Diagnosis and monitoring of oncogene addicted cancers

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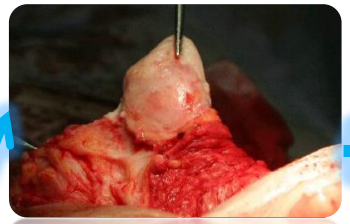
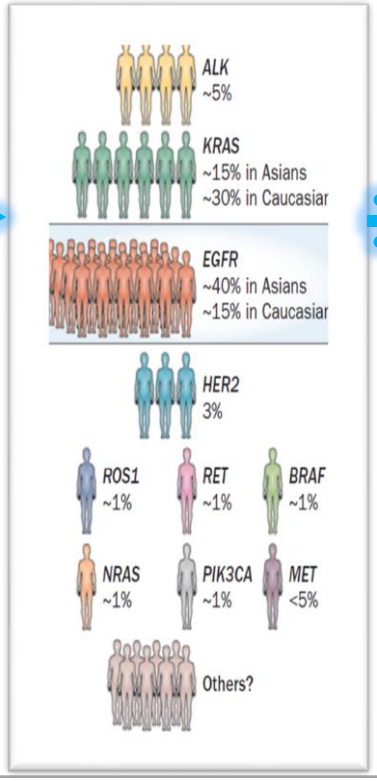
Management of Lung Cancer

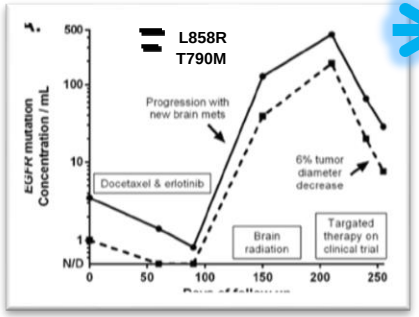
Heterogeneity in LC pts.



tissue

liquid



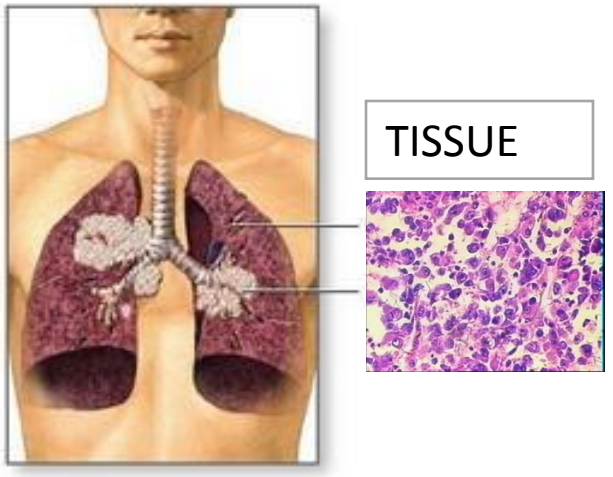


OS

Detection → Diagnosis → Treatment → Monitoring

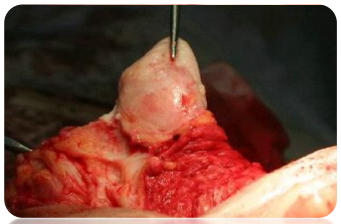
The tissue is the issue.
BUT Challenging

- **Challenging:**
  - ***Invasive***--high risk, unpleasant, painful;
  - ***Selection bias***--Intra-tumoral heterogenity, primary versus metastase;
  - ***Re-biopsy difficult***--monitoring treatment and resistance;
  - ***Insufficient–*** too small to more molecular analysis;



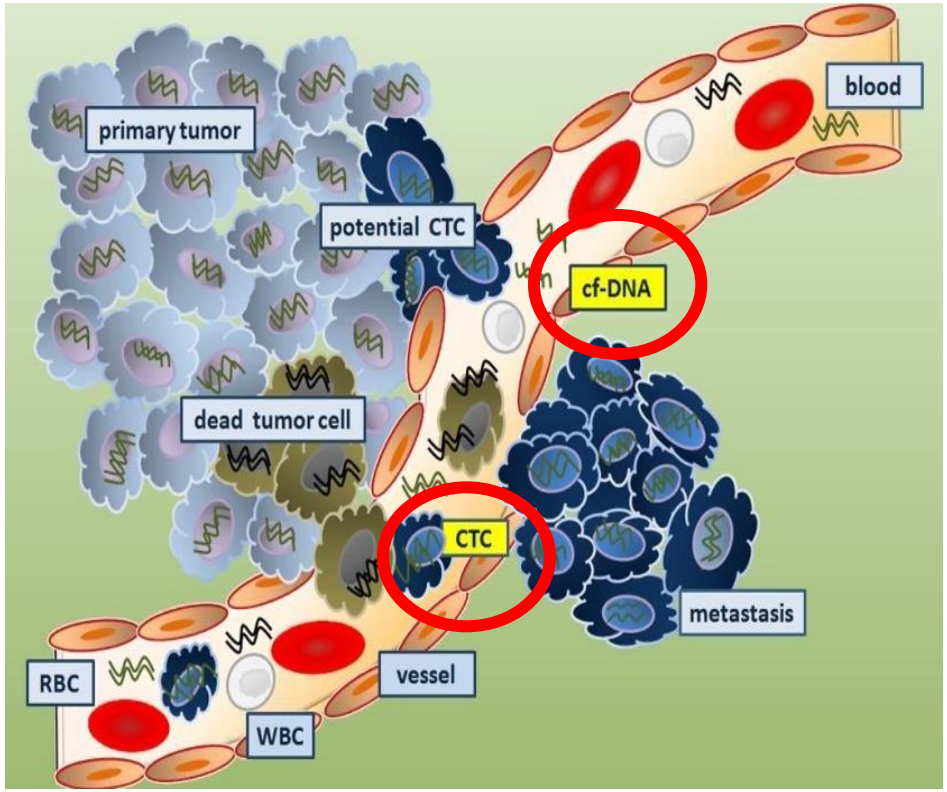
Liquid biopsy may be the Solution

- Simple and less invasive
- More representative for the overall disease, avoids intra-tumoral and inter-metastatic tumor heterogeneity associated with tissue.
- Performed coherence for monitoring therapy and dynamic changes in the tumor.



Liquid biopsy

- **ctDNA**
  - **Low concentration: average 17 ng/ml plasma in advanced-stage cancers**
  - **Low proportion: tumor DNA can range between 0.01% and 93%**
- **CTC**
  - **range 0~several thousands**



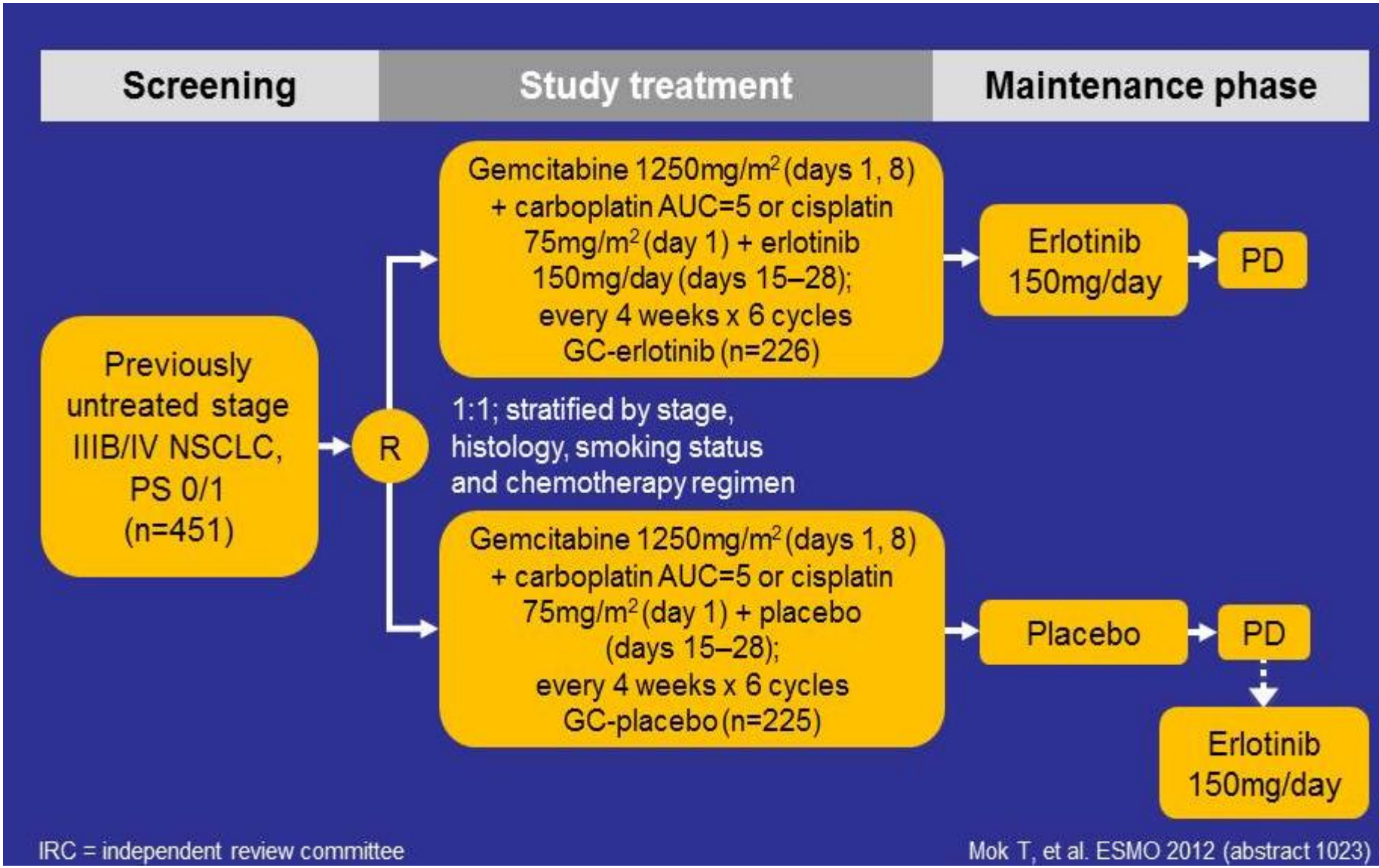
Crowley, E. *et al. Nat. Rev. Clin. Oncol. 2013, 10: 472–484*

Applications of ctDNA

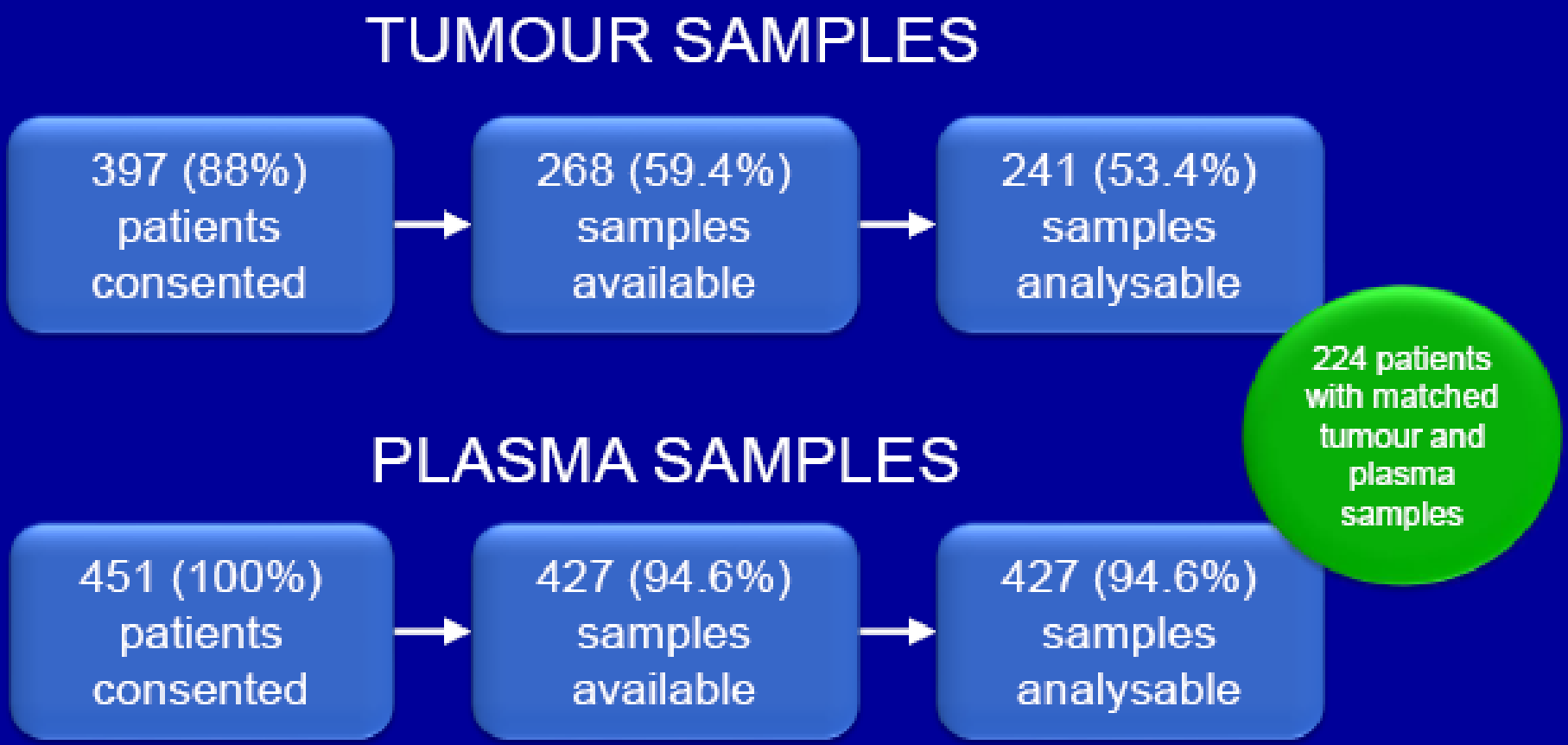
Application 1

Molecular Diagnosis & Predicting Efficacy

FASTACT-2 study



t*EGFR* and p*EGFR* mutation analysis of FASTACT-2 (ASCO 2013)



EGFR = epidermal growth factor receptor; tEGFR = tumour EGFR; pEGFR = plasma EGFR

Wu YL, et al. Lancet Oncol 2013;14:777–86
Mok T, et al. J Clin Oncol 2013;31 (suppl 15 pt I):Abs 8021

Concordance between tumour and plasma samples using the cobas® 4800 system

- A total of 224 patients had both tumour and baseline plasma samples with available *EGFR* mutation analysis results
  - sensitivity: 77% (69/90)
  - specificity: 96% (129/134)
  - positive predictive value: 93% (69/74)
  - negative predictive value: 86% (129/150)
  - overall concordance: 88% (198/224)

PFS

pEGFR+ tEGFR+ pEGFR- tEGFR-

Figure 2. PFS of (a) p-EGFR Mut+ and (b) t-EGFR Mut+ Patients by Treatment Arm

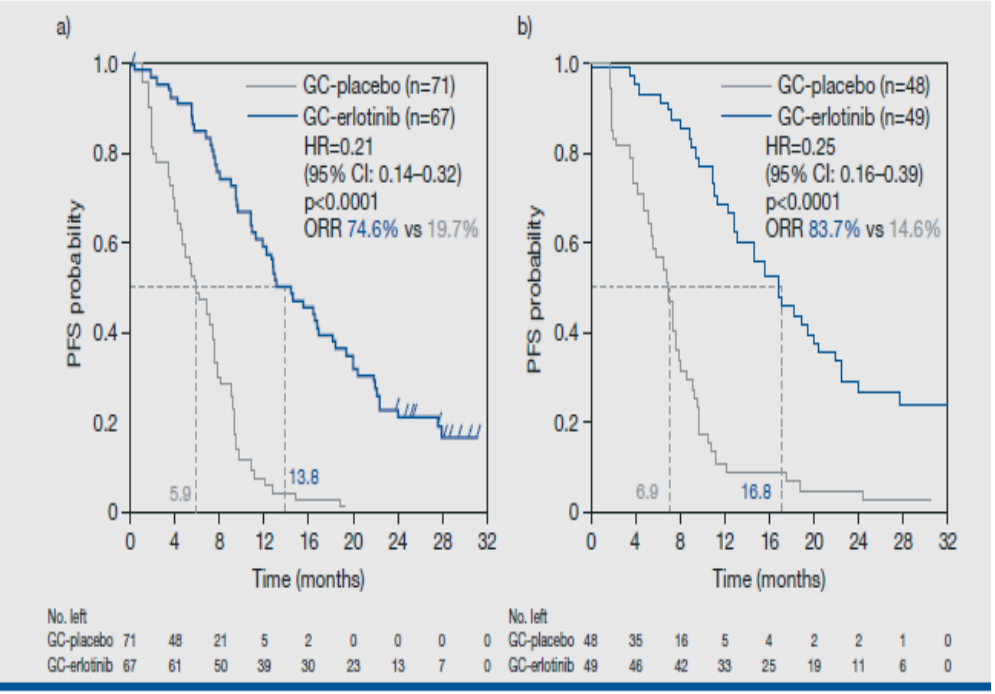
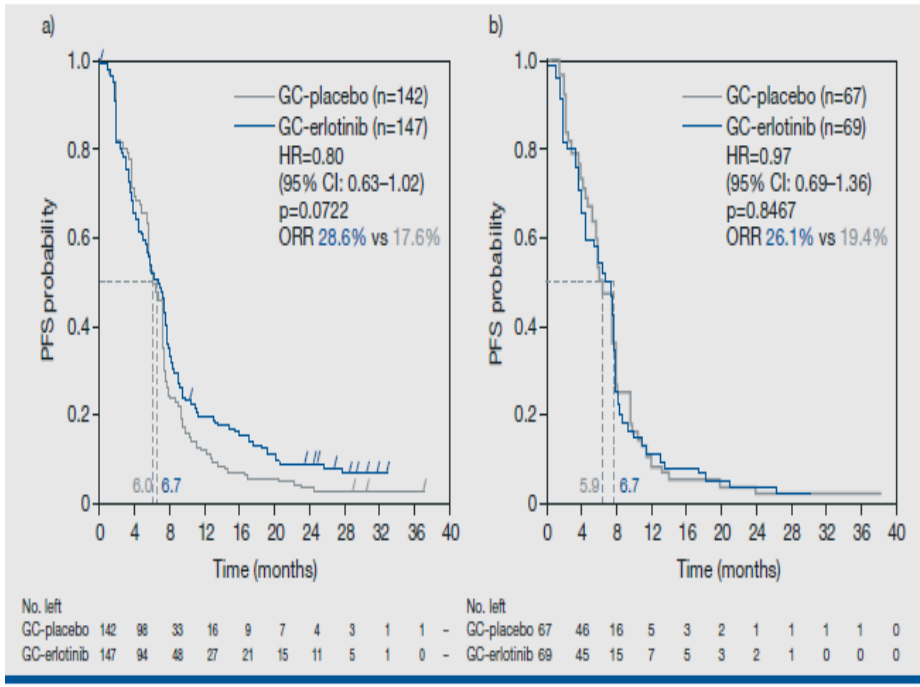


Figure 3. PFS of (a) p-EGFR Mut– and (b) t-EGFR Mut– Patients by Treatment Arm



Other techniques detecting plasma EGFR mutation

ARMS

EGFR mutation		serum +	serum -	Total
tissue	+	**6**	**2**	8
	-	**1**	**33**	34
Total		7	35	**42**

Sensitivity : 75%; Specificity: 97%.

（ps.：Tissue using Sequencing ）

Kimura et al. Br J Cancer 2007; 97(6): 778-784

ddPCR

EGFR mutation		serum +	serum -	Total
tissue	+	**15**	**4**	19
	-	**0**	**16**	16
Total		15	20	**35**

Sensitivity : 79%; Specificity : 100%.

（ps.Tissue using ddPCR and Sequencing ）

Yung et al. Clin Cancer Res 2009;15(6): 2076-2084

PCR/dHPLC

EGFR mutation		plasma +	plasma -	Total
tissue	+	**63**	**14**	77
	-	**16**	**137**	153
Total		79	151	**230**

Sensitivity : 82%; Specificity : 90%.

Bai et al. J Clin Oncol 2009; 27:2653-2659

2015 年 02 月 13 日

吉非替尼片说明书

请仔细阅读说明书并在医师指导下使用。

[药品名称]

通用名称：吉非替尼片
商品名称：易瑞沙
英文名称：Gefitinib Tablets
汉语拼音：Jifeitini Pian

[注意事项]

当考虑本品用于晚期或转移性 NSCLC 患者的一线治疗时，推荐对所有患者的肿瘤组织进行 EGFR 突变检测。如果肿瘤标本不可评估，则可使用从血液（血浆）标本中获得的循环肿瘤 DNA（ctDNA）。

只能使用经论证可用于测定肿瘤或 ctDNA 的 EGFR 突变状态的检测方法，检测方法须稳定、可靠并且灵敏，以避免出现假阴性或假阳性的测定结果。

非吸烟、组织学类型为腺癌、女性或亚裔更可能从本品的治疗中获益。这些临床特点也和较高的肿瘤 EGFR 突变阳性率相关。

4.4 Special warnings and precautions for use

When considering the use of IRESSA as a treatment for locally advanced or metastatic NSCLC, it is important that EGFR mutation assessment of the tumour tissue is attempted for all patients. If a tumour sample is not evaluable, then circulating tumour DNA (ctDNA) obtained from a blood (plasma) sample may be used.

Application 2

Detect resistant biomarkers

AURA study (AZD 9291)

- 72 paired FFPET/ctDNA samples
- Detected by cobas and BEAMing

	Cobas AS-PCR	BEAMing digital PCR
Exon 19 deletion assays		
Sensitivity	82% (22/27)	85% (22/26)
Specificity	97% (29/30)	97% (29/30)
L858R assay		
Sensitivity	88% (21/24)	88% (21/24)
Specificity	97% (31/32)	97% (31/32)
T790M assay		
Sensitivity	74% (31/42)	81% (33/41)
Specificity	70% (16/23)	61% (14/23)

*8 with no T790M tissue result, 16 with no 19del/L858R result

Thress K., et al. ASCO 2014

AURA study (AZD 9291)

Different results between tumor and plasma

	Tissue	Plasma	
Patient	cobas AS-PCR	BEAMing dPCR (% mutant)	cobas AS-PCR
1	Positive	Positive (0.021%)	Negative
2	Positive	Positive (0.048%)	Negative
3	Positive	Positive (0.064%)	Negative
4	Positive	Positive (0.202%)	Negative
5	Positive	Negative	Negative
6	Positive	Negative	Negative
7	Positive	Negative	Negative
8	Positive	Negative	Negative
9	Positive	Negative	Negative
10	Positive	Negative	Positive
11	Positive	Negative	Negative
12	Positive	Negative	Negative
13	Negative	Positive (0.025%)	Negative
14	Negative	Positive (0.027%)	Positive
15	Negative	Positive (0.056%)	Positive
16	Negative	Positive (0.060%)	Negative
17	Negative	Positive (0.063%)	Positive
18	Negative	Positive (0.340%)	Positive
19	Negative	Positive (0.344%)	Positive
20	Negative	Positive (0.491%)	Positive
21	Negative	Positive (1.113%)	Positive

'False' negatives by AS-PCR plasma

'False' positives by BEAMing

AURA study (AZD 9291)

Correlation between T790M status and efficacy of AZD9291

- T790M+：

  Tissue vs. Plasma： 62% vs. 63%

- T790M-:

  Tissue vs. Plasma: 26% vs. 29%

	Tissue		Plasma*	
	T790M +	T790M-	T790M +	T790M-
Response Rate (CR & PR)	62% (26/42)	26% (6/23)	63% (24/38)	29% (10/34)
Disease control Rate (CR, PR, & SD)	95% (40/42)	70% (16/23)	89% (34/38)	76% (26/34)

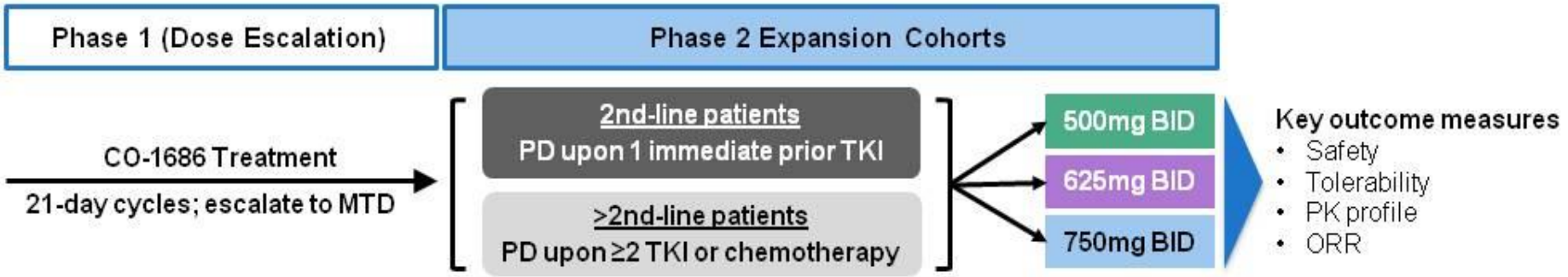
-April 2nd 2014 data cut off
-includes confirmed responses & responses awaiting confirmation

*AS-PCR assay

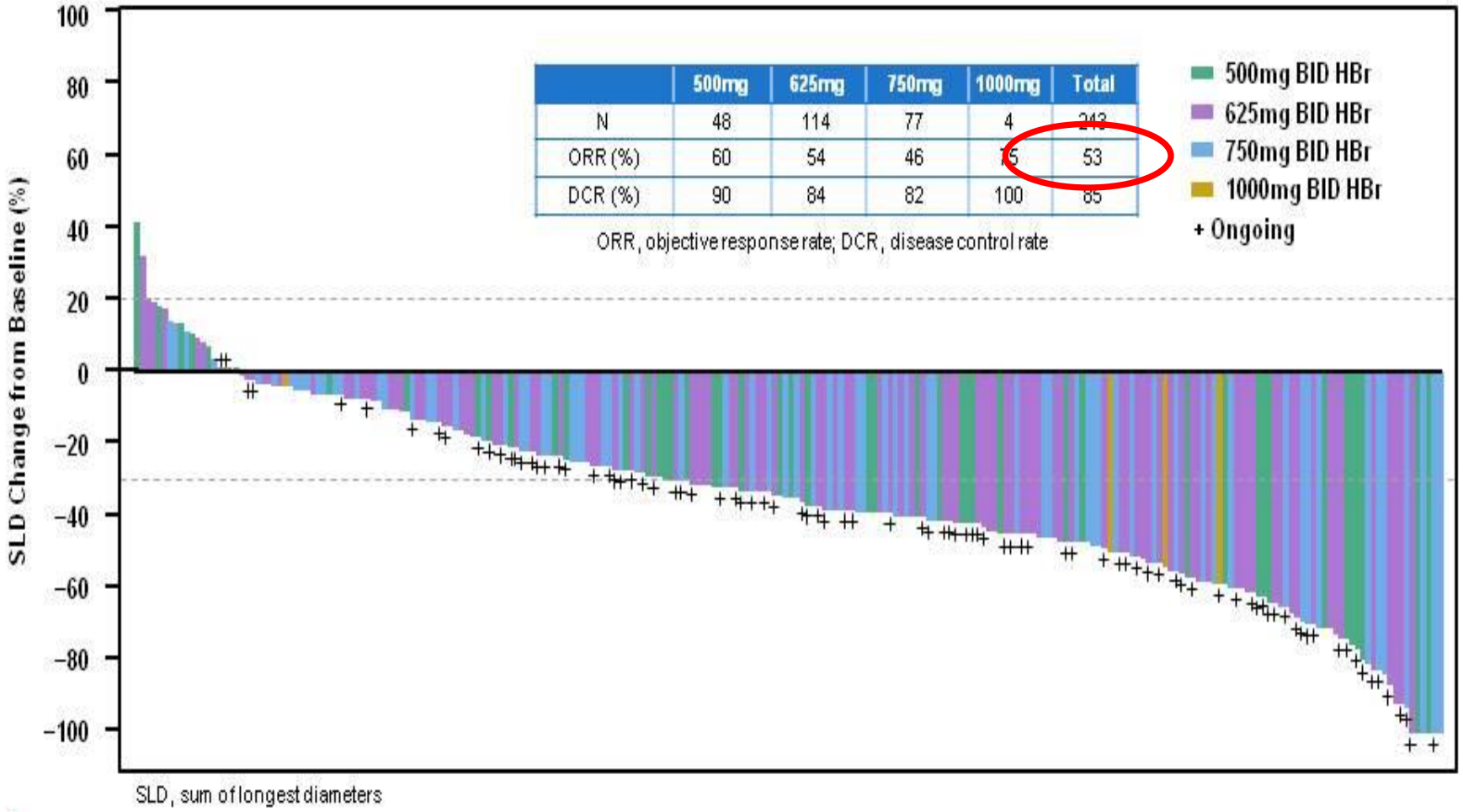
TIGER-X: Phase 1/2 Trial of Rociletinib

Key eligibility criteria

- Advanced or recurrent NSCLC with a documented activating EGFR mutation
- Prior treatment with EGFR-directed therapy
- Recent biopsy available or willing to undergo a new on-study biopsy; plasma samples collected
- Phase 2 only
  - Disease progression while on treatment with EGFR-directed therapy
  - T790M-positive biopsy at the time of entering study
  - Treated stable CNS metastases are allowed



Best Response to Rociletinib (All Doses) in 243 Centrally Confirmed Tissue T790M+ Patients

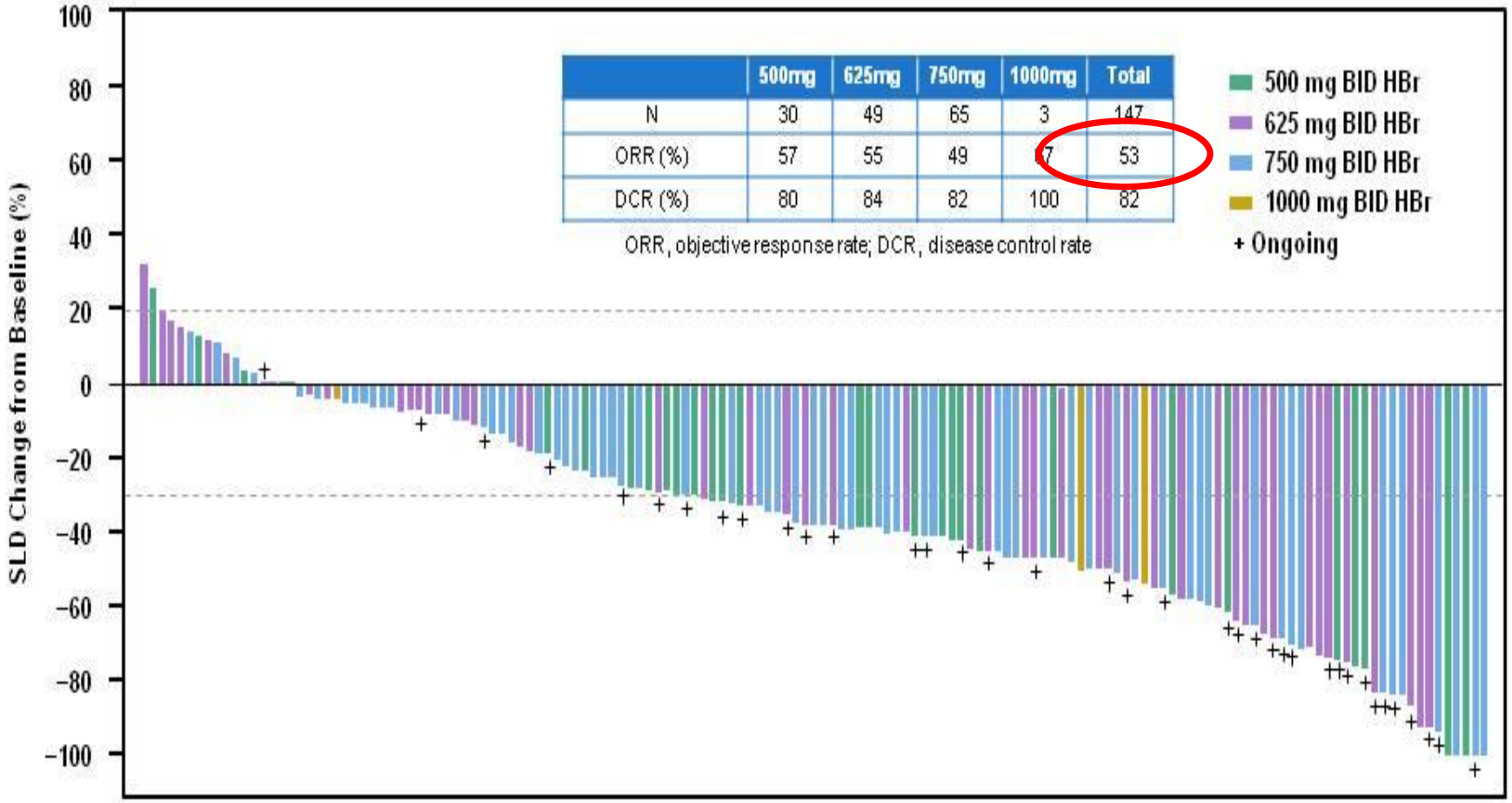


	500mg	625mg	750mg	1000mg	Total
N	48	114	77	4	243
ORR (%)	60	54	46	75	53
DCR (%)	90	84	82	100	85

ORR, objective response rate; DCR, