Clinical Application of NGS in Lung Cancer Management

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2019 12 08

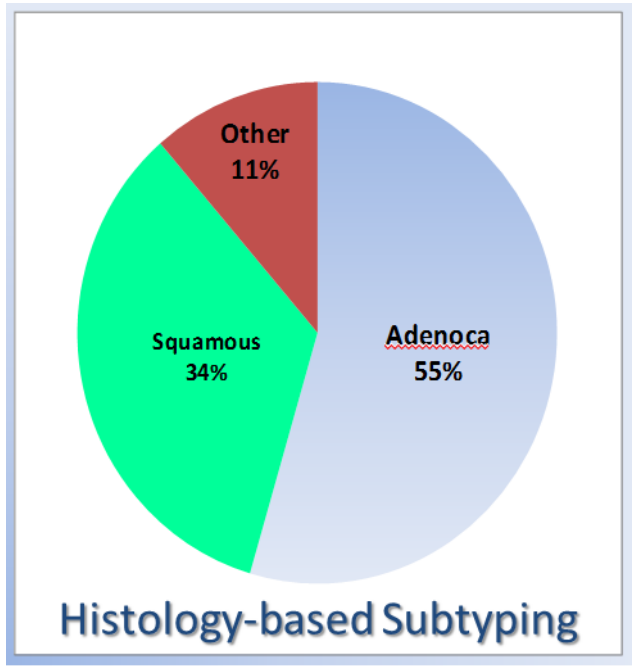
20 years ago

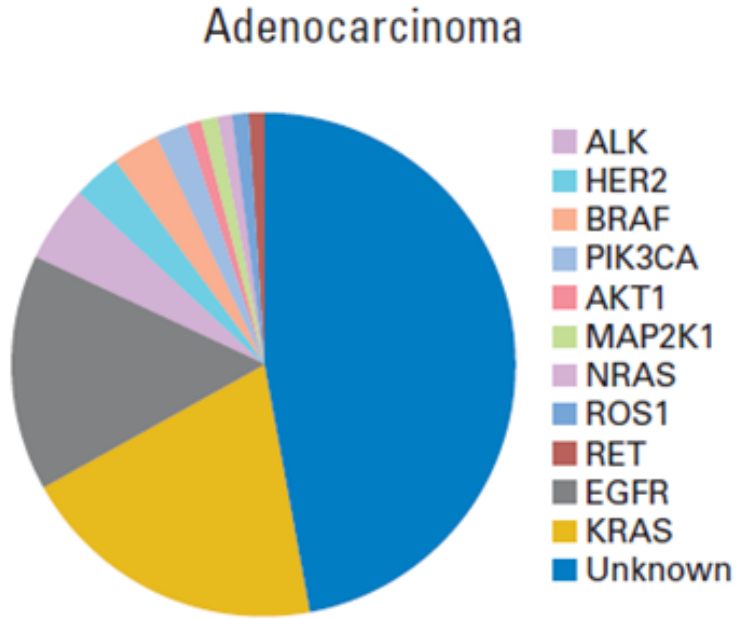
NSCLC → Platinum-based doublets

10 years ago

NSCLC
- ADC
  - EGFRm → EGFR TKI
  - EGFR WT → Platinum-based doublets
- SqCC → Platinum-based doublets (no pemetrexed)

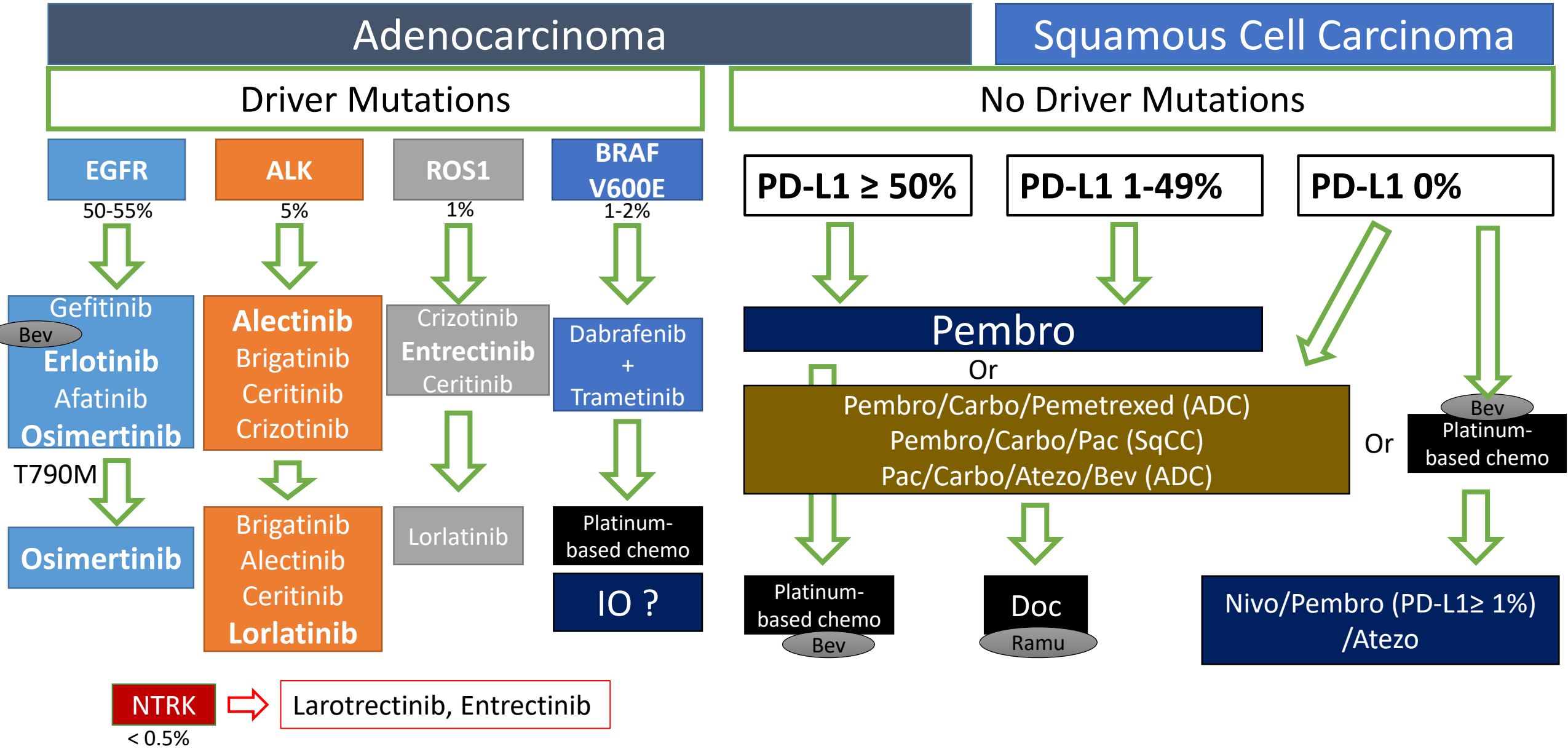
NSCLC as one disease



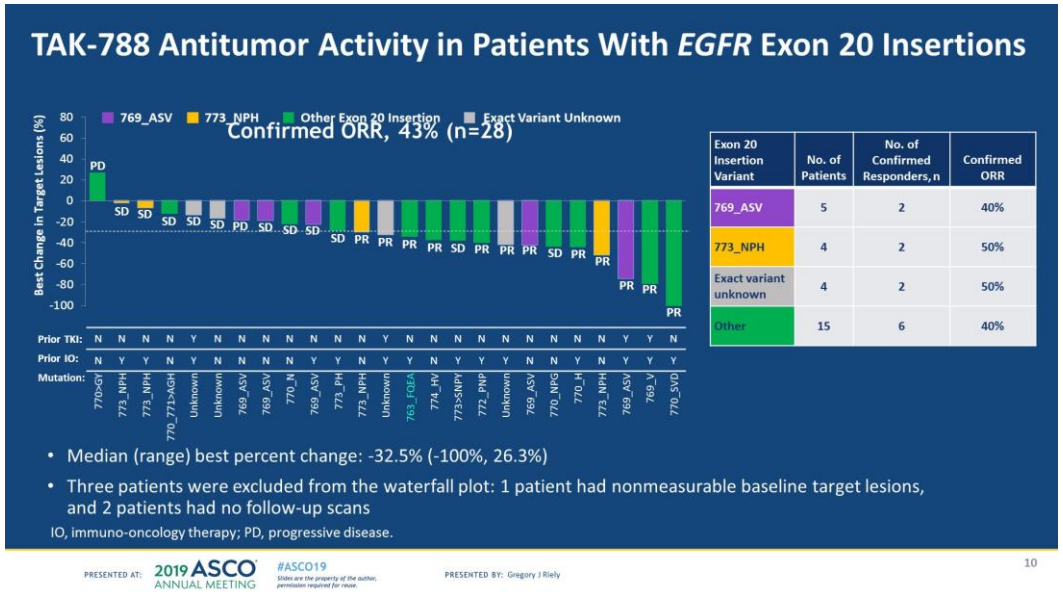


Li, Mack, Kung, Gandara: JCO 2013

NSCLC treatment algorithm in 2019 (adapted from NCCN guidelines)

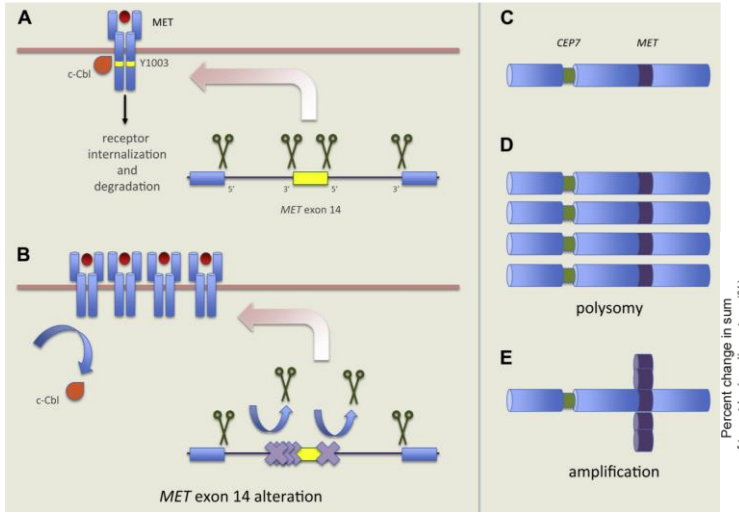


Exon 20 insertion



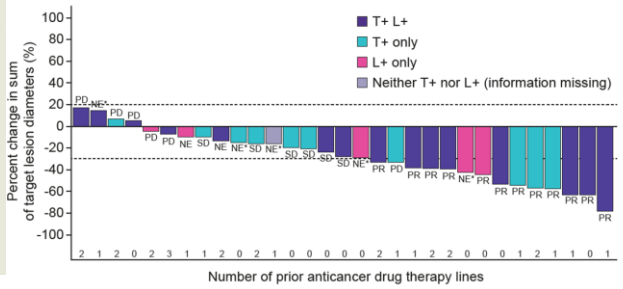
Exon 20 Insertion Variant	No. of Patients	No. of Confirmed Responders, n	Confirmed ORR
769_ASV	5	2	40%
773_NPH	4	2	50%
Exact variant unknown	4	2	50%
Other	15	6	40%

MET exon 14 skipping

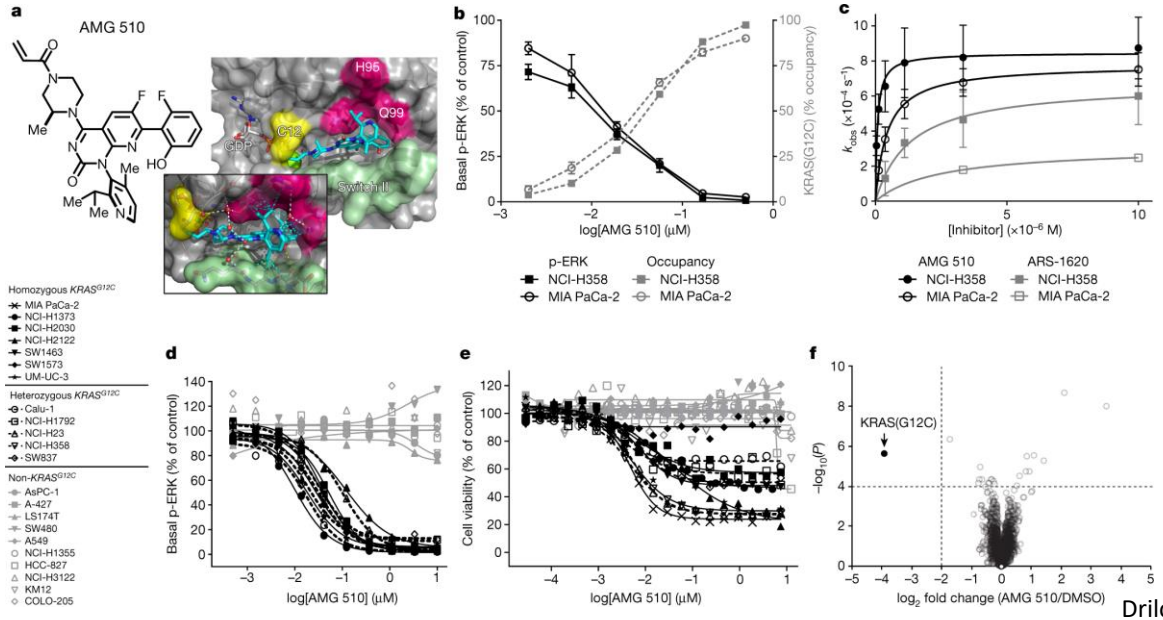


Tepotinib

ORR 42%, mDOR 12.4m

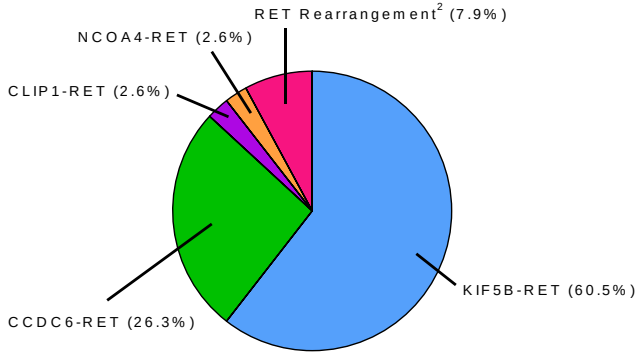


KRAS G12C



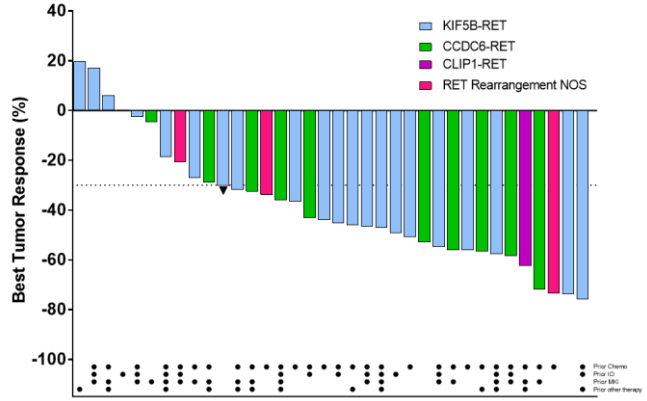
RET fusion

***RET* fusion partner[3]**



LOXO-292

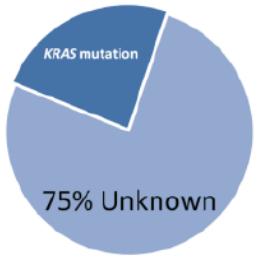
ORR 68%



Drilon et al. WCLC 2016 Felip E et al. ASCO 2018; Awad et al. JCO 2016; Oxnard et al. WCLC 2018; Pasi A Janne et al. ASCO 2019;

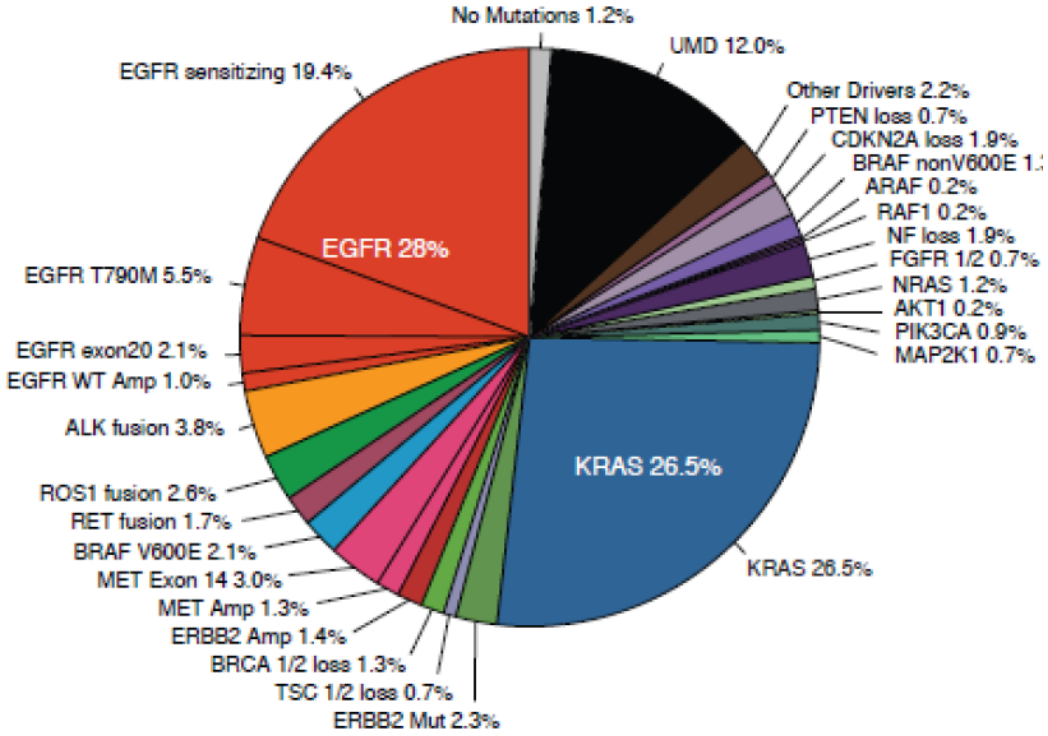
Increasing needs for comprehensive genomic profiling in the era of precision medicine

Driver mutations

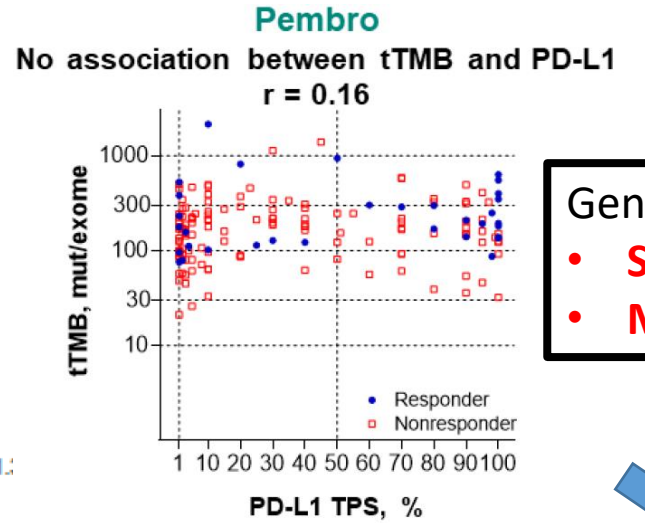


1999

2018

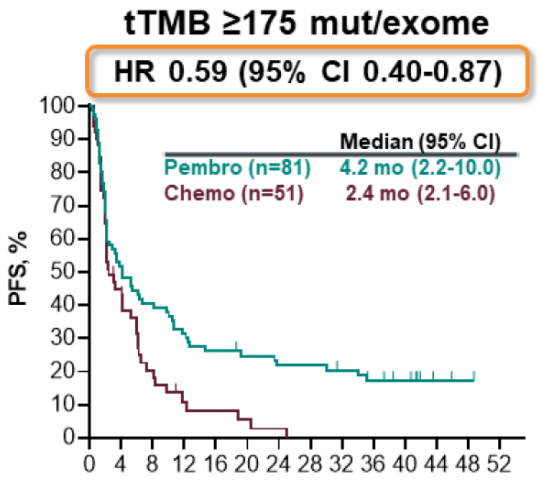


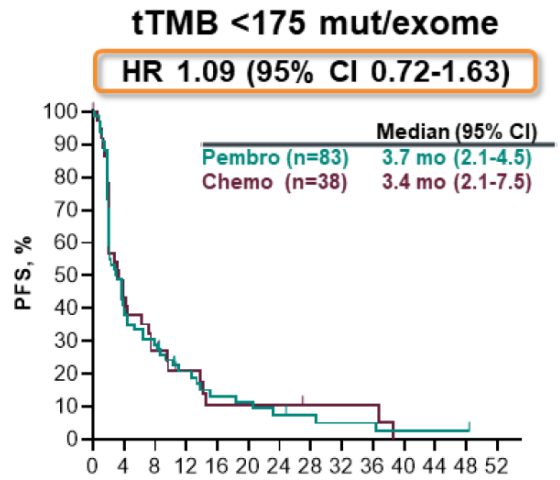
Immunotherapy



Genetic alterations affecting IO:

