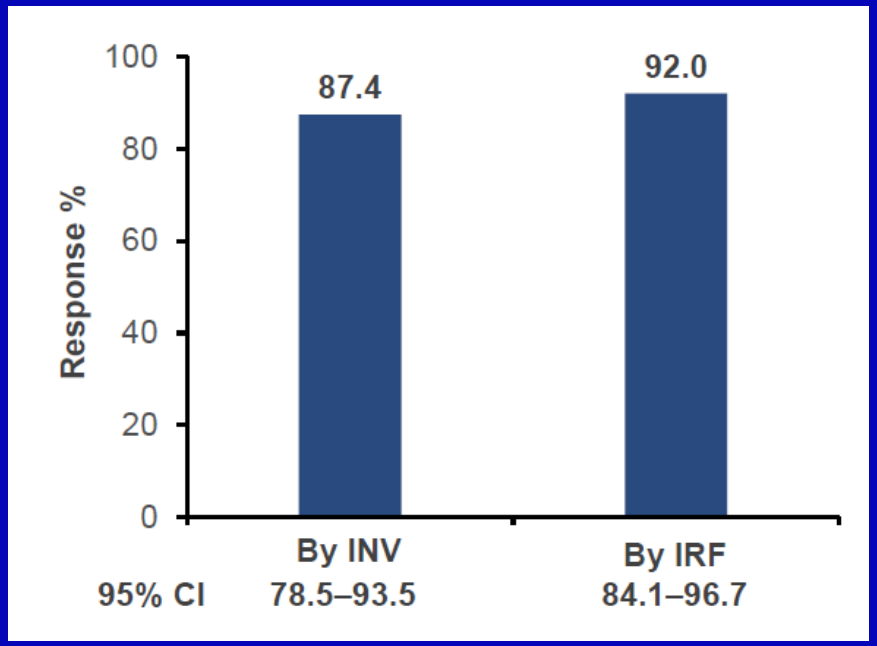


Gadgeel SM. ESMO 2019

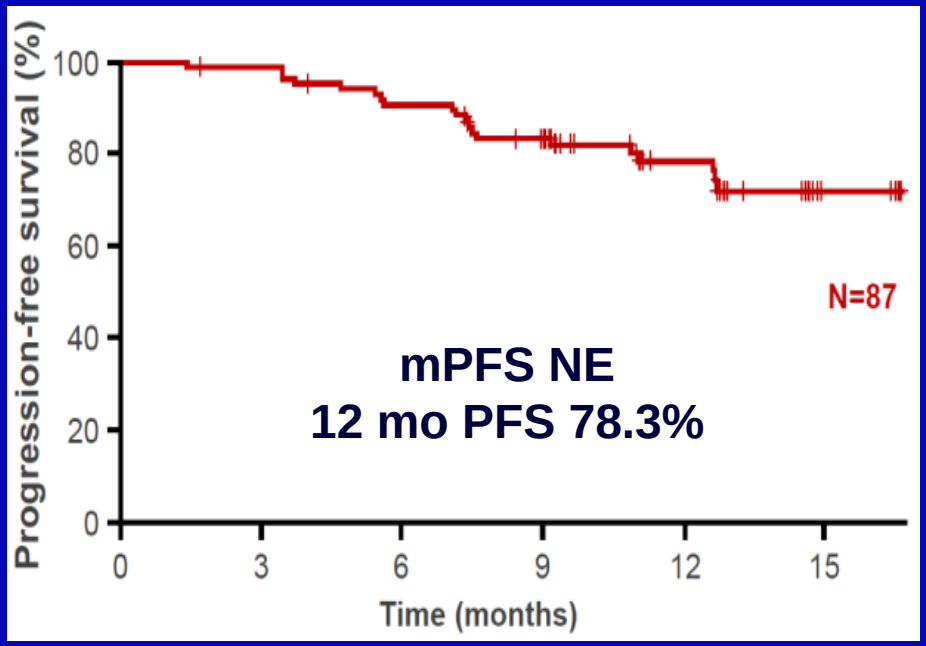


Positive ctDNA result represents sufficient evidence to initiate targeted treatment

ORR*



PFS*



*Efficacy similar to those from ALEX[1] (VENTANA D5F3)

[1]Peters S. NEJM 2017

VISION: Single-arm, Phase II trial of tepotinib in patients with NSCLC harboring MET exon14 skipping mutation (Guardant360)

Key inclusion criteria

- **Stage IIIB/IV NSCLC**
  All histologies
- ***MET*ex14 skipping**
  Tissue- (T+) and/or blood-based (L+)
- **First, second or third line of therapy**
  Prior immunotherapy allowed

Tepotinib 500 mg once daily

Selected endpoints

Primary endpoint

- ORR, RECIST v1.1 (by IRC)

Secondary endpoints include:

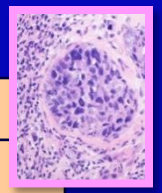
- ORR (investigator)
- Safety
- DOR
- PFS

Predefined analysis sets for efficacy:
*MET*ex14 detected by liquid biopsy or by tissue biopsy

Liquid biopsy positive n = 57; efficacy* n = 48

Tissue biopsy positive n = 58; efficacy* n = 51

Data cut-off: February 18, 2019

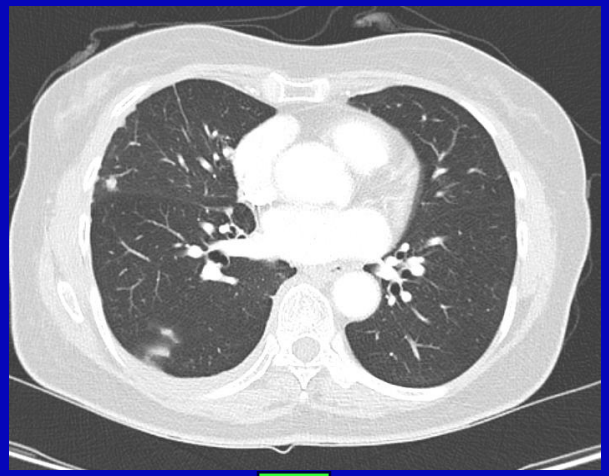


	Liquid biopsy positive		Tissue biopsy positive	
	IRC (n = 48)	**Investigator (n = 47)**	**IRC (n = 51)**	**Investigator (n = 51)**
ORR,* n (%) [95% CI]	24 (**50.0**) [35.2, 64.8]	26 (**55.3**) [40.1, 69.8]	23 (**45.1**) [31.1, 59.7]	28 (**54.9**) [40.3, 68.9]
mDOR, months [95% CI]	**12.4** [5.8, ne]	**17.1** [7.1, ne]	**15.7** [9.0, ne]	**14.3** [5.7, ne]
12-month event-free rate [95% CI]	**58%** [30, 78]	**55%** [28, 76]	**70%** [40, 87]	**59%** [32, 79]

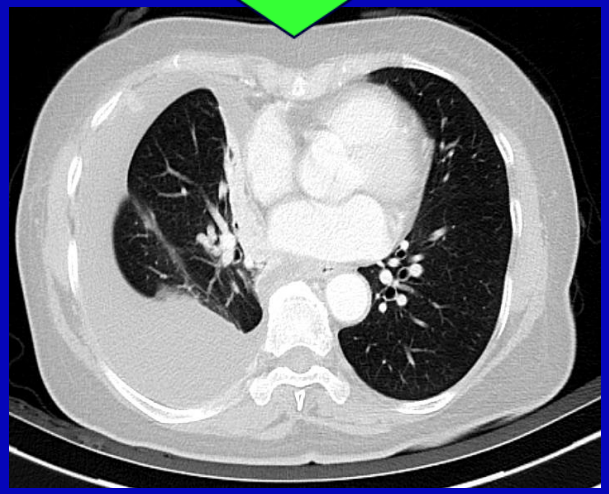
*ORR: confirmed complete response or partial response; †mDOR for first-line treatment not mature at time of analysis.
CI, confidence interval; IRC, Independent Review Committee; mDOR, median duration of response; ne, not estimable; ORR, objective response rate

Park K. ESMO Asia 2019

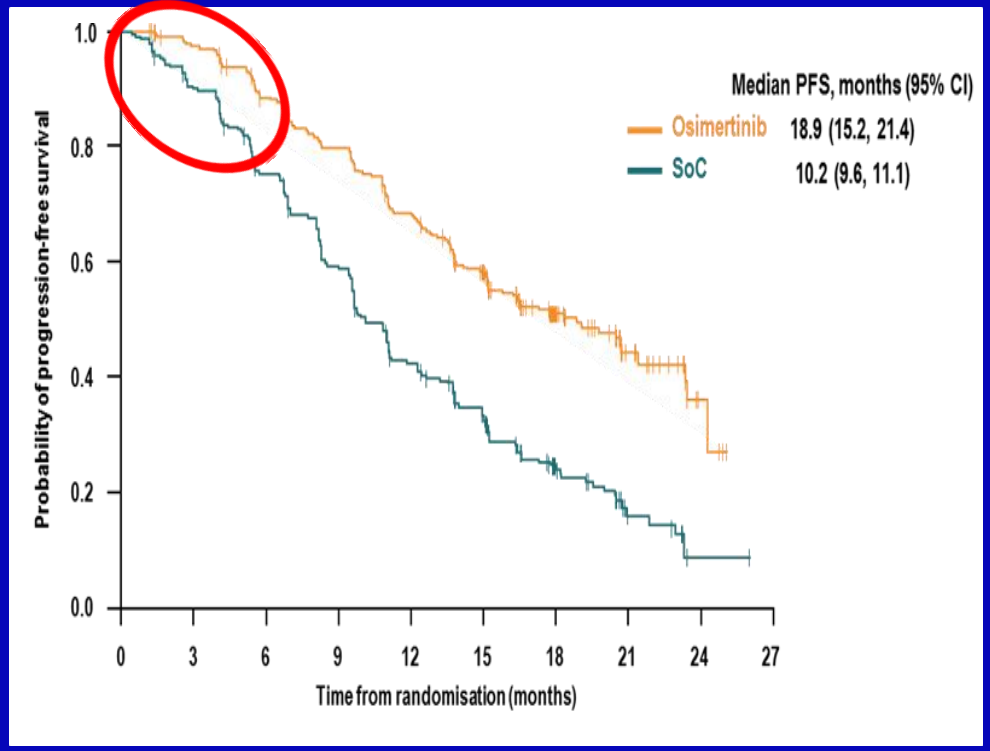
Not all EGFR mutant patients respond well to EGFR-TKI



Gefitinib 1 mo



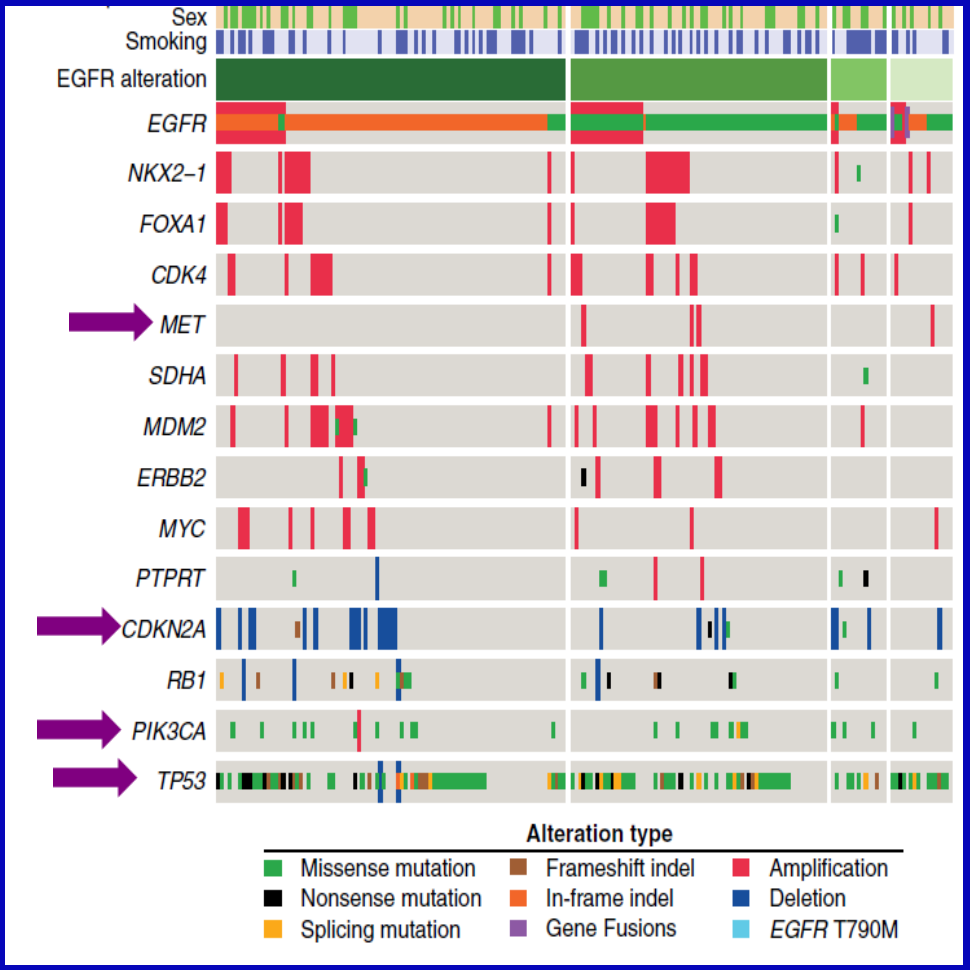
FLAURA

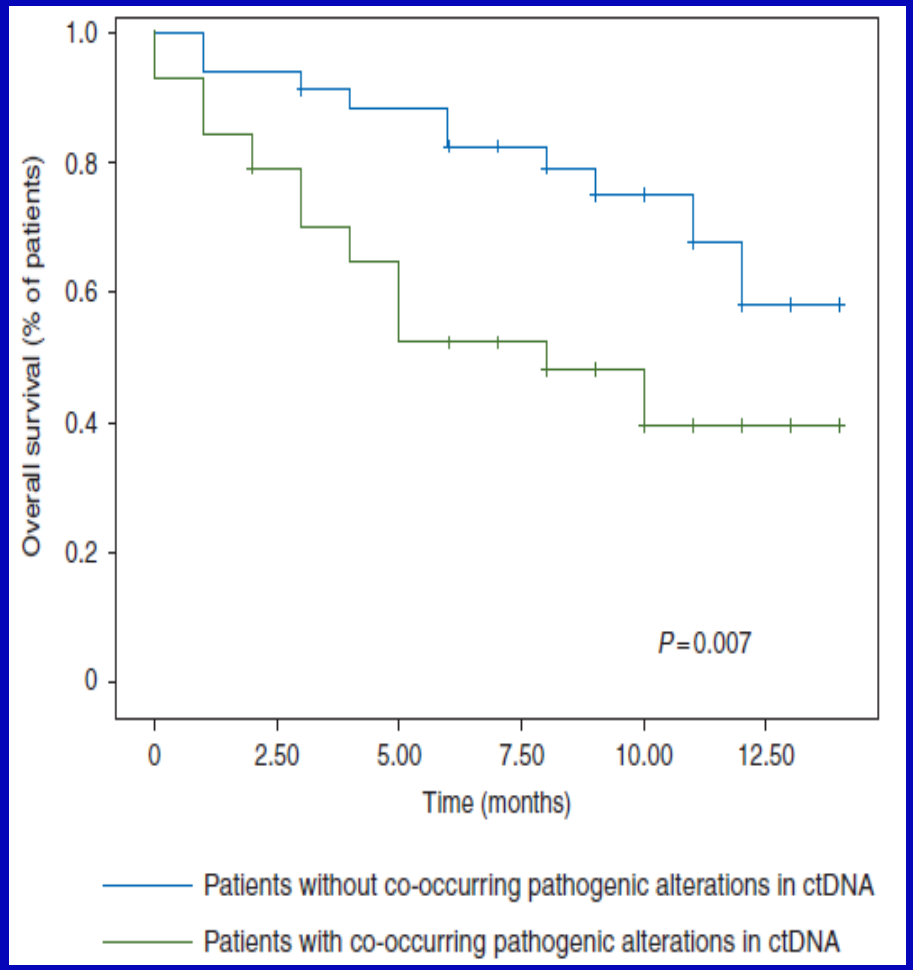


22% of patients in SoC arm did not achieve responses (**5%** had PD)

Ramalingam SS. NEJM 2017

Concurrent Genomic Alterations in ctDNA May Provide Prognostic Information

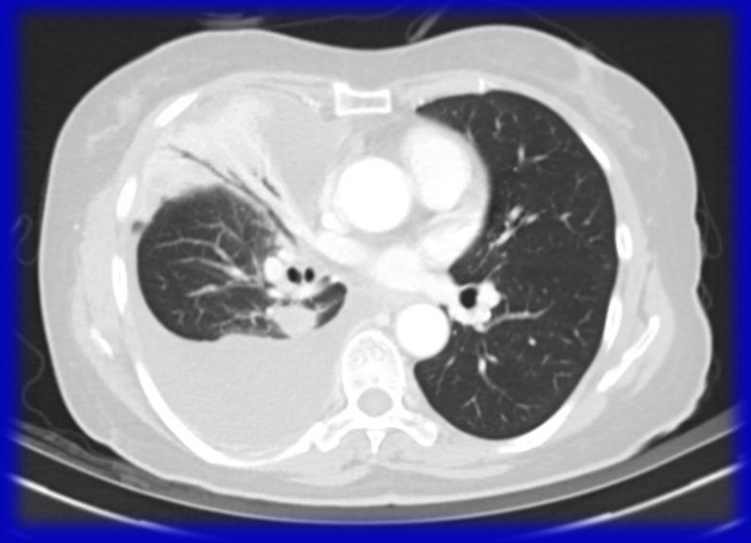


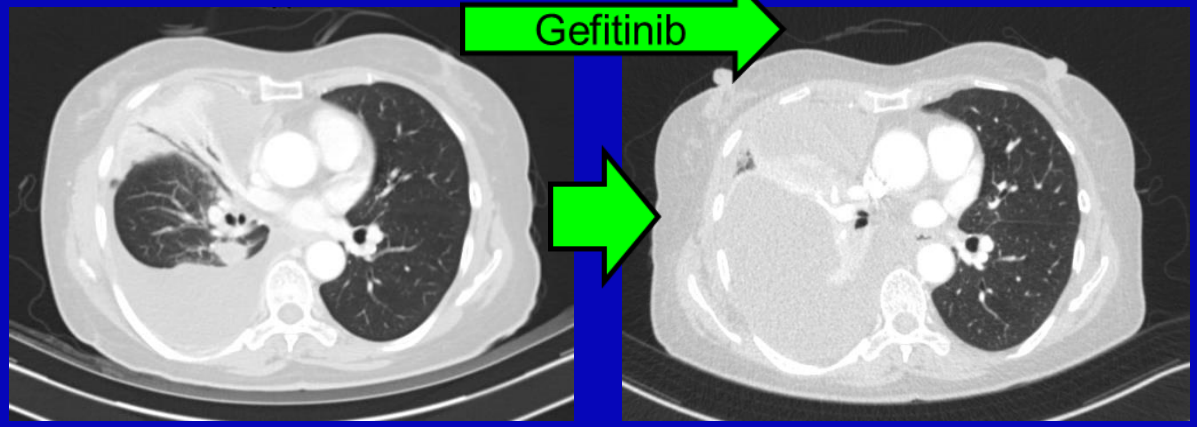


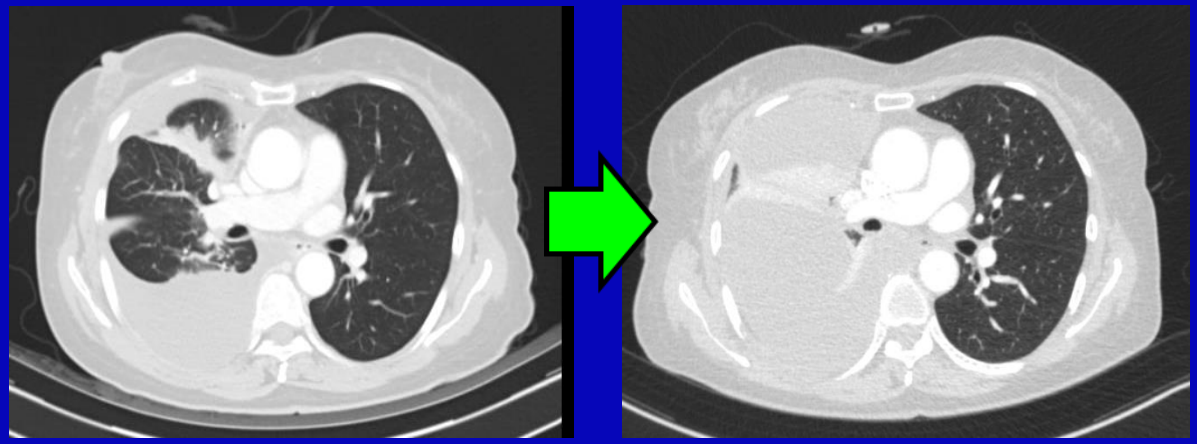
Zugazagoitia J. Ann Oncol 2019

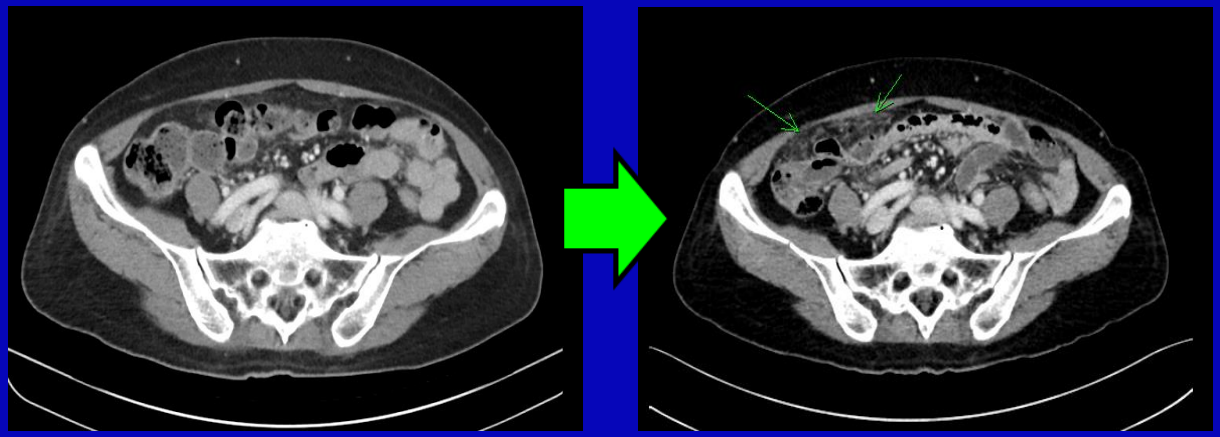
Case #2: 63 year-old Lady with dyspnea and chest pain

- Never smoker
- VATS RLL pleural biopsy
- Metastatic adenocarcinoma (TTF1+) cT2aN0M1a
- EGFR exon 19 deletion by PANAMutyper™
- Started gefitinib (No doubt!!)




Gefitinib





Not all EGFR Mutations Created Equally

Cobas® v2 Plasma

Whole blood	EGFR mutation [plasma cfDNA]	G719X	▦	Not detected
		Ex19Del	▦	Mutant(9.98)
		S768I	▦	Not detected
		T790M	▦	Not detected
		Ex20Ins	▦	Not detected
		L858R	▦	Not detected
		L861Q	▦	Not detected

FoundationOne Plasma

HISTORIC PATIENT FINDINGS		TEST 1 MAF%
EGFR	L747P	1.0%
TP53	I255N	0.78%

TruSight™ Tumor 170

1. Variants of clinical significance

- SNVs & Indels :

GENE	MUTATION TYPE	AA CHANGE	VAF	HGVSc	HGVSp
EGFR	Missense mutation	p.L747P	18.1%	NM_005228.3:c.2239_2240delTTinsCC	NP_005219.2:p.Leu747Pro
TP53	Missense mutation	p.I255N	17.5%	NM_000546.5:c.764T>A	NP_000537.3:p.Ile255Asn

- Fusion gene : None
- Copy number variation : None

A rare point mutation in exon 19

Int J Clin Exp Pathol 2015;8(7):8603-8606
www.ijcep.com /ISSN:1936-2625/IJCEP0010373

Case Report

***EGFR* mutation L747P led to gefitinib resistance and accelerated liver metastases in a Chinese patient with lung adenocarcinoma**

Thoracic Cancer
Open Access
Thoracic Cancer ISSN 1759-7706

CASE REPORT

Non-small cell lung cancer harboring a rare *EGFR* L747P mutation showing intrinsic resistance to both gefitinib and osimertinib (AZD9291): A case report

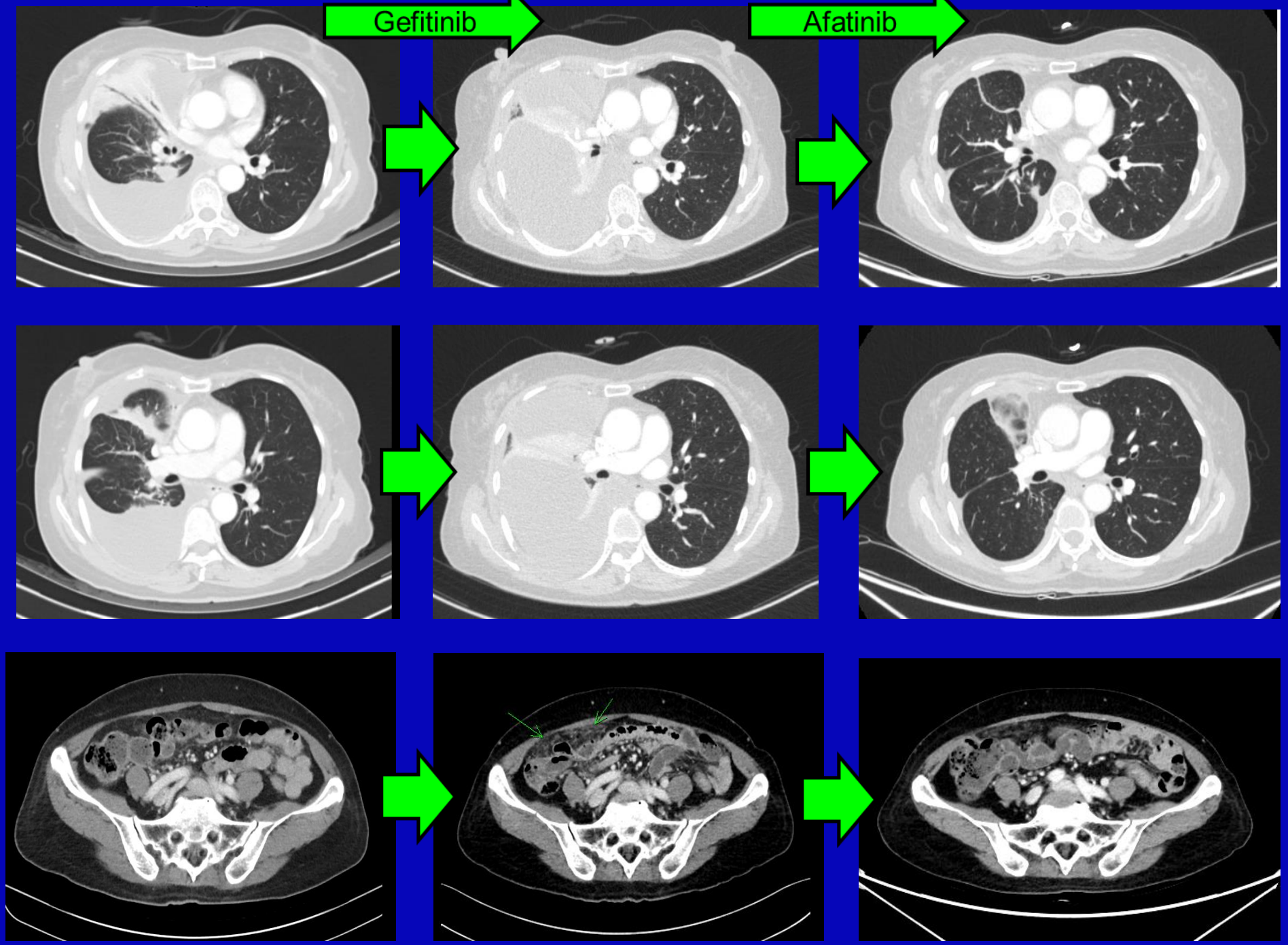
Case Report

Exon 19 L747P mutation presented as a primary resistance to EGFR-TKI: a case report

Case Report

EGFR L747P mutation in one lung adenocarcinoma patient responded to afatinib treatment: a case report

Tong Zhou[1], Xiaoyue Zhou[1], Peng Li[1], Chuang Qi[2], Yang Ling[1]


Gefitinib
Afatinib

Treatment monitoring with repeated liquid biopsies

Treatment monitoring

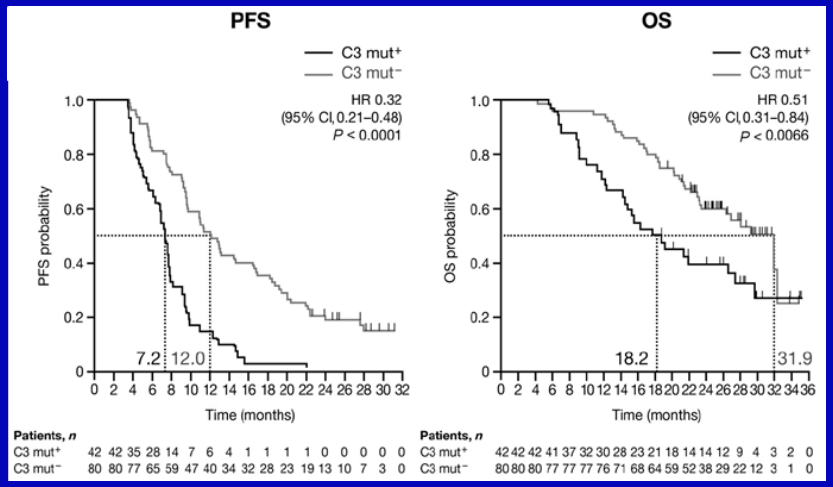
1. Clearance of founder mutation
2. Early detection of resistant clones prior to radiological progression

Modifying treatment

1. Intensifying therapy
2. Switching therapy

Early plasma ctDNA dynamics can identify poorly responding patients

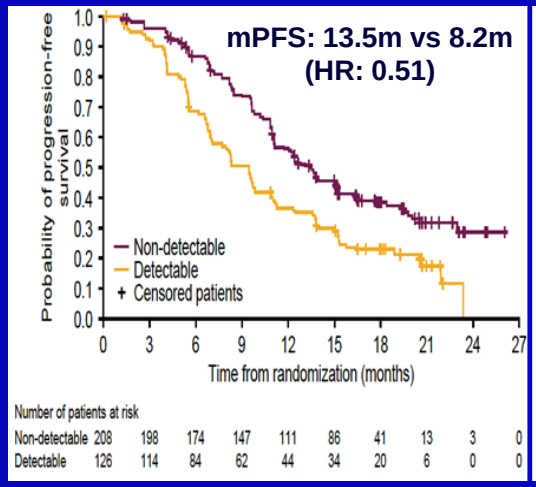
FASTACT-2



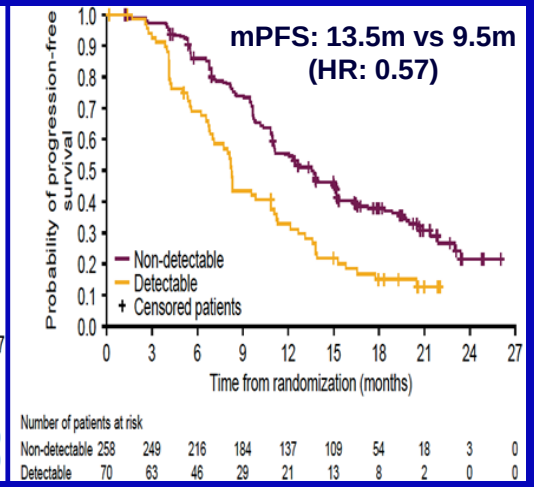
Presence of EGFR mt at Cycle 3 is associated with worse PFS and OS

FLAURA

Week 3



Week 6



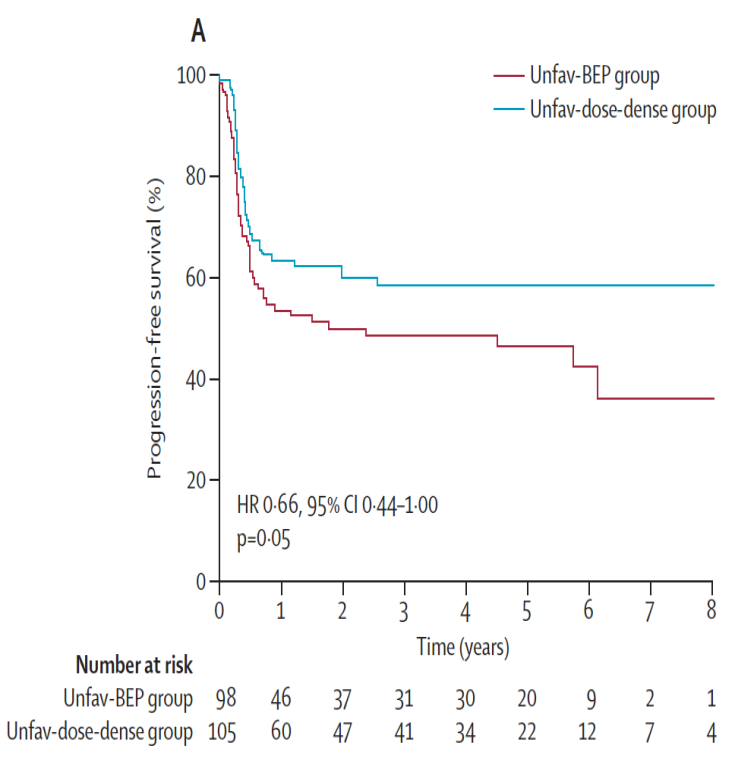
Presence of EGFR mt at week 3 and 6 is associated with worse PFS and lower ORR.

Mok CCR 2015; Zhou ASCO 2019

Modifying treatment at earlier timepoints enables individualization of treatment

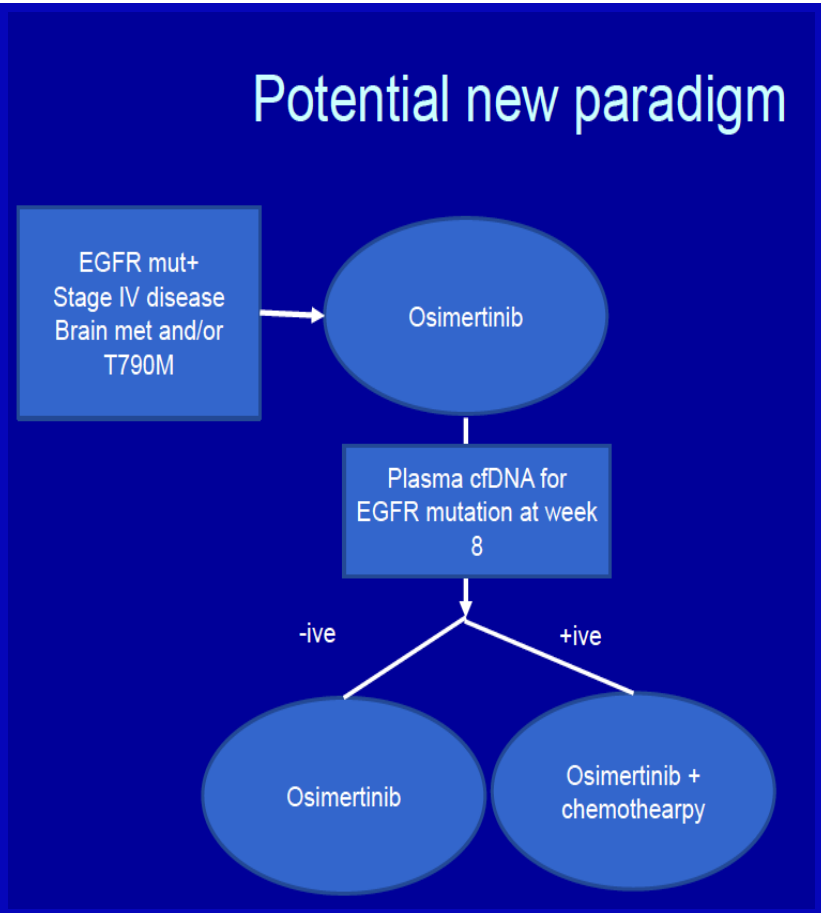
Personalised chemotherapy based on tumour marker decline in poor prognosis germ-cell tumours (GETUG 13): a phase 3, multicentre, randomised trial

Karim Fizazi, Lance Pagliaro, Agnes Laplanche, Aude Fléchon, Josef Mardiak, Lionnel Geoffrois, Pierre Kerbrat, Christine Chevreau, Remy Delva, Frederic Rolland, Christine Theodore, Guilhem Roubaud, Gwenaëlle Gravis, Jean-Christophe Eymard, Jean-Pierre Malhaire, Claude Linassier, Muriel Habibian, Anne-Laure Martin, Florence Journeau, Maria Reckova, Christopher Logothetis, Stephane Culine



Fizazi. TLO 2014

Modifying therapy by integrating ctDNA dynamics



Mok ESMO 2019

Recurrence Risk Assessment: ctDNA analysis to detect Minimal Residual Disease (MRD)

LUNAR assay

SNVs

AKT1	*ALK*	***APC***	*ATM*	*BRAF*
CTNNB1	*EGFR*	*ERBB2*	*ESR1*	*GATA3*
KIT	***KRAS***	*MET*	***MYC***	*NRAS*
PIK3CA	*PTEN*	***STK11***	*TERT*	***TP53***

Bolded genes indicate genes with complete exon coverage.

Indels

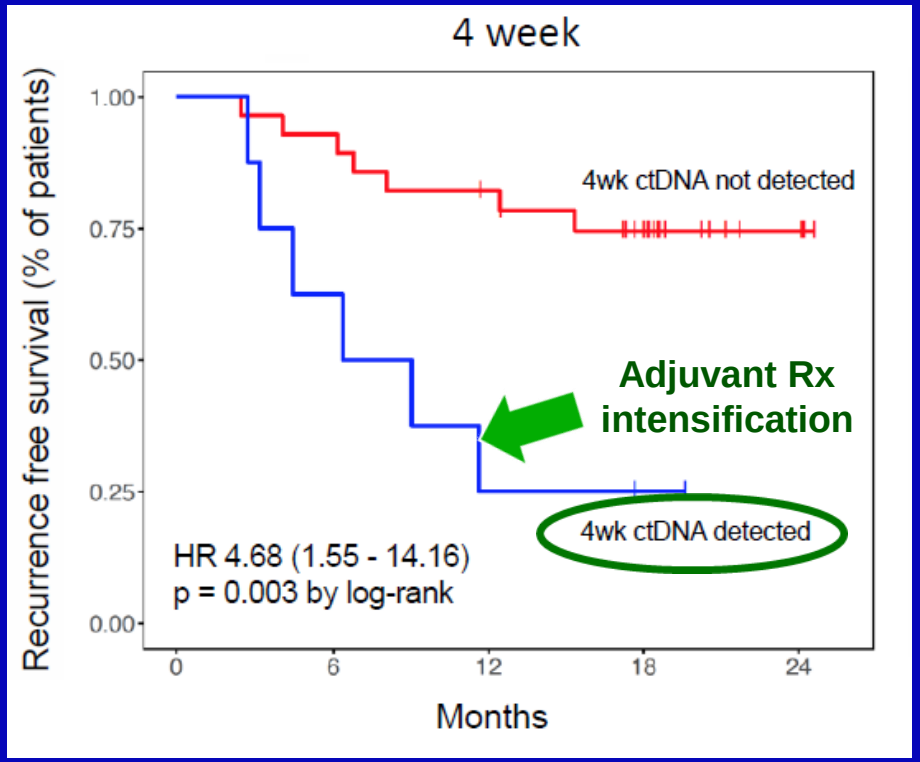
APC	*ATM*	*EGFR*	*ERBB2*
MET	*PTEN*	*STK11*	*TP53*

Fusions

ALK

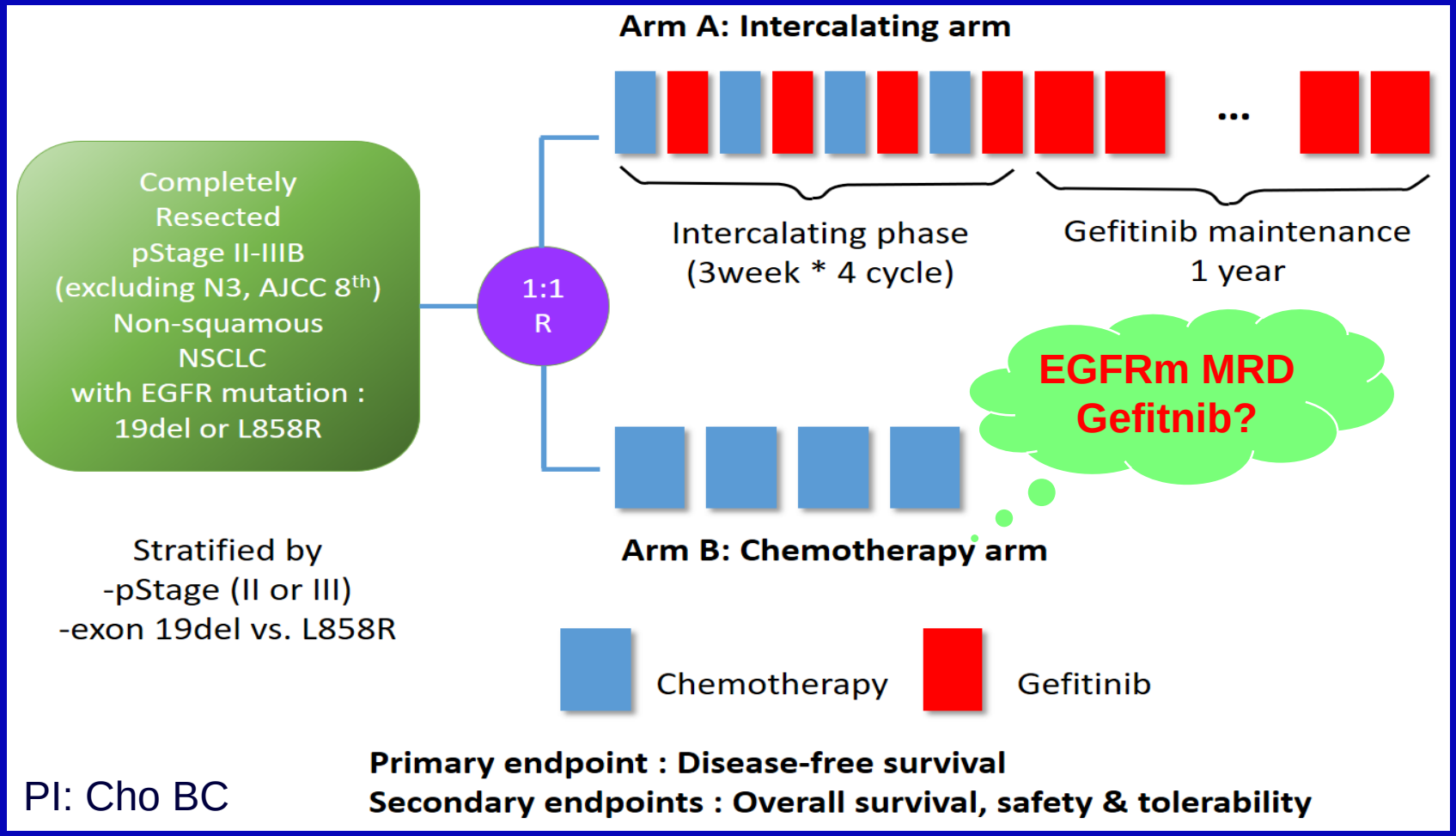
Multigene panel designed for >90% sensitivity across major cancer types

Resected early-stage NSCLC



Lam VK. WCLC 2018

A RAndomized PHase 3 Adjuvant gEfitinib EGFR-Mutant Non-small Cell Lung Cancer (RAPHAEL)

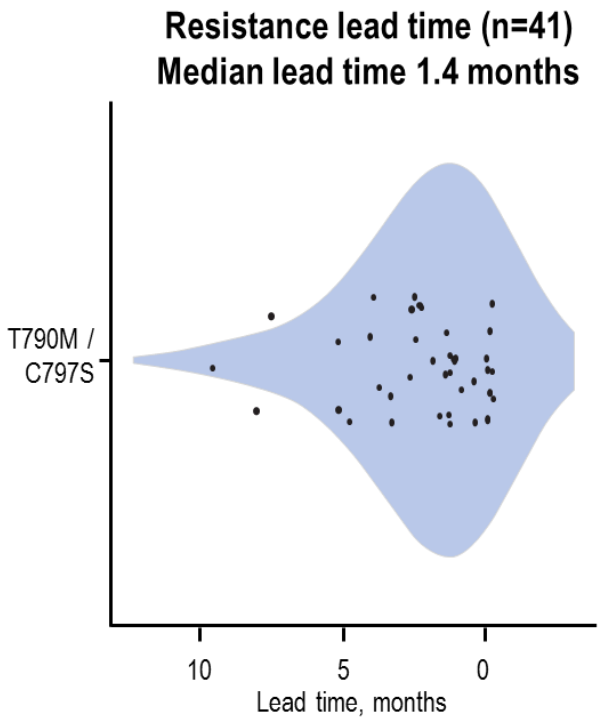


Blood sample every 4 months (up to 3 years) during follow-up for detection of ctDNA recurrence by LUNAR assay

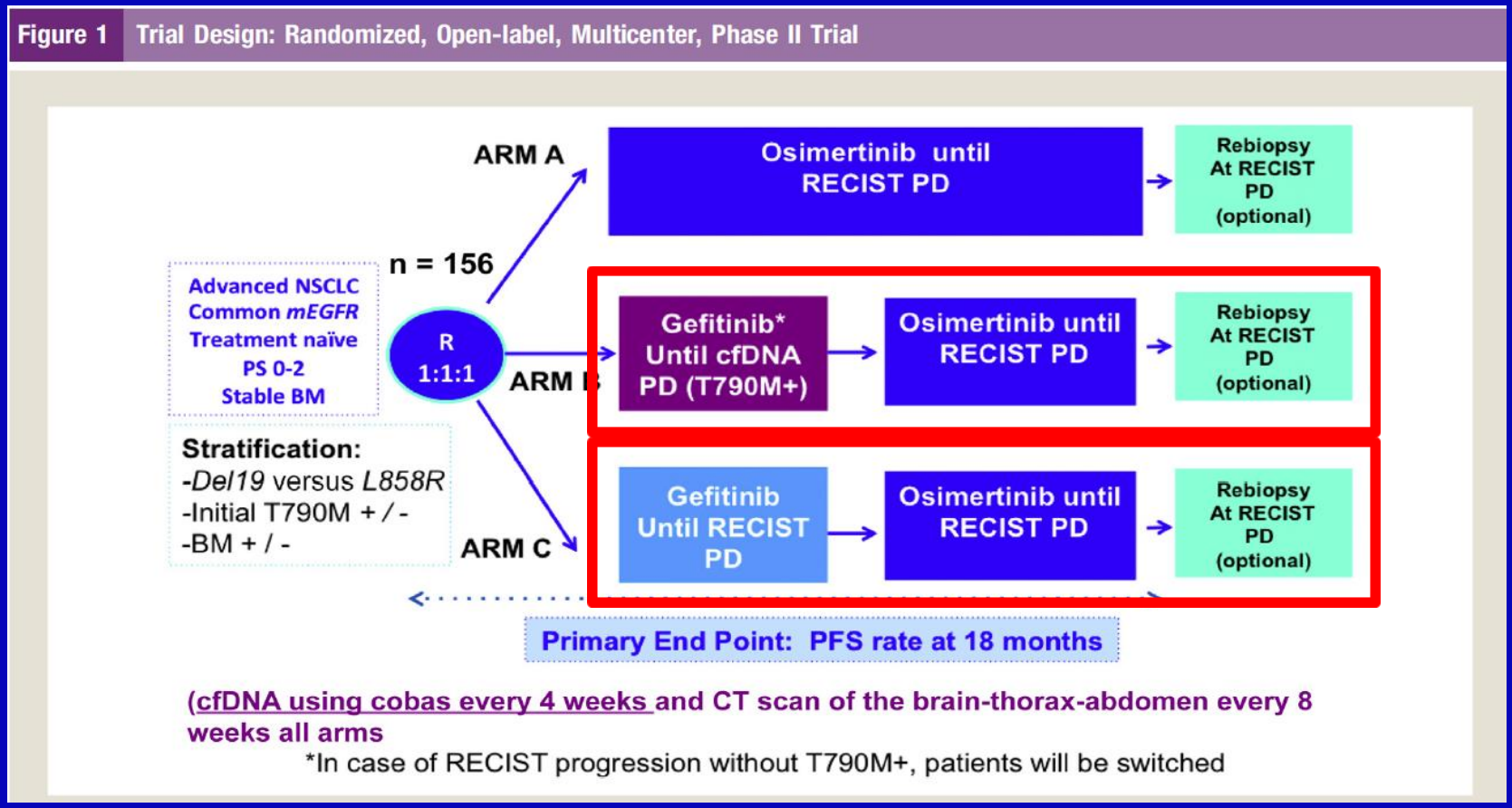
FLAURA ctDNA analysis: Early Detection of T790M or C797S EGFR mutation Before RECIST progression

- Acquired C797S and T790M resistance mutations were detected in 8% and 74% of patients with ctDNA PD in the osimertinib and comparator EGFR-TKI arms, respectively
- Median lead time to acquired C797S or T790M in patients with ctDNA PD and RECIST PD was 1.4 months (IQR 0.5–3.4 months)

Patients with ctDNA PD	Osimertinib (n=50)	Comparator EGFR-TKI (n=72)	Overall (n=122)
Resistance mutation detected, n (%)	4 (8%) C797S	53 (74%) T790M	57 (47%)
Median time to detection (IQR), months	16.7 (12.6–19.7)	8.4 (5.6–12.4)	8.4 (5.6–14.0)
Patients with ctDNA PD and RECIST PD	**Osimertinib (n=39)**	**Comparator EGFR-TKI (n=67)**	**Overall (n=106)**
Resistance mutation detected, n (%)	2 (5%) C797S	50 (75%) T790M	52 (49%)
ctDNA resistance lead time equivalent* or earlier, n (%)	2 (5%)	39 (60%)	41 (39%)



Modifying treatment prior to radiological progression



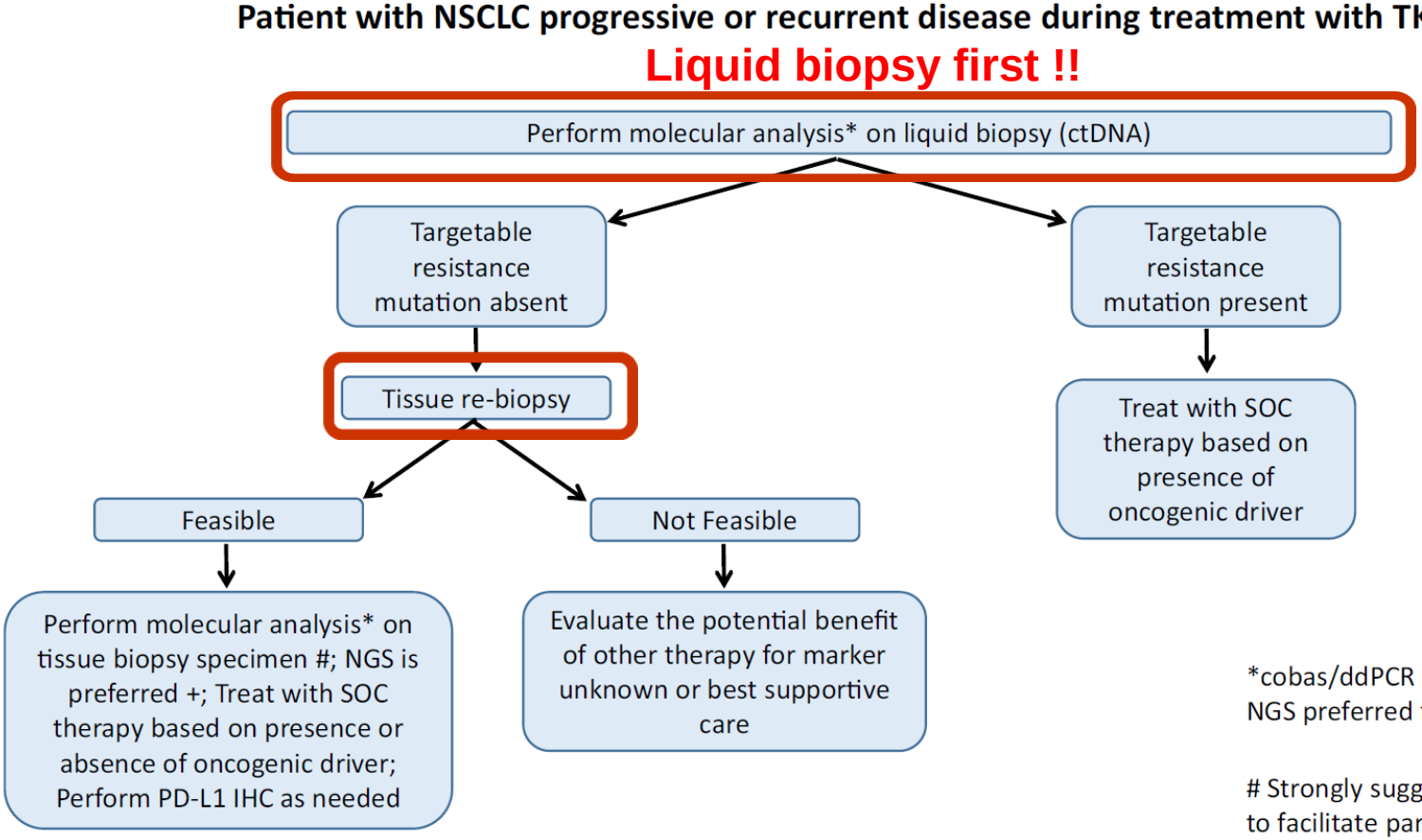
APPLE Trial: Feasibility and Activity of Osimertinib on Positive Plasma T790M in EGFR-mutant NSCLC Patients (EORTC 1613)

Remon J. Clin Lung cancer 2017

Key message: Liquid Biopsy in Treatment-naïve Patients

- Same criteria as molecular testing from tissue
  - ✓ Advanced nonsquamous NSCLC or squamous NSCLC with clinical features of a molecular driver
- Particularly recommended when tumor tissue is scarce or a significant delay (> 2 weeks) is expected in obtaining tumor tissue and in patients for whom invasive procedure may be contraindicated or with bone biopsy
- A negative ctDNA result should be followed up with tumor biopsy
  - ✓ False negative liquid biopsy result (sensitivity max ~85%)
- Patients should be drawn before any treatment

Liquid Biopsy for Advanced NSCLC: Consensus Statement from the IASLC (Rolfo C. JTO 2018)

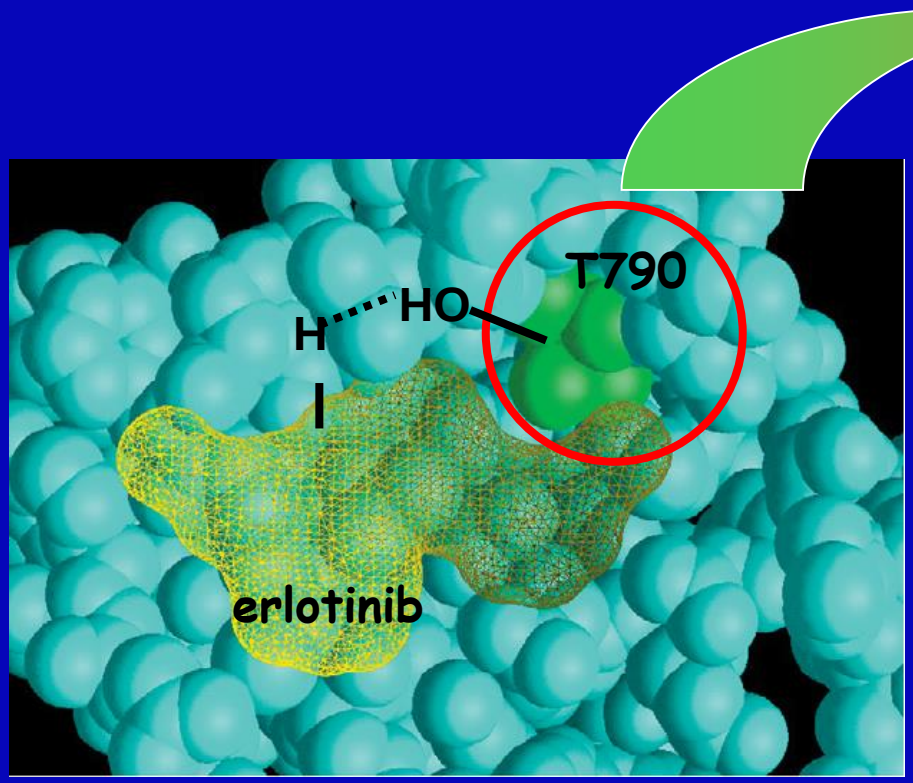


*cobas/ddPCR for *EGFR* mutation
NGS preferred for *ALK* and *ROS1*

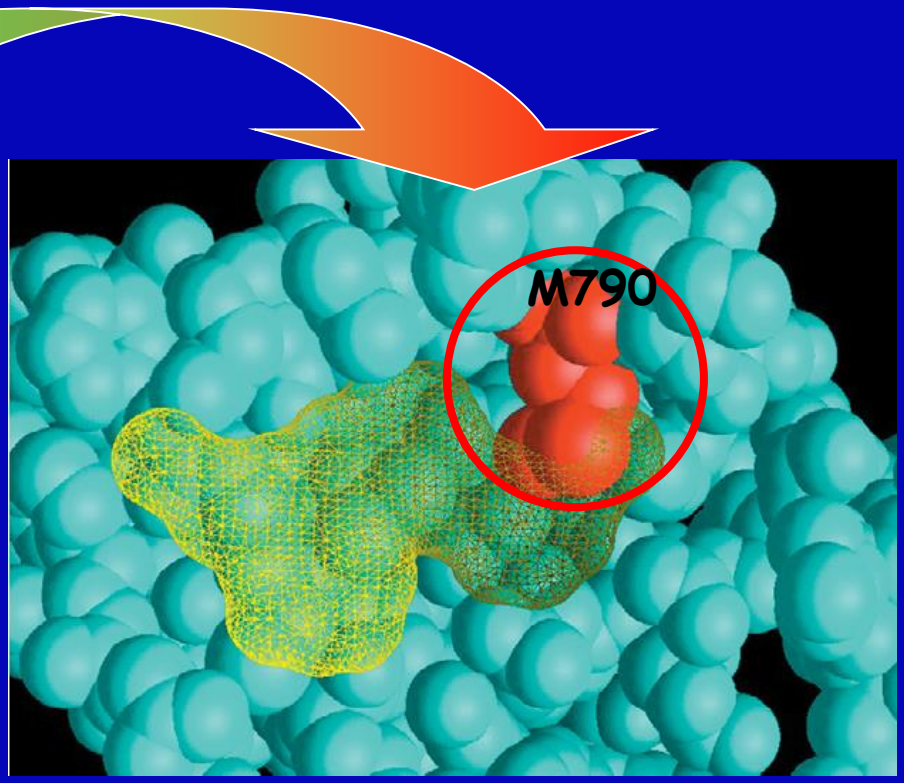
Strongly suggest tissue sparing to facilitate participation in clinical trials

+ While NGS is preferred, based on availability, other validated assays are acceptable

T790M Gatekeeper Mutation



Erlotinib bound to EGFR



Steric hindrance inhibits binding of erlotinib to catalytic site

EGFR T790M found in ~50% of patients who become resistant to gefitinib/erlotinib/afatinib

Why is the detection T790M important?

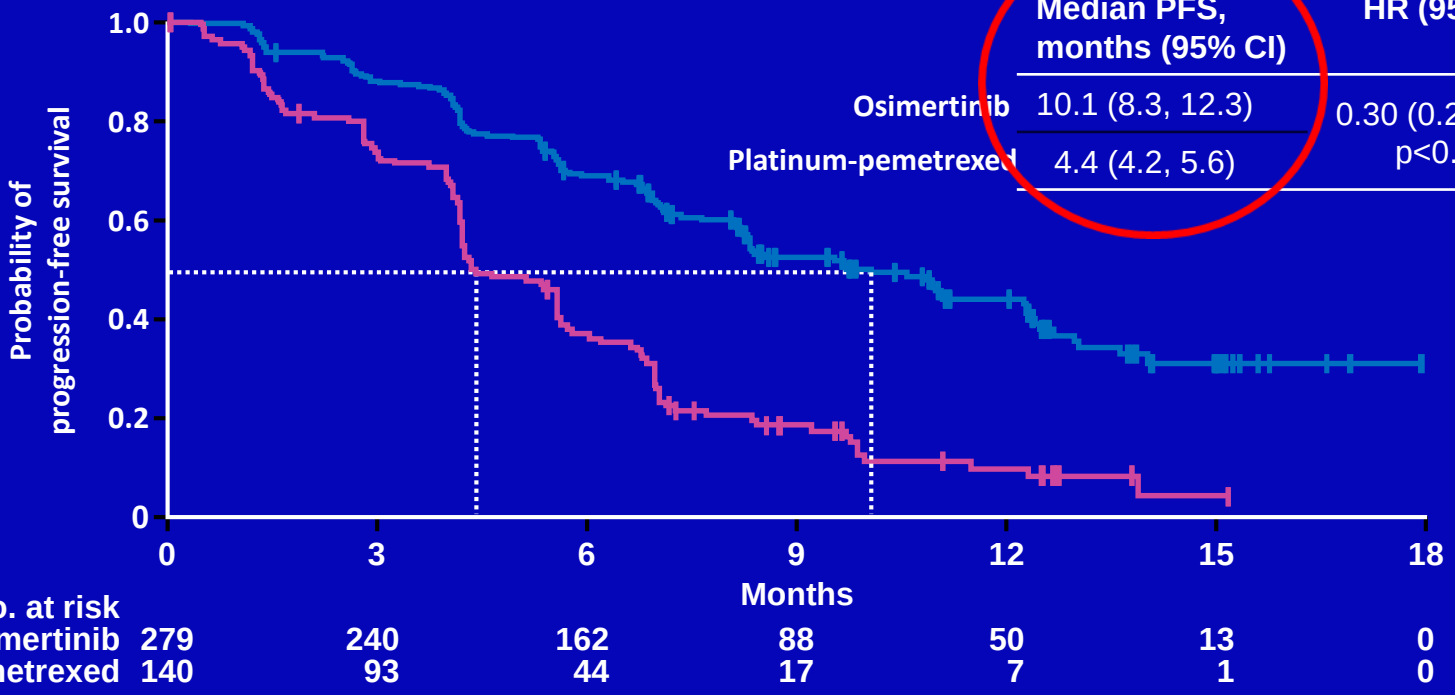
ORIGINAL ARTICLE

Osimertinib or Platinum–Pemetrexed in *EGFR* T790M–Positive Lung Cancer

T.S. Mok, Y.-L. Wu, M.-J. Ahn, M.C. Garassino, H.R. Kim, S.S. Ramalingam, F.A. Shepherd, Y. He, H. Akamatsu, W.S.M.E. Theelen, C.K. Lee, M. Sebastian, A. Templeton, H. Mann, M. Marotti, S. Ghiorghiu, and V.A. Papadimitrakopoulou, for the AURA3 Investigators*

Osimertinib- the only 3G EGFR TKI approved for patients with progression after EGFR TKI and harboring T790M

	Median PFS, months (95% CI)	HR (95% CI)
Osimertinib	10.1 (8.3, 12.3)	0.30 (0.23, 0.41) p<0.001
Platinum-pemetrexed	4.4 (4.2, 5.6)	



Why Should Liquid Biopsy be First in Lung Cancer Progressing during Targeted Therapy?

- Biopsy feasibility (~60%[1])
- Faster turnaround time
  - ✓2 (1-4) vs. 27 days (1-146)[2]
- HETEROGENEITY

[1]Kawamura T. Cancer Sci. 2016, Hong MH. YMJ 2019; [2]Sacher AG. JAMA Oncol 2016

Detecting T790M mutations in plasma

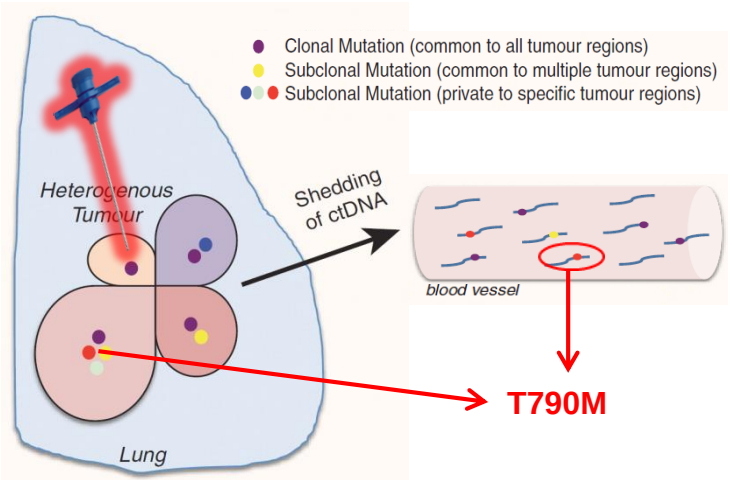
- Challenges:

✓ Very low concentration of the mutations

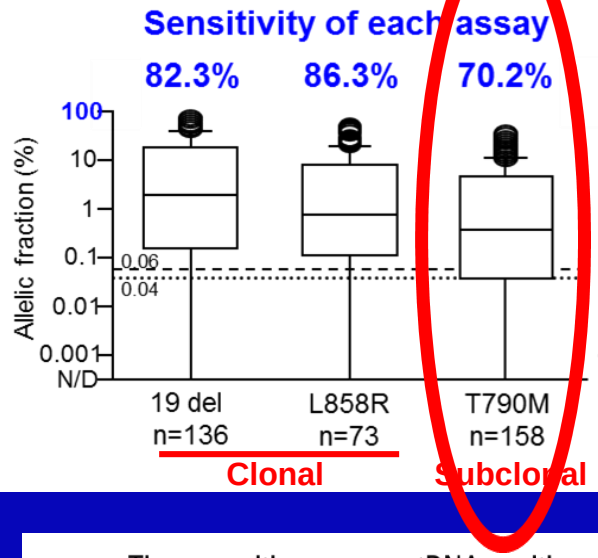
✓ High concentration of wild-type sequences from non-malignant tissues

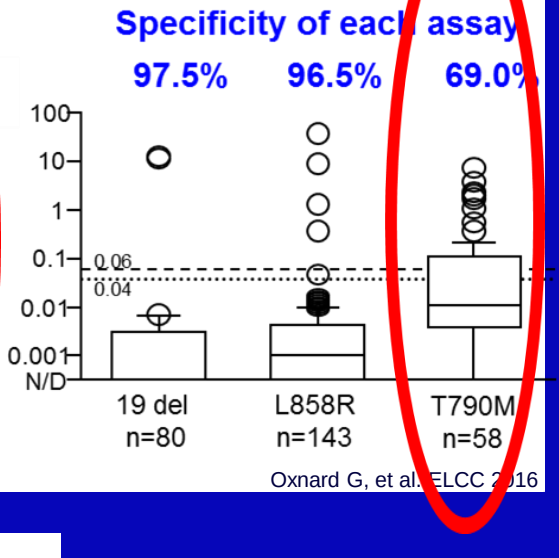
✓ Single nucleotide difference T790M

Clonal Mutations are More likely to be Detected than Subclonal Mutations in Plasma

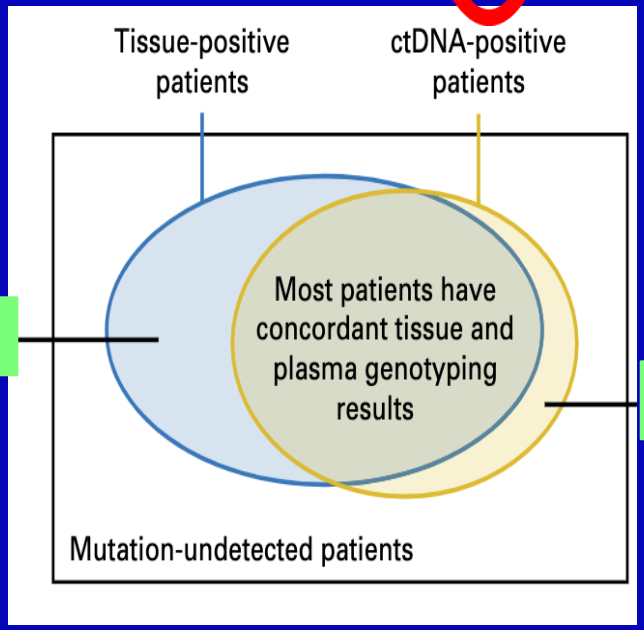


Murphy DJ. Cell Death & Differentiation 2017





Oxnard G, et al. ELCC 2016

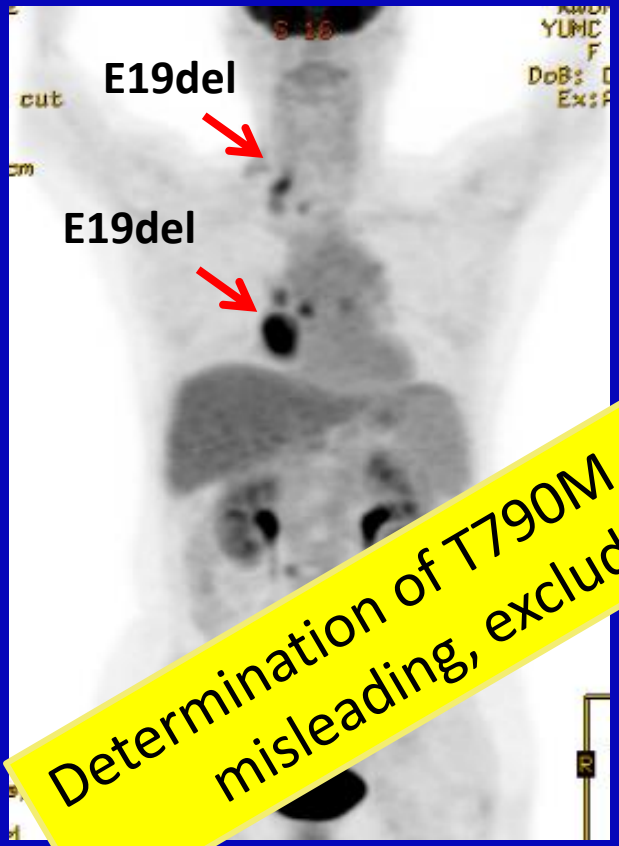


Tumor+/ctDNA-

ctDNA+/Tumor-

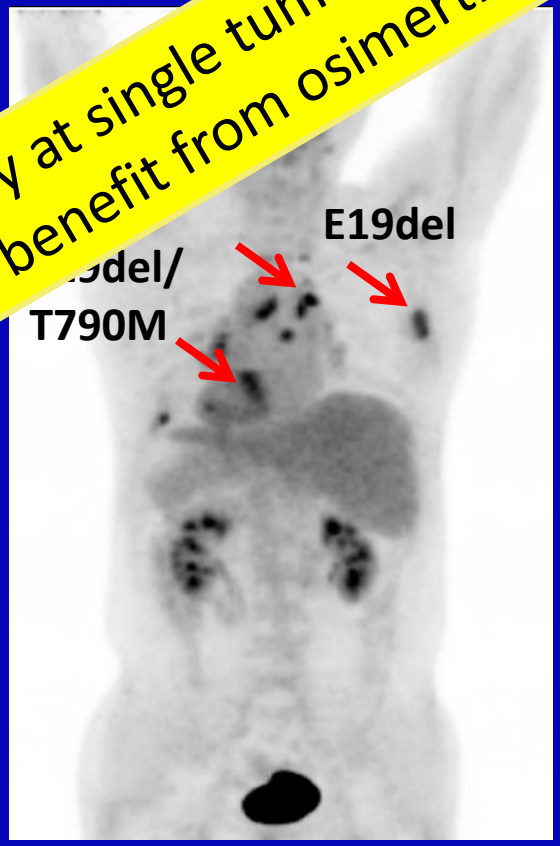
A single biopsy specimen may not represent intrinsic heterogeneity of a resistant cancer

Homogenous/Concordant



At diagnosis

Heterogenous/



At progression

Determination of T790M status based on biopsy at single tumor site may be misleading, excluding potential pts who benefit from osimertinib

Plasma assay performance for T790M detection using tissue test as reference

Platform	Sensitivity	Specificity	Reference
cobas	61	79	Jenkins, JTO 2017
cobas	51	77	Wu, WCLC 2016
cobas	73	67	Thress, Lung Cancer 2015
cobas	64	98	Karlovich, CCR 2016
ddPCR	77	63	Sacher, JAMA Oncol 2016
BEAMing	70	69	Oxnard, JCO 2016
BEAMing	73	50	Karlovich, CCR 2016
BEAMing	80	58	Thress, Lung Cancer 2015

- **50-77%** patients can have T790M mutation status determined without an invasive procedure
- Specificity issues in plasma assay likely from tumor heterogeneity (“False-positive plasma”)

Sensitivity of 3 Technologies for T790M detection (AURA 3)

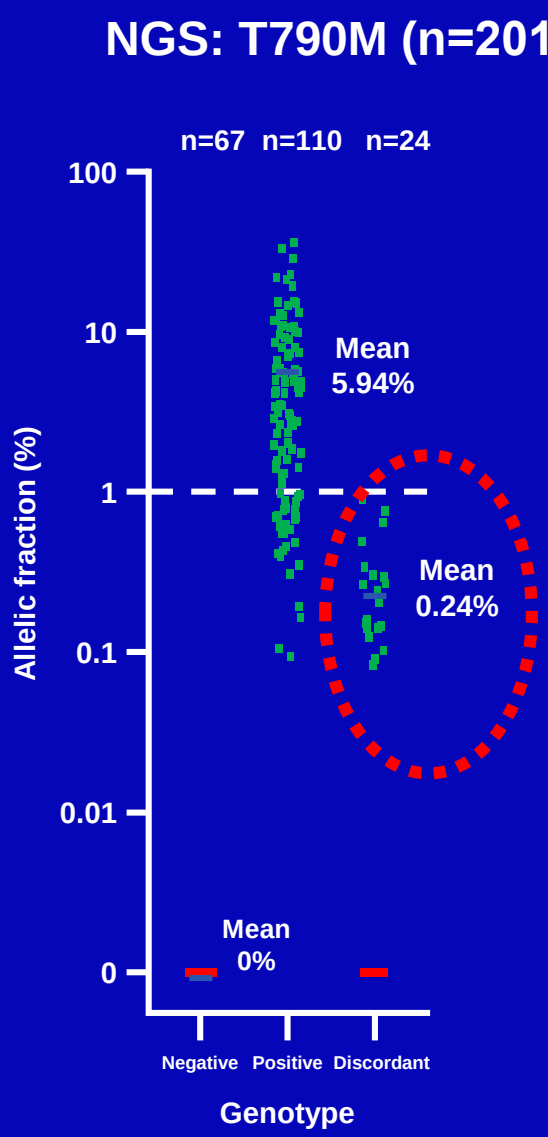
	T790M		Exon 19 deletion		L858R	
	PPA	NPA*	PPA	NPA	PPA	NPA
AS-PCR (Cobas® v2, n=226)	**51%** (115/226)	NA	**85%** (132/155)	**99%** (70/71)	**59%** (40/68)	**100%** (158/158)
ddPCR (Biodesix, n=208)	**57%** (118/208)	NA	**72%** (102/142)	**100%** (66/66)	**69%** (44/64)	**99%** (141/143)
NGS (Guardant Health, n=227)	**65%** (148/227)	NA	**81%** (126/156)	**99%** (70/71)	**62%** (42/68)	**98%** (156/159)

- Using the cobas tissue test as a reference, sensitivity for the detection of T790M was increased for ddPCR and NGS compared with AS-PCR
- Specificity of the tests for T790M detection could not be assessed because all patients were T790M positive by tissue test*

Population: osimertinib-dosed patients with a valid cobas tissue T790M-positive result and matched plasma samples
*Specificity for the detection of T790M was not evaluable as all patients enrolled in AURA3 were T790M positive
NA, not applicable; NPA, negative percent agreement (specificity); PPA, positive percent agreement (sensitivity)

Ahn et al WCLC 2017

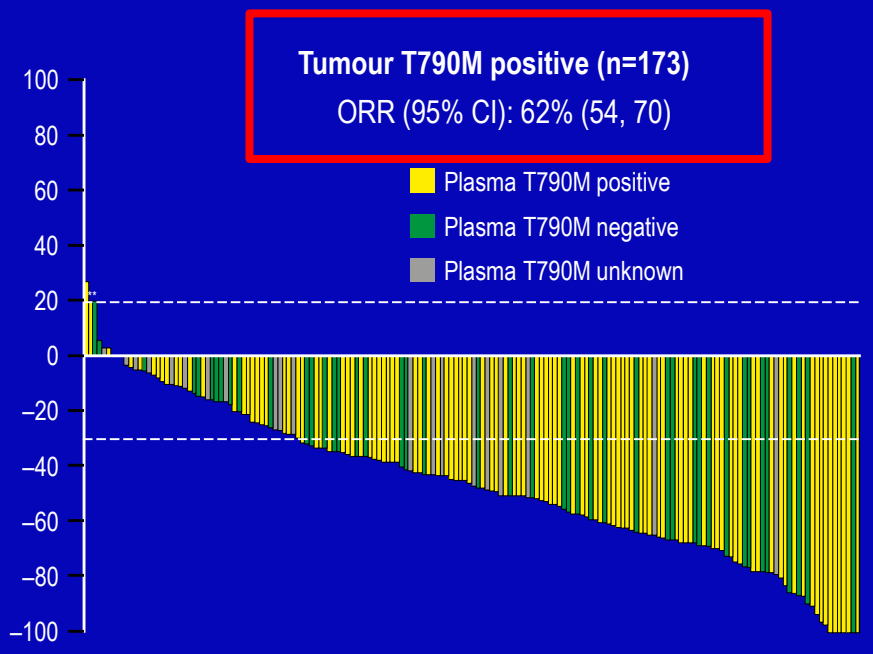
T790M discordance by patient: ddPCR vs NGS

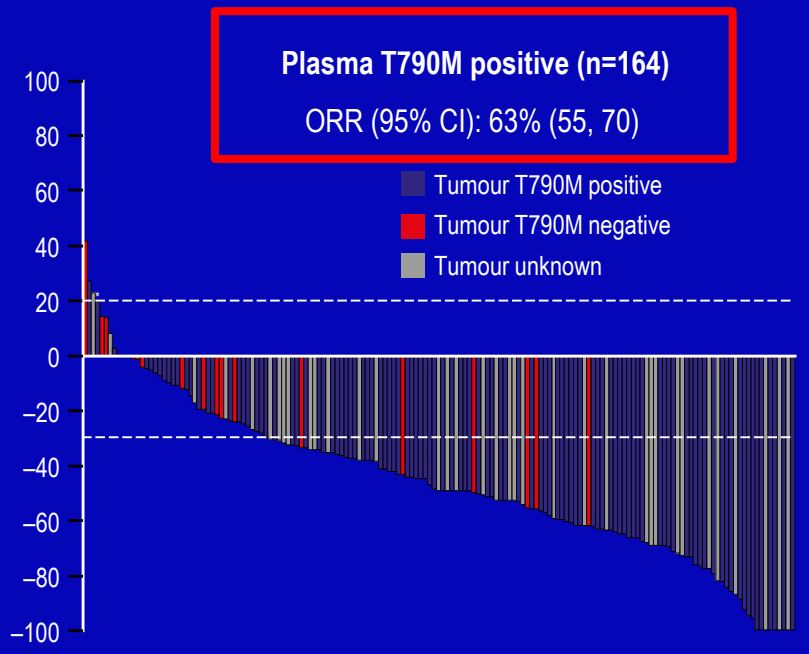


- 201 patients had a valid ddPCR and NGS plasma T790M test result
- 24 patients (12%) with a discordant result (i.e. differing mutation status by ddPCR and NGS) are shown here
- 100% of discordant samples had allelic fractions <1% in both assays
- 19/24 (79%) of discordant samples were ddPCR negative but NGS positive

Green = NGS positive result, red = NGS negative result; population: osimertinib-dosed patients with a valid cobas tissue T790M-positive result and matched plasma samples.

Plasma cfDNA positivity in T790M is predictive of tumor response (AURA)



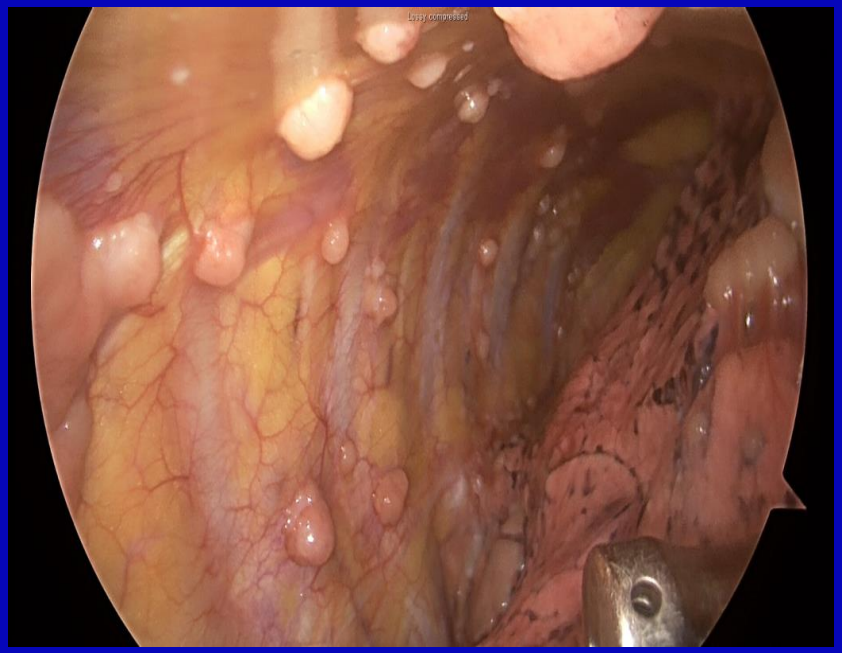


		ORR (%)		PFS (months)	
	Platform	Plasma	Tumor	Plasma	Tumor
AURA, AURA 2	Cobas	64	66	NR	NR
AURA 3	Cobas	77	71	8.2	10.1
AURA	Beaming	63	62	9.7	9.7

Jenkins, JTO 2017; Wu, WCLC 2016; Oxnard G, JCO 2016

Case #2: 43 year-old never smoking woman

- Diagnosis of stage IV lung adenocarcinoma with EGFR E19del
- Gefitinib for 1 year, symptomatic disease progression at lung and brain
  - ctDNA with cobas EGFR WT
  - Bronchoscopy nondiagnostic
  - Wedge resection E19 del
- Started lazertinib (a novel third-generation EGFR TKI)



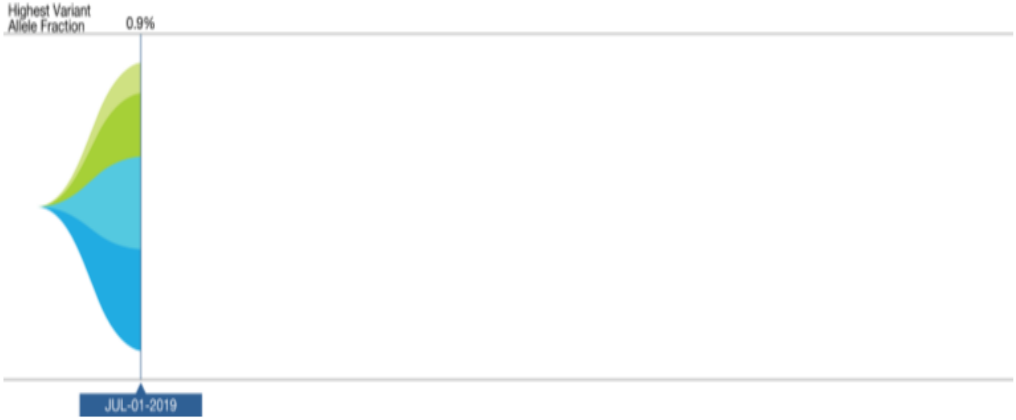
Pleural biopsy under VATS

Lazertinib in patients with *EGFR* mutation-positive advanced non-small-cell lung cancer: results from the dose escalation and dose expansion parts of a first-in-human, open-label, multicentre, phase 1–2 study

Myung-Ju Ahn, Ji-Youn Han, Ki Hyeong Lee, Sang-We Kim, Dong-Wan Kim, Yun-Gyoo Lee, Eun Kyung Cho, Joo-Hang Kim, Gyeong-Won Lee, Jong-Seok Lee, Young Joo Min, Jin-Soo Kim, Sung Sook Lee, Hye Ryun Kim, Min Hee Hong, Jin Seok Ahn, Jong-Mu Sun, Heung Tae Kim, Dae Ho Lee, Sohee Kim, Byoung Chul Cho

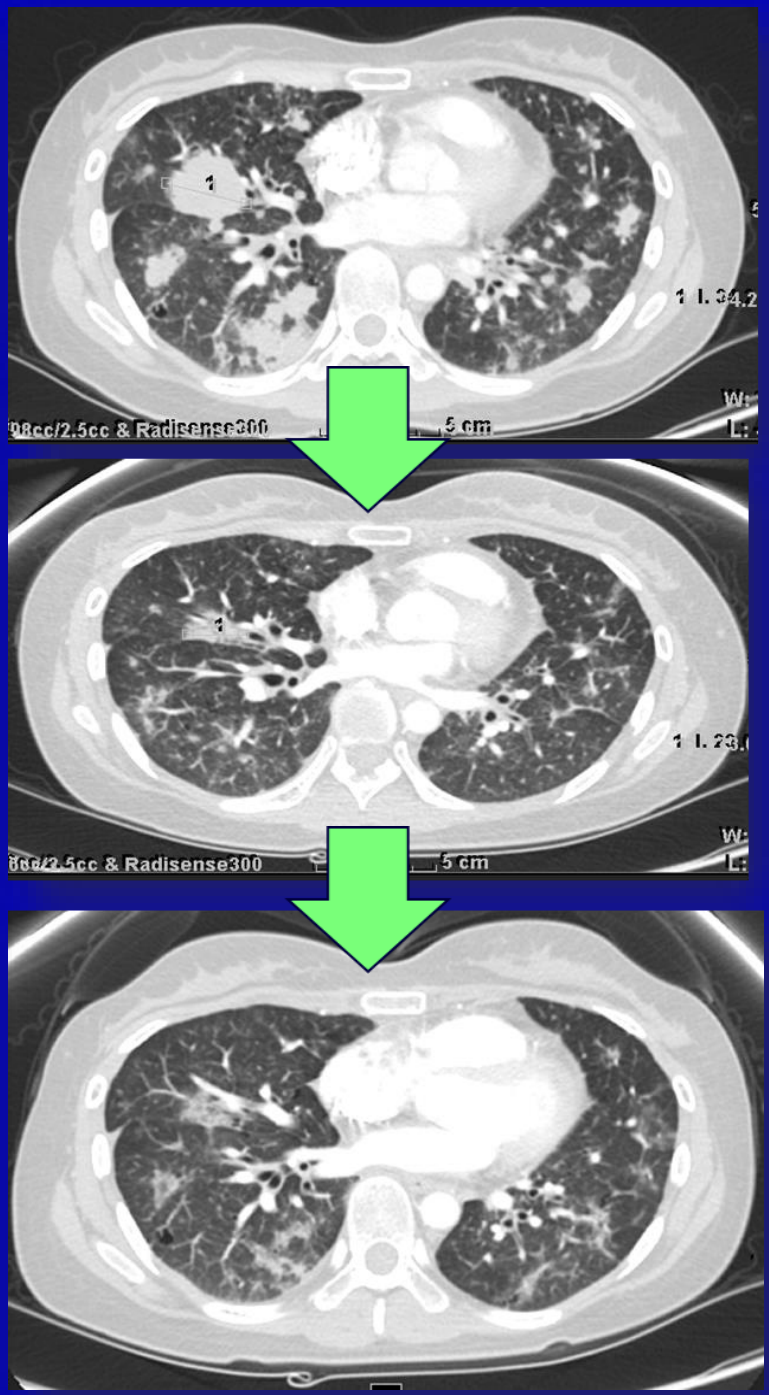
Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the variant allele fraction (% cfDNA) of observed somatic variants at each sample submission time point. Amplifications are not plotted, and only the first and last five test dates are plotted. Please see the Physician Portal (portal.guardanthealth.com) for the Tumor Response Map with all test dates.



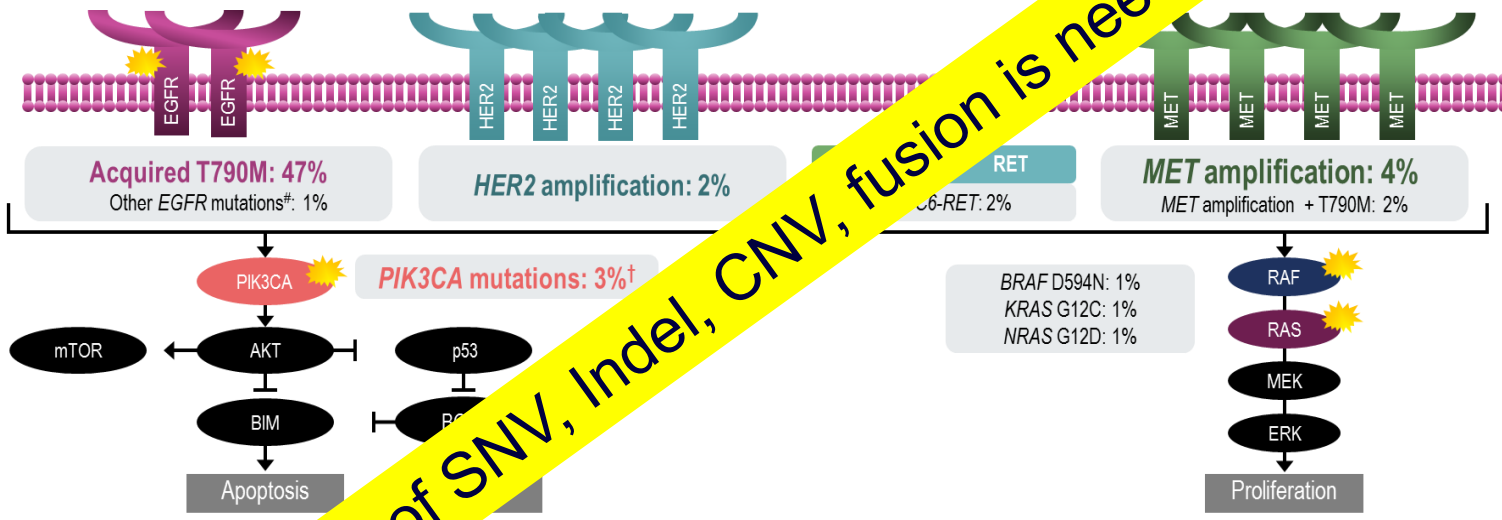
Alteration	% cfDNA or Amp
PIK3CA E110del	0.9%
TP53 R248Q	0.8%
EGFR E746_A750del (Exon 19 deletion)	0.5%
EGFR T790M	0.2%

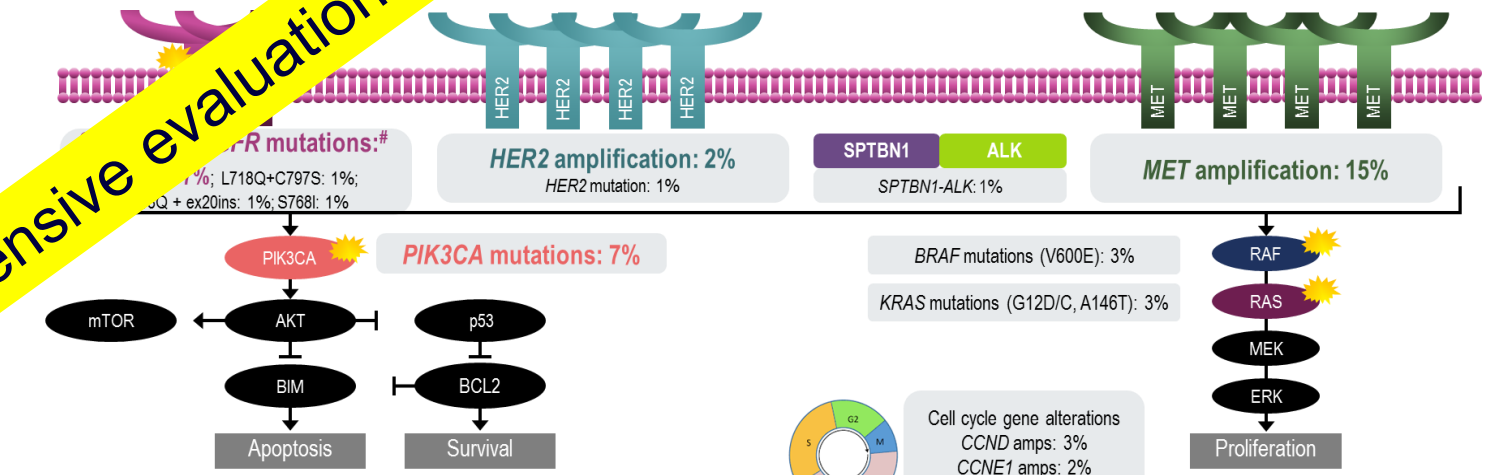
The table above annotates the variant allele fraction (% cfDNA) detected in this sample, listed in descending order.



Why is NGS Preferred in Patients with NSCLC Progressive during EGFR TKI Treatment

FLAURA





Comprehensive evaluation of SNV, Indel, CNV, fusion is neeed!!

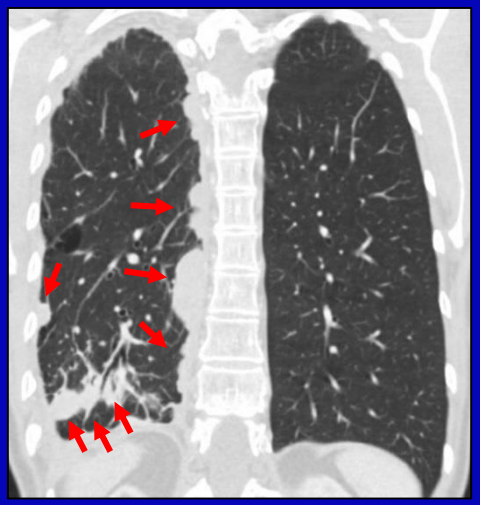
Guardant360 assay (73 genes) or GuardantOMNI assay (500 genes)

Ramalingam SS. ESMO 2018

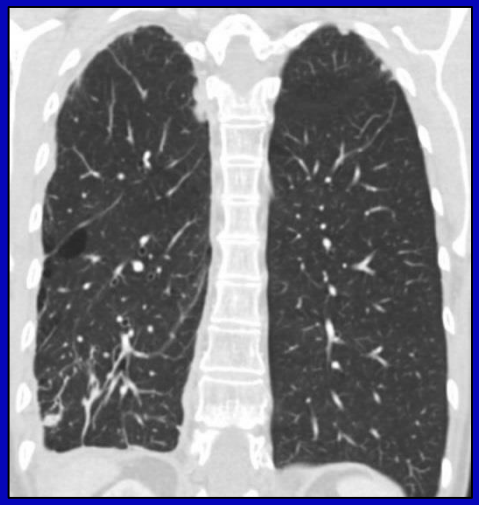
Combination of Osimertinib and Pralsetinib (RET inhibitor) Shows Response in EGFR mutant Patients with Acquired RET Fusion

- 60-year old female with EGFR del19 NSCLC received afatinib for one year, then osimertinib for 18 months
- Biopsy post-osimertinib shows *CCDC6-RET* fusion, T790M "lost"
- Patient treated with osimertinib + pralsetinib
- Osimertinib 80mg QD; pralsetinib 200mg QDx2 weeks, then 300 mg QD

RECIST 1.1 Partial Response (-78%)



Baseline



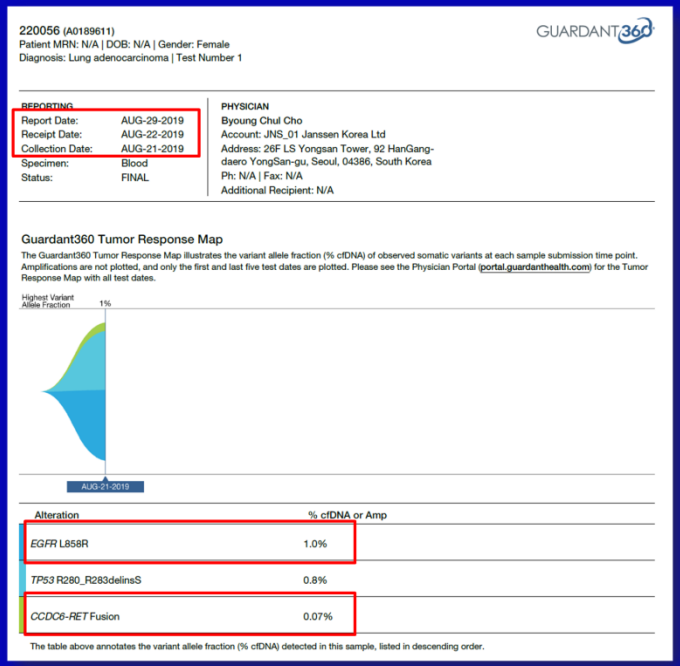
8 weeks

Zofia Piotrowska, et al. IASLC. 2018. MA26.03

Case #3 47 year-old never smoker woman

- Stage IV lung adenocarcinoma with EGFR L858R mutation
- Afatinib for 2 years, disease progression
- Chemotherapy with gemcitabine-cisplatin
- Palliative radiotherapy at T-, L-spine
- Cobas ctDNA confirmed L858R/T790M
- Osimertinib with response for 13 months
- Symptomatic lung disease progression
- Lung rebiopsy NGS still pending
- G360 confirmed CCDC6-RET fusion as acquired resistance mechanism to osimertinib

It is Real!



220056 (A0189611)
Patient MRN: N/A | DOB: N/A | Gender: Female
Diagnosis: Lung adenocarcinoma | Test Number 1

GUARDANT360

REPORTING	
Report Date:	AUG-29-2019
Receipt Date:	AUG-22-2019
Collection Date:	AUG-21-2019
Specimen:	Blood
Status:	FINAL

PHYSICIAN
Byoung Chul Cho
Account: JNS_01 Janssen Korea Ltd
Address: 26F LS Yongsan Tower, 92 HanGang-daero YongSan-gu, Seoul, 04386, South Korea
Ph: N/A | Fax: N/A
Additional Recipient: N/A

Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the variant allele fraction (% cfDNA) of observed somatic variants at each sample submission time point. Amplifications are not plotted, and only the first and last five test dates are plotted. Please see the Physician Portal (portal.guardanthealth.com) for the Tumor Response Map with all test dates.

Alteration	% cfDNA or Amp
EGFR L858R	1.0%
TP53 R280_R283delinsS	0.8%
CCDC6-RET Fusion	0.07%

The table above annotates the variant allele fraction (% cfDNA) detected in this sample, listed in descending order.

Liquid Biopsy: NCCN Guideline & Recommendations

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

- CAP/IASLC/AMP 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

Use of cfDNA testing can be considered in specific clinical circumstances, most notably:

- If a patient is medically unfit for invasive sampling
- In the initial diagnostic setting, if following pathologic confirmation of a NSCLC diagnosis.. there is insufficient material for molecular analysis.. cfDNA should be used only if follow-up tissue-based analysis is planned for patients in which an oncogenic driver is not identified

- NCCN Treatment Guidelines 1.2019 Non-Small Cell Lung Cancer

Lindeman et al JTO 2018; Burstein et al. JCO 2017; NCCN Guideline, NSCLC

Our Experience of Guardant360 in Lung Cancer

Patients Demographics

Characteristic	N=203 (%)
Age--yr	
Median (range)	65 (25-85)
Sex — no. (%)	
Male	149 (73.4%)
Smoking status — no. (%)	
Current or former smoker	137 (67.5%)
Never smoked	66 (32.5%)
Tumor histologic type — no. (%)	
Adenocarcinoma	160 (78.8%)
Squamous cell carcinoma	35 (17.2%)
Others	8 (4.0%)
Tissue pathology mutation — no. (%)	
EGFR/ALK/ROS1 WT	188 (92.6%)
EGFR mutation	4 (2.0%)
ALK fusion	3 (1.5%)
ROS1 fusion	8 (3.9%)
Status at cfDNA NGS — no. (%)	
Stage IV or relapsed	195 (96.1%)
Treatment history at G360 — no. (%)	
Treatment naïve	64 (31.5%)
Post TKIs	1 (0.5%)
Post chemo-Immunotherapy	126 (62.1%)
Post cutative therapy	12 (5.9%)

Patients who Became Candidates for Targeted Therapy Based on G360 Results

No	Actionable mutation identified	VAF (%)	Line of therapy	Previous Treatment	Treatment (context)	Best Response
1	EGFR T725M	0.8	Second-line	Pemetrexed/Cisplatin	Erlotinib (SoC)	SD
2	EGFR exon 19 del	1.0	Second-line	Pemetrexed/Cisplatin	Gefitinib (SoC)	SD
3	EGFR L858R	0.9	Third-line	Pemetrexed/Cisplatin Docetaxel	Gefitinib (SoC)	PR
4	EGFR L858R	1.2	Second-line	Keytruda	Gefitinib (SoC)	PR
5	EGFR exon 20 ins	0.09	Third-line	Pemetrexed/Cisplatin Gemcitabine/Carboplatin	JNJ-61186372 (Clinical trial)	SD
6	KIF5B-RET fusion	1.7	Second-line	Pemetrexed/Cisplatin	BLU-667 (Clinical trial)	SD
7	NCOA4-RET fusion	5.6	Second-line	Gemcitabine/Cisplatin	Loxo-292 (Clinical trial)	PR
8	ERBB2 G660D	6.2	Second-line	Pemetrexed/Cisplatin	Neratinib/Herceptin (Clinical trial)	SD

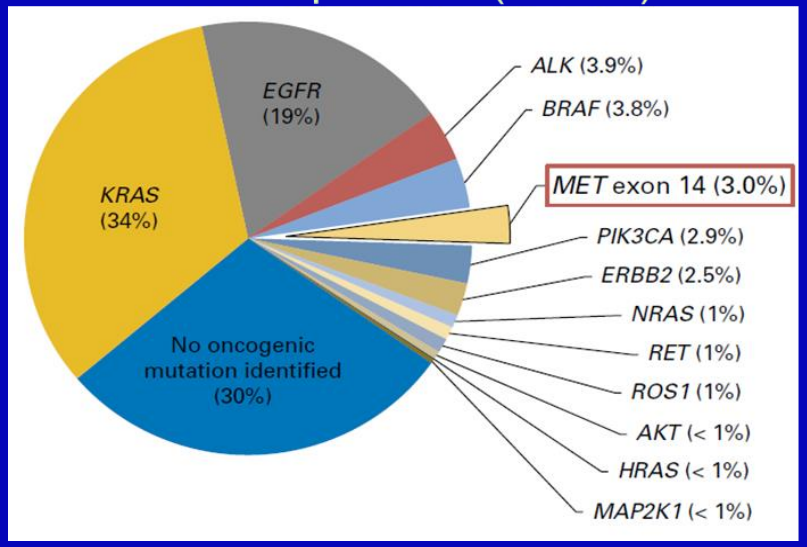
In these patients, tissue results were wild-type for actionable mutations or unavailable due to tissue insufficiency

Can We Identify Actionable Mutation using Guardant 360?

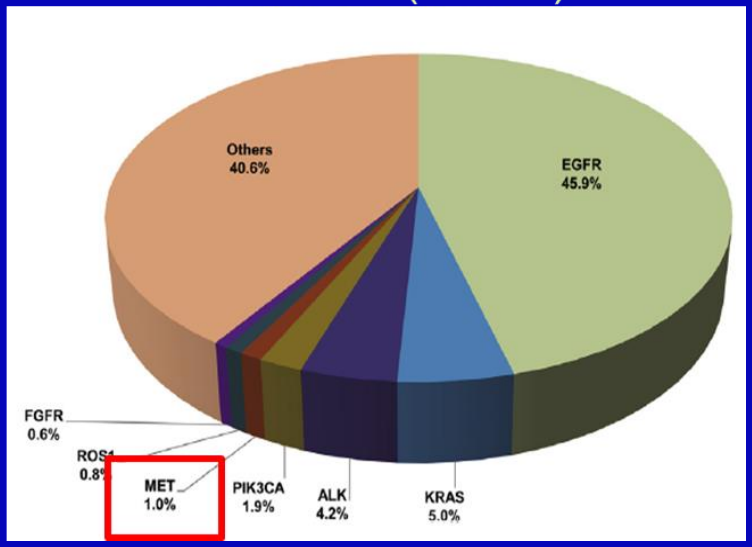
	Mutation	N (%)
Level 1 FDA-approved biomarker predictive of response to an FDA-approved drug in lung cancer	EGFR ALK fusion ROS1 fusion	8/203 (3.9%)
Level 2A standard of care biomarkers for FDA-approved drugs in lung cancer	MET amplification/Exon 14 skipping BRAF V600E RET fusion	9/203 (4.4%)
Level 2B standard of care biomarkers for FDA-approved drugs in other cancer	ERBB2 amplification BRCA 1/2 loss TSC 1/2 loss CDK 4 amplification IHD1	20.1%
Level 3 alterations with promising clinical evidence for drug response but not currently standard of care in any cancer type	ERBB2 mutation EGFR exon 20 insertion FGFR 1/2 amplification PIK3CA MAP2K1 ARAF	15/203 (7.4%)

MET exon 14 skipping represents a unique subset of NSCLC

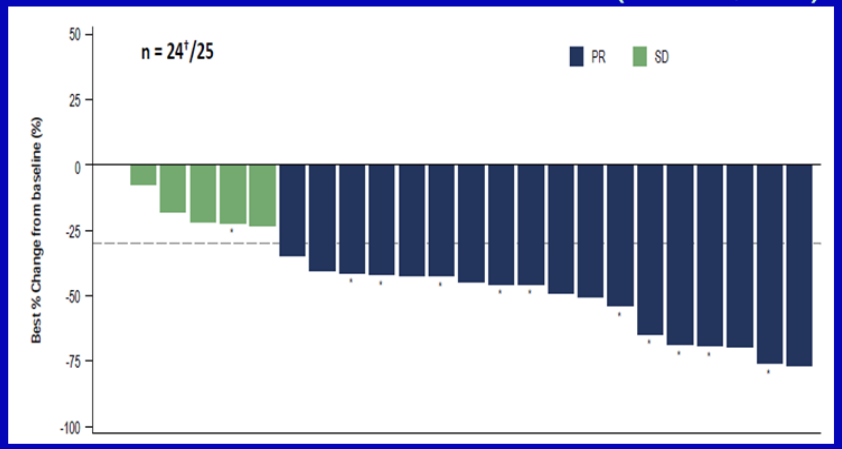
White patients (n=933)



Chinese (n=968)

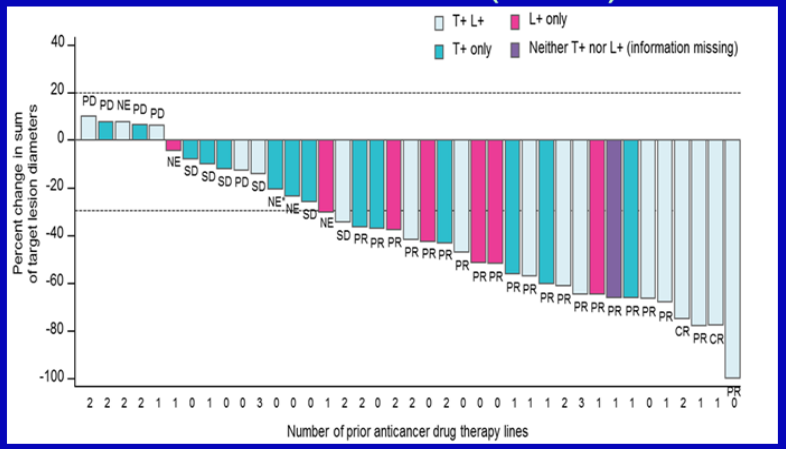


GEOMETRY MONO-1 Ph II (n=25, 1L)



ORR 72.0% (95% CI: 50.6-87.9)

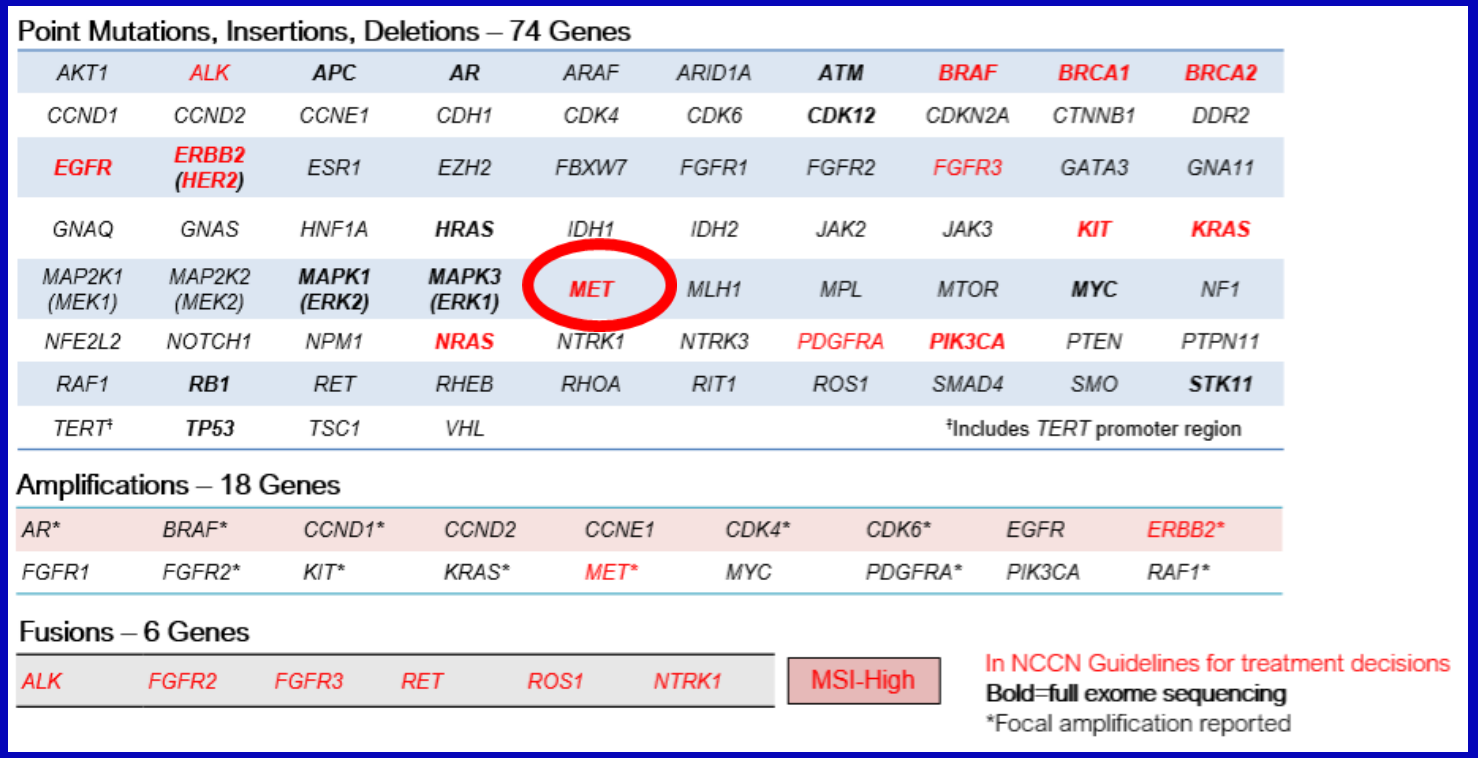
VISION Ph II (n=69)



ORR 57.5% (95% CI: 40.9, 73.0)

Awad MM. JCO 2016; Liu SY. JTO 2016; Liu X. JCO 2016

Guardant360: 74 cancer-associated genes



Point Mutations, Insertions, Deletions – 74 Genes

AKT1	*ALK*	***APC***	***AR***	*ARAF*	*ARID1A*	***ATM***	***BRAF***	***BRCA1***	***BRCA2***
CCND1	*CCND2*	*CCNE1*	*CDH1*	*CDK4*	*CDK6*	***CDK12***	*CDKN2A*	*CTNNB1*	*DDR2*
EGFR	***ERBB2 (HER2)***	*ESR1*	*EZH2*	*FBXW7*	*FGFR1*	*FGFR2*	*FGFR3*	*GATA3*	*GNA11*
GNAQ	*GNAS*	*HNF1A*	***HRAS***	*IDH1*	*IDH2*	*JAK2*	*JAK3*	***KIT***	***KRAS***
MAP2K1 (MEK1)	*MAP2K2 (MEK2)*	***MAPK1 (ERK2)***	***MAPK3 (ERK1)***	***MET***	*MLH1*	*MPL*	*MTOR*	***MYC***	*NF1*
NFE2L2	*NOTCH1*	*NPM1*	***NRAS***	*NTRK1*	*NTRK3*	*PDGFRA*	***PIK3CA***	*PTEN*	*PTPN11*
RAF1	***RB1***	*RET*	*RHEB*	*RHOA*	*RIT1*	*ROS1*	*SMAD4*	*SMO*	***STK11***
TERT‡	***TP53***	*TSC1*	*VHL*				‡Includes *TERT* promoter region		

Amplifications – 18 Genes

*AR**	*BRAF**	*CCND1**	*CCND2*	*CCNE1*	*CDK4**	*CDK6**	*EGFR*	*ERBB2**
FGFR1	*FGFR2**	*KIT**	*KRAS**	*MET**	*MYC*	*PDGFRA**	*PIK3CA*	*RAF1**

Fusions – 6 Genes

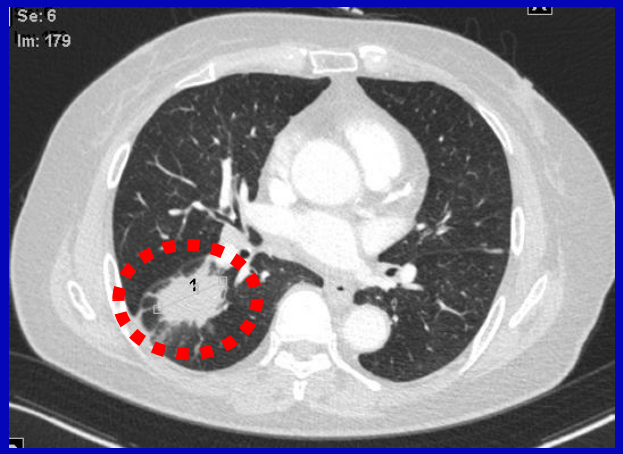
ALK	*FGFR2*	*FGFR3*	*RET*	*ROS1*	*NTRK1*

MSI-High

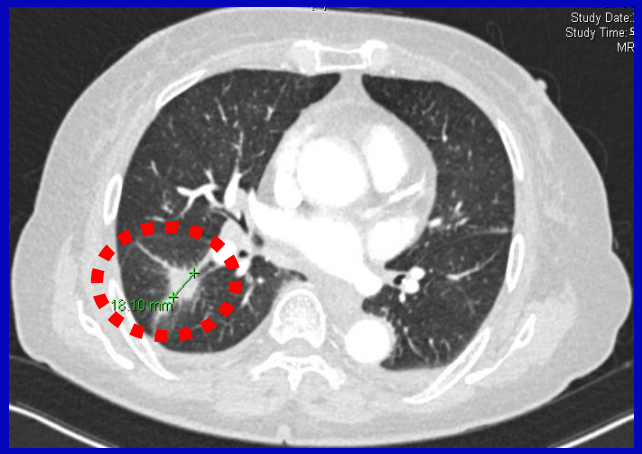
In NCCN Guidelines for treatment decisions
Bold=full exome sequencing
*Focal amplification reported

Guardant360 reports insertion/deletion variants and amplification of MET gene

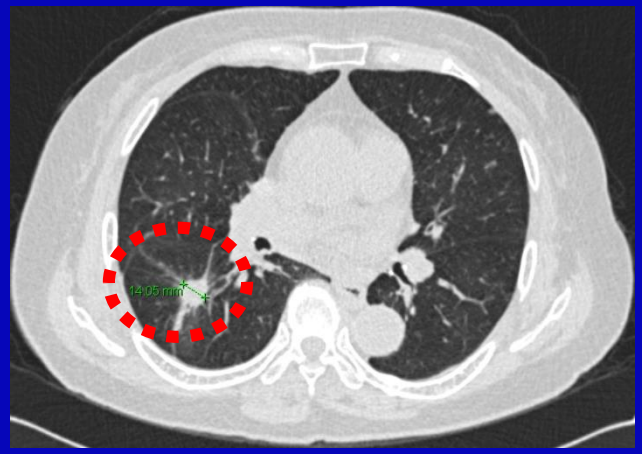
82/F Never smoker, Lung adenocarcinoma harboring MET ex14 skipping mutation



Apr 2019



May 2019



Jul 2019

Capmatinib

Same Results but Right on Time!

TAT 10 days

GUARDANT360™

Alteration	% cfDNA or Amp
TP53 M246I	5.4%
MET Exon 14 Skipping SNV	4.8%
STK11 K48fs	4.1%
EGFR Amplification Amplifications not graphed above	Low (+) Plasma Copy Number: 2.2

TAT 6 weeks

Illumina TruSight™ Tumor 170

검체 번호	성별	나이	Unit NO.	환자명	장기명/진단	검체 유형
SS18-78809 C	남	63	8777506	서○진	LN/Lung,Metastaitc adenocarcinoma	FFPE
의뢰의	의뢰의 소속			검체의 적절성여부	검체 접수일	결과보고일
조병철	종양내과			적합 (Tumor%: 10 %)	2019.03.14	2019.04.24

▣ 검사결과

1. Variants of clinical significance

MAF 14%

- Splice variant: MET exon 14 skipping

GENE	AFFECTED EXON(S)	TRANSCRIPT	BREAKPOINT START	BREAKPOINT END	SPLICE SUPPORTING READS
MET	14	ENST00000318493	chr7:116411710	chr7:116414933	1174

- SNVs & Indels

GENE	MUTATION TYPE	AA CHANGE	VAF	HGVSc	HGVSp
TP53	Missense mutation	p.M246I	3.86%	NM_000546.5:c.738G>C	NP_000537.3:p.Met246Ile

- Fusion gene : None

- Copy number variation : None

검사기관 : 세브란스병원 병리과 분자병리 검사실
서울시 서대문구 연세로 50-1 (신촌동 134) 세브란스병원 병리과
Tel: 02-2228-2625

세브란스병원 SEVERANCE HOSPITAL

M/48 stage IV lung adenocarcinoma

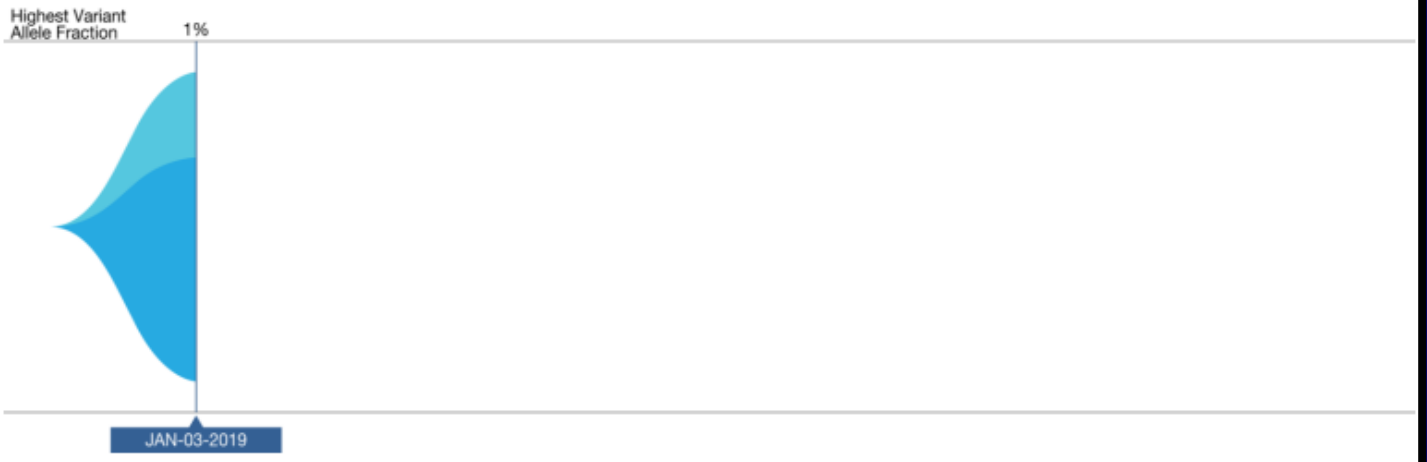
- Referred from another hospital
- Current smoker (30 PYS)
- EGFR cobas/ALK/ROS1 (-/-/-), SP263 10%
- Tissue insufficient for NGS
- s/p Pemetrexed/cisplatin (Apr 2018 ~ Jan 2019)
- Having progressive disease on Feb 2019

- What do you recommend to this patient?

Guardant360 Result

Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the variant allele fraction (% cfDNA) of observed somatic variants at each sample submission time point. Amplifications are not plotted, and only the first and last five test dates are plotted. Please see the Physician Portal (**portal.guardanthealth.com**) for the Tumor Response Map with all test dates.

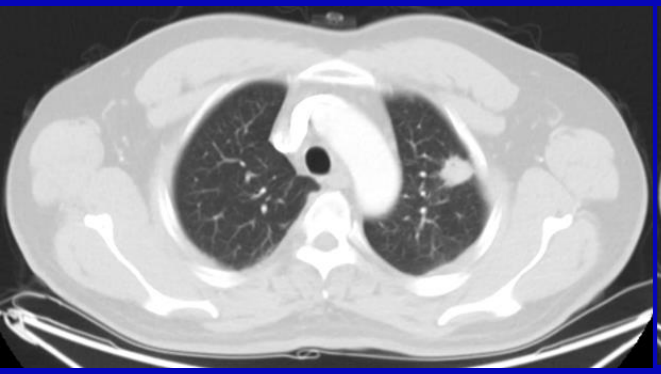


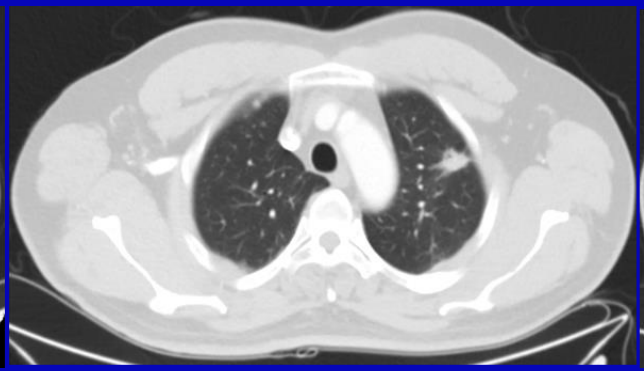
Alteration	% cfDNA or Amp
EGFR T751_I759delinsD (Exon 19 deletion)	1.0%
TP53 H168L	0.3%

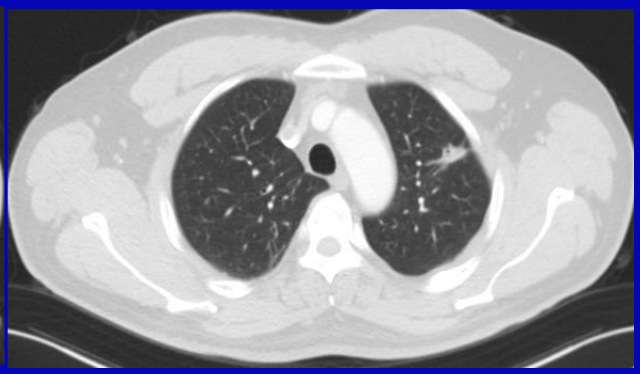
The table above annotates the variant allele fraction (% cfDNA) detected in this sample, listed in descending order.

Treatment course

Gefitinib 250 mg/day







Feb 2019 Mar 2019 May 2019

What if this patient was not lucky enough to have Guardant360?
Docetaxel, gemcitabine……. All ineffective therapies

F/69 stage IV lung adenocarcinoma

- Never smoker
- EGFR cobas WT, ALK/ROS1 (-/-), PD-L1 SP263 0%
- Oncomine comprehensive assay® : WT

No Tier I/II genetic alteration : EGFR, KRAS, BRAF, MET, PIK3CA, HRAS, NRAS, ERBB2, TSC1/2, FGFR1/2/3, ALK, RET, ROS1, NTRK1/3

Mapped Reads: 6,932,713 On Target: 98.05%
Mean Depth: 2,918 Uniformity: 97.65%
Total quality score: very good

- s/p Pemetrexed/cisplatin (Oct 2018.10~Dec 2018)
- After 2 cycles of docetaxel, she had to stop the chemotherapy due to severe toxicities (referred)

Guardant360 Result

Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the variant allele fraction (% cfDNA) of observed somatic variants at each sample submission time point. Amplifications are not plotted, and only the first and last five test dates are plotted. Please see the Physician Portal (portal.guardanthealth.com) for the Tumor Response Map with all test dates.

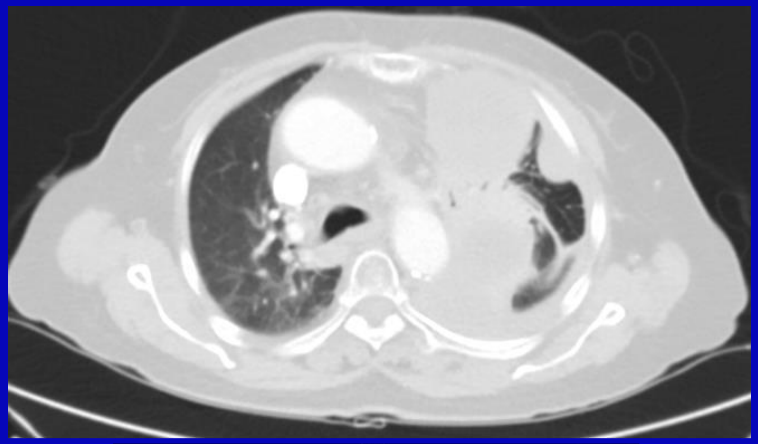
Alteration	% cfDNA or Amp	
EGFR L858R	0.9%	
APC M949V	0.5%	
TP53 R282G	0.4%	
ALK H976H	0.3%	Synonymous Alteration §
EGFR L1017F	0.3%	

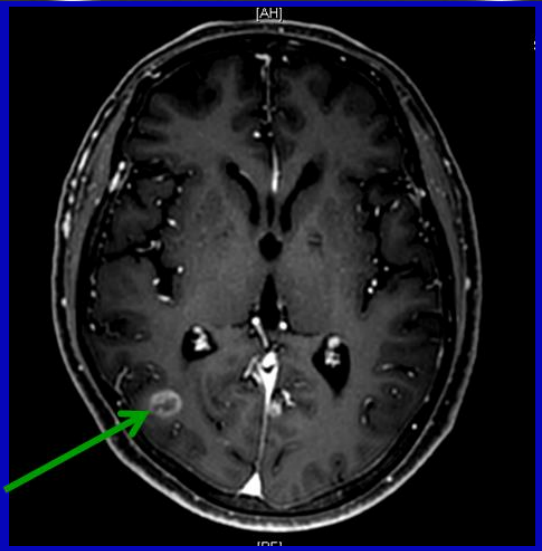
*Tumor tissue NGS may have low sensitivity in low tumor purity

Treatment course

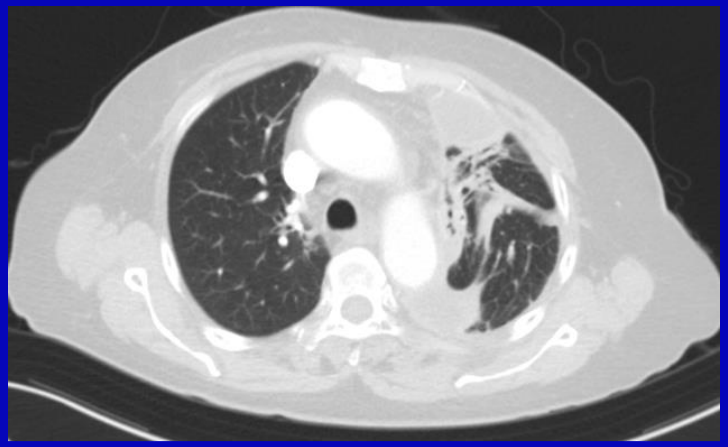
Gefitinib 250 mg/day

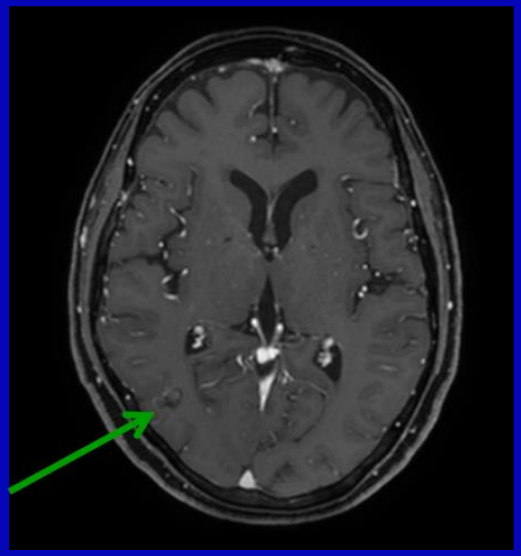
Feb 2019





Oct 2019





M/50 stage IV lung adenocarcinoma

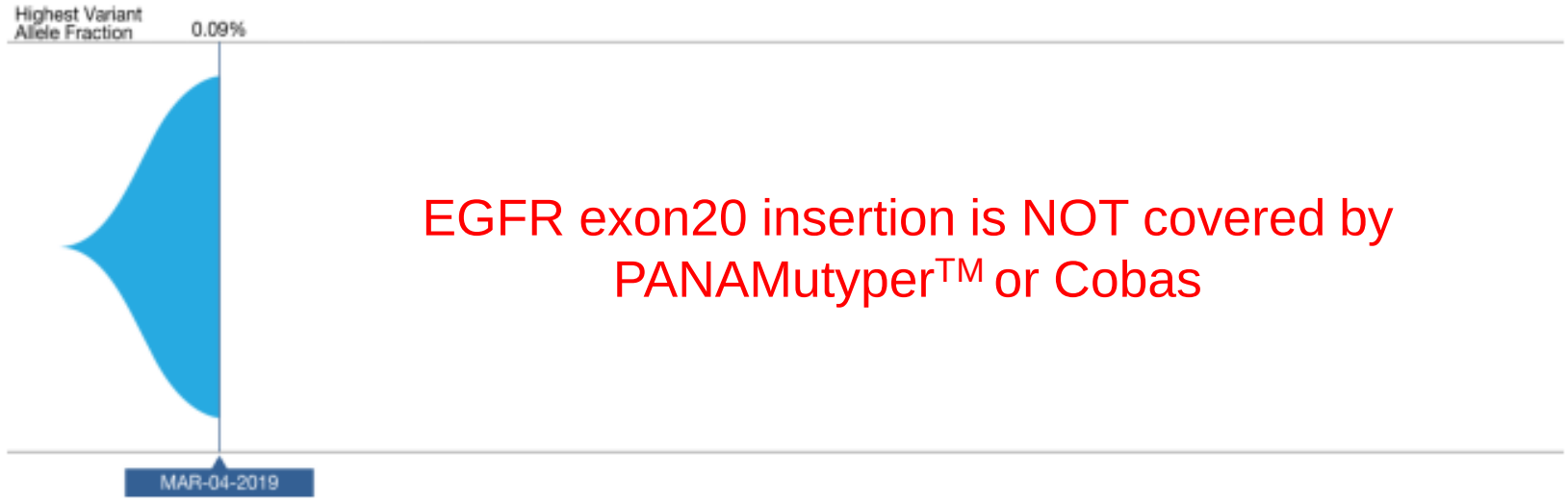
- Current smoker (10 PYS)
- EGFR/ALK (-/-), PD-L1 SP263 0%
- Tissue for NGS: not done
- s/p 2cycles pemetrexed/cisplatin (PD)
- s/p GKS
- s/p 2 cycles gemcitabine/carboplatine (PD)
- Referred

- What do you recommend to this patient?

Guardant360 Result

Guardant360 Tumor Response Map

The Guardant360 Tumor Response Map illustrates the variant allele fraction (% cfDNA) of observed somatic variants at each sample submission time point. Amplifications are not plotted, and only the first and last five test dates are plotted. Please see the Physician Portal (portal.guardanthealth.com) for the Tumor Response Map with all test dates.



EGFR exon20 insertion is NOT covered by PANAMutyper™ or Cobas

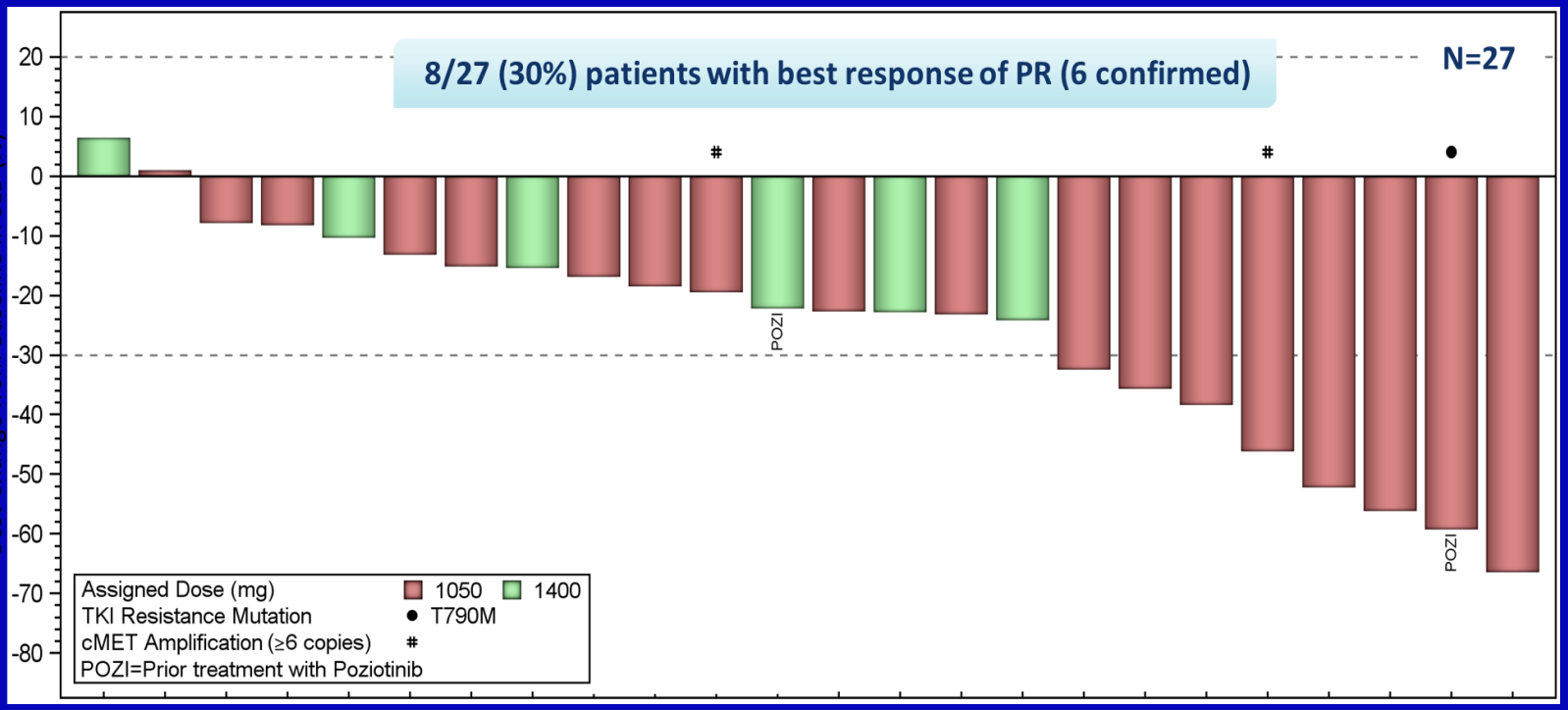
Alteration	% cfDNA or Amp
EGFR A763_Y764insFQEA (Exon 20 insertion)	0.09%

The table above annotates the variant allele fraction (% cfDNA) detected in this sample, listed in descending order.

JNJ-61186372 (JNJ-372), an EGFR-cMet bispecific antibody, in EGFR-driven advanced non-small cell lung cancer (NSCLC)

Eric B. Haura,[1] Byoung Chul Cho,[2] Jong-Seok Lee,[3] Ji-Youn Han,[4] Ki Hyeong Lee,[5] Rachel E. Sanborn,[6] Ramaswamy Govindan,[7] Eun Kyung Cho,[8] Sang-We Kim,[9] Karen L. Reckamp,[10] Joshua K. Sabari,[11] Catherine A. Shu,[12] Dong-Wan Kim,[13] Jorge E. Gomez,[14] Aaron S. Mansfield,[15] Alexander Spira,[16] Pasi A. Jänne,[17] Santiago Viteri,[18] Jose Manuel Trigo,[19] Martin Curtis,[20] Patricia A. Lorenzini,[20] Meena Thayu,[20] Amy Roshak,[20] Kyounghwa Bae,[20] Roland E. Knoblauch,[20] Joshua C. Curtin,[20] Nahor Haddish-Berhane,[20] Matthew V. Lorenzi,[20] Keunchil Park,[21] Joshua M. Bauml[22]

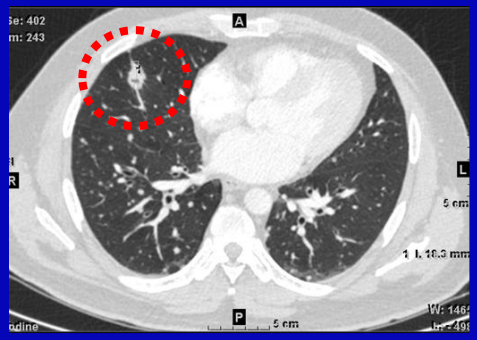
Activity of JNJ-372 in Patients with Exon20ins Disease



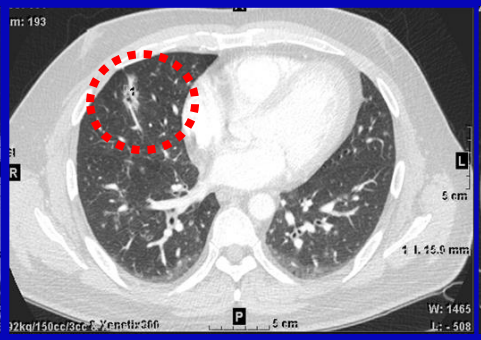
Haura EB. ASCO 2019

Treatment course

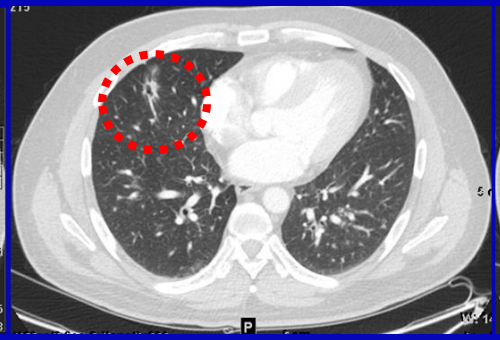
JNJ-61186372 **PFS 7+ months**



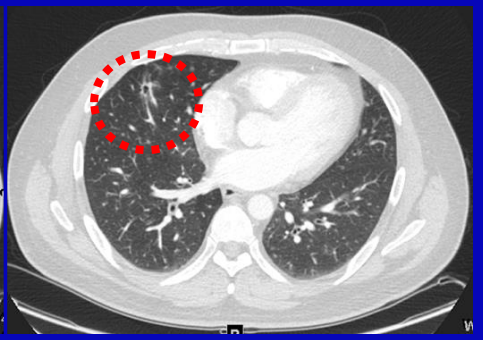
Apr 2019



Jun 2019



Sep 2019

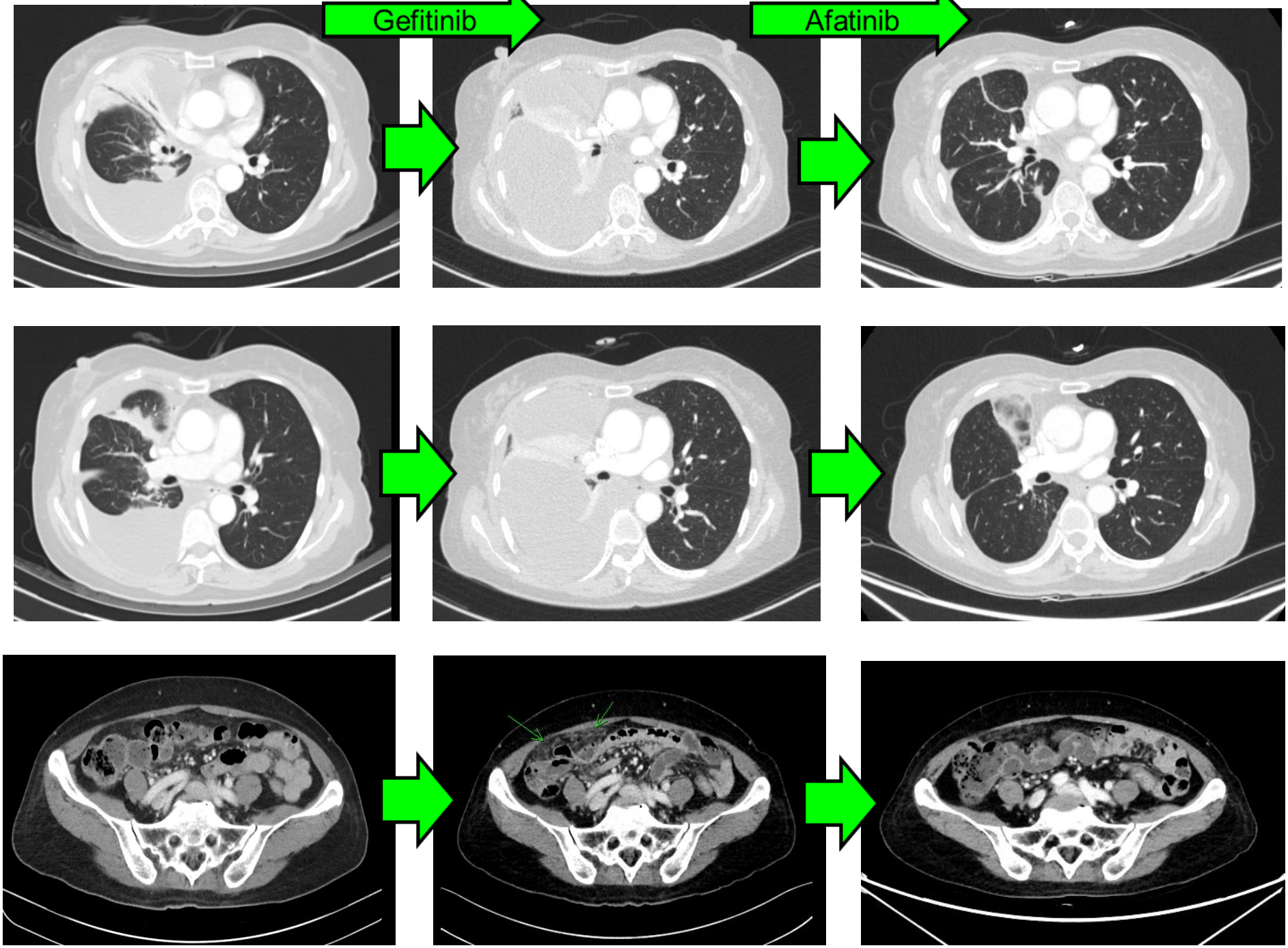


Nov 2019

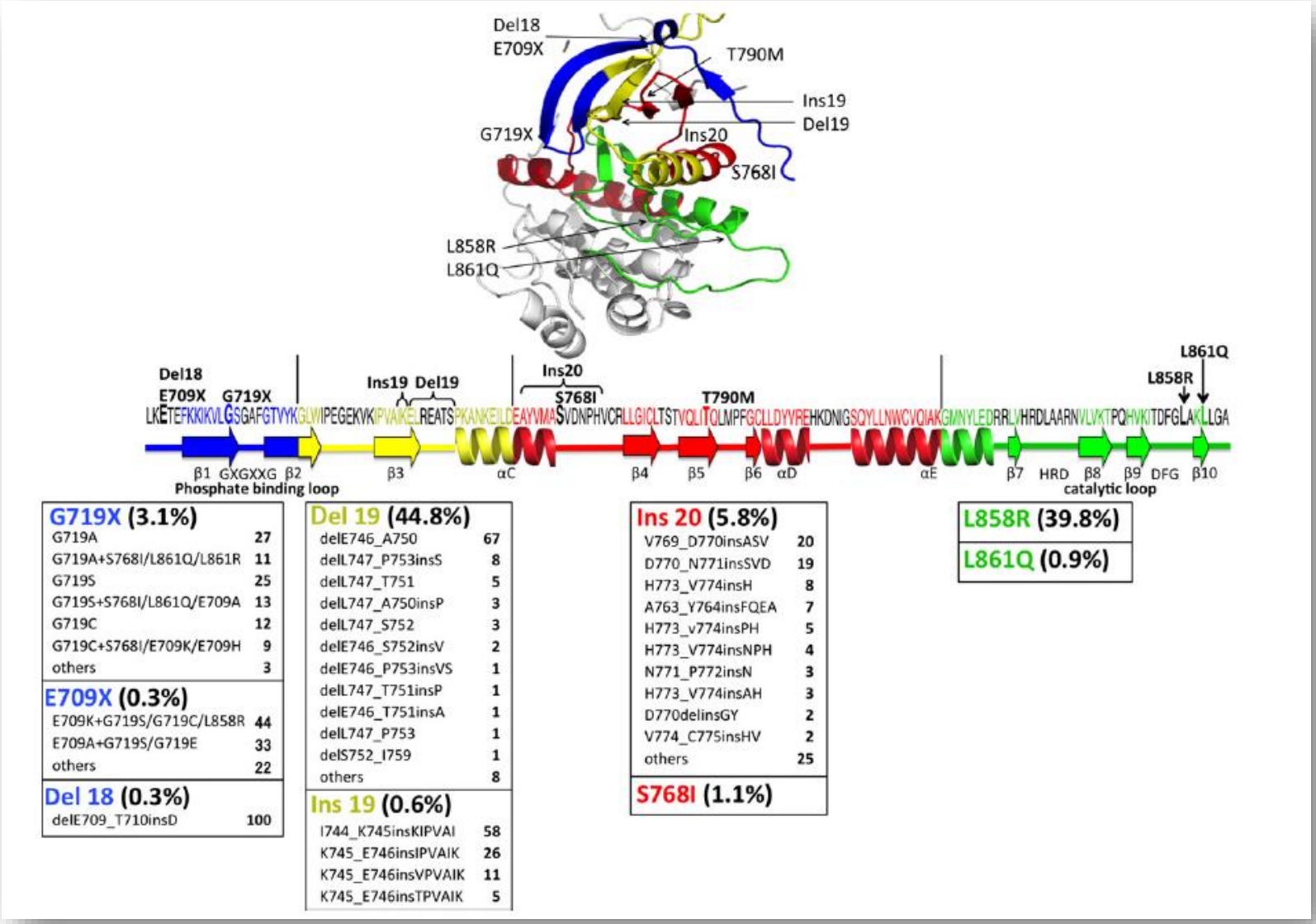
My Experience with Guardant360…….

- Satisfied with TAT and quality of report (simple and clear)
- Help find a new potentially effective treatment right on time
- Notably, help find level I/IIA biomarkers (EGFR, ALK, ROS1, RET, HER2 mutation etc) not detected by tissue PCR and NGS
- Useful in cases with insufficient tissue
- Help “laserpoint” the best EGFR-TKI

Gefitinib
Afatinib
Dacomitinib
Osimertinib
Lasertinib


Gefitinib
Afatinib

Not all EGFR mutations are created equally



Kobayashi Y. Cancer Sci. 2016

In Vitro Sensitivity of Ba/F3 cells expressing each EGFR mutation to various TKI

Exon	Category	Mutations	First generation		Second generation			Third generation	
			Gefitinib	Erlotinib	Afatinib	Dacomitinib	Neratinib	Osimertinib	Rociletinib
18	Del18	delE709_T710insD	882	884	1.7	29	27	93	999
	E709X	E709K	187	215	0.7	16	6	62	706
	G719X	G719A	213	167	0.9	6	1.1	53	214
19	Del19	delE746_A750	4.8	4.9	0.9	<1	60	1.1	19
	Del19	delE746_S752insV	306	14	0.2	1.4	86		
	Del19	delL747_A750insP	7.4	13	1	1.6	30		
	Del19	delL747_P753insS	4.1	5.4	2	1.9	38		
	Del19	delS752_I759	35	7.9	0.2	2	6.7		
	Ins19	I744_K745insKIPVAI	400		7				
	Ins19	K745_E746insTPVAIK	100		0.9				
20	Ins20	A763_Y764insFQEA	174	48	3.7			44	673
	Ins20	Y764_V765insHH	>1000	3845	79			237	1730
	Ins20	M766_A767insAI		3403	79				
	Ins20	V769_D770insASV	3100	4400	72	230	48	333	5290
	Ins20	D770_N771insNPG	3356	3700	72		230	42	262
	Ins20	D770_N771insSVD		3187	86				
	Ins20	H773_V774insH		>10 000	268		550		
	S768I	S768I	315	250	0.7			49	
	T790M	T790M+delE746_A750	8300	>10 000	64	140		3	28
	T790M	T790M+L858R	>10 000	>10 000	119	300		21	13
21	L858R	L858R	26	16	4	2.6	1.4	9	140
	L861Q	L861Q	170	103	0.5		3.3	9	
EGFR wild type with interleukin-3			3350	>10 000	>100	>1000	>1000	3078	1543
Plasma drug concentration			(448–2717)	(2717–4040)	(69–130)	(166–238)	(N/A–132)	(400–600)	N/A–N/A

Kobayashi Y. Cancer Sci. 2016

8821999 유O렬 F

Case Presentation

Pathology report Parietal pleural biopsy

Metastatic adenocarcinoma, Moderately differentiated
EGFR 19del mutant, ALK (-), ROS1(-), PDL1(22C3) 60%

Lung cancer, cT2aN0M1a – 2018.12
s/p VATS RLL wedge resection (2018.12.05, at 서울성모병원)
NGS; TP53muta-p.Ile255Asn(c.764T>A)(variant allele frequency
EGFRmuta-p.Leu747Pro(c.2239_2240TT>CC)variant allele

s/p Gefitinib monotherapy (2019.05.08 ~ 2019.07.17)
→ Rt. Pleural effusion 증가 (Malignant pleural effusion)

s/p #2 Gemcitabine/carboplatin (비급여) + Gefitinib (2019.07.18 ~
s/p #5 Gemcitabine/carboplatin (비급여) + Afatinib (2019.08.28 ~
on #7 Gemcitabine/carboplatin (비급여) + Gefitinib (2019.11.12 ~

NGS, 고형암 panel (Level II) Analysis Report

▣ 검체 정보

검체 번호	성별	나이	Unit NO.	환자명	장기명/진단	검체 유형
SR19-05032	여	62	8821999	유O렬	Pleura/Lung,Metastatic adenocarcinoma	FFPE
의뢰의	의뢰의 소속			검체의 적절성여부	검체 접수일	결과보고일
조병철	종양내과			적합 (Tumor%: 20 %)	20190802	20190926

▣ 검사결과

1. Variants of clinical significance

- SNVs & Indels :

GENE	MUTATION TYPE	AA CHANGE	VAF	HGVSc	HGVSp
EGFR	Missense mutation	p.L747P	18.1%	NM_005228.3:c.2239_2240delTTinsCC	NP_005219.2:p.Leu747Pro
TP53	Missense mutation	p.I255N	17.5%	NM_000546.5:c.764T>A	NP_000537.3:p.Ile255Asn

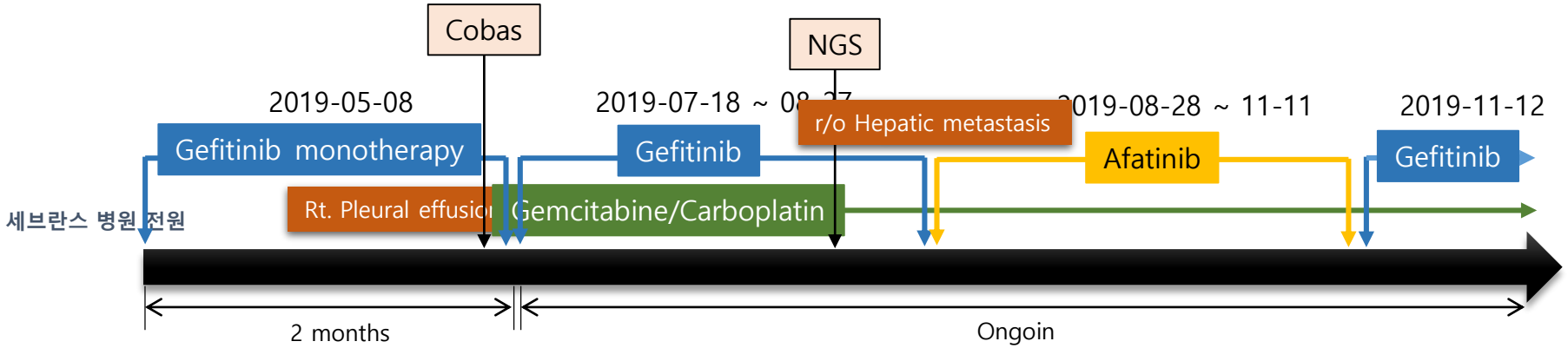
- Fusion gene : None

- Copy number variation : None

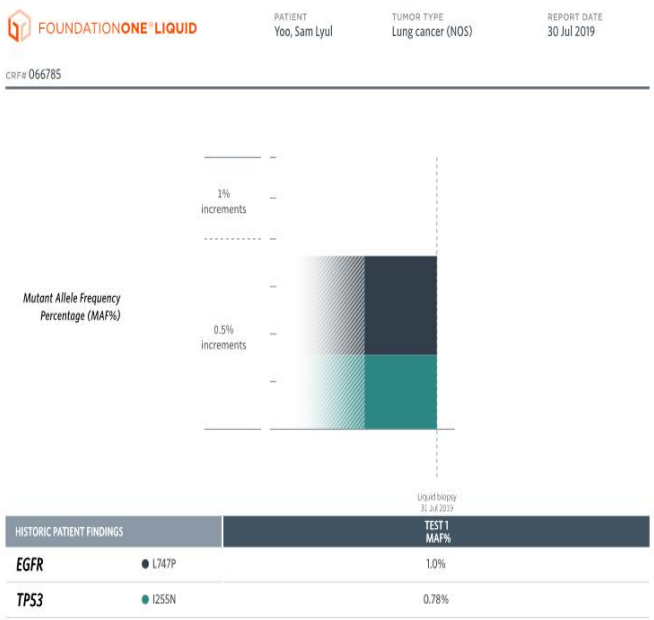
2. Variants of unknown significance

- SNVs & Indels :

GENE	MUTATION TYPE	AA CHANGE	VAF	HGVSc	HGVSp
IDH2	Missense mutation	p.M397V	49%	NM_002168.2:c.1189A>G	NP_002159.2:p.Met397Val
MPL	Frameshift insertion	p.E576Rfs*37	47%	NM_005373.2:c.1725dupA	NP_005364.1:p.Glu576ArgfsTer37
NOTCH1	Missense mutation	p.D2239N	43.7%	NM_017617.3:c.6715G>A	NP_060087.3:p.Asp2239Asn
BRIP1	Missense mutation	p.C350S	40.9%	NM_032043.2:c.1049G>C	NP_114432.2:p.Cys350Ser

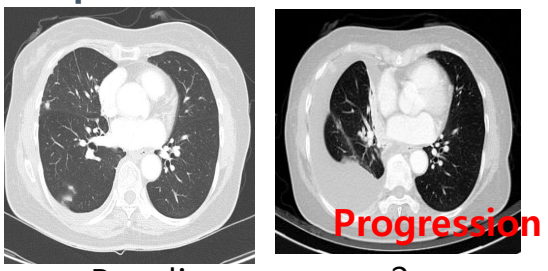


Case Presentation



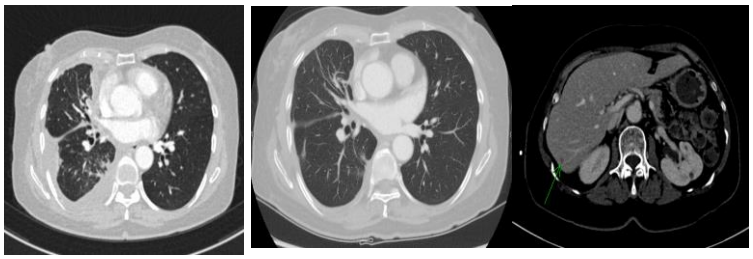
Whole blood	EGFR mutation [plasma cfDNA]	G719X	⊞	Not detected
		Ex19Del	⊞	Mutant(9,98)
		S768I	⊞	Not detected
		T790M	⊞	Not detected
		Ex20Ins	⊞	Not detected
		L858R	⊞	Not detected
		L861Q	⊞	Not detected

Response of Gefitinib

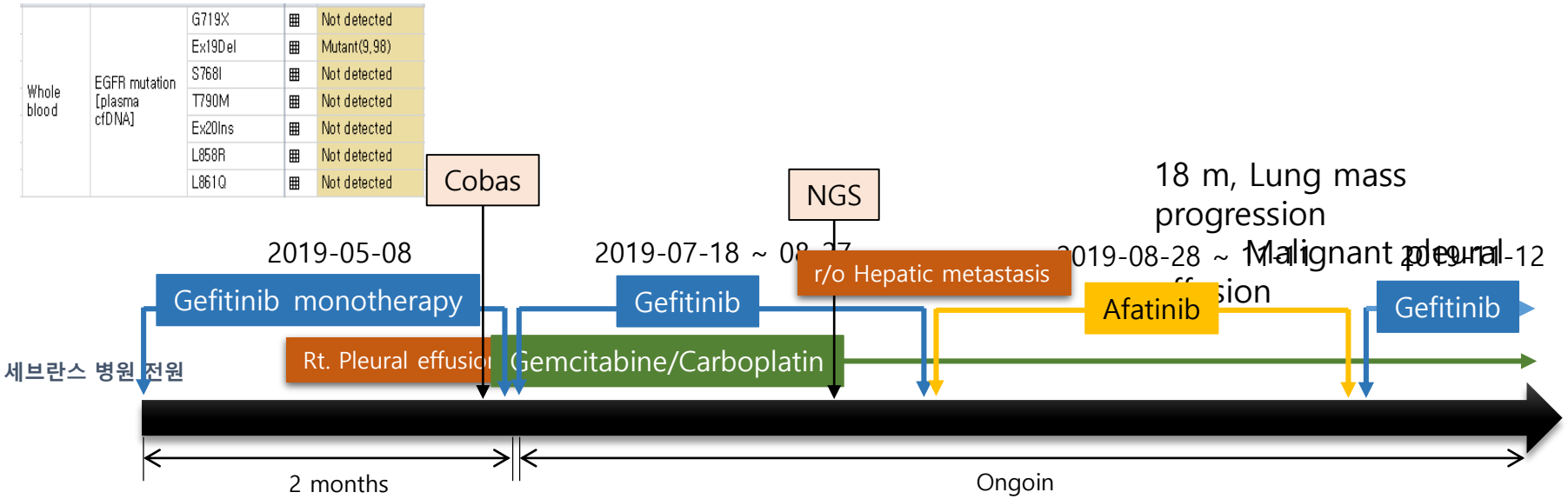


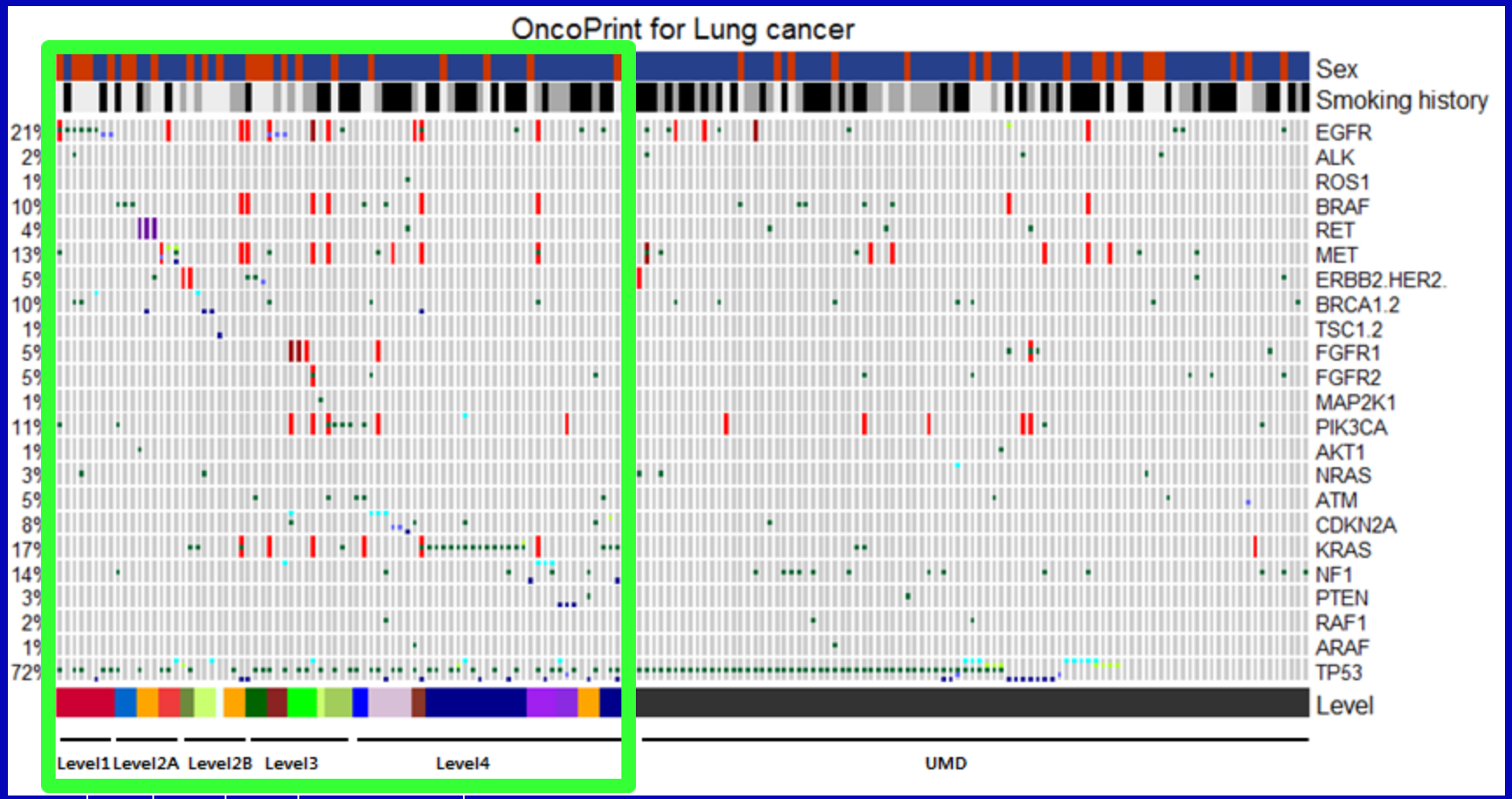
Baseline 2 m

Response of Gemcitabine/Carboplatin



1 m 3 m





Sex
F
M

Smoking history
Ex
Current
Never

Genetic alterations
Amplification
Gain
Missense mutation
Inframe mutation
nonsense mutation
Frameshift mutation
Splice site mutation
Fusion

Level 1
EGFR mutation

Level 2A
BRAF V600E
RET Fusion
MET exon 14

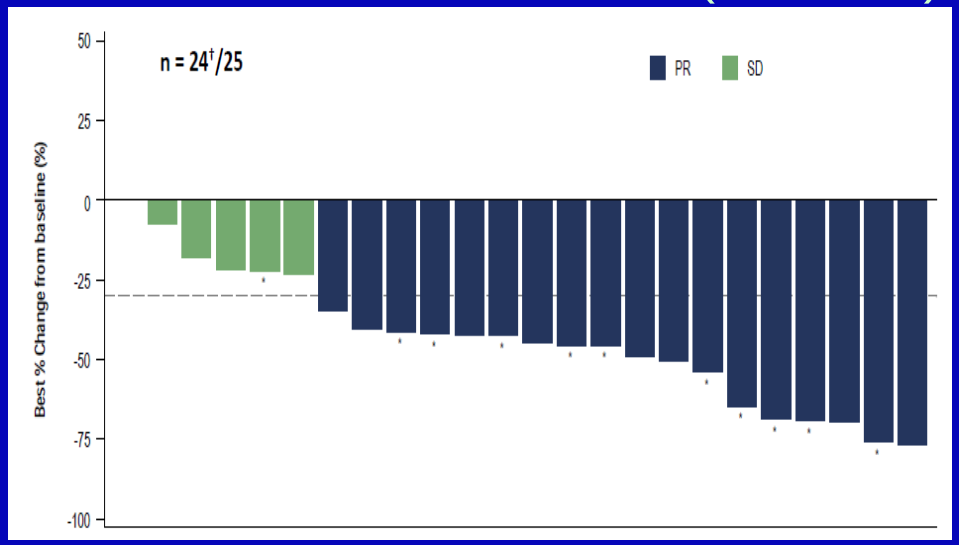
Level 2B
ERBB2 amp
BRCA 1/2 loss
MET exon 14
Others

Level 3
ERBB2 mutation
EGFR exon 20
FGFR 1/2 amp
MAP2K1
PIK3CA

Level 4
ATM
CDKN2A loss
EGFR WT amp
KRAS
NF1 loss
PTEN loss
Others
Combined level 4

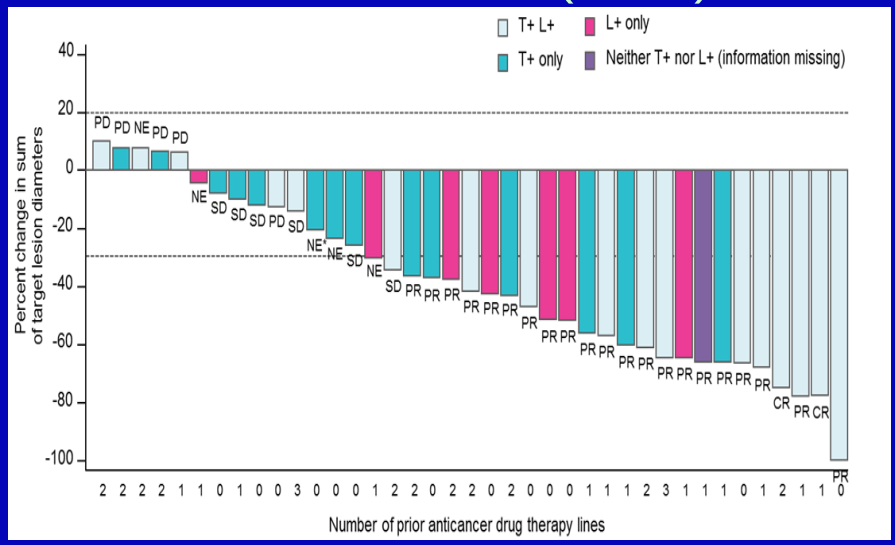
Efficacy of Capmatinib and Tepotinib in MET exon 14 skipping

GEOMETRY MONO-1 Ph II (n=25, 1L)



ORR 72.0% (95% CI: 50.6-87.9)

VISION Ph II (n=69)

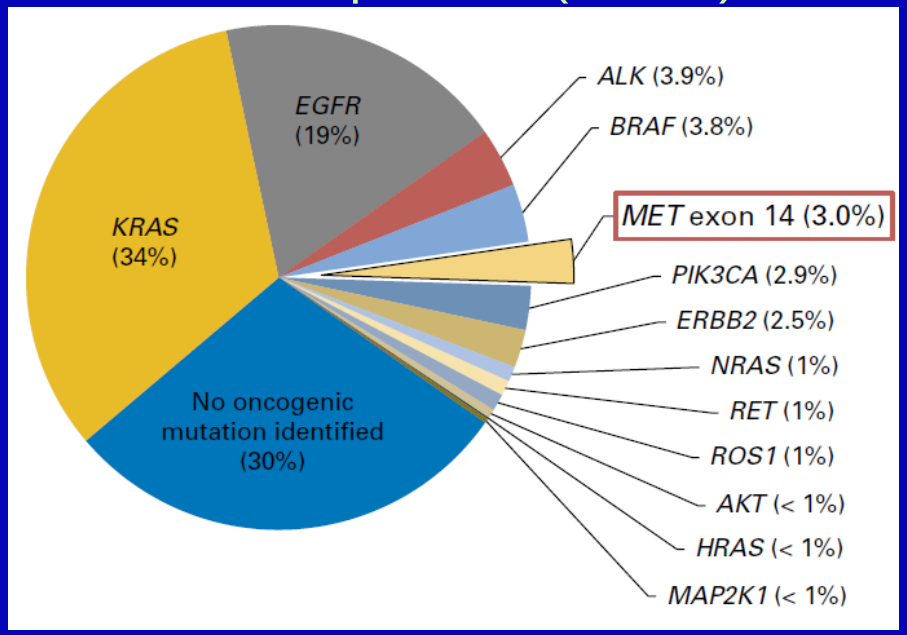


ORR 57.5% (95% CI: 40.9, 73.0)

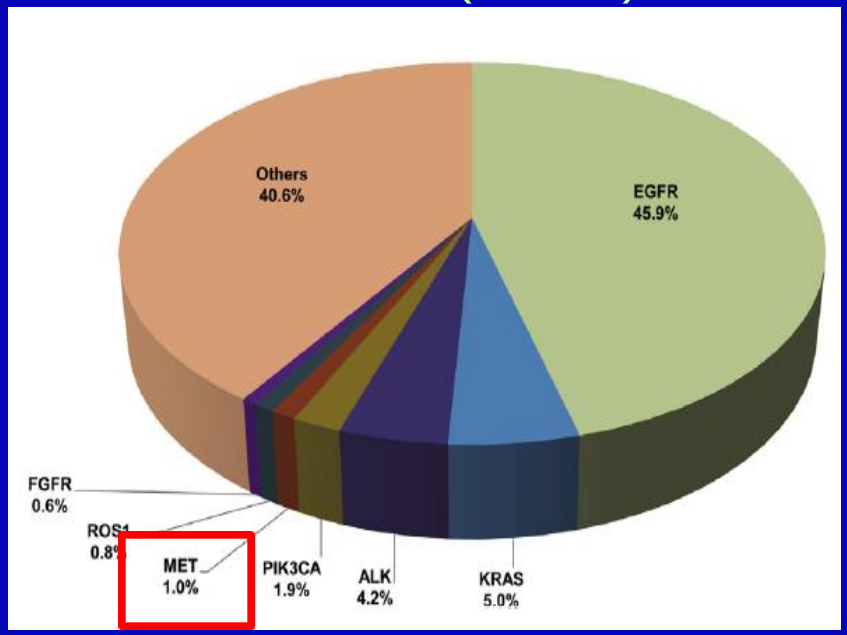
Wolf J. ESMO 2018; Felip E. WCLC 2018

MET exon 14 skipping represents a unique subset of NSCLC

White patients (n=933)



Chinese (n=968)



- Significantly older than EGFR/KRAS mutant patients/~60% smoker
- Occur predominantly in adenocarcinoma; enriched in sarcomatoid carcinoma (~20%)
- Up to 20% with concurrent high-level MET amplification
- Mutually exclusive with other oncogenic drivers (EGFR/KRAS/ERBB2)
- Diagnosis: DNA-based NGS

Awad MM. JCO 2016; Liu SY. JTO 2016; Liu X. JCO 2016