- **STK11/LKB1** → Poor response
- **MSI-H**, **POLE/POLD1** → Good response





Jordan, et al. Cancer Discovery 2017; Roy S. Herbst, et al. ESMO 2019

Clinical Application of NGS in lung cancer

- **Druggable driver mutation not routinely checked in local lab.**

Case 1

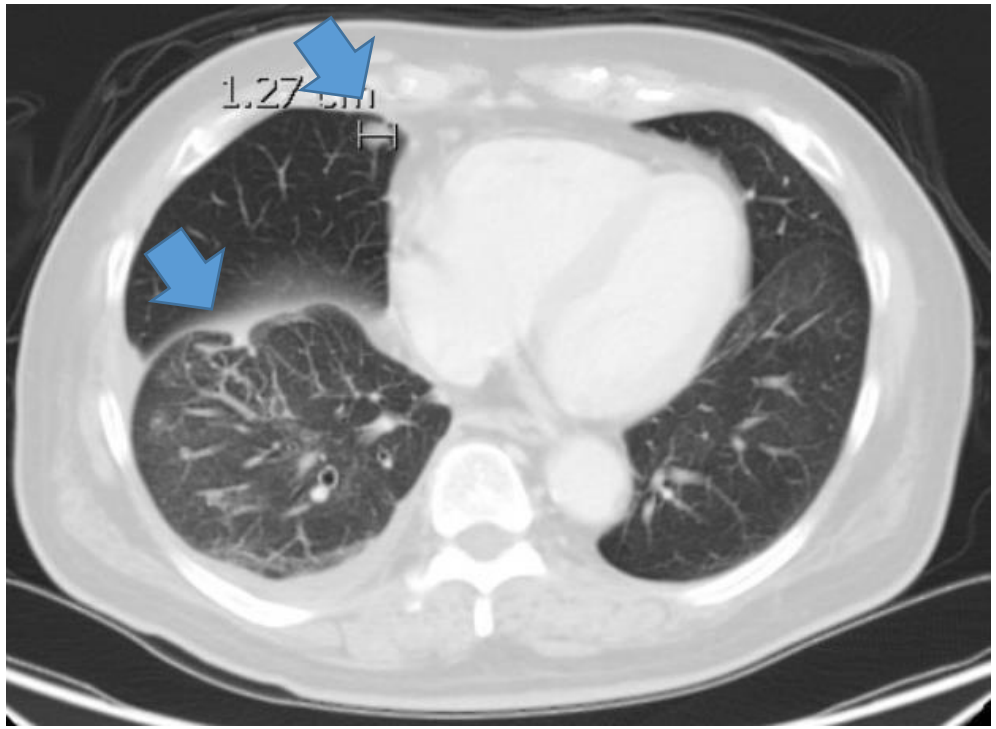
71 M

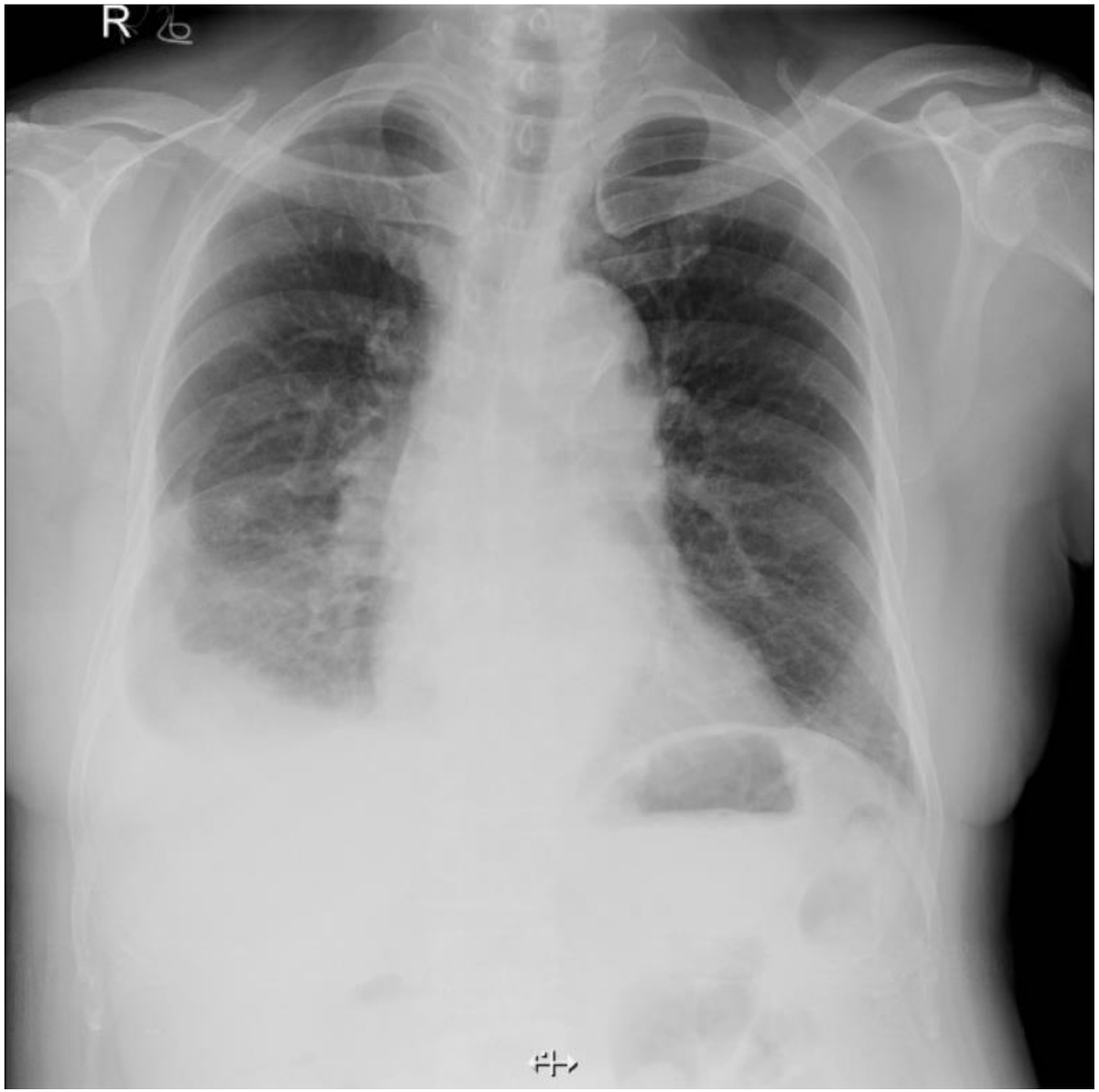
CAD, DM, HTN

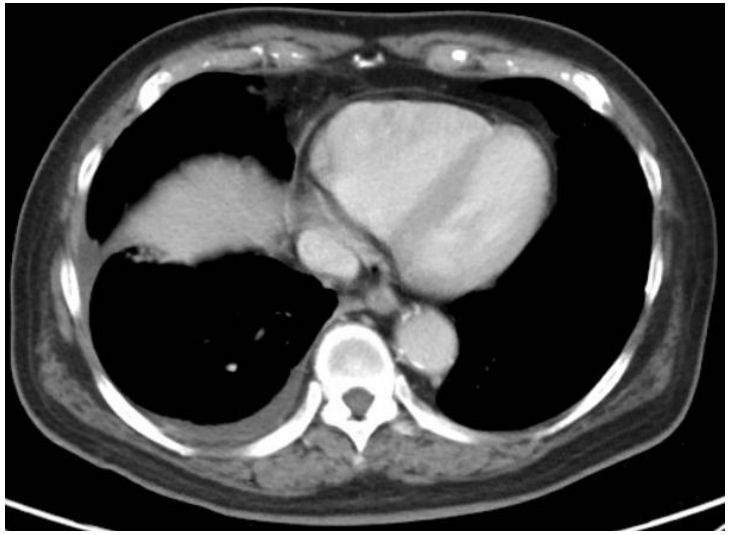
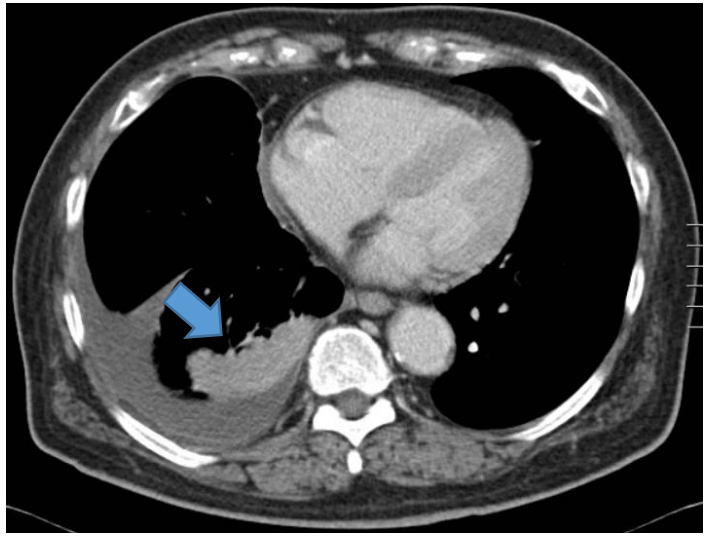
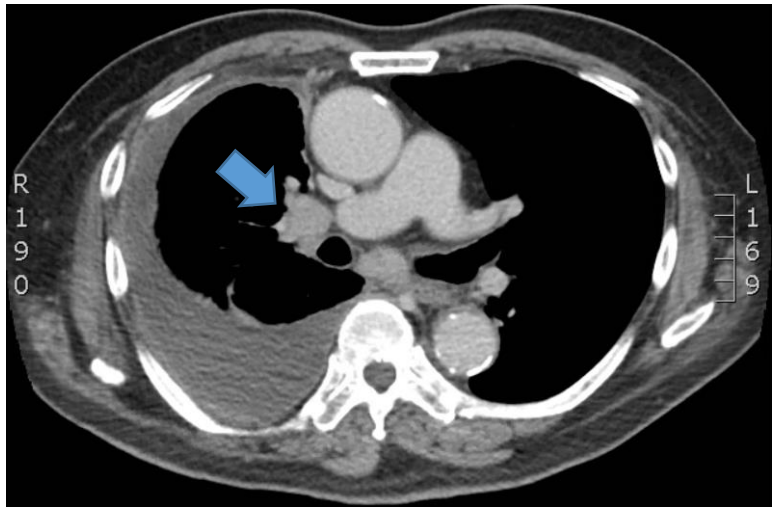
Heavy smoker: 2PPD for 30 yrs

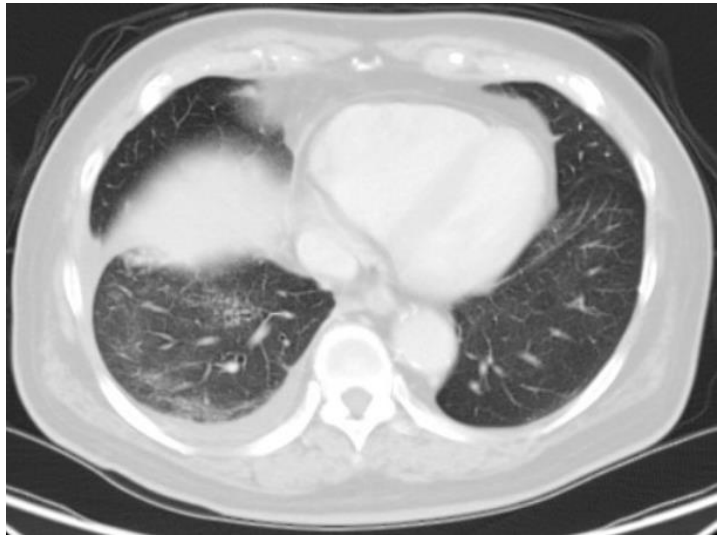
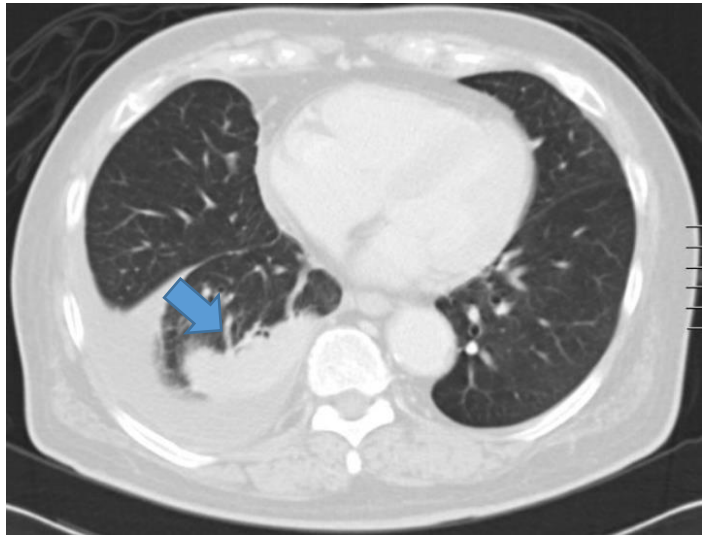
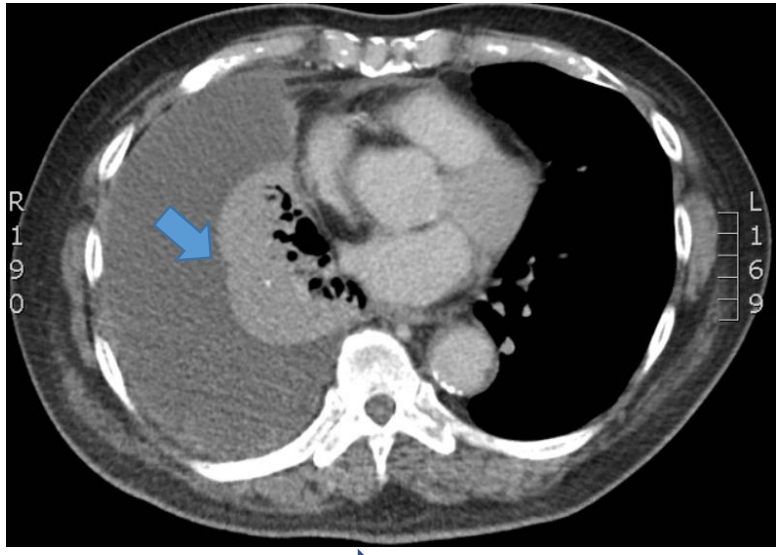
NSCLC, ADC, cT1aN2M1a, stage IVA

EGFR/ALK/ROS1: WT





Pemetrexed/Cisplatin x6 -> Pemetrexed x 3

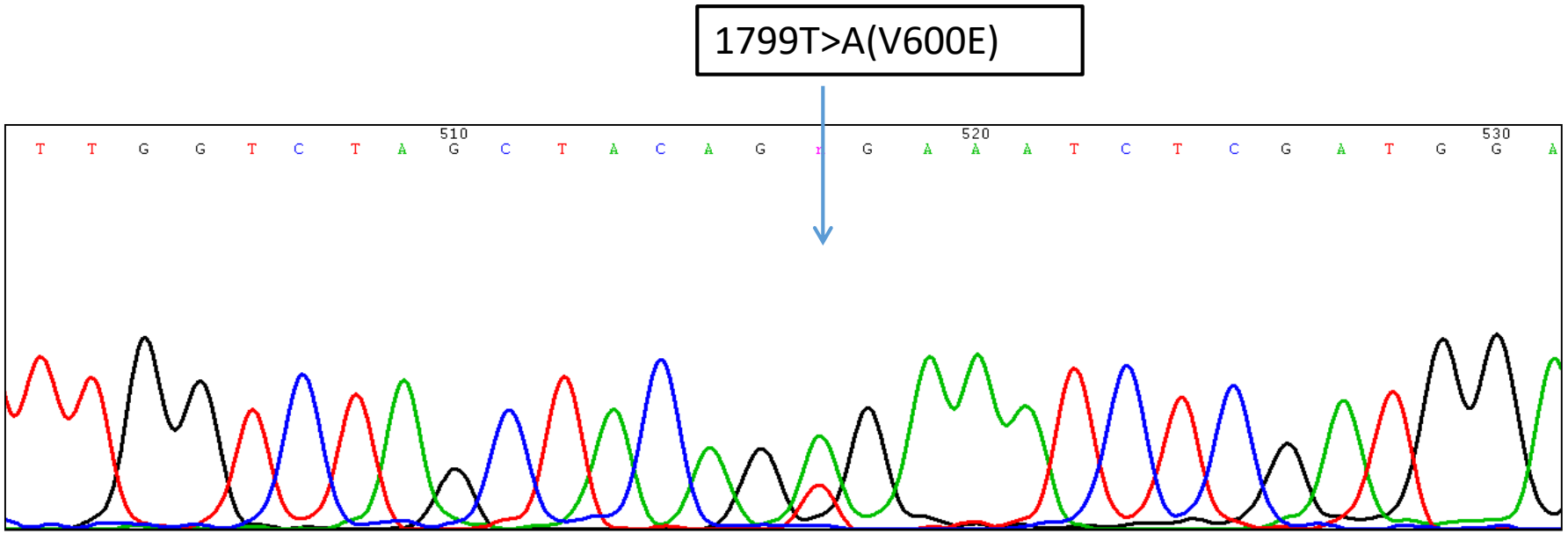
Docetaxel x3

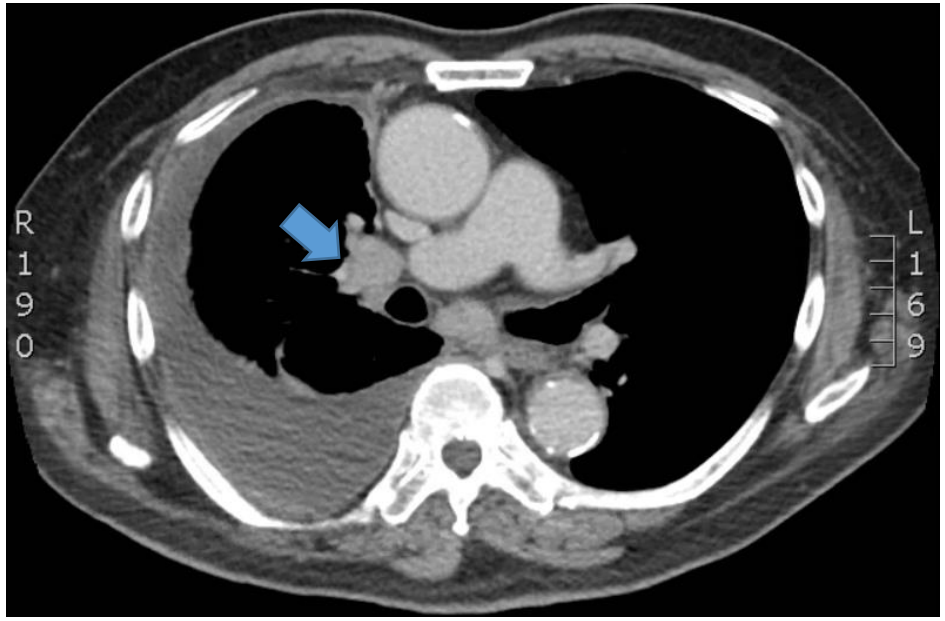
2017/10

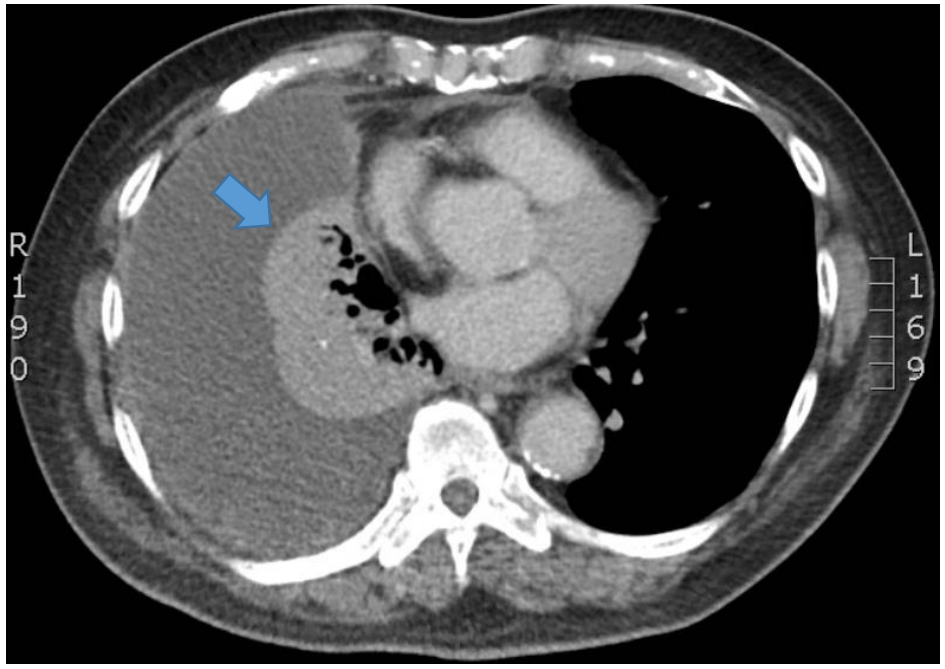
2018/6 PD

2018/9 PD

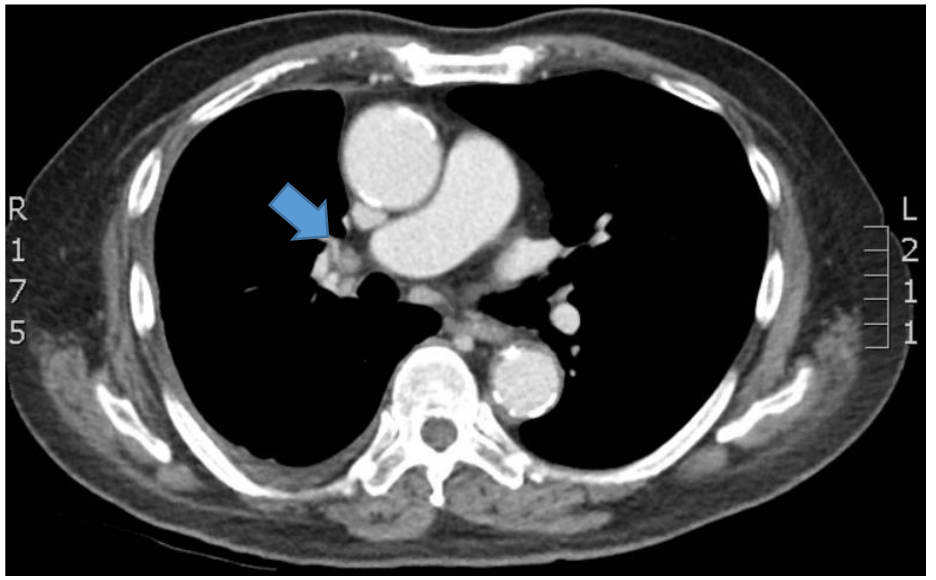
BRAF V600E mutation was detected by **RT-PCR** and **MALDI-TOF** from pleural effusion

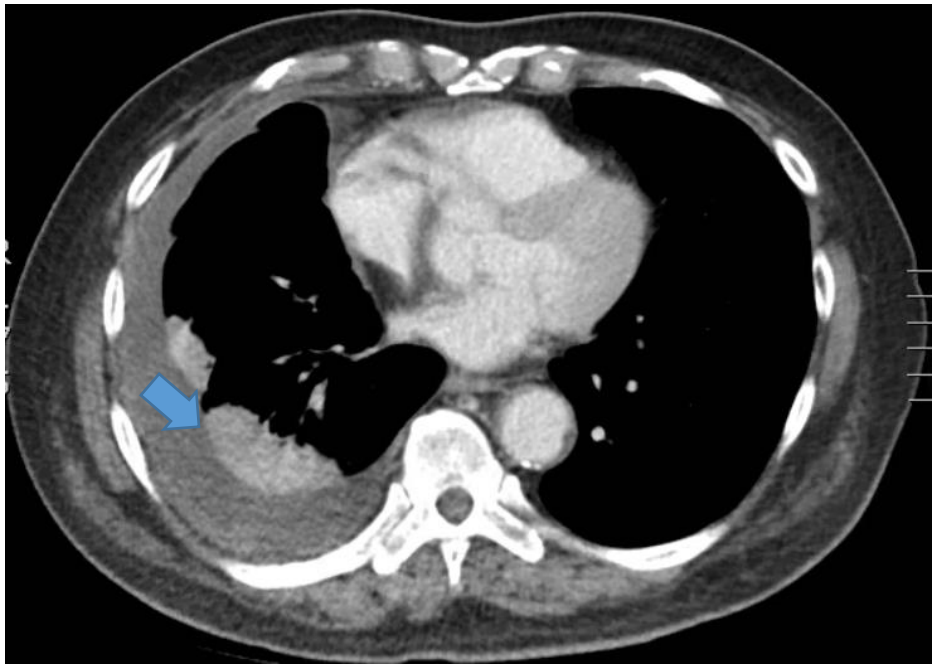






Dabrafenib
Trametinib

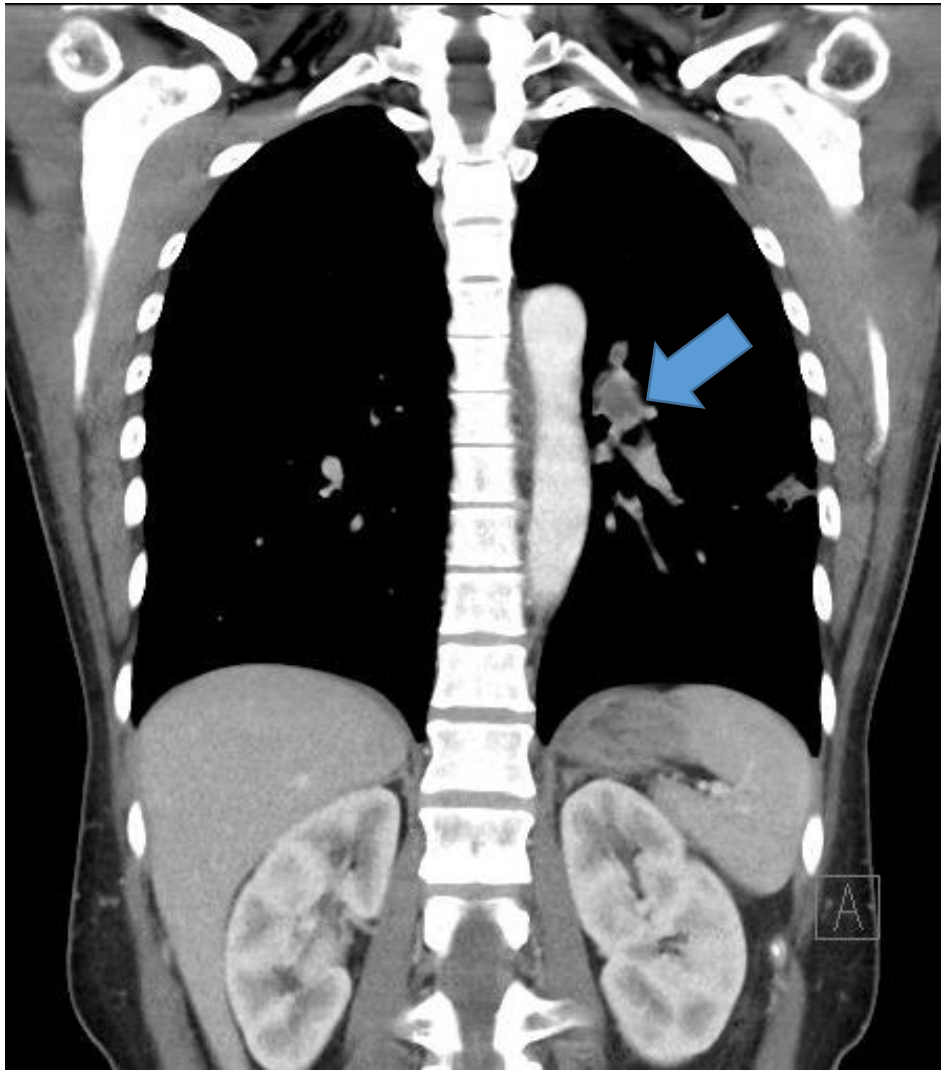




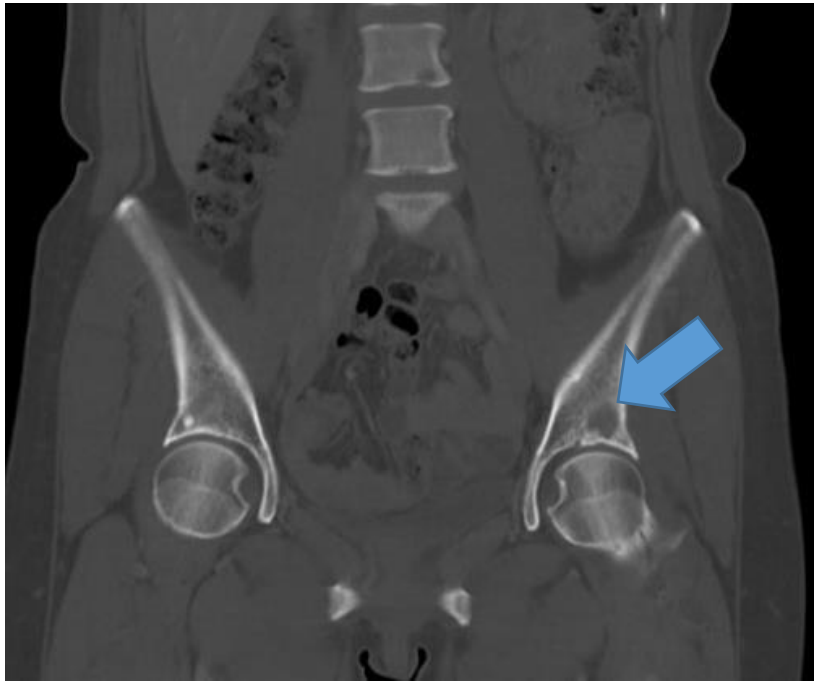
2018/9

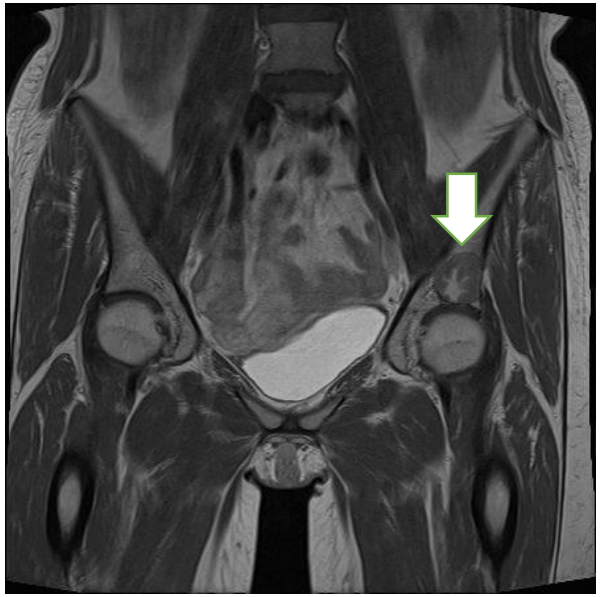
2018/12

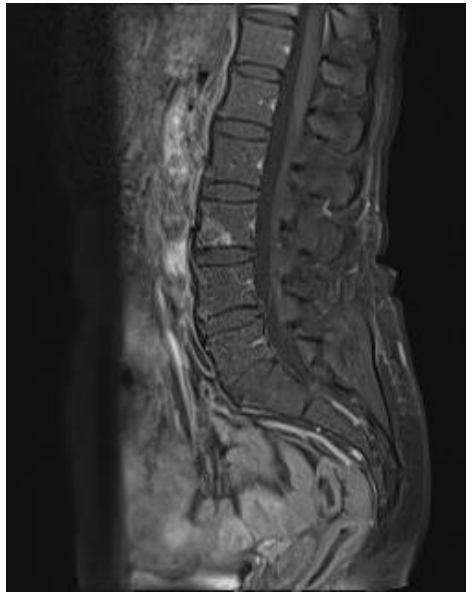
Case 2



50 y/o woman with left hip pain
NSCLC, cT1bN3M1b, bone mets
EGFR (MALDI-TOF)/ALK/ROS1: WT
PD-L1 40%







NGS report:

EGFR amplification, duplication exons 18-25

ARAF R188H

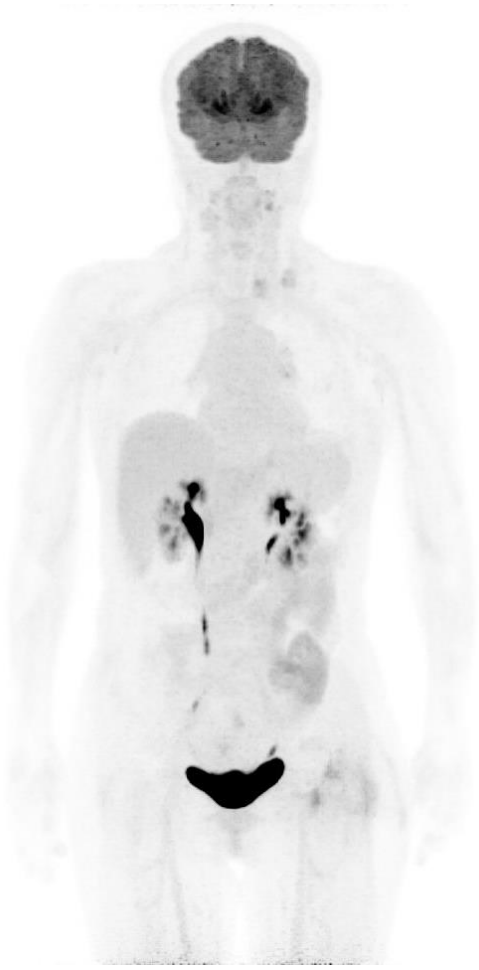
TP53 C176W

Tumor Mutation Burden TMB-Low; 4 Muts/Mb

- Progressive bone pain after 2 cycles of **pembrolizumab + Pemetrexed + Carboplatin**
- MRI showed progressive bone metastasis
- CEA : 383 -> 807 ng/ml

Afatinib

X 5 weeks

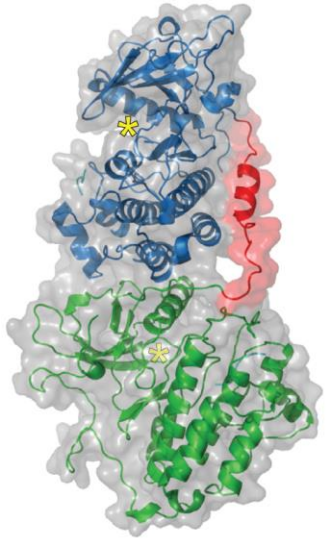


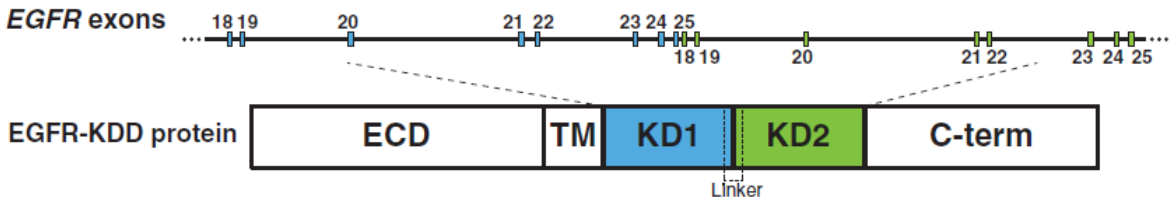
* Left THR for intractable pain
* CEA : 1653 -> 118 ng/ml

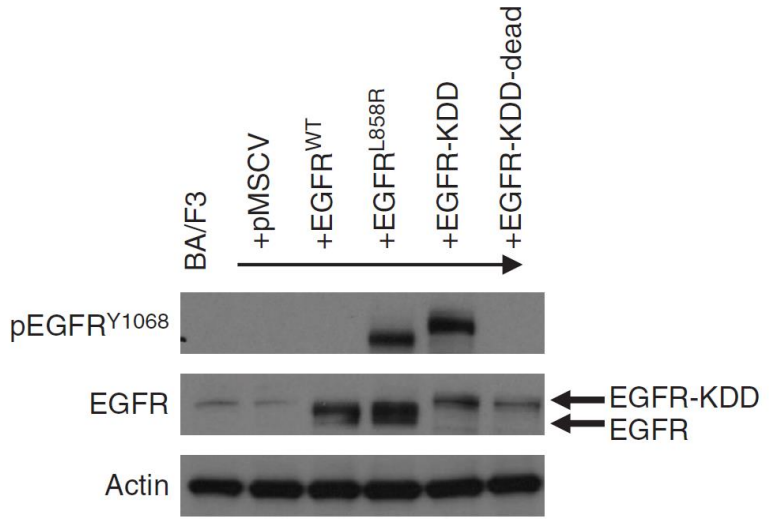
RESEARCH BRIEF

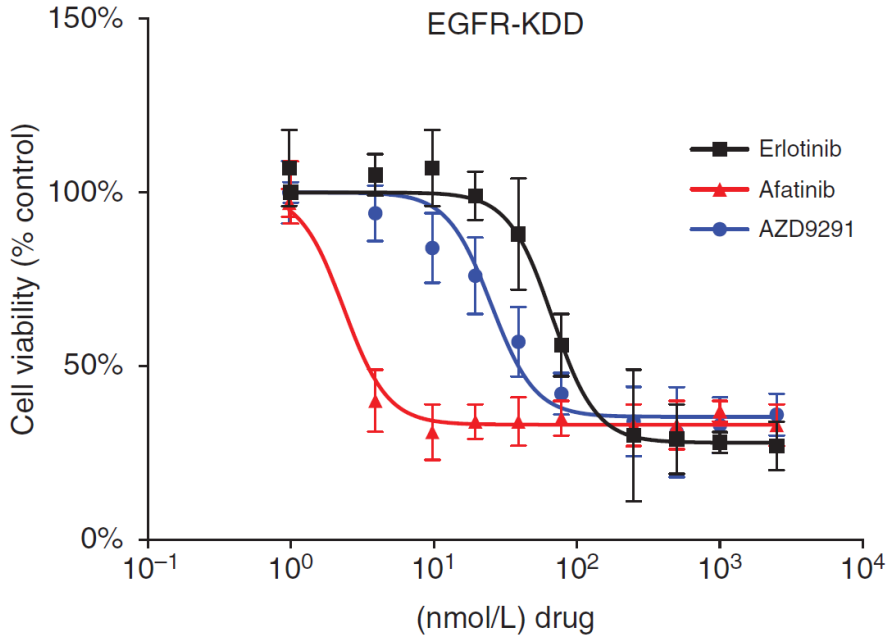
EGFR Kinase Domain Duplication (*EGFR*-KDD) Is a Novel Oncogenic Driver in Lung Cancer That Is Clinically Responsive to Afatinib

Jean-Nicolas Gallant[1,2], Jonathan H. Sheehan[3,4], Timothy M. Shaver[2,3], Mark Bailey[5], Doron Lipson[5], Raghu Chandramohan[6], Monica Red Brewer[2,7], Sally J. York[2,7], Mark G. Kris[8], Jennifer A. Pietenpol[2,3], Marc Ladanyi[6], Vincent A. Miller[5], Siraj M. Ali[5], Jens Meiler[4,9], and Christine M. Lovly[1,2,7]





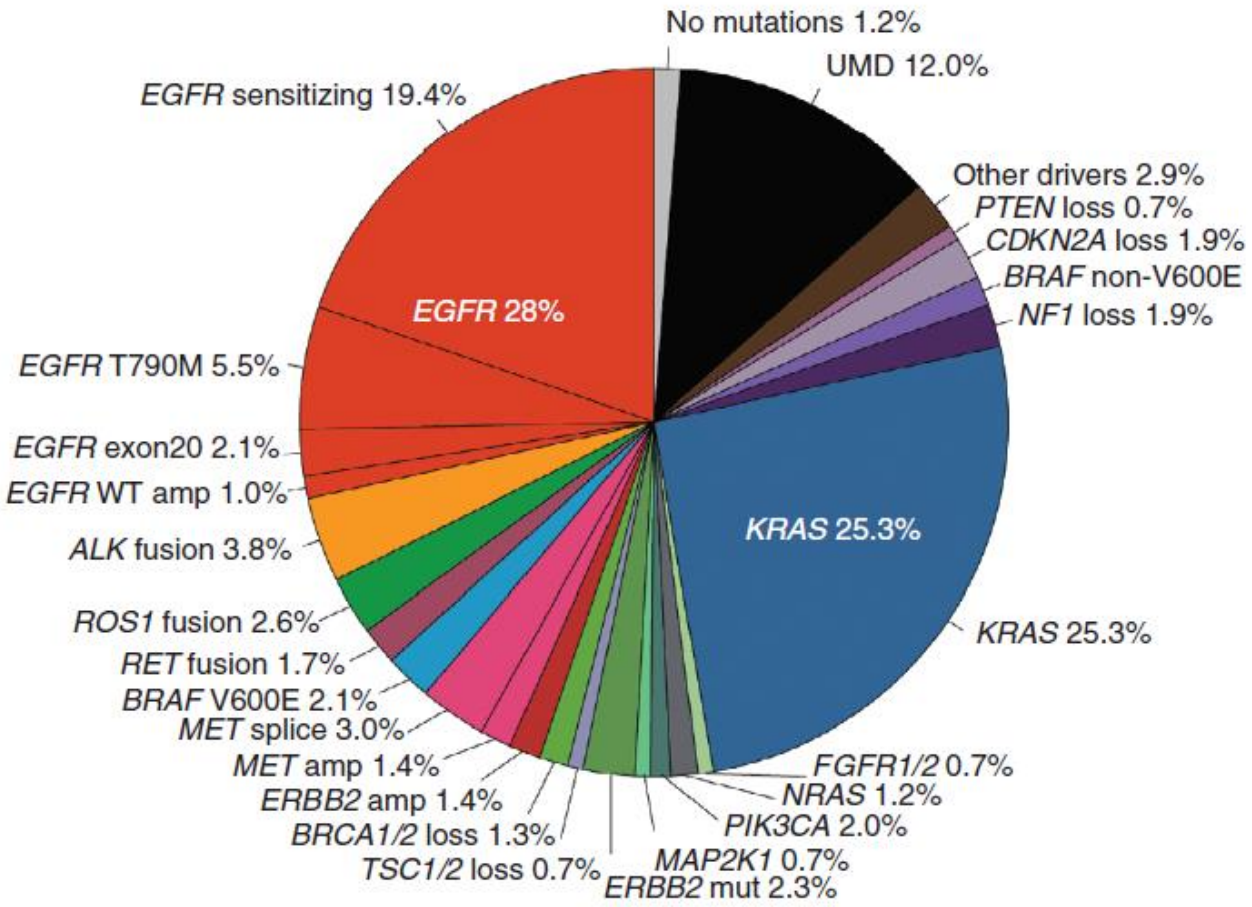




Gallant et al. Cancer Discov. 2015 Nov;5(11):1155-63.

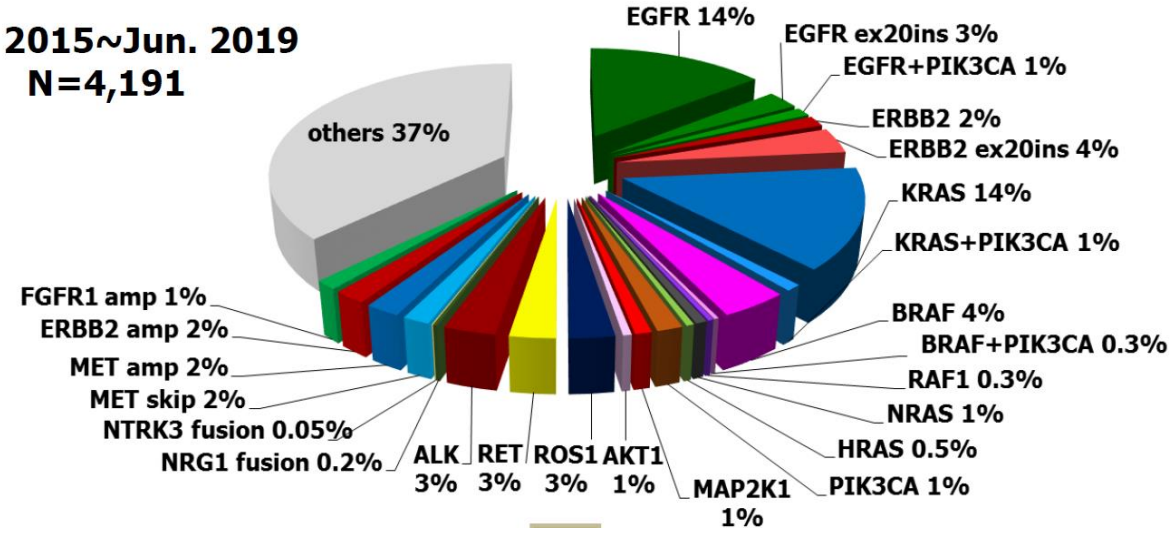
USA

MSK-IMAPCT in 860 NSCLC patients



Japan

Oncomine™ in LC-SCRUM: 4191 Japanese NSCLC patients



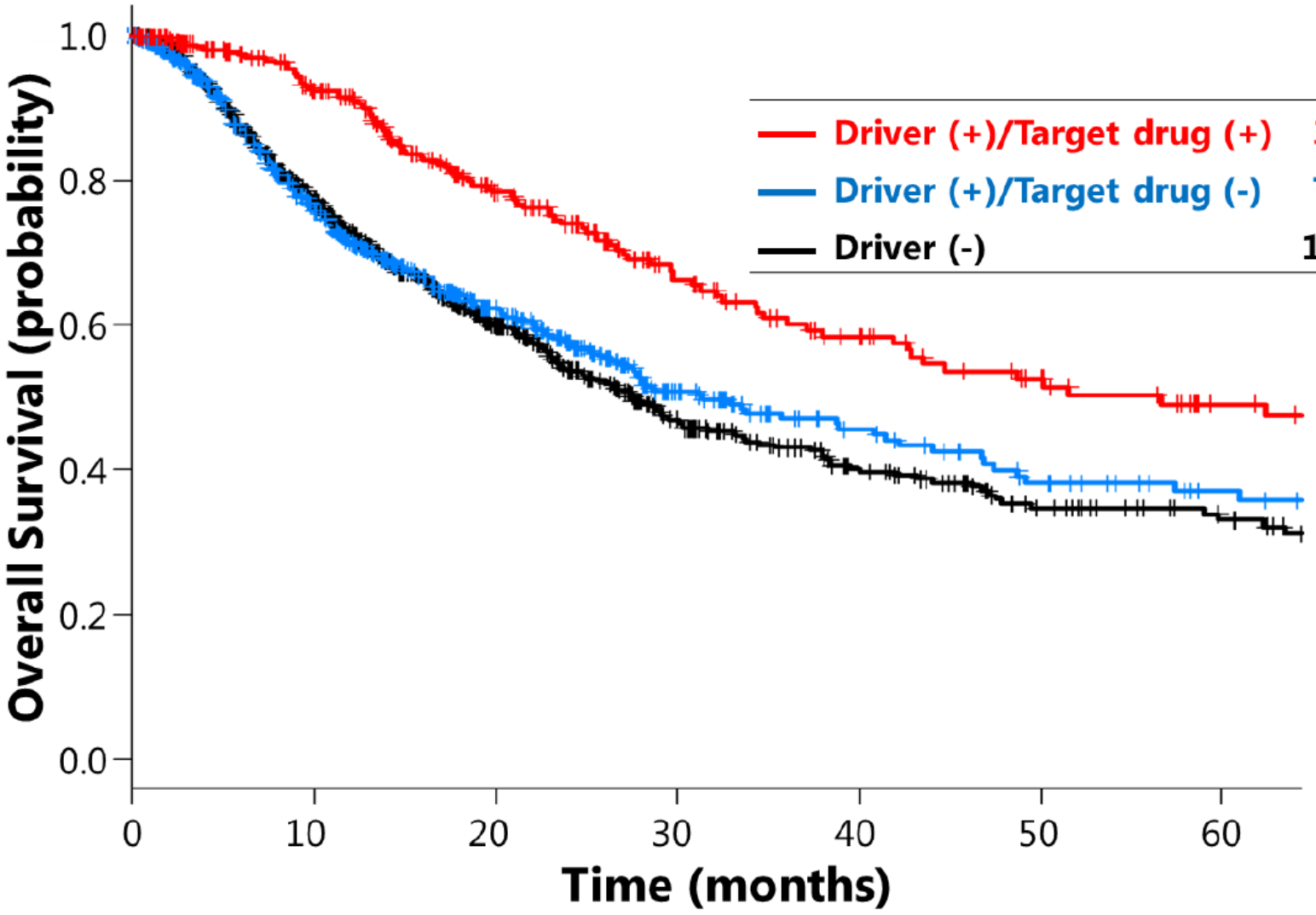
EGFRm detected in local lab were excluded in LC-SCRUM project

More than 60% of NSCLC patients had driver mutations

Jordan, et al. Cancer Discovery 2017
Miyamoto et al. ESMO 2019

Better outcomes in “Druggable” Driver Mutations

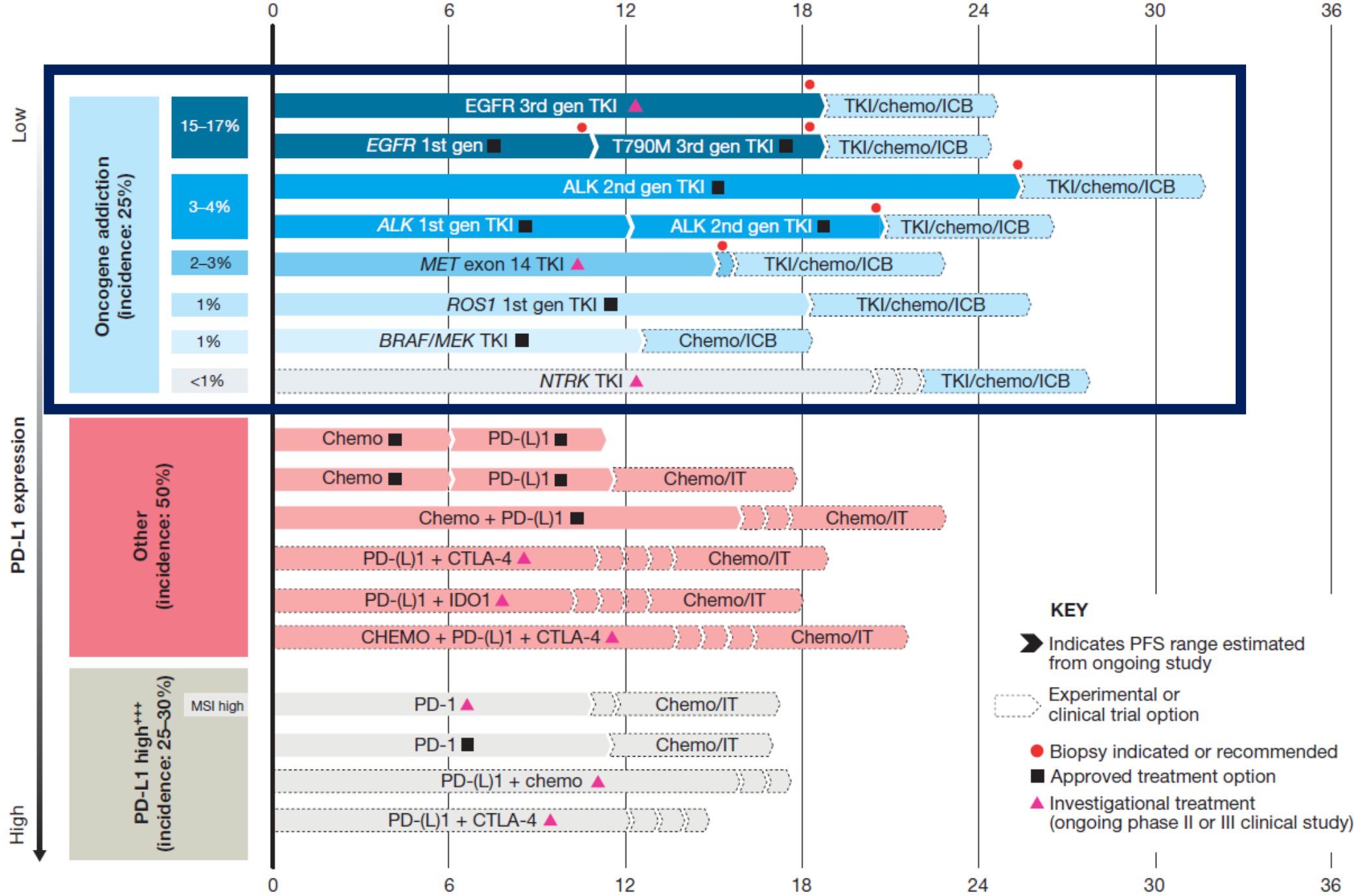
LC-SCRUM-JAPAN



	N	mOS (months)	[95% C.I.]
Driver (+)/Target drug (+)	335	56.6	[41.9-117.3]
Driver (+)/Target drug (-)	714	31.4	[26.4-41.5]
Driver (-)	1159	27.6	[24.0-30.4]

Logrank, p<0.001

Takashi Seto. ASCO 2018

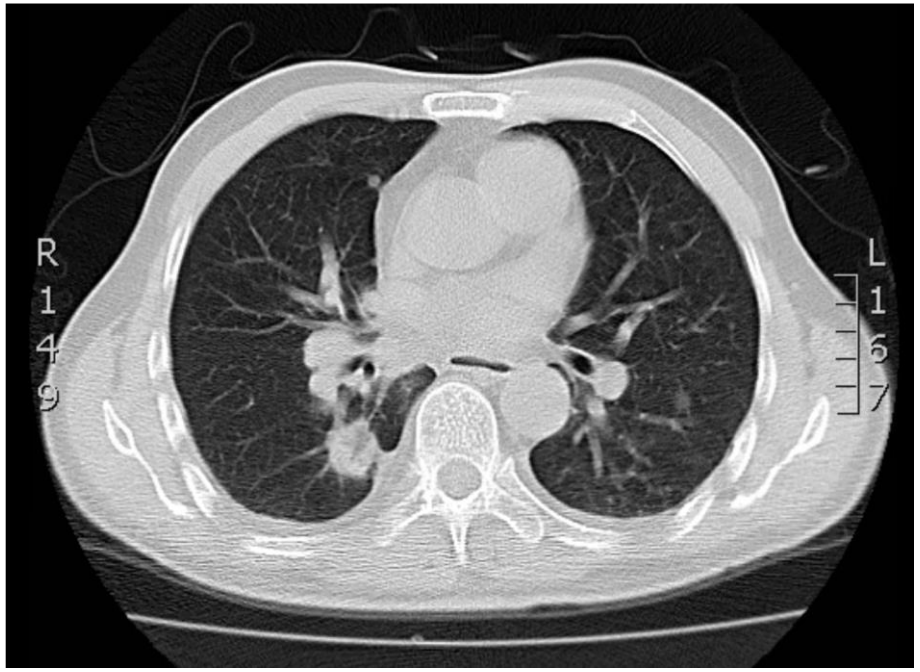


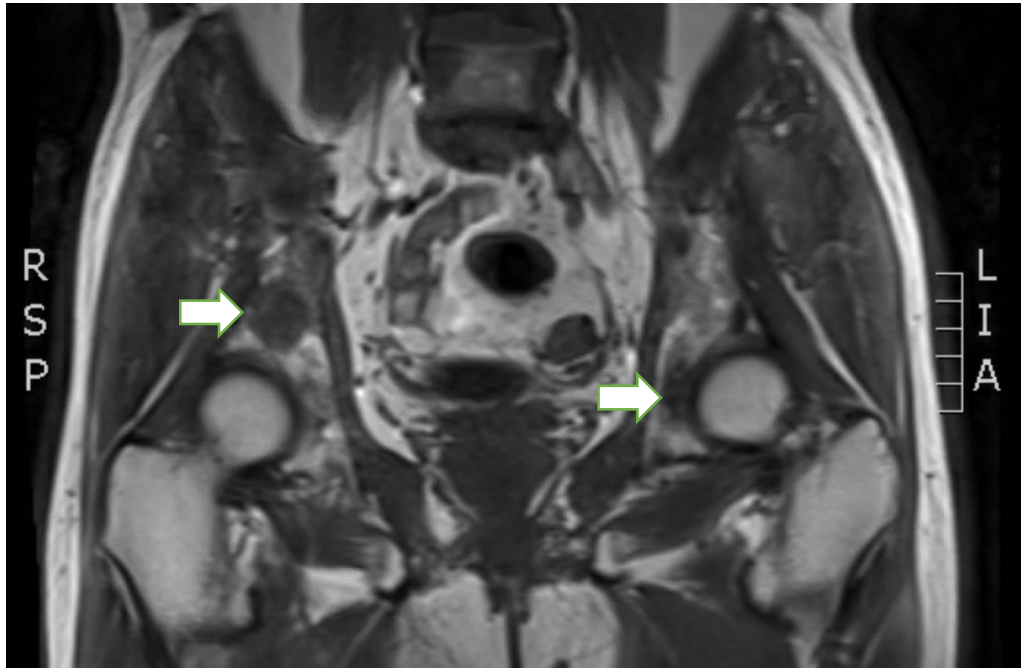
Roy S. Herbst et al. Nature Review 2018

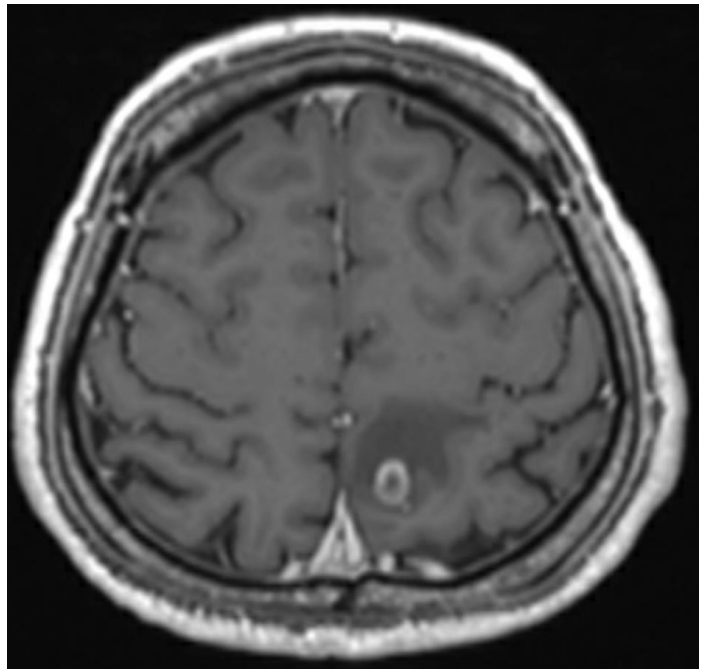
Clinical Application of NGS in lung cancer

- Druggable driver mutation not routinely checked in local lab.
- **Copy number variation which can be effectively targeted.**

Case 3

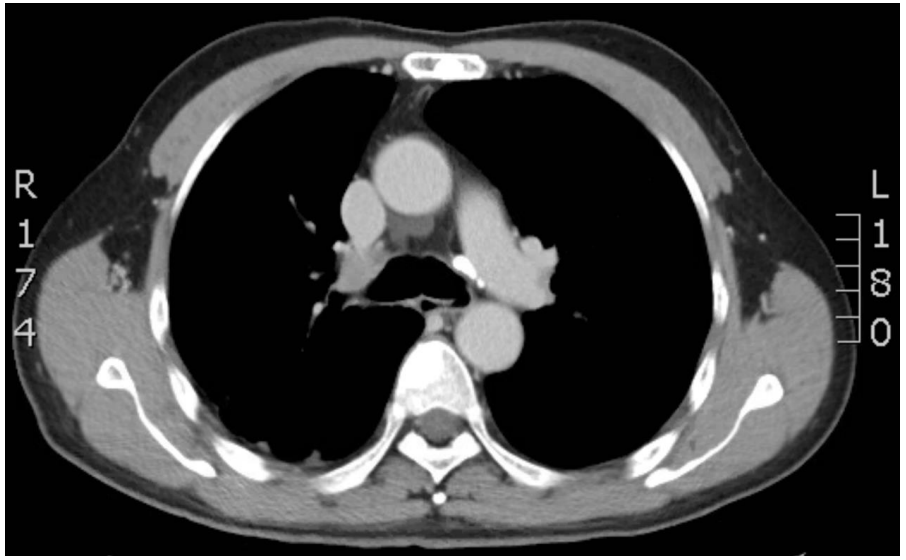


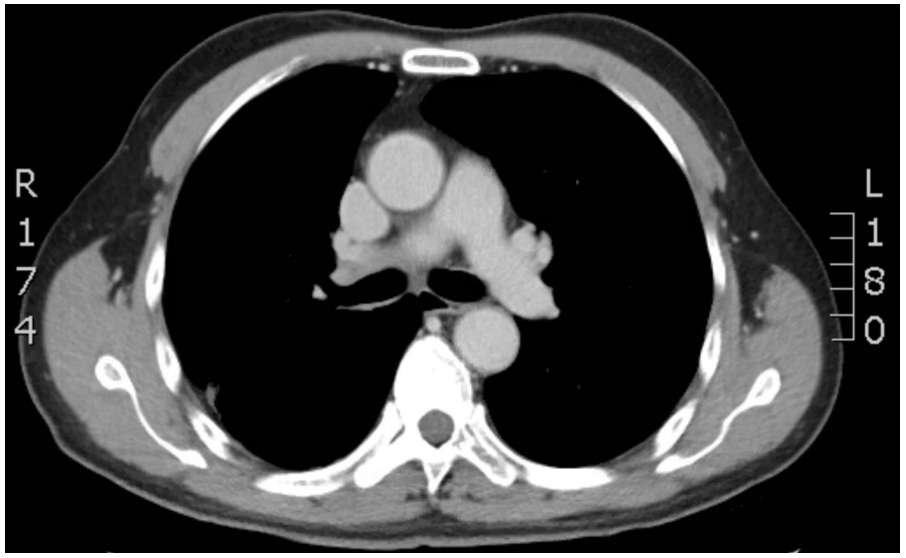




58 y/o man, with severe hip pain
NSCLC, ADC, cT4N3M1c, stage IVB
Bronchoscopy EBUS biopsy: adenocarcinoma, TTF-1 (+)
EGFR: del19; ALK, ROS-1 WT
PD at left lung tumor after **erlotnib/Bevacizumab** for 8m
T790M neg

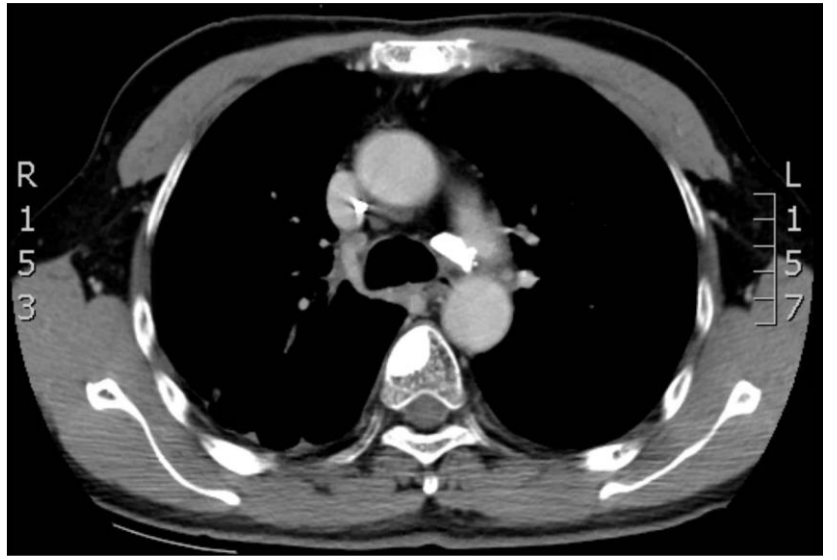
2018/5

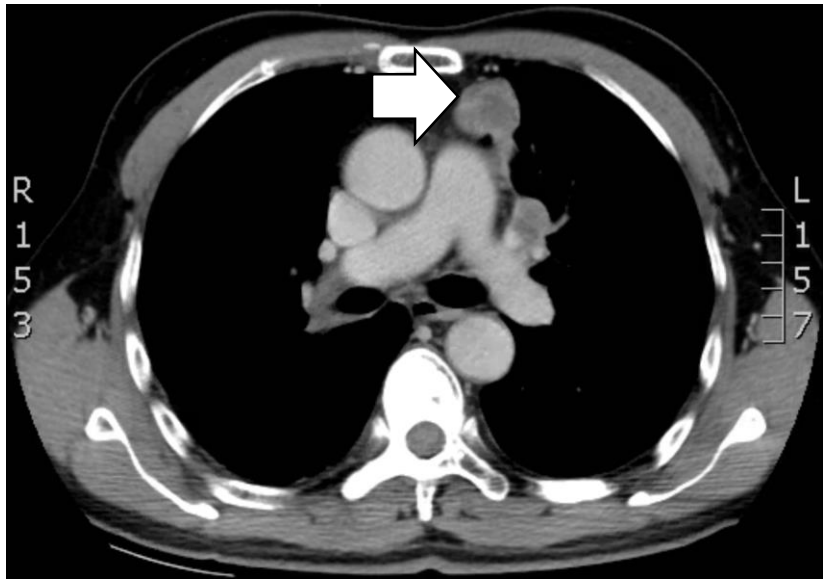




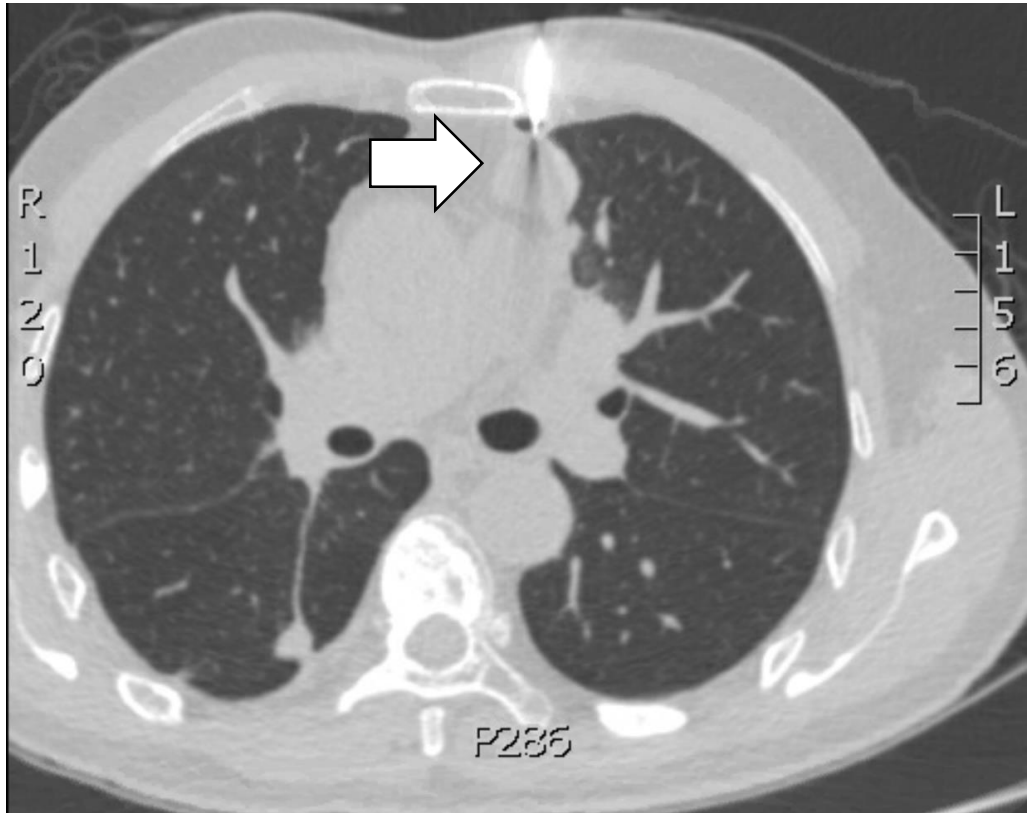
Pemetrexed/Carbo x4

2018/8



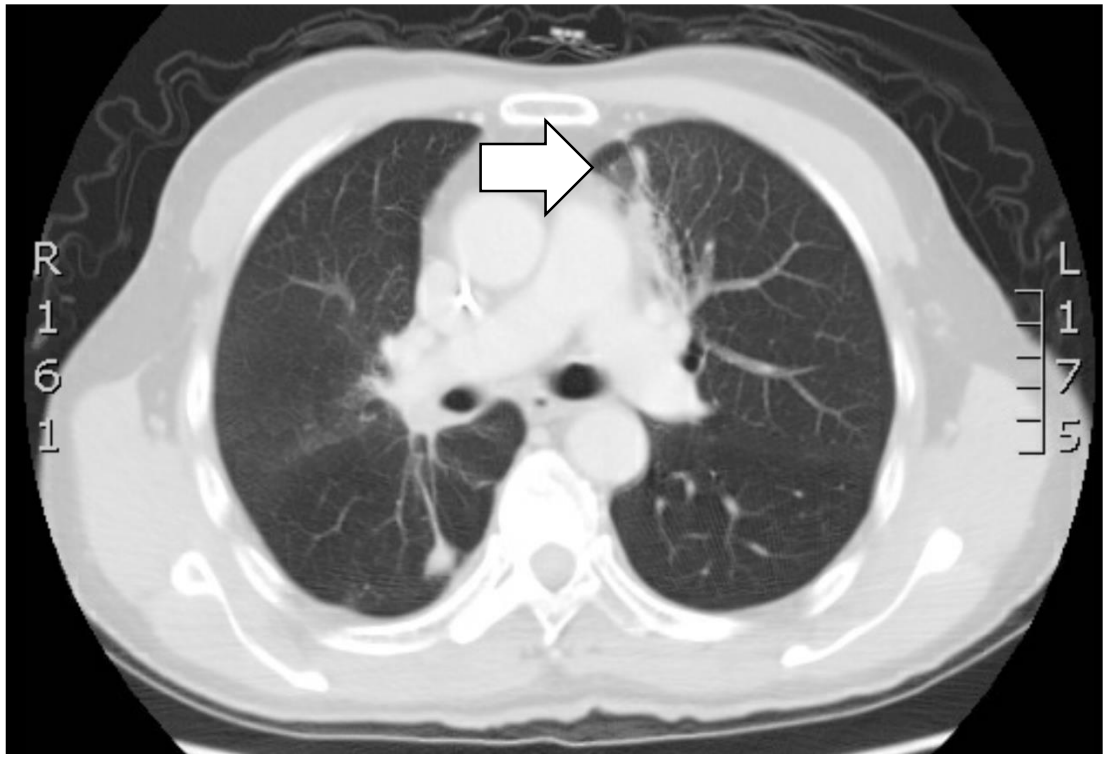


2018/8



Osimertinib
+ **Crizotinib**

2018/11

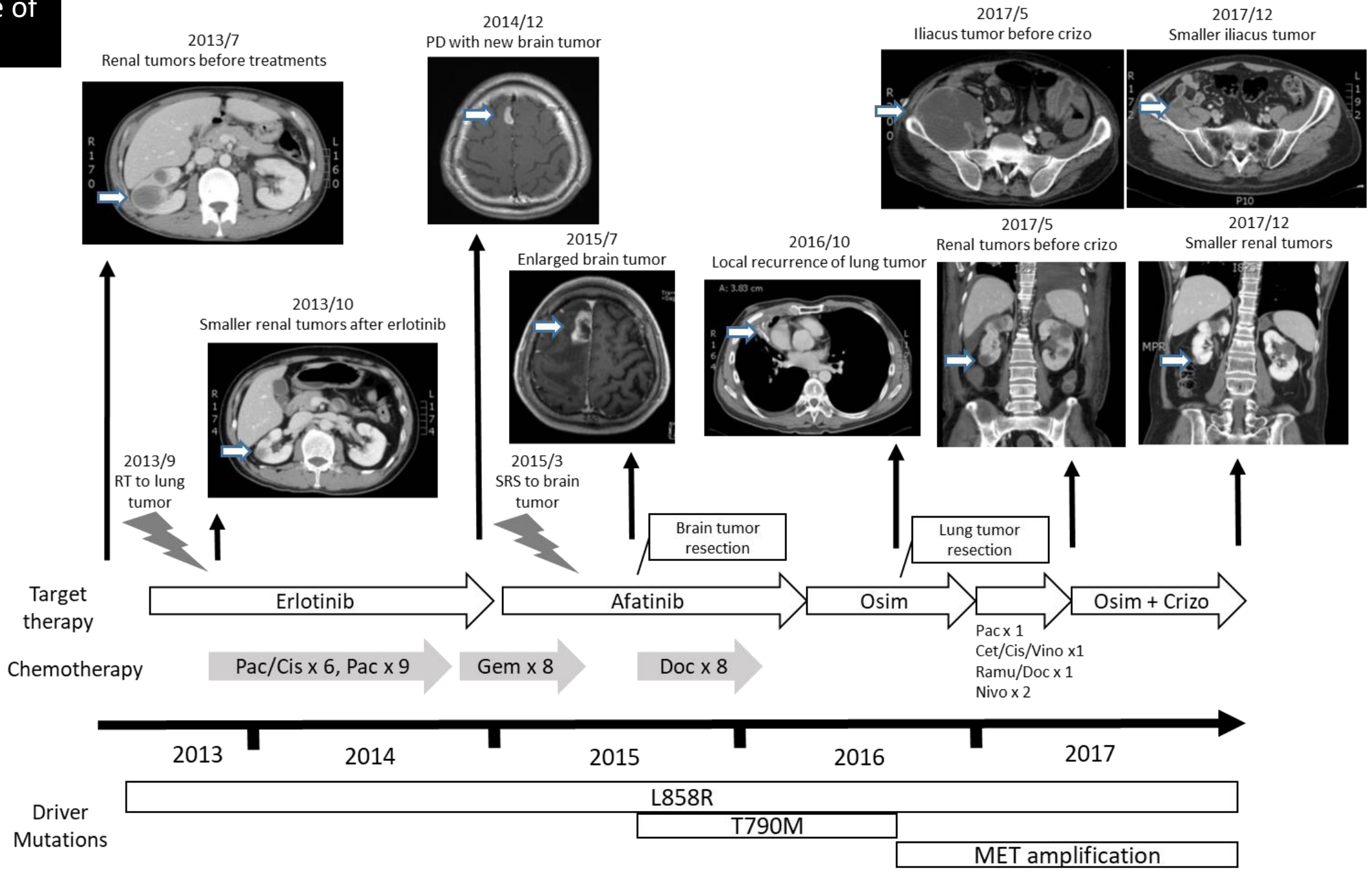


CT-guided biopsy of left lingular lobe tumor:
Adenocarcinoma, **EGFR del19, no T790M**
FoundationOne:
MET amplification, **copy number 8**

Good response of left lung tumors

EGFR	AMPLIFICATION	COPY NUMBER= 16
MET	AMPLIFICATION	COPY NUMBER= 8
PIK3CG	AMPLIFICATION	COPY NUMBER= 10

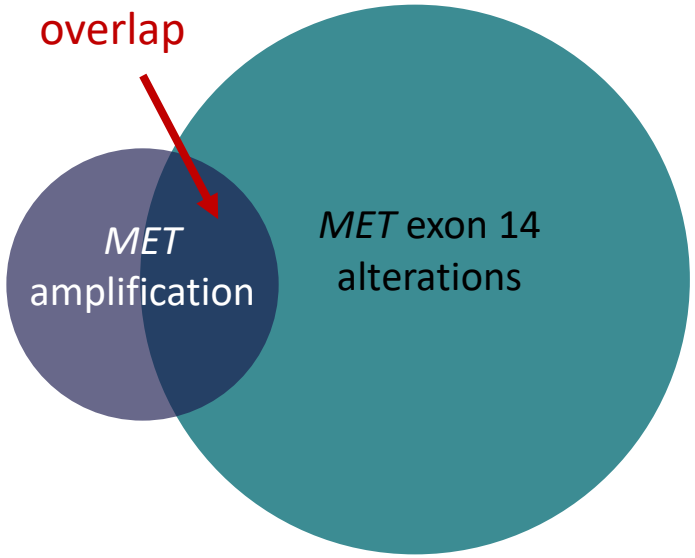
Another Case of METamp

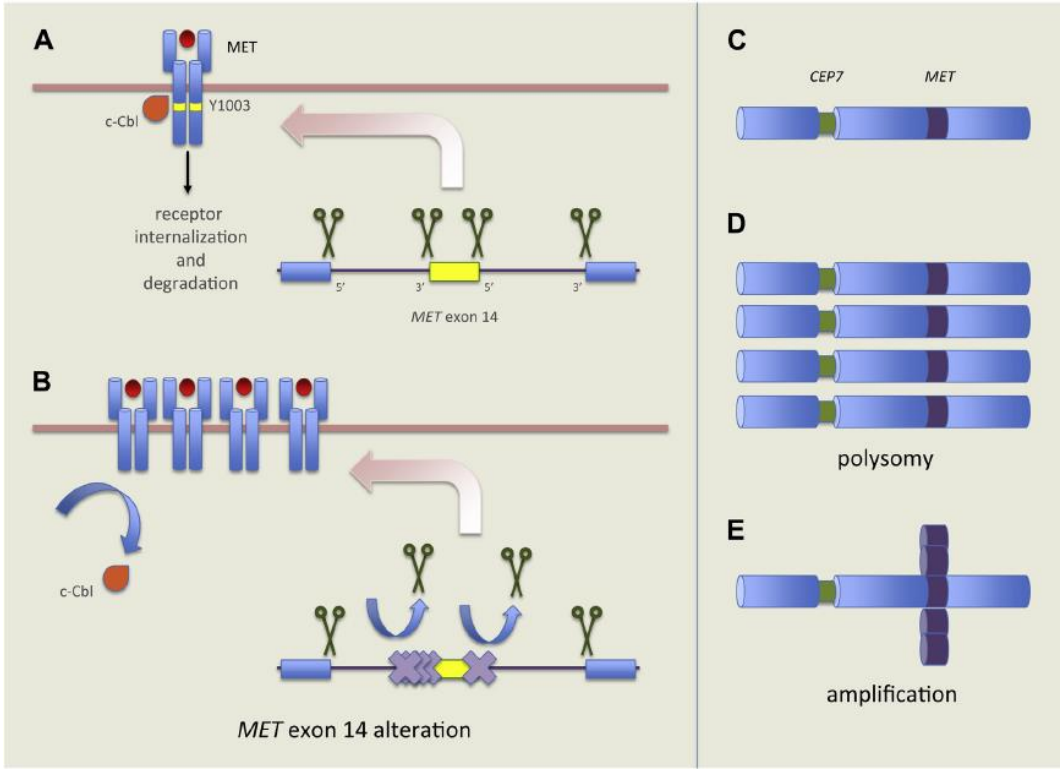


Yang et al. Journal of Oncology Practice 2019

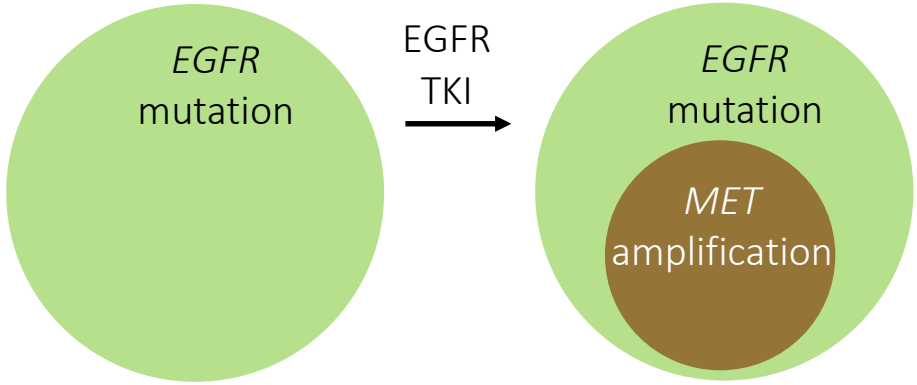
MET as a primary driver

15-20% overlap

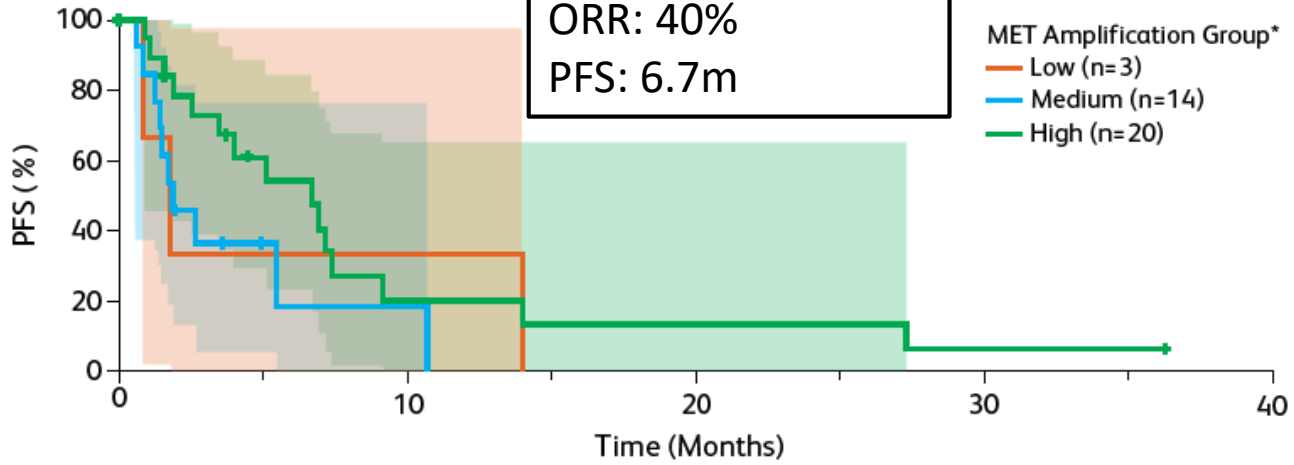




MET as a secondary/co-driver



Crizo in High MET (n=20):
ORR: 40%
PFS: 6.7m



Drilon et al. JTO 2017; Camidge, et al. ASCO 2018

Clinical Application of NGS in lung cancer

- Druggable driver mutation not routinely checked in local lab.
- Copy number variation which can be effectively targeted.
- **Genetic alterations affecting TKI or ICI responses.**

Referred to Ph.D. Chen's talk !

Case 4

42 y/o woman
NSCLC, ADC, **EGFR: L858R, PD-L1 0%**
Right MPE, liver and spleen metastases

2018/3-8 **Erlotinib, SD -> PD**

PFS of Erlotinib is only 5 months

NGS

2018/9-12 Pemetrexed/Cisplatin x6, Pemetrexed x1

2019/1 PD with Increased liver metastases
s/p liver tumor biopsy and RFA

2019/2-3 Brain metastases
s/p tumor resection and WBRT

What shall we give as the subsequent treatment ?

Report highlights include the following:

1. Variants of clinical relevance:

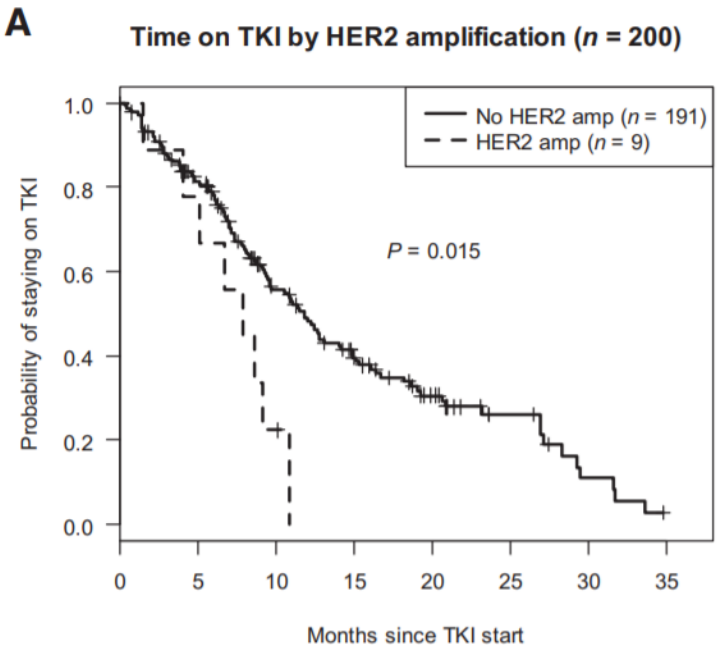
Mutation	EGFR E709G, EGFR L858R, TP53 V73fs
Amplification	Not detected
Homozygous deletion	Not detected
Heterozygous deletion	CDKN2A, CHEK2, FBXW7, FLCN, NF2, PTCH1, PTEN, RAD51, STK11, TSC1

2. Tumor mutational burden(TMB): 21.4 mutations / megabase (High TMB)
3. MSI status: MSI-Low
4. Fusion gene: Not detected

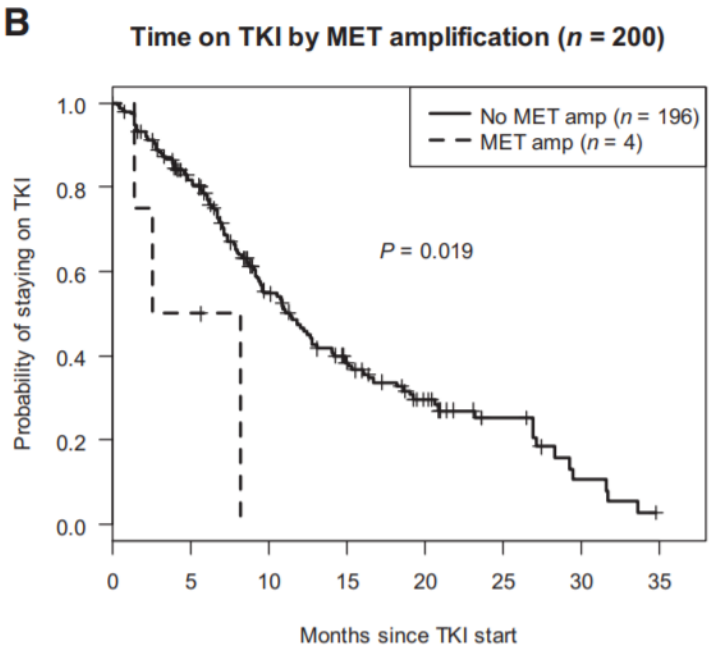
EGFR mutation
TP53
High TMB
STK11 (hetero)

Concurrent genetic alterations associated with EGFR TKI response

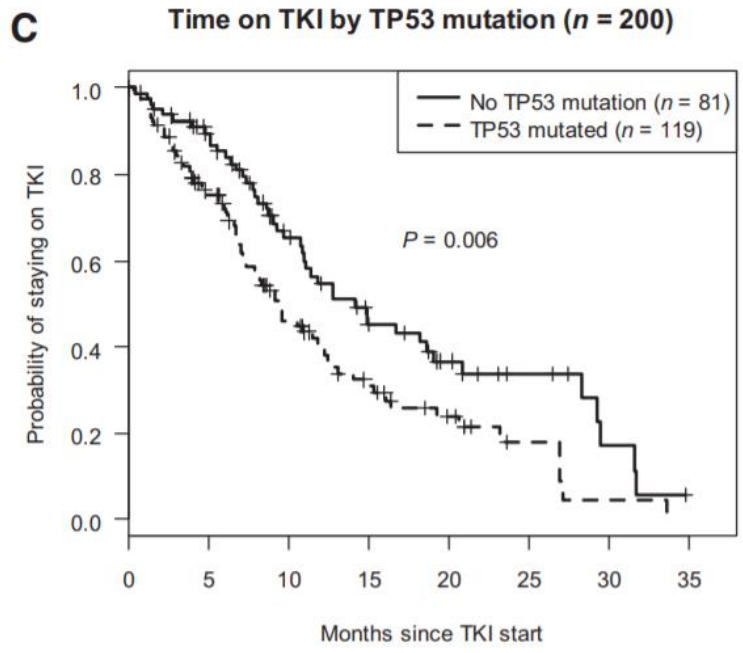
HER2 amp



MET amp



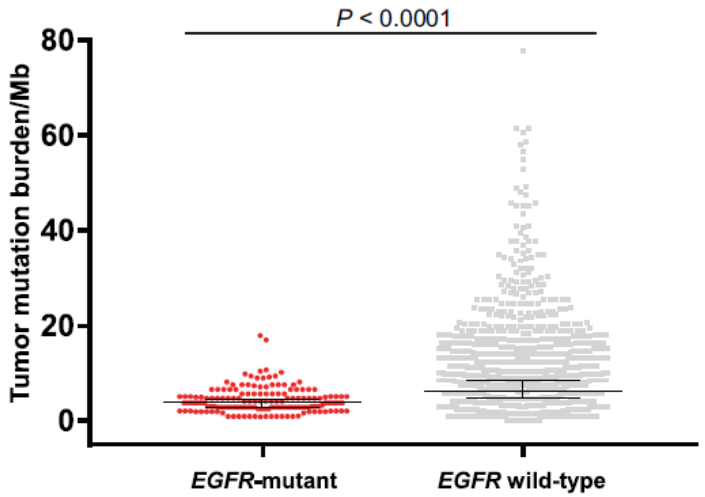
TP53 mutation

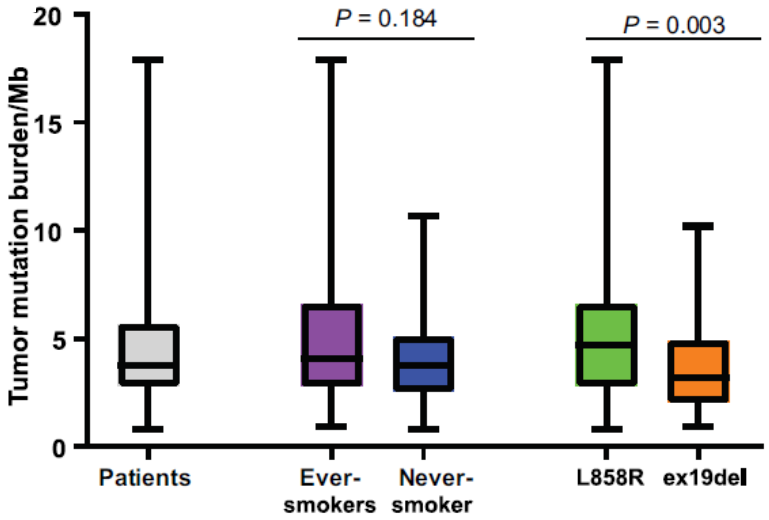


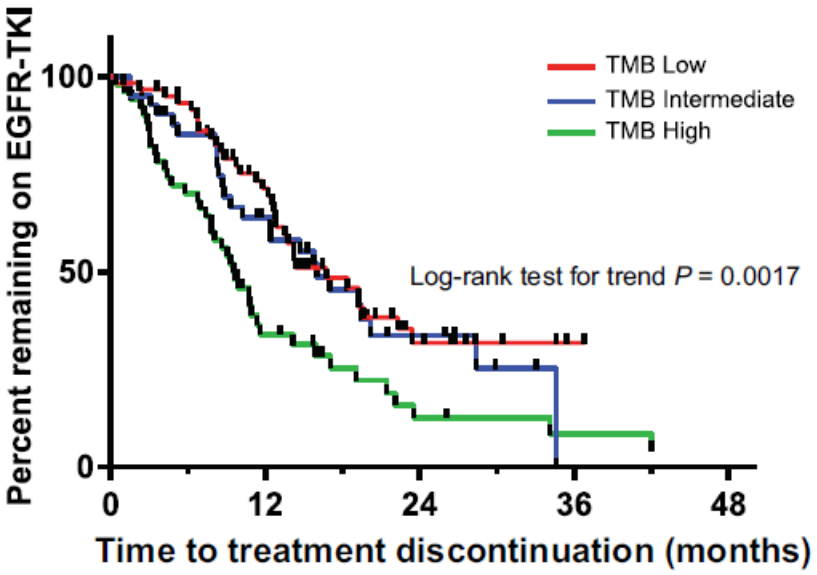
200 pre-treatment EGFRm tumors tested by NGS

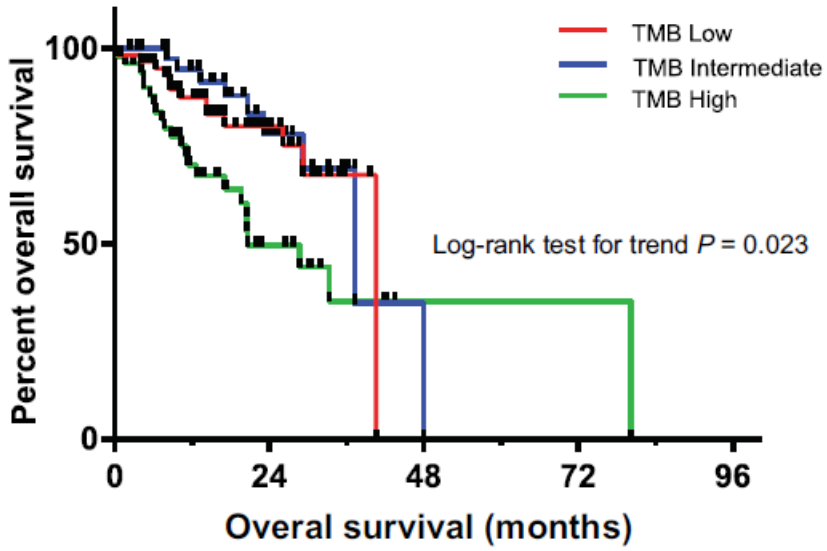
Helena A. Yu et al. CCR 2018

Tumor mutation burden in EGFRm ADC patients



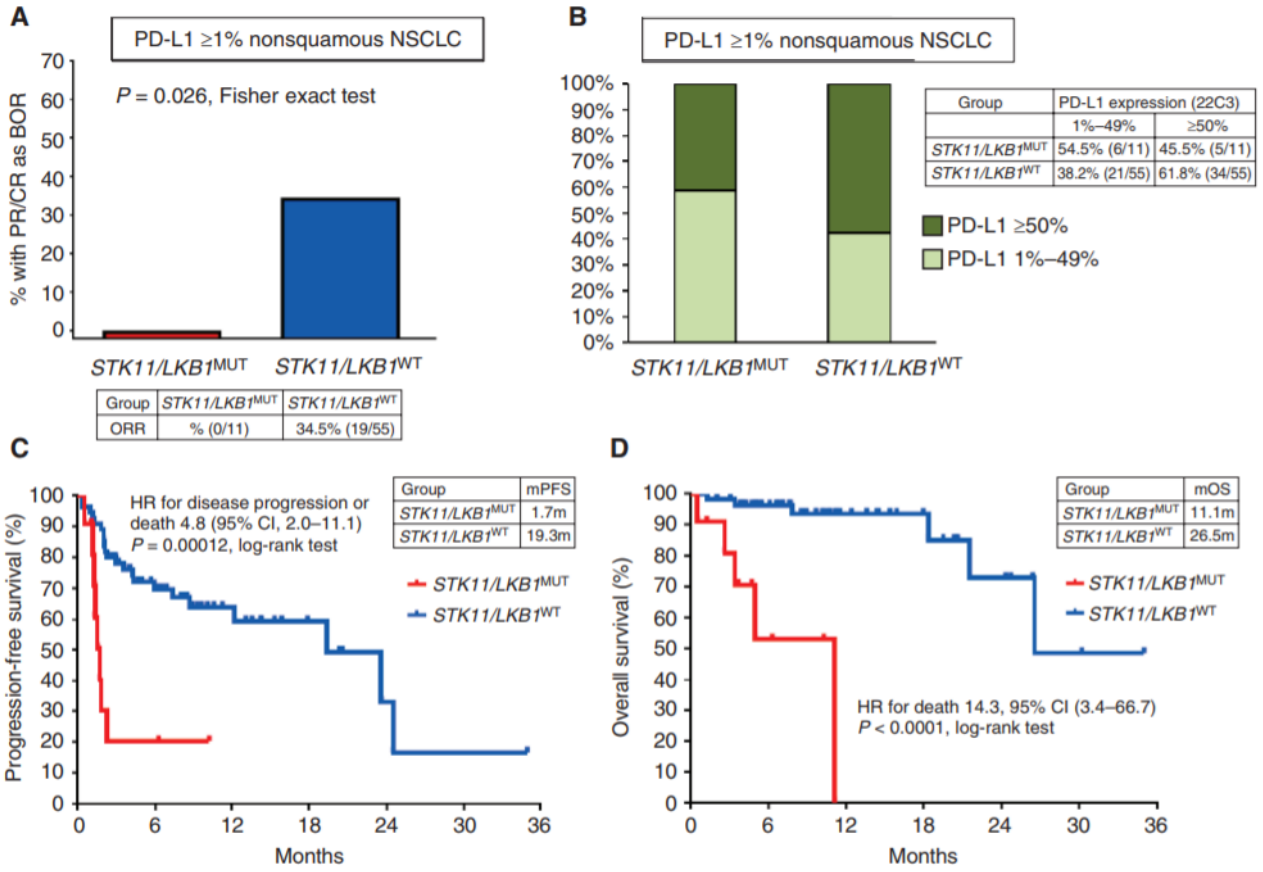






Offin et al. CCR 2019

STK-11/LKB1m: poor IO response



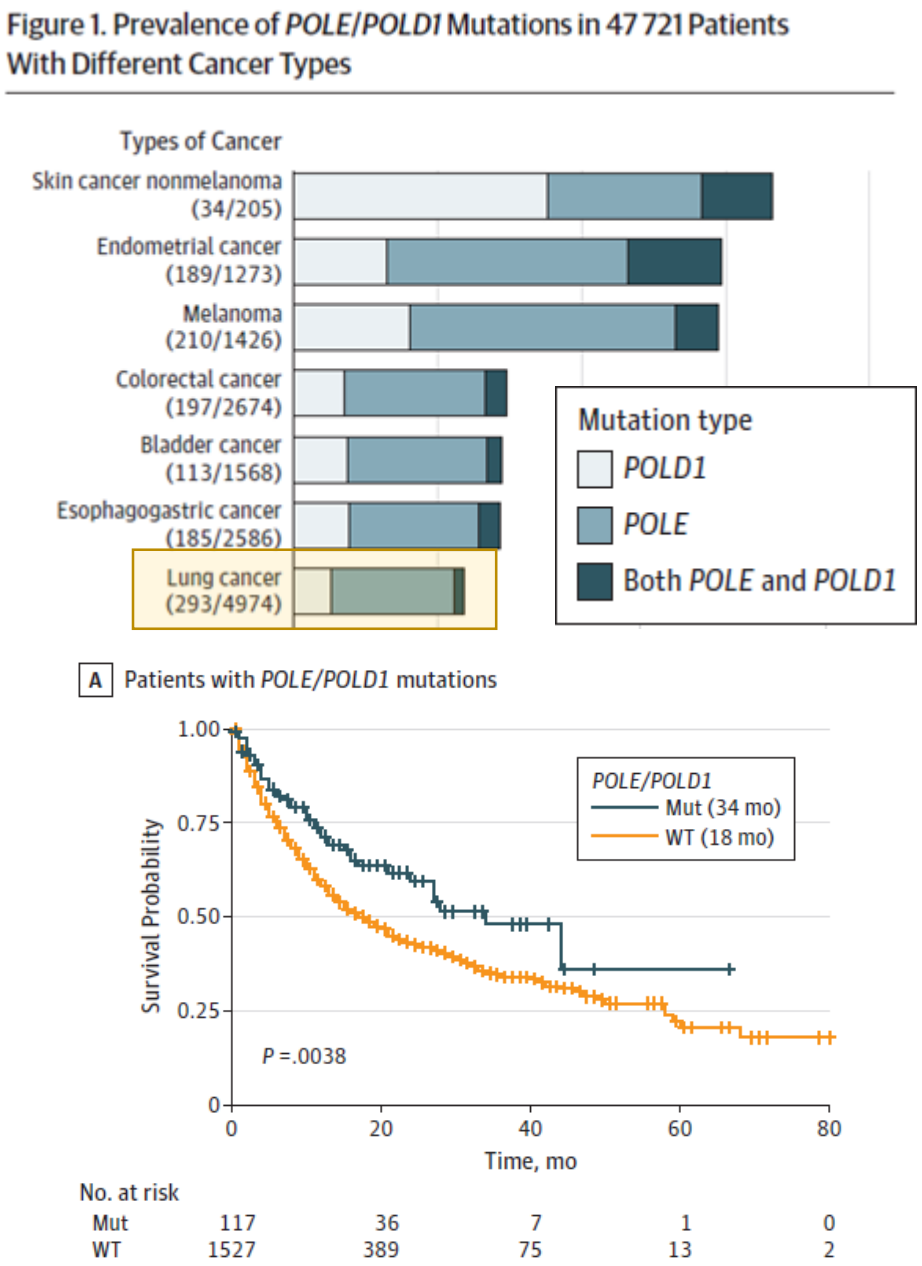
Group	STK11/LKB1MUT	STK11/LKB1WT
ORR	% (0/11)	34.5% (19/55)

Group	PD-L1 expression (22C3)	
	1%–49%	≥50%
STK11/LKB1MUT	54.5% (6/11)	45.5% (5/11)
STK11/LKB1WT	38.2% (21/55)	61.8% (34/55)

Group	mPFS
STK11/LKB1MUT	1.7m
STK11/LKB1WT	19.3m

Group	mOS
STK11/LKB1MUT	11.1m
STK11/LKB1WT	26.5m

POLE/POLD1m: good IO response



Skoulidis et al. Cancer Discovery 2018
Wang et al. JAMA Oncol 2019

Clinical Application of NGS in lung cancer

- Druggable driver mutation not routinely checked in local lab.
- Copy number variation which can be effectively targeted.
- **Genetic alterations affecting TKI or ICI responses.**

Referred to Ph.D. Chen's talk !

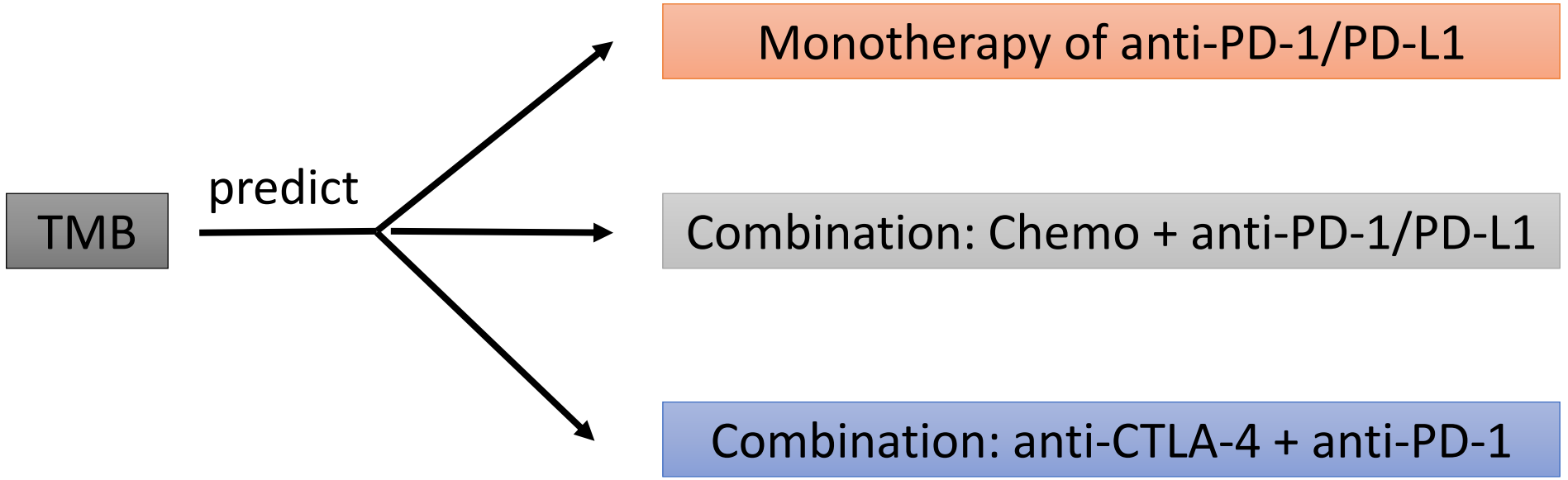
TP53, HER2amp (?), METamp, high TMB: poor response to TKI

STK11/LTB1: poor response to ICI

POLE/POLD1, MSI-H: good response to ICI

Clinical Application of NGS in lung cancer

- Druggable driver mutation not routinely checked in local lab.
- Copy number variation which can be effectively targeted.
- Genetic alterations affecting TKI or ICI responses.
- **Tumor mutation burden as a biomarker of ICI.** Referred to Ph.D. Chen's talk !



Summary of TMB in prediction of IO response

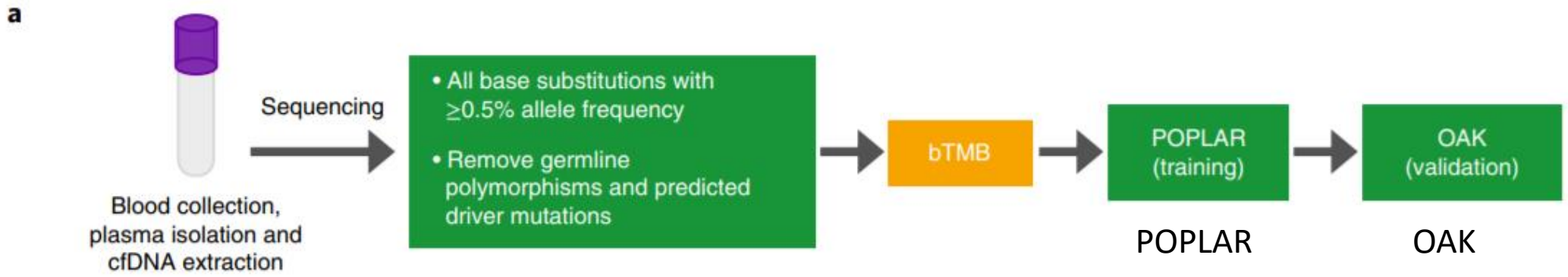
VS chemo	TMB high (vs low)			
Nivo	PFS	↑	OS	— ?
Nivo/chemo	PFS	↑ (PD-L1 < 1%)	OS	??
Pembro	PFS	↑	OS	↑
Pembro/chemo	PFS	—	OS	—
Ipi/Nivo	PFS	↑ ?	OS	—

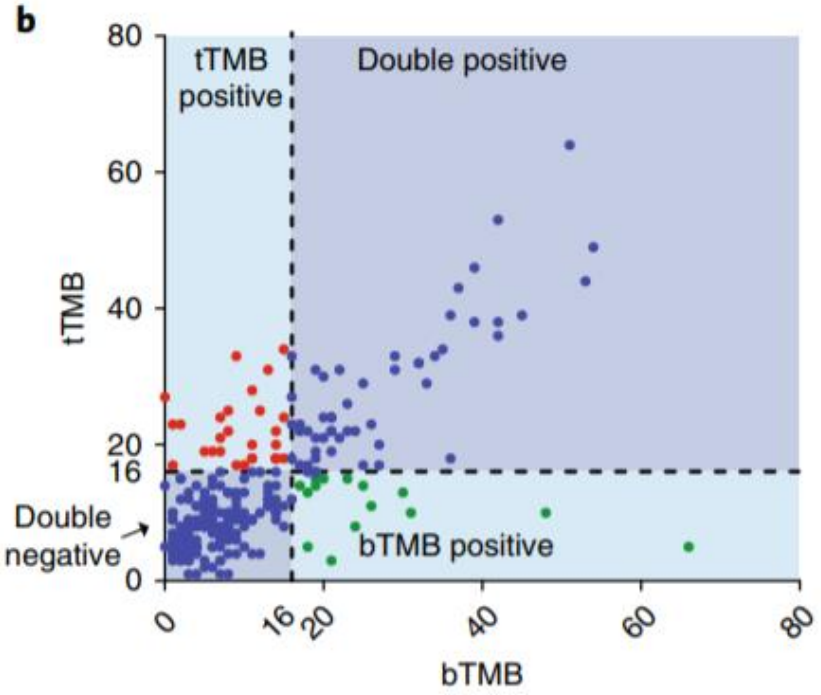
Solange Peters, et al. AACR 2017; ESMO 2019
Paz-Ares, et al. ESMO 2019
Roy S. Herbst et al. ESMO 2019

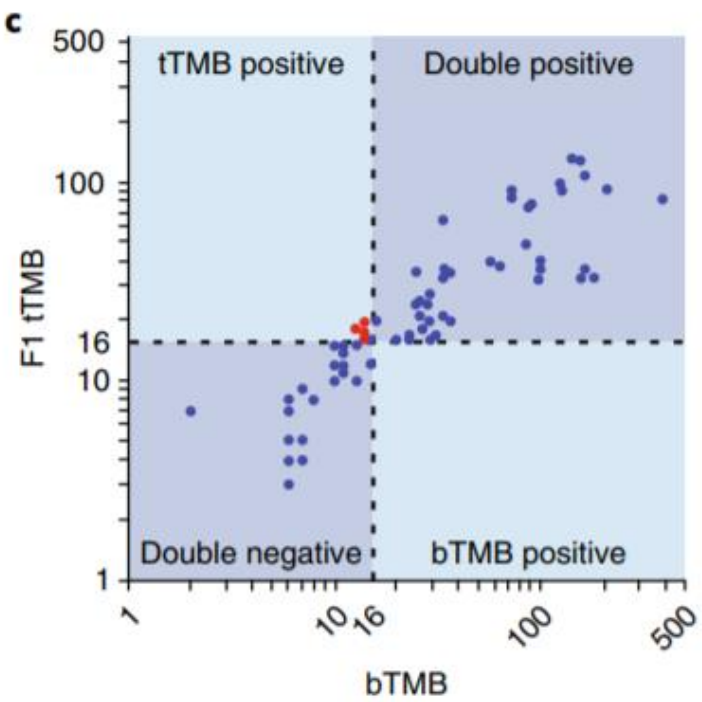
Clinical Application of NGS in lung cancer

- Druggable driver mutation not routinely checked in local lab.
- Copy number variation which can be effectively targeted.
- Genetic alterations affecting TKI or ICI responses.
- Tumor mutation burden as a biomarker of ICI.
- **Tissue versus liquid based NGS.**

bTMB and tTMB: are they the same ?



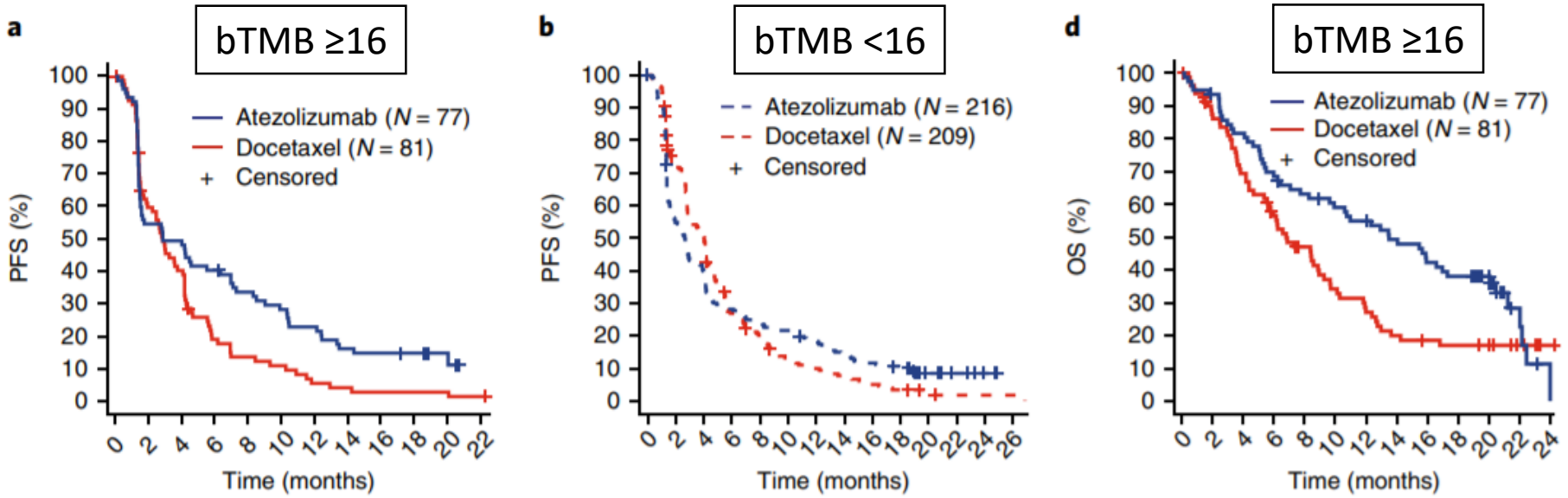


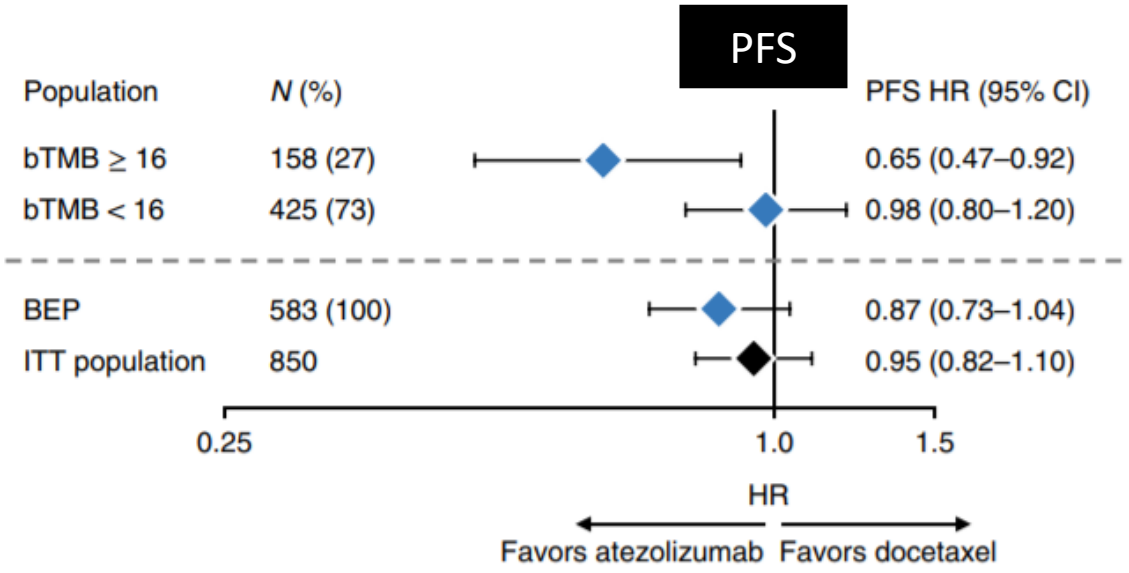


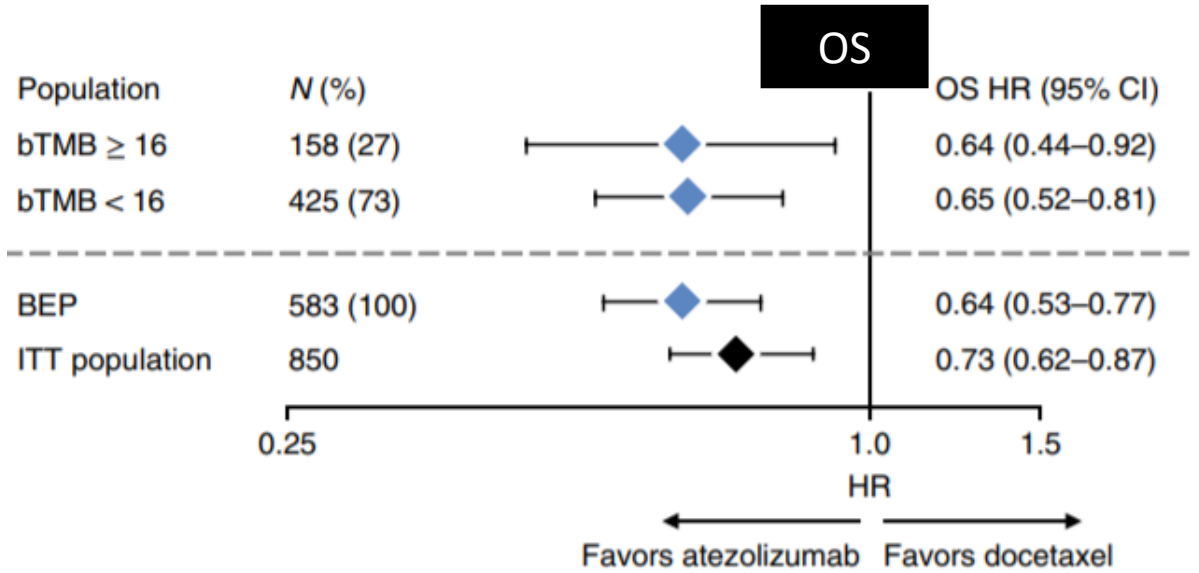
bTMB	PPA (%)	NPA (%)
≥10	100	100
≥11	94.6	92.3
≥12	89.1	92.9
≥13	94	84.2
≥14	90	89.5
≥15	85.7	90
≥16	89.1	100
≥17	90.2	89.3
≥18	92.1	83.9
≥19	94.4	81.8
≥20	97.1	82.4

David Gandara et al. Nat Med 2018

bTMB to predict ICI responses

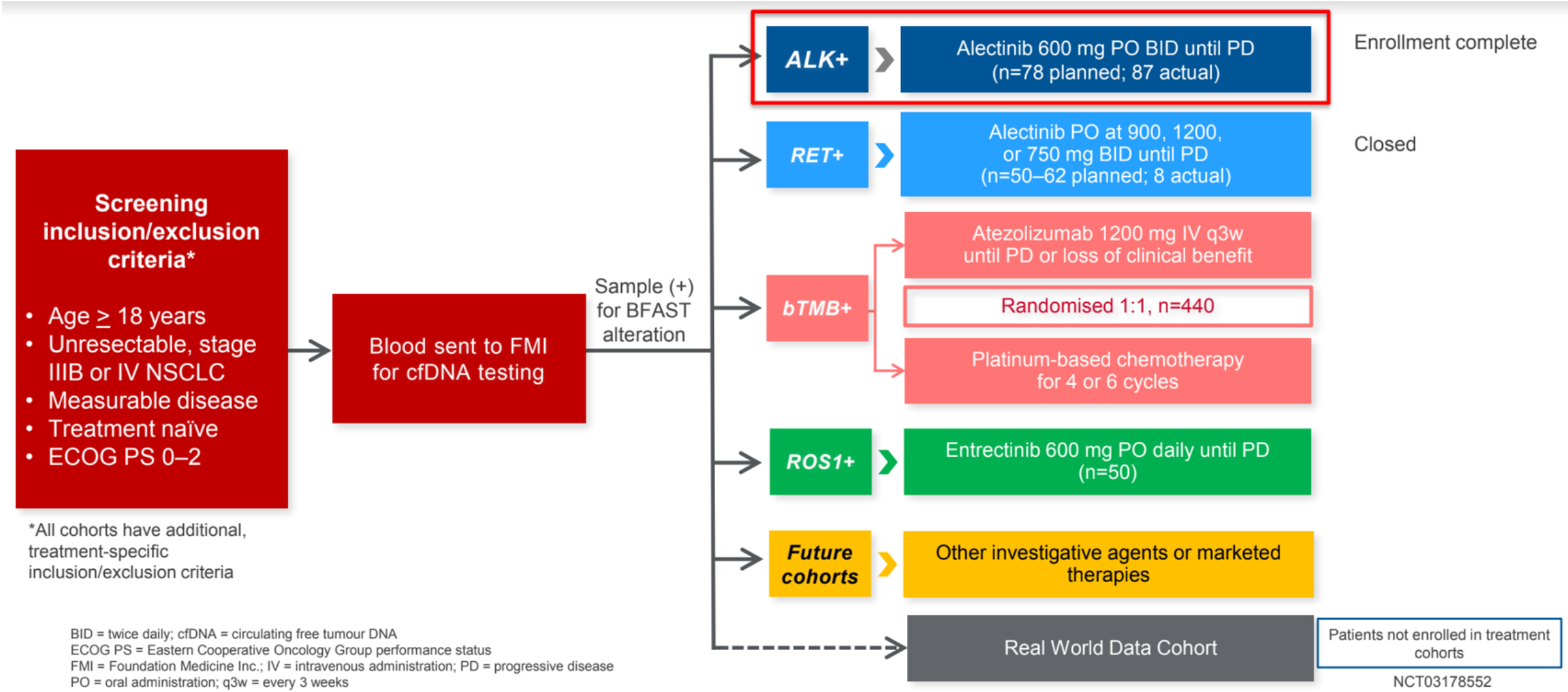




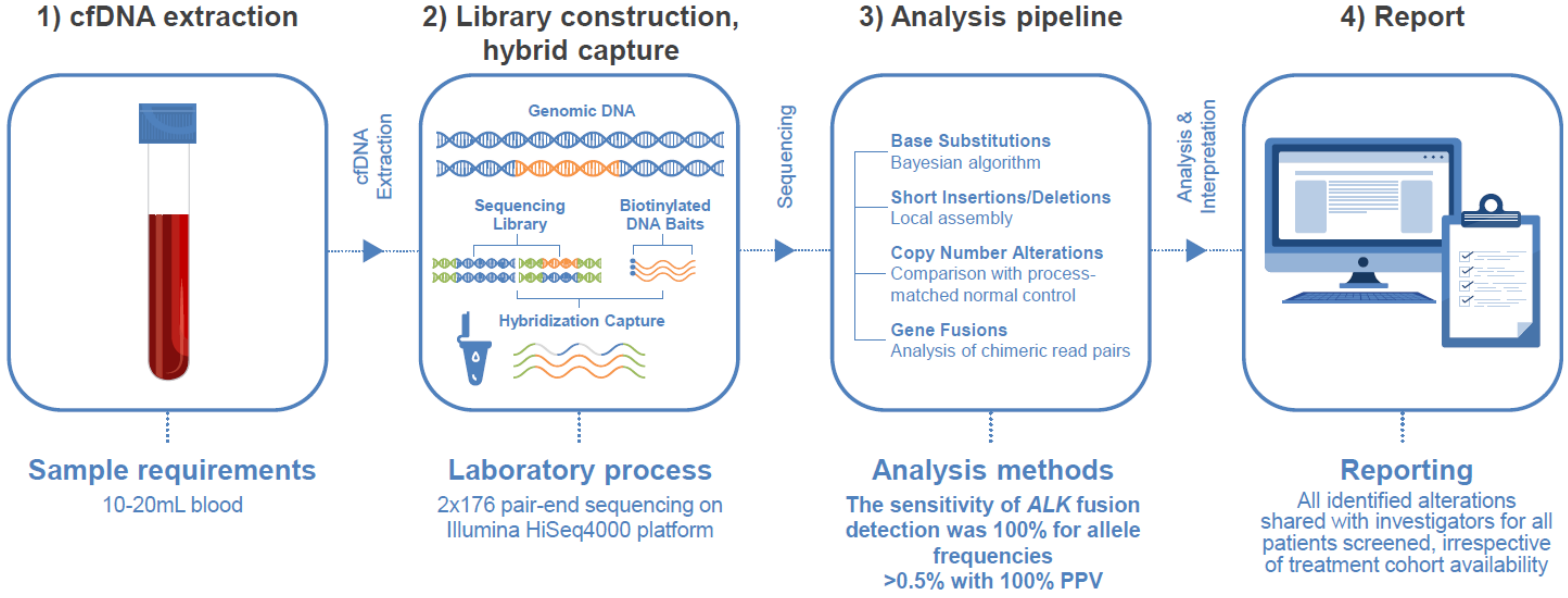


David Gandara et al. Nat Med 2018

Is BFAST **fast** and **efficacious** enough ?

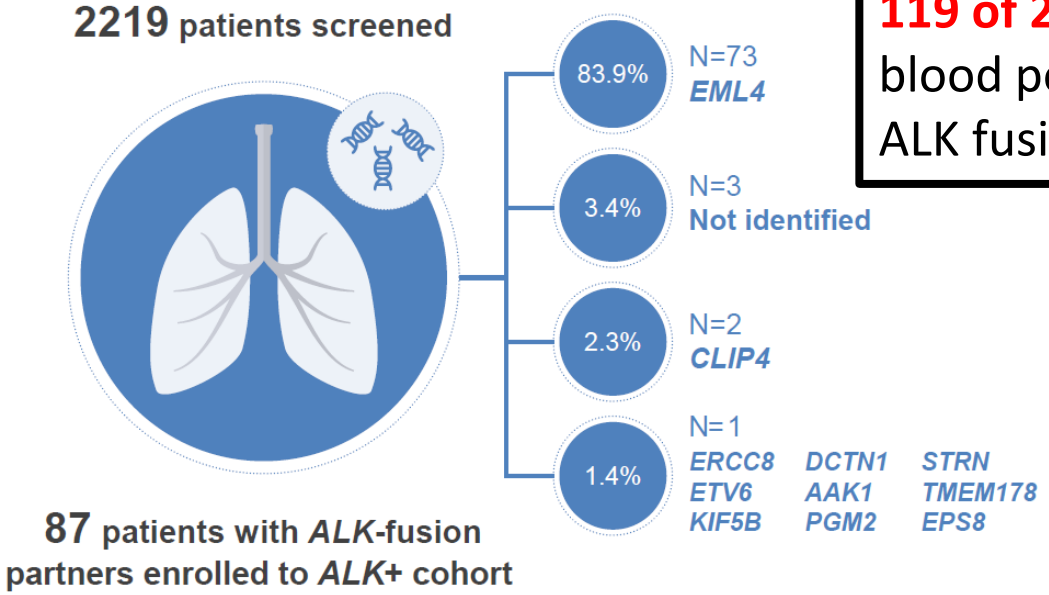


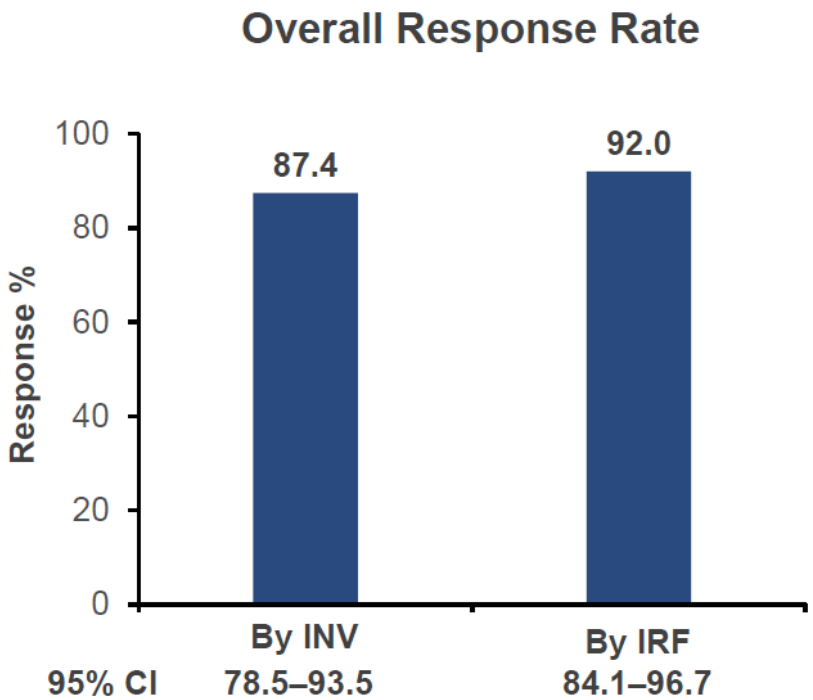
Gadgeel et al. ESMO 2019



10-14 calendar days

119 of 2219 (5.4%) blood positive for ALK fusion





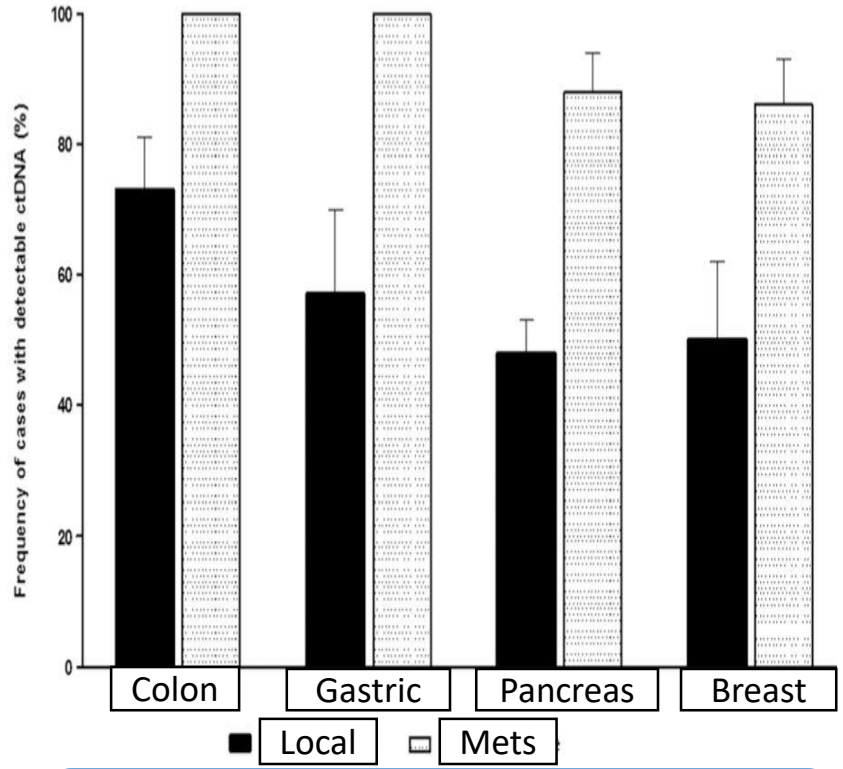
Gadgeel et al. ESMO 2019

Biological considerations of liquid biopsy

	Golden standard	
	Tumor positive	Tumor negative
ctDNA positive	True positive	**False positive**
ctDNA negative	**False negative**	True negative

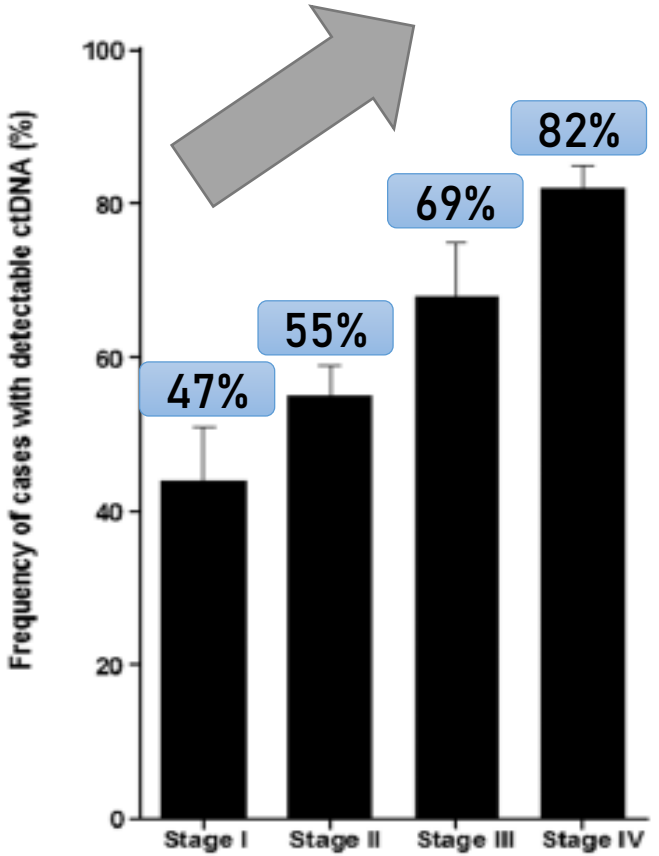
Detectable cfDNA differ among different **cancer type** and **stage**

Fraction of patients with ctDNA in localized(stage I-III) and metastatic (stage IV) maligancies



Detectable levels of ctDNA :
Localized tumor-49 to 78 %
Metastatic tumor-86 to 100 %

Fractions of patients with detectable ctDNA

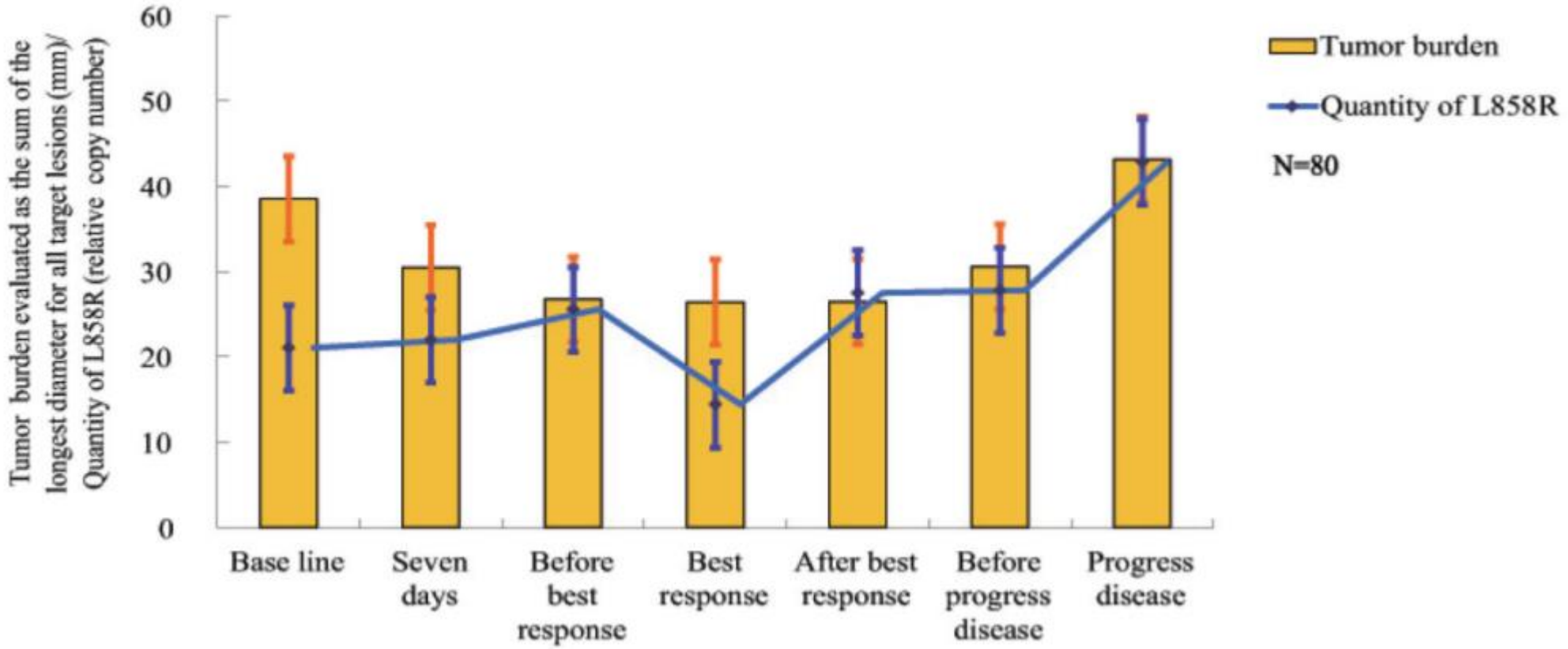


Detection of ctDNA depends on locations and metastatic spread

Bettegowda et al. Science Translational Medicine 2014

False Negative

Exploratory analysis of CTONG-0901 patients: cfDNA decreased after effective EGFR TKI treatment



Zhou et al. Journal of Hematology and Oncology 2016

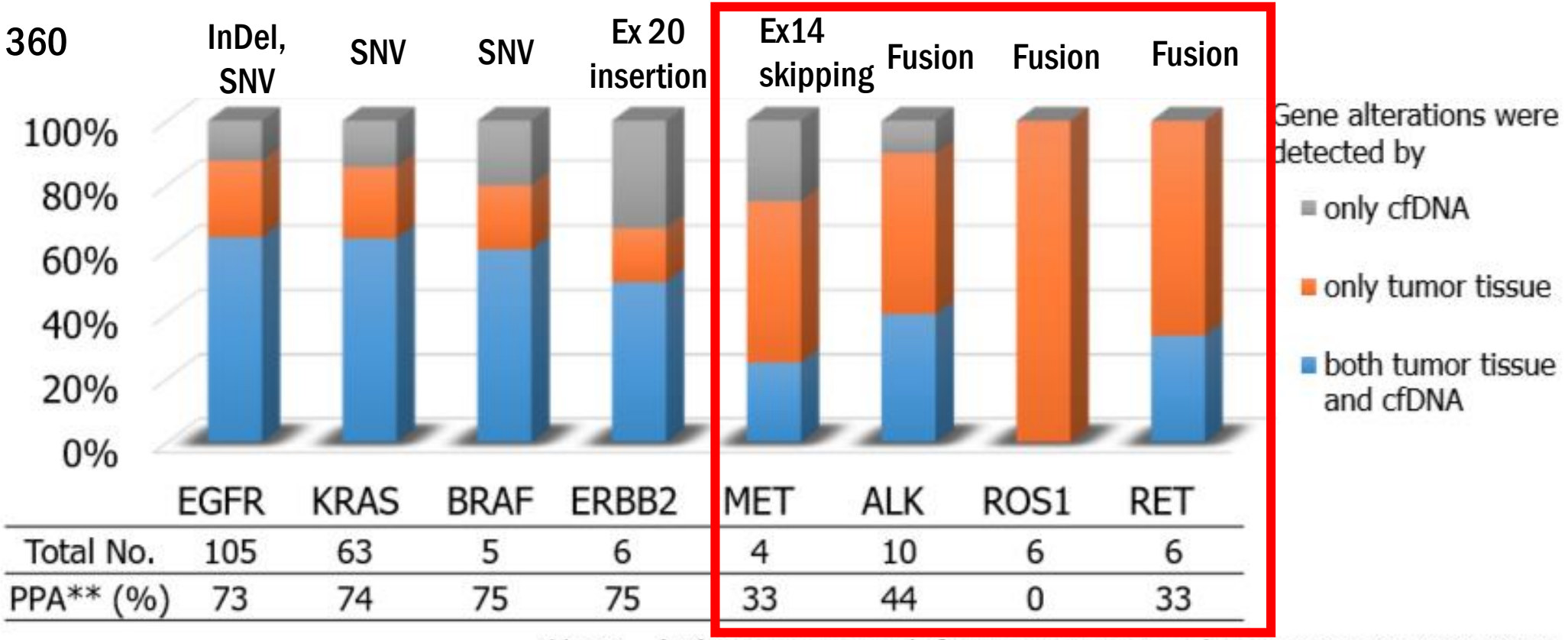
Limitation of fusion detection in liquid biopsy

Tissue: Oncomine comprehensive assay, version 3

Liquid: Guardant 360

N=363

LC-SCRUM-Japan



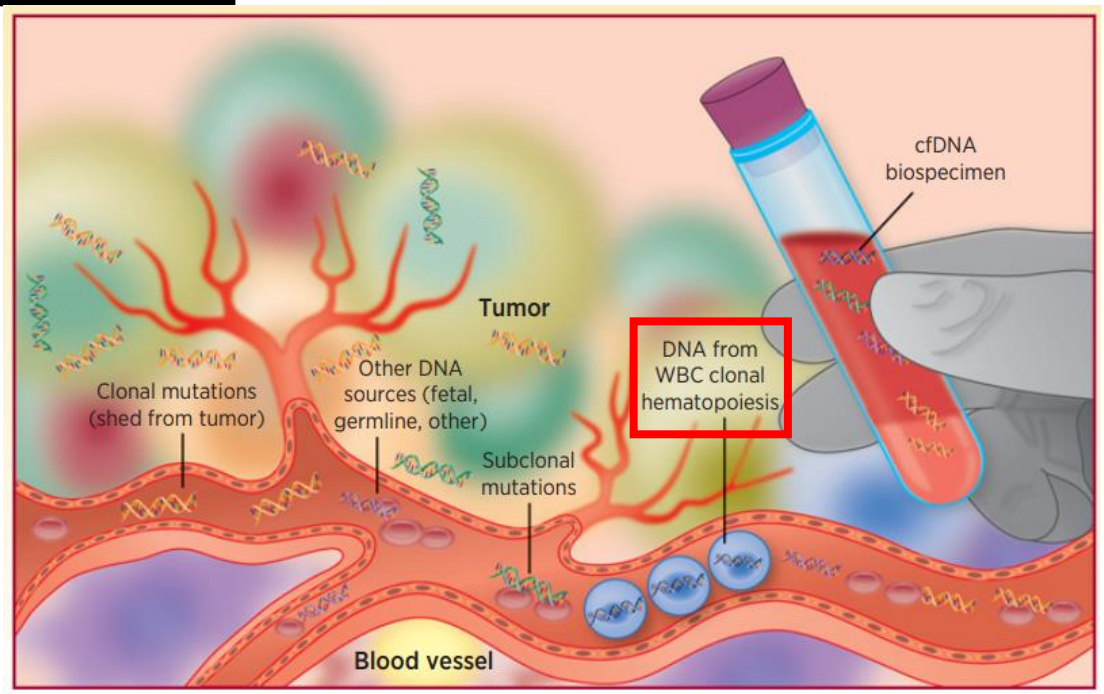
	EGFR	KRAS	BRAF	ERBB2	MET	ALK	ROS1	RET
Total No.	105	63	5	6	4	10	6	6
PPA** (%)	73	74	75	75	33	44	0	33

** PPA = both tumor tissue and cfDNA positive patients / tumor tissue positive patients.

Detection of fusion genes by ctDNA is limited.

Annals of Oncology (2019) 30 (suppl_5): v602-v660. 10.1093/annonc/mdz260

False Positive



Clonal hematopoiesis (CHIP) (複製性造血作用)- development of somatic mutations in hematopoietic cells

advanced NSCLC (n=122)

ID	Age	Plasma NGS result (AF %)	PBC testing method	Tumor NGS result	PBC testing result (AF %)
JAK2 analysis					
4433	58	*JAK2* V617F (19.5)	ddPCR	Not detected	Detected (4.66)
4379	63	*JAK2* V617F (2.5)	ddPCR	Not available	Detected (0.19)
4014	59	*JAK2* V617F (0.5)	ddPCR	Not detected	Detected (0.68)
349	84	*JAK2* V617F (0.3)	ddPCR	Not detected	Detected (0.14)
4447	87	*JAK2* V617F (0.2)	ddPCR	Not available	Detected (0.13)

False-positive plasma genotyping due to clonal hematopoiesis (JAK2, some TP53, rare KRAS).

Clin Cancer Res. 2018 Sep 15;24(18):4352-4354.

Biological considerations of liquid biopsy

	Golden standard	
	Tumor positive	Tumor negative
ctDNA positive	True positive	**False positive:** **CHIP**
ctDNA negative	**False negative** **(non-shedder):** **Low grade/stage** **Respond to Tx** **Liquid for fusion**	True negative

Service Providers of Next-Generation Sequencing in Taiwan

Tissue

	ACT Genomics ACTDrug / ACTOnco	Foundation Medicine FoundationOneCDx	OncoDNA OncoDeep	Thermo Fisher Oncomine Focus/Comprehensive
Cost	NT 60,000 /NT 105,000	~NT 105,500	~ NT 200,000	~NT 90,000/~NT 160,000
Testing content	• 40 or 440 genes (optional) • CNV • RNA-Seq Fusion (31 genes)	• 324 genes • CNV • DNA-Seq Fusion (28 genes)	• 313 genes • CNV • RNA-SeqFusion (11 genes)	• 52 or 143 genes (optional) • CNV • RNA-Seq Fusion (22 or 23 genes)
Immunotherapy	• TMB, MSI • Resistance info. to IO (ACTOnco)	• TMB, MSI	• TMB, MSI, IHC • More slides required	N/A
Pathology evaluation	Y	N/A	Y	N/A
Mutation frequency	Y	N/A	Y	Y
FDA approved status	Application is ongoing (FDA IVD medical device, class II)	8 indications in lung cancer (FDA IVD medical device, class III)	N/A	4 indications in lung cancer (FDA IVD medical device, class III)

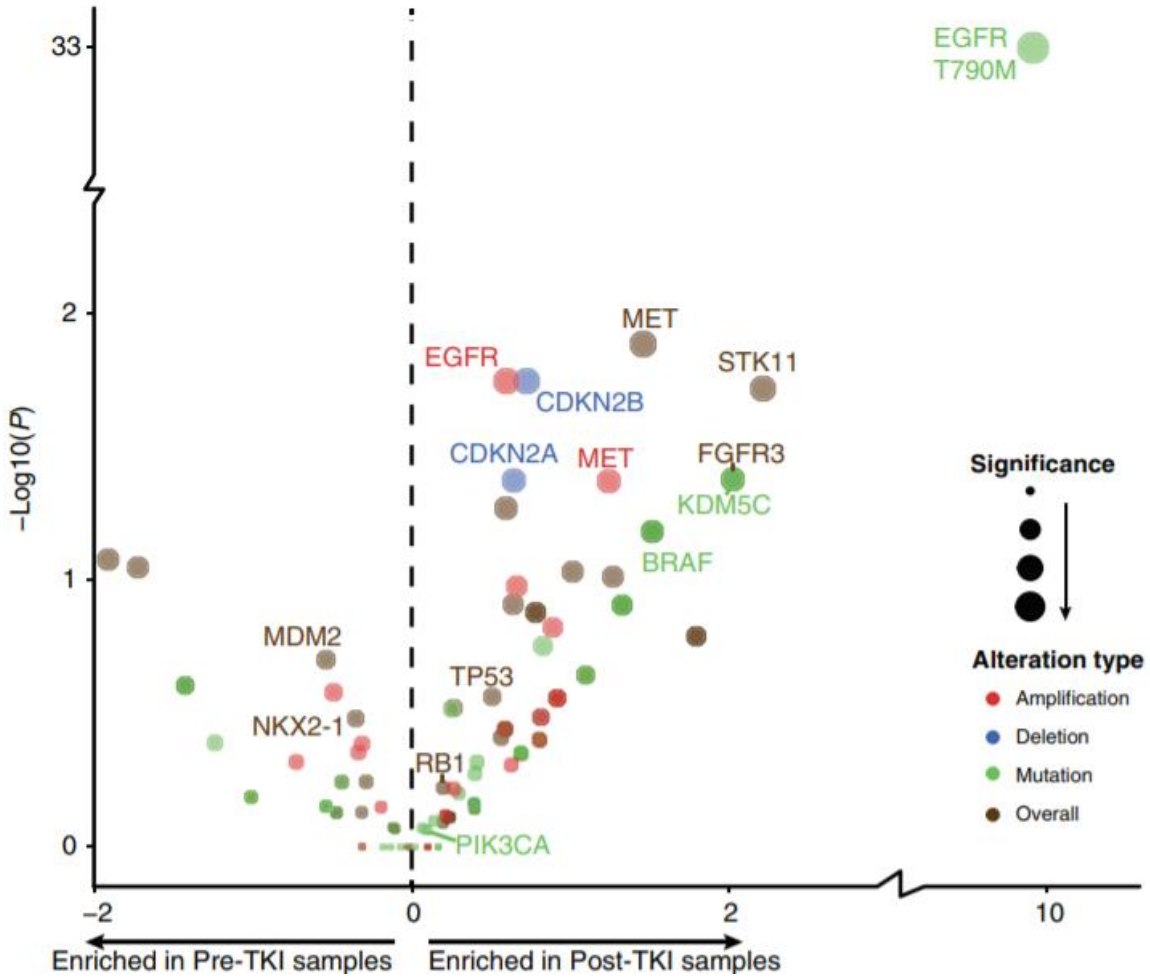
Plasma

	ACT Genomics ACTMonitor+	Foundation Medicine FoundationOne Liquid	OncoDNA OncoSELECT	Sofiva Drug+	CellMax OncoLBx	Guardant Health G360
Cost	• NT 30,000 (11 genes) • NT 60,000 (50 genes)	NT 105,500	NT 55,000	NT 59,900	NT 160,000	~NT 160,000
Testing content	• 11 or 50 genes (optional)	• 70 genes • CNV (partial) • Fusion (7 genes) • MSI	• 12 genes • CNV (partial) • Fusion (5 genes)	• 77 genes • CNV (partial) • Fusion (6 genes)	• 73 gene • CNV (partial) • Fusion (5 genes)	• 73 genes • CNV (partial) • Fusion (5 genes) • MSI
Effectiveness/ Turn around time	• Fast (local test) • 2 weeks	• Medium (overseas test) • 3-4 weeks	• Medium (overseas test) • 3-4 weeks	• Medium-Fast (local test) 2-4 weeks	• Medium (overseas test) • 3-4 weeks	• Fast (overseas test) • 2 weeks

Clinical Application of NGS in lung cancer

- Druggable driver mutation not routinely checked in local lab.
- Copy number variation which can be effectively targeted.
- Genetic alterations affecting TKI or ICI responses.
- Tumor mutation burden as a biomarker of ICI.
- Tissue versus liquid based NGS.
- **NTUH experiences of NGS in EGFRm ADC PD after 1L TKI.**

Enrichment of genetic alterations after EGFR TKI treatment



Before (n=200) vs After (n=136):

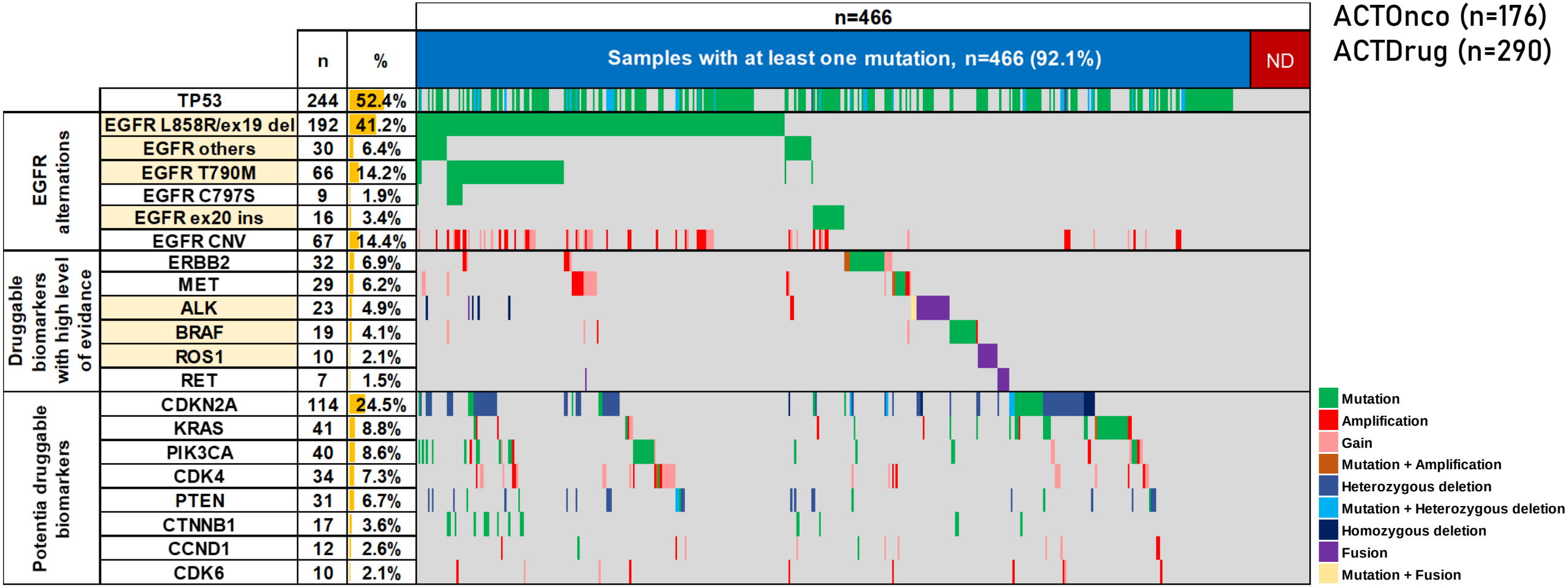
- **EGFR T790M**: 0% vs 52%
- **BRAF** alterations: 1% vs 5.1%
- CDKN2A loss: 12.5% vs 21.3%
- CDKN2B loss: 10.5% vs 19.9%
- EGFR amp: 23% vs 35%
- FGFR3 alteration: 0.5% vs 3.7%
- **MET amp**: 2% vs 6%

Acquired resistance in paired cases (n=38):

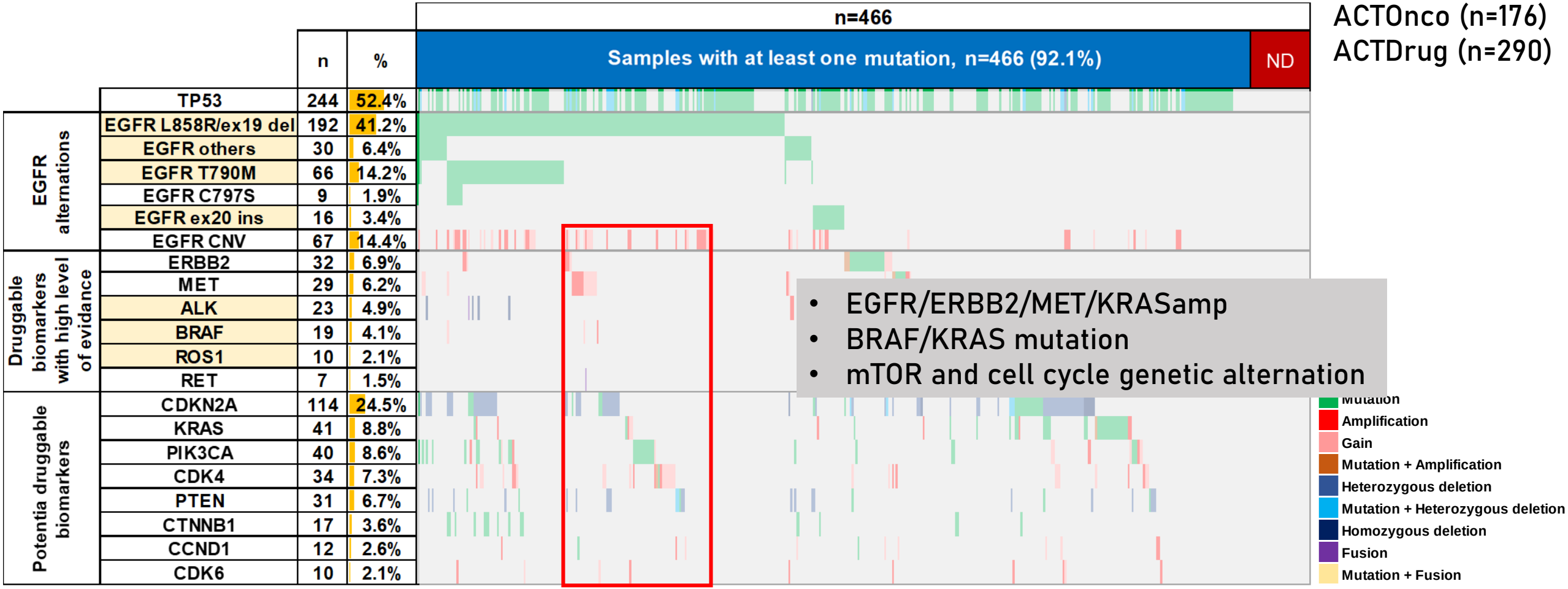
- **T790M**: 16/38 (42%)
- EGFR amp: 6/38 (16%)
- **MET amp**: 3/38 (8%)
- ERBB2 amp: 2/38 (5%)

Helena A. Yu et al. CCR 2018

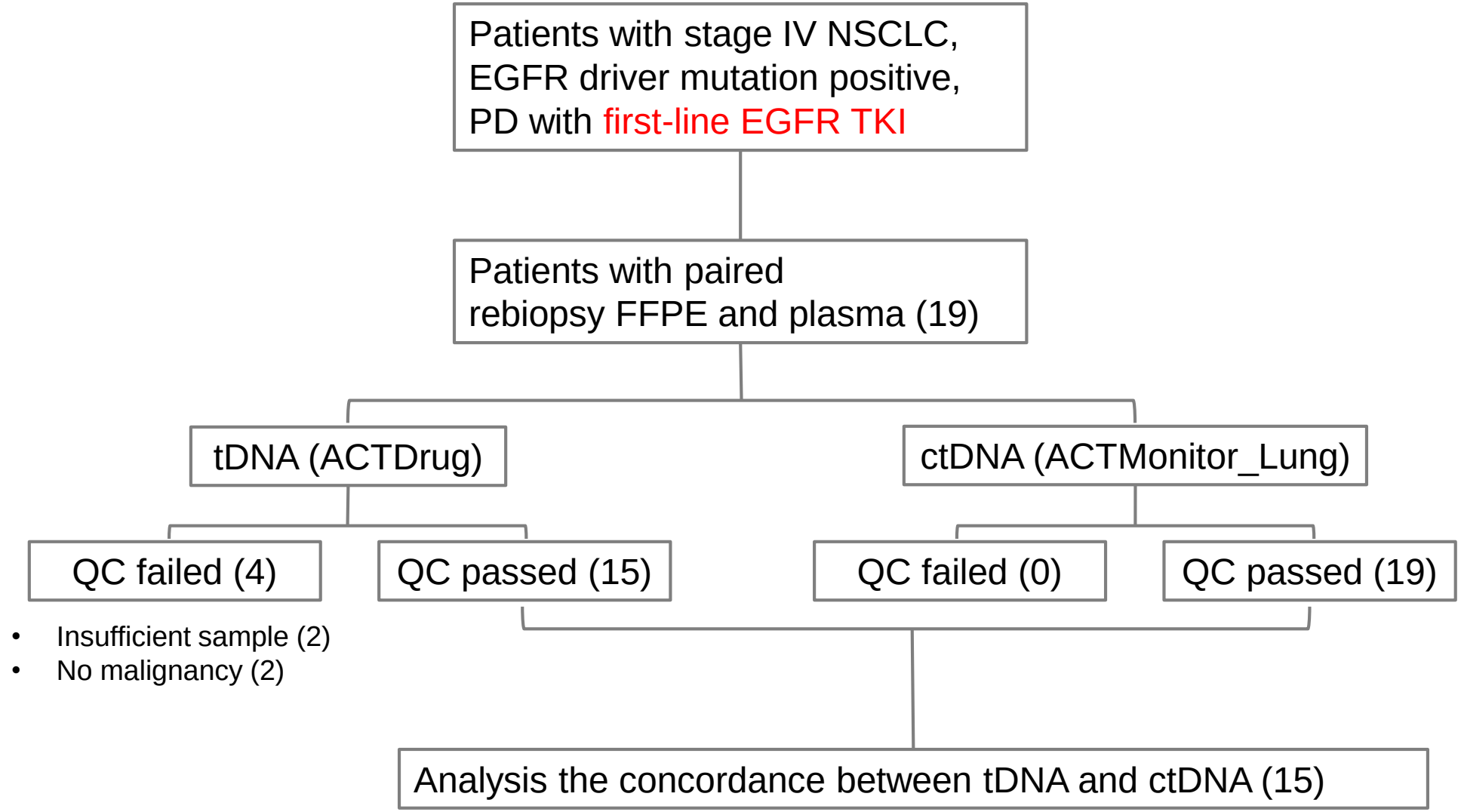
The Real World Experience of Lung Cancer (total cases)



The EGFR TKI Resistance Mechanism **other than T790M**

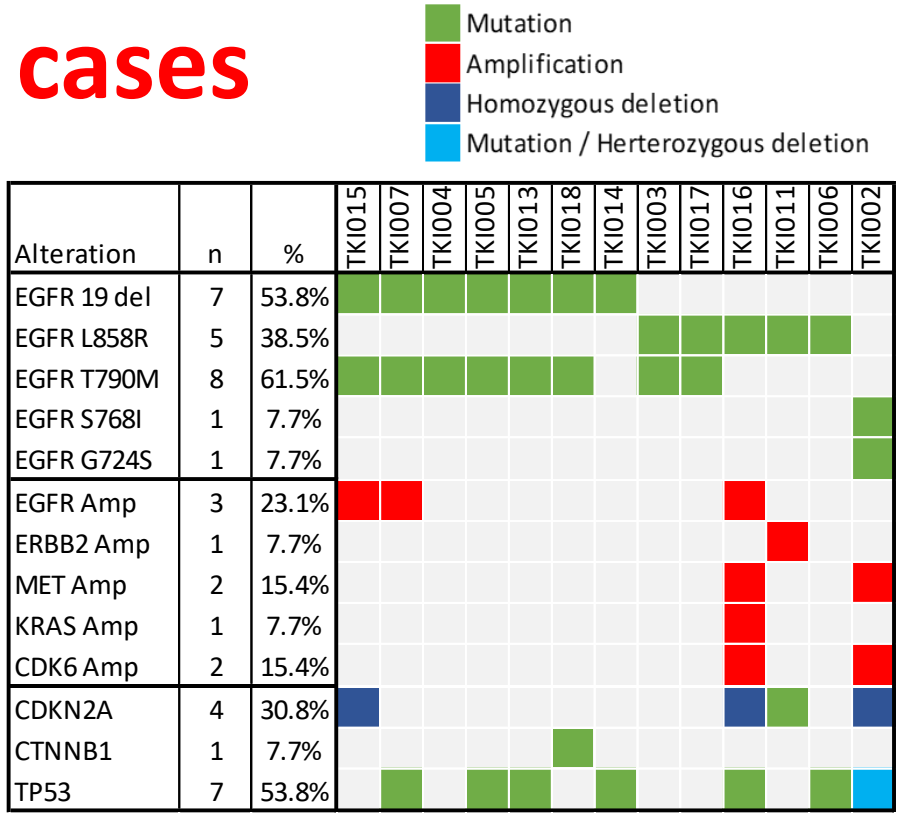


Study Design



The Results of ACTDrug, 13 cases

Patient ID	Tumor purity	Macrodissection	ACTDrug, SNV/InDel	ACTDrug, CNV	Treatment
TKI016	65%	No	EGFR L858R TP53 R181P	EGFR 6.5 CDK6 3.5 MET 5.5 CDKN2A 1 KRAS 5.5	Gefitinib
TKI002	60%	No	EGFR S768I / G724S TP53 D259Y	TP53 1 CDK6 3.5 MET 4.5 CDKN2A 0	Afatinib / Avastin
TKI015	55%	No	EGFR 19 del / T790M	EGFR 5.5 CDKN2A 1	Erlotinib
TKI018	55%	No	EGFR 19 del / T790M CTNNB1 S37C		Erlotinib
TKI004	40%	No	EGFR 19 del / T790M	CDKN2A 0	Gefitinib
TKI007	40%	No	EGFR 19 del / T790M TP53 D208V	EGFR 7.5	Afatinib
TKI014	35%	Yes	EGFR 19 del TP53 C242fs		Gefitinib
TKI011	25%	No	EGFR L858R CDKN2A P81L	ERBB2 24.5	Gefitinib
TKI003	15%	Yes	EGFR L858R / T790M		Erlotinib
TKI013	15%	No	EGFR 19 del/ T790M TP53 Y163C		Gefitinib
TKI017	15%	Yes	EGFR L858R / T790M		Erlotinib
TKI006	10%	Yes	EGFR L858R TP53 R209fs		Erlotinib
TKI005	5%	Yes	EGFR 19 del / T790M TP53 H179Q		Afatinib



1L EGFR TKI:
- Gefitinib: 5
- Erlotinib: 5
- Afatinib: 3

Resistance:
- **T790M: 8 of 13**
- ERBB2 amp: 1 of 13
- MET amp: 2 of 13
- CDK6 amp: 2 of 13

Macrodissection may Overcome the Sample with **Low Tumor Purity**

Patient ID	Tumor purity	Macrodissection	EGFR driver mut. (AF%)	EGFR T790M (AF%)
TKI016	65%	No	L858R (63.2%)	ND
TKI002	60%	No	S768I (49.5%) G724S (45.9%)	ND
TKI015	55%	No	19 del (83.2%)	T790M (21.5%)
TKI018	55%	No	19 del (31.6%)	T790M (7.2%)
TKI004	40%	No	19 del (50.6%)	T790M (9.3%)
TKI007	40%	No	19 del (76.5%)	T790M (31.1%)
TKI014	35%	Yes	19 del (54.2%)	ND
TKI011	25%	No	L858R (14.0%)	ND
TKI003	15%	Yes	L858R (28.9%)	T790M (28.2%)
TKI013	15%	No	19 del (9.8%)	T790M (6.6%)
TKI017	15%	Yes	L858R (24.8%)	T790M (13.9%)
TKI006	10%	Yes	L858R (35.1%)	ND
TKI005	5%	Yes	19 del (51.4%)	T790M (5.9%)

Total cases: 13

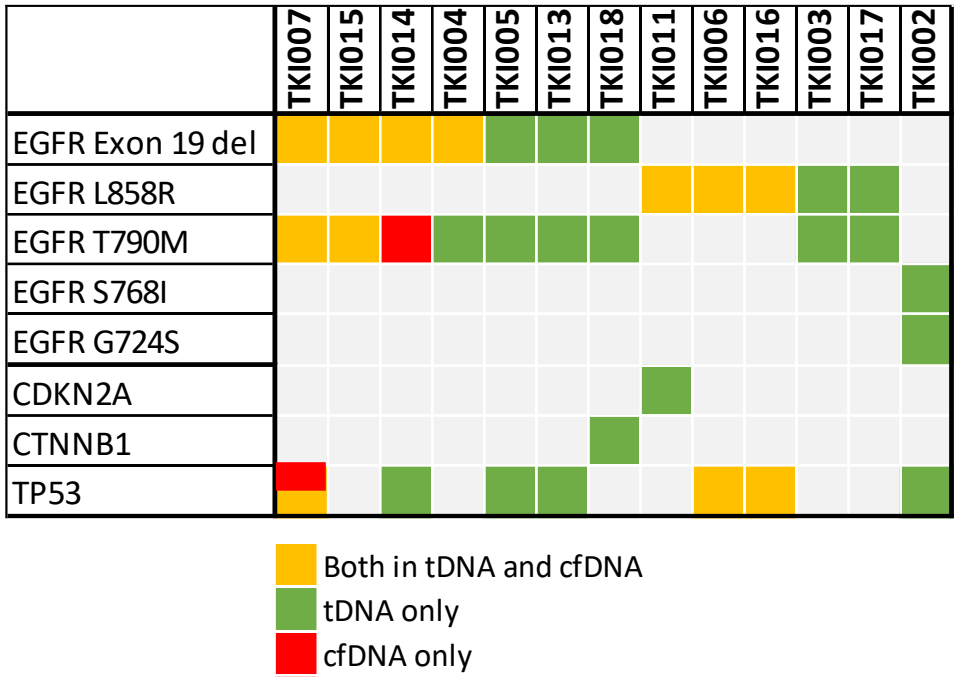
Tumor purity≥30%: 7

Tumor purity<30%: 6

→Macrodissection Yes: 4
No: 2

The Concordance between ACTDrug (tissue) and ACTMonitor_Lung (liquid)

Patient ID	ACTDrug, SNV/InDel	ACTMonitor_Lung	cfDNA Amount (ng)	T	N	M
TKI016	EGFR L858R TP53 R181P	EGFR L858R TP53 R181P	352.8	T4	N3	M1a
TKI015	EGFR 19 del / T790M	EGFR 19 del / T790M	246	T4	N3	M1c
TKI014	EGFR 19 del TP53 C242fs	EGFR 19 del / T790M	216	T2	N0	M1c
TKI004	EGFR 19 del / T790M	EGFR 19 del	92.5	T2	N3	M1b
TKI006	EGFR L858R TP53 R209fs	EGFR L858R TP53 R209fs	82.8	T4	N0	M1a
TKI007	EGFR 19 del / T790M TP53 D208V	EGFR 19 del / T790M TP53 D208V / R273C	72.8	T4	N2	M1a
TKI003	EGFR L858R / T790M	No variant	72	T1	N1	M0
TKI018	EGFR 19 del / T790M CTNNB1 S37C	No variant	32.5	T4	N0	M1c
TKI005	EGFR 19 del / T790M TP53 H179Q	No variant	31	T4	N2	M1b
TKI013	EGFR 19 del/ T790M TP53 Y163C	No variant	29.9	T3	N0	M1b
TKI002	EGFR S768I / G724S TP53 D259Y	No variant	22	T2	N0	M1a
TKI017	EGFR L858R / T790M	No variant	22	T1	N3	M1a
TKI011	EGFR L858R CDKN2A P81L	EGFR L858R	13	T1	N1	M1b



For EGFR driver mut. by patients

Sensitivity: 53.8%

For all mutations

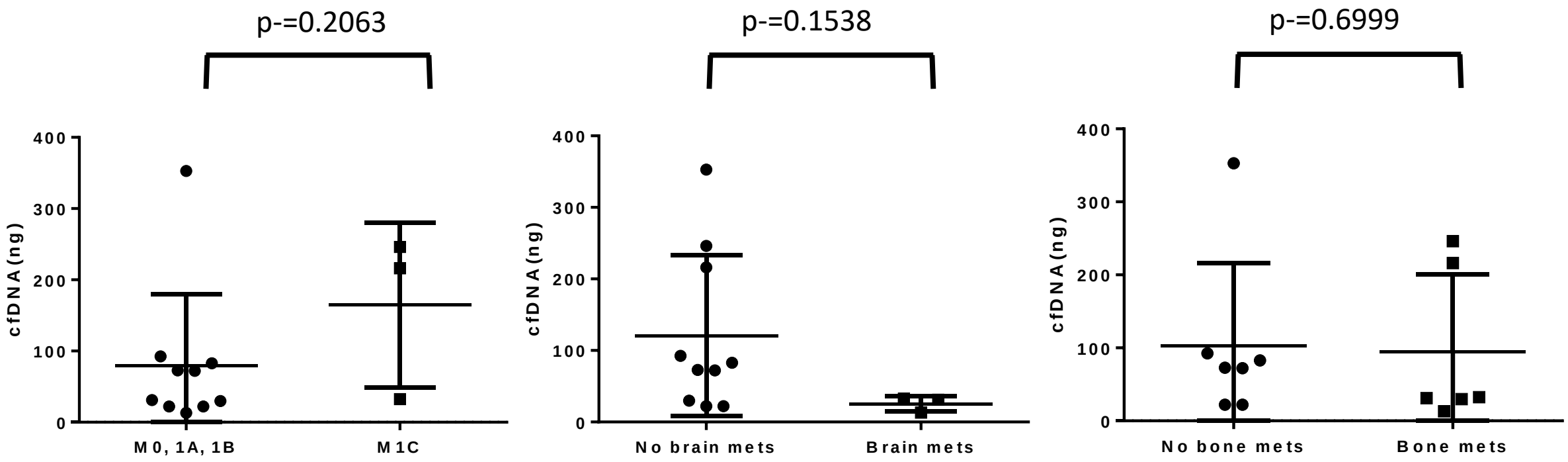
Sensitivity : 40.6%

The Amount of cfDNA Correlated with the Sensitivity of Liquid NGS Test

ACT GENOMICS

Patient ID	ACTDrug, SNV/InDel	ACTMonitor_Lung	cfDNA (ng)	T	N	M
TKI016	EGFR L858R (63.2%) TP53 R181P (42.9%)	EGFR L858R (54.08%) TP53 R181P (32.65%)	352.8	T4	N3	M1a
TKI015	EGFR 19 del (83.2%) T790M (21.5%)	EGFR 19 del (39.91%) T790M (5.20%)	246	T4	N3	M1c
TKI014	EGFR 19 del (54.2%) TP53 C242fs (18.9%)	EGFR 19 del (49.74%) T790M (17.82%)	216	T2	N0	M1c
TKI004	EGFR 19 del (50.6%) T790M (9.3%)	EGFR 19 del (1.43%)	92.5	T2	N3	M1b
TKI006	EGFR L858R (35.1%) TP53 R209fs (19.1%)	EGFR L858R (0.57%) TP53 R209fs (0.24%)	82.8	T4	N0	M1a
TKI007	EGFR 19 del (76.5%) T790M (31.1%) TP53 D208V (27.4%)	EGFR 19 del (33.76%) T790M (8.71%) TP53 D208V (4.95%) R273C (1.81%)	72.8	T4	N2	M1a
TKI003	EGFR L858R (28.9%) T790M (28.2%)	No variant	72	T1	N1	M0
TKI018	EGFR 19 del (31.6%) T790M (7.2%)	No variant	32.5	T4	N0	M1c
TKI005	EGFR 19 del (51.4%) T790M (5.9%) TP53 H179Q (55.5%)	No variant	31	T4	N2	M1b
TKI013	EGFR 19 del (9.8%) T790M (6.6%) TP53 Y163C (7.4%)	No variant	29.9	T3	N0	M1b
TKI002	EGFR S768I (49.5%) G724S (45.9%) TP53 D259Y (59.9%)	No variant	22	T2	N0	M1a
TKI017	EGFR L858R (24.8%) T790M (13.9%)	No variant	22	T1	N3	M1a
TKI011	EGFR L858R (14.0%) CDKN2A P81L (16.1%)	EGFR L858R (1.52%)	13	T1	N1	M1b

Tumor burden (at test) and the detected concentration of cfDNA



The Sensitivity of Liquid Biopsy in Clinical Trials

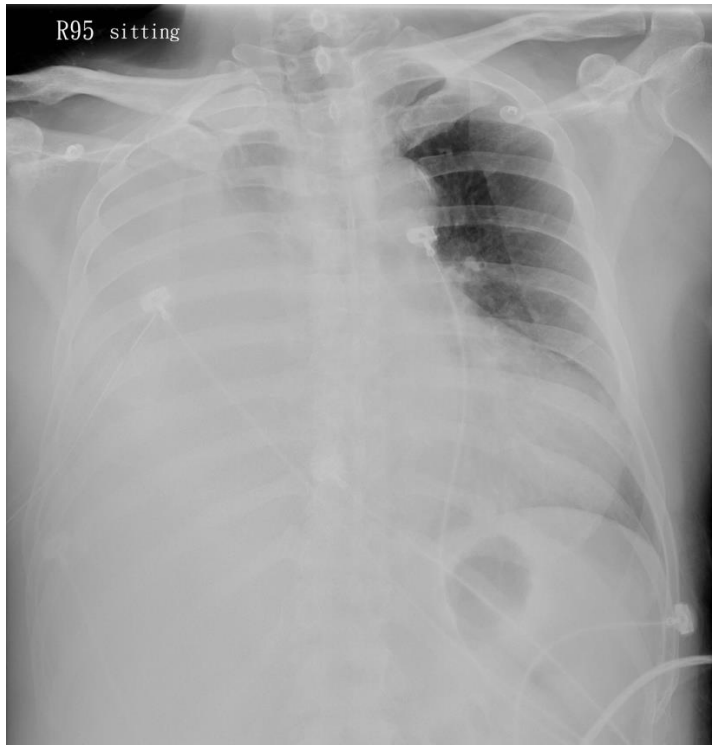
Trial	Cancer	Mutation	Method	Case (n)	Sensitivity	Specificity	Reference
AURA	Advanced NSCLC	EGFR T790M	BEAMing	216	70.3%	69.0%	Oxnard GR, et al. J Clin Oncol. 2016
AURA Extension and AURA2	Advanced NSCLC	EGFR T790M	cobas v2	551	61%	79%	Jenkins S, et al. J Thorac Oncol. 2017
AURA3	Advanced NSCLC	EGFR T790M	cobas v2	359	51.3%	NA	Mok TS, et al. N Engl J Med. 2017
BELLE-2	Advanced HR+/Her2- BC	15 PIK3CA activating mutations	BEAMing	582	71%	79.2%	Baselga J, et al. Lancet Oncol. 2017
SANDPIPER	Advanced HR+/Her2- BC	PIK3CA activating mutations	Foundation OneLiquid	508	66.7%	NA	Vasan N, et al. Science. 2019

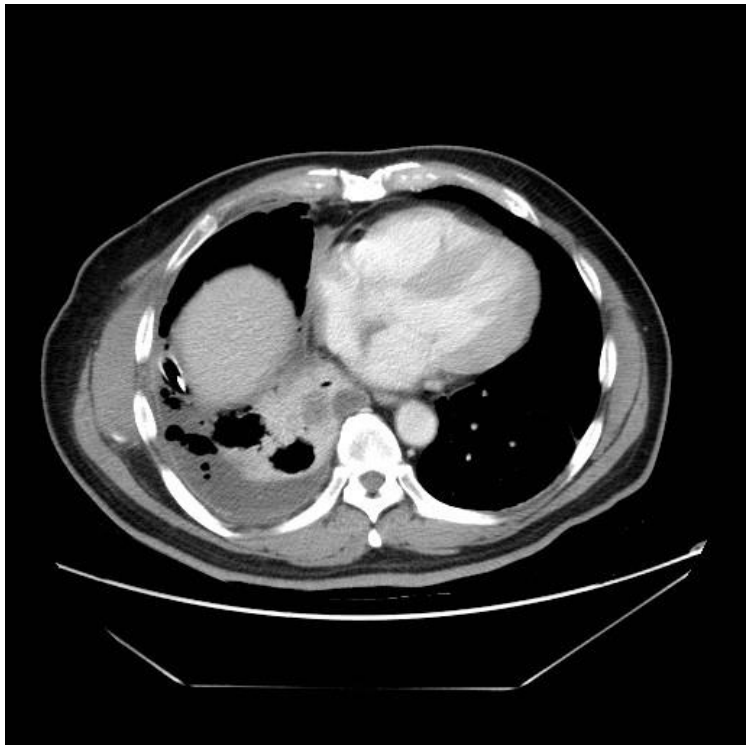
~30% false-negative rate for variant detection

Case 5

Alteration	n	%	TKI015	TKI007	TKI004	TKI005	TKI013	TKI018	TKI014	TKI003	TKI017	TKI016	TKI011	TKI006	TKI002
EGFR 19 del	7	53.8%													
EGFR L858R	5	38.5%													
EGFR T790M	8	61.5%													
EGFR S768I	1	7.7%													
EGFR G724S	1	7.7%													
EGFR Amp	3	23.1%													
ERBB2 Amp	1	7.7%													
MET Amp	2	15.4%													
KRAS Amp	1	7.7%													
CDK6 Amp	2	15.4%													
CDKN2A	4	30.8%													
CTNNB1	1	7.7%													
TP53	7	53.8%													

55 year-old male, never smoker
cT2aN0M1a, with malignant pleural effusion stage IVA
EGFR (MALDI-TOF)/ALK/ROS1/BRAF: WT





Bronchoscopic biopsy: not feasible for molecular test

檢查報告

Lung, labeled as RB10, biopsy, congestion with mild inflammation

The specimen submitted consists of six tissue fragments measuring up to 0.2 x 0.1 x 0.1 cm in size fixed in formalin.

Grossly, they are brown and soft.

All for section. Jar 0

Microscopically, it shows congestion with mild inflammation and mild inflammatory cell infiltration.
Immunohistochemically, no carcinoma can be seen in the cytokeratin and TTF-1 stains.

NTUH Liquid biopsy (plasma EGFR Cobas V2): EGFR S768I

FoundationOne® Liquid:

EGFR: G724S + S768I

CTNNB1 S45F

TP53: D259Y

EGFR Cobas V2

表 1 羅氏 EGFR 檢測設計可偵測以下突變

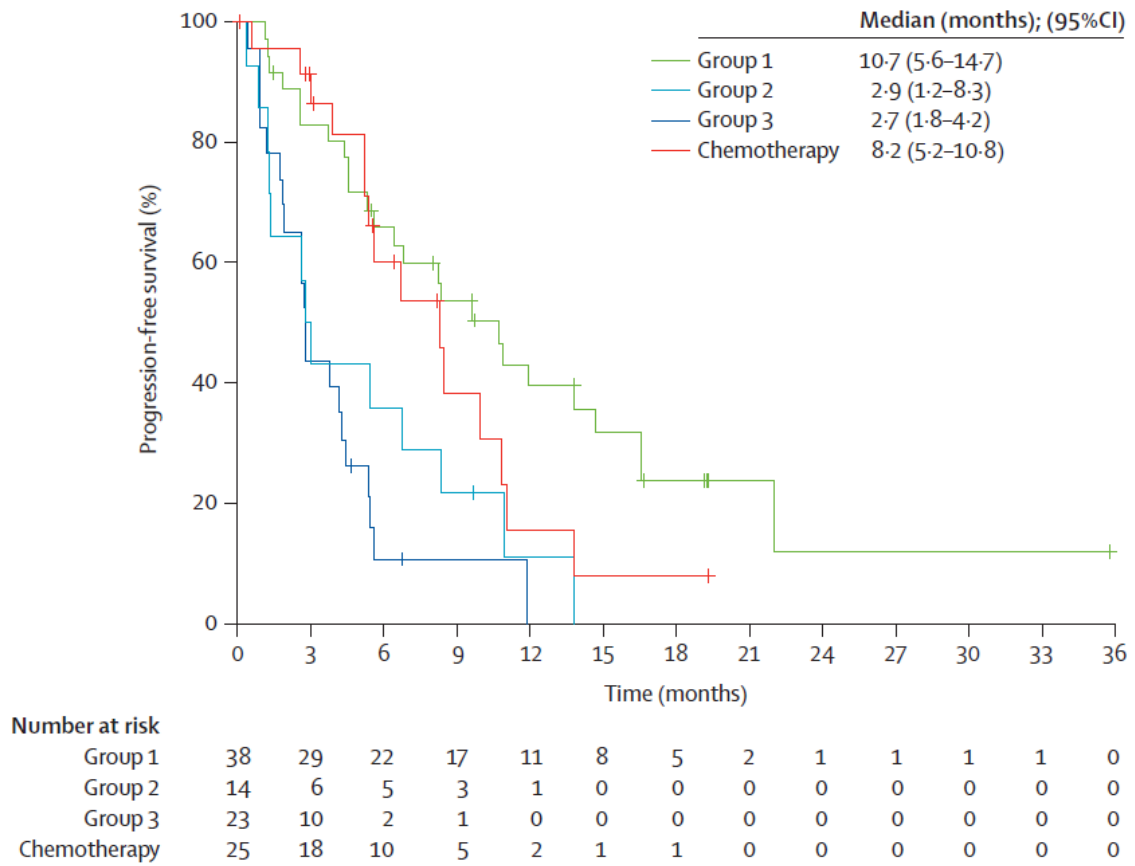
Exon	EGFR Mutation Group	EGFR 核酸序列	COSMIC ID[6]
Exon 18	G719X	2156G>C	6239
		2155G>A	6252
		2155G>T	6253
Exon 19	Ex19Del	2240_2251del12	6210
		2239_2247del9	6218
		2238_2255del18	6220
		2235_2249del15	6223
		2236_2250del15	6225
		2239_2253del15	6254
		2239_2256del18	6255
		2237_2254del18	12367
		2240_2254del15	12369
		2240_2257del18	12370
		2239_2248TTAAGAGAAG>C	12382
		2239_2251>C	12383
		2237_2255>T	12384
		2235_2255>AAT	12385
		2237_2252>T	12386
		2239_2258>CA	12387
		2239_2256>CAA	12403
		2237_2253>TTGCT	12416
		2238_2252>GCA	12419
		2238_2248>GC	12422
		2237_2251del15	12678
		2236_2253del18	12728
		2235_2248>AATTC	13550
		2235_2252>AAT	13551
		2235_2251>AATTC	13552
		2253_2276del24	13556
		2237_2257>TCT	18427
		2238_2252del15	23571
		2233_2247del15	26038

- Uncommon mutations: Cobas V2 只能驗 **G719X, S768I and L861Q.**
- Other potential **TKI-sensitive EGFR mutations** are not detected:
  - **Exon 18: E709 complex, L747P, G724S**
  - **Exon 19: Exon 19 insertion**
  - **Exon 20: A763_Y764 insFQEA**
  - **EGFR-KDD**
  - Others not reported…

羅氏 EGFR 基因突變檢驗 v2 仿單 https://www.fda.gov.tw › MLMS › ShowFile

Clinical activity of afatinib in patients with advanced non-small-cell lung cancer harbouring uncommon *EGFR* mutations: a combined post-hoc analysis of LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6

James C-H Yang, Lecia V Sequist*, Sarayut Lucien Geater, Chun-Ming Tsai, Tony Shu Kam Mok, Martin Schuler, Nobuyuki Yamamoto, Chong-Jen Yu, Sai-Hong I Ou, Caicun Zhou, Daniel Massey, Victoria Zazulina, Yi-Long Wu*



38 group 1: point mutations and duplications, or both, in exons 18–21
12 Leu861Gln alone
8 Gly719Xaa alone
5 Gly719Xaa + Ser768Ile
3 Gly719Xaa + Leu861Gln
2 Glu709Gly or Val + Leu858Arg
2 Ser768Ile + Leu858Arg
1 Ser768Ile alone
1 Leu861Pro alone
1 Pro848Leu alone
1 Arg776His + Leu858Arg
1 Leu861Gln + del19
1 Lys739_1744dup6

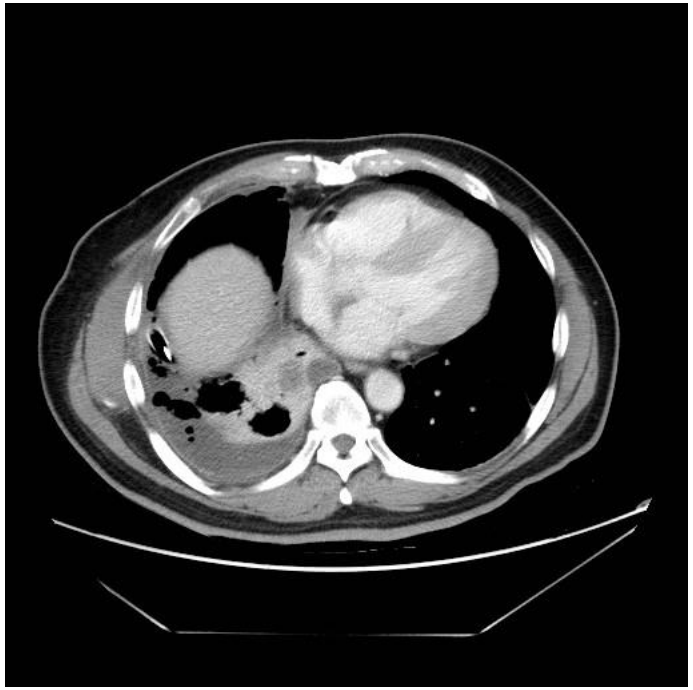
第二代 TKI 對於**uncommon mutation**的效果，較第一代TKI好

Median PFS **10-11個月**

Afatinib is approved by FDA for **G719X, L861Q, and S768I EGFR mutation.**

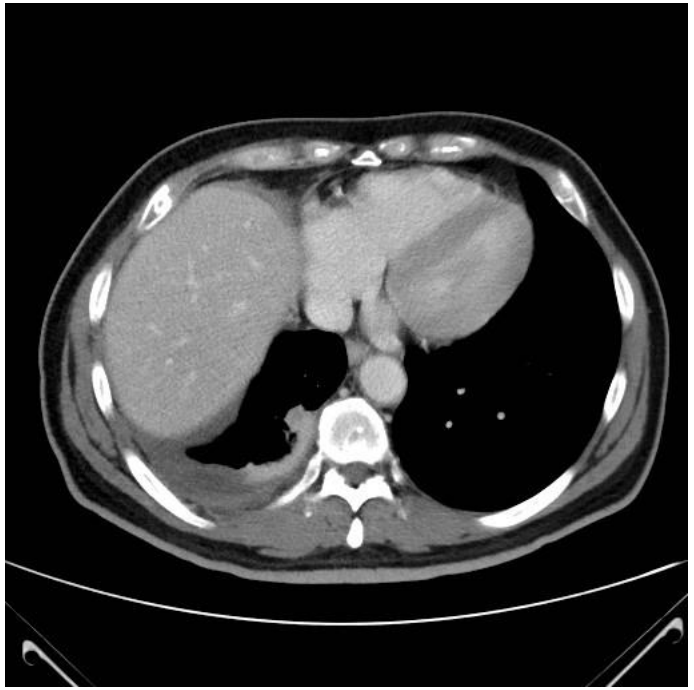
Yang, et al. Lancet Oncol 2016

Good partial response to afatinib



2018/09

Afatinib



2018/11

ARTICLE

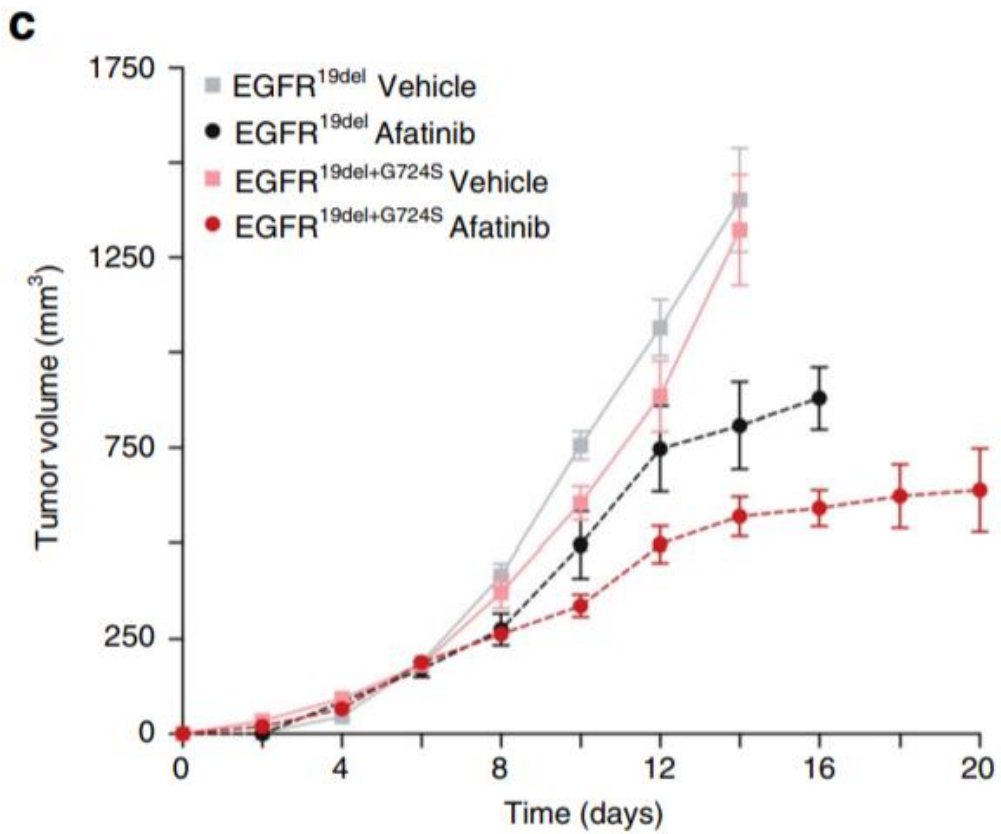
DOI: 10.1038/s41467-018-07078-0 OPEN

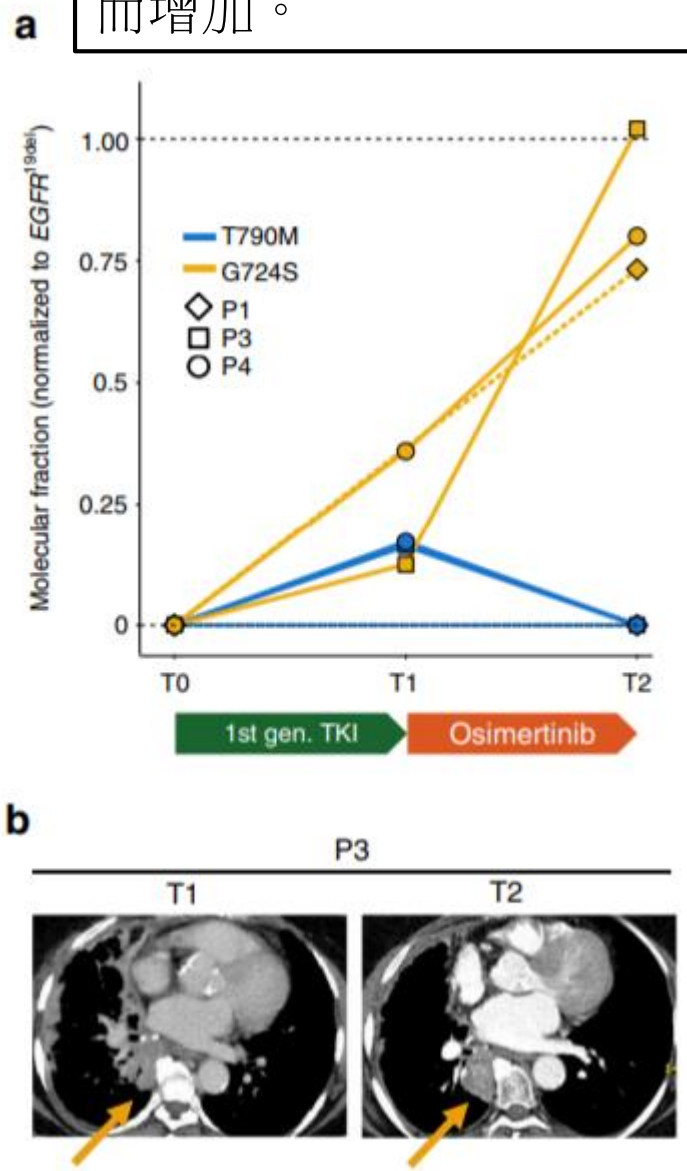
Overcoming *EGFR*^{G724S}-mediated osimertinib resistance through unique binding characteristics of second-generation EGFR inhibitors

Jana Fassunke et al.#

Plasma cfDNA中**G724S**的比率，隨著1G->3G TKI的使用而增加。

產生新的**G724S** 合併**失去T790M突變**，可能對**afatinib** 治療有效





Fassunke et al. Nature Communication 2018

Application of NGS in lung cancer treatment

Driver mutations

- Explore more driver mutations not checked at local lab.

Eg: EGFR-KDD, EGFR exon20ins, BRAF, ROS1, RET, NF1, NTRK…

- Give appropriate available targeted therapy

Eg:
Afatinib for EGFR-KDD, G724S
Crizotinib for MET exon 14
Dabra/Trame for BRAF V600E
……

- Enroll in suitable clinical trial

Eg:
MET exon 14- > VISION
Exon 20ins -> TAK-788
KRAS G12C -> AMG-510
……

Copy number variation

- **METamp** as a resistant mechanism to EGFR TKI responding to crizotinib
- Clinical trials ?
- ERBB2? B2M? Others ?

Tumor mutation burden (TMB)

- Relatively consistent data among each platforms
- Predict **mono-ICI** rather than ICI-chemo combo or dual-ICI
- In the era of combination therapy, the future of TMB ??
  I don't know at this time…

Thanks for your Listening and Comments！

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National Taiwan University Hospital

Chingyao.yang@gmail.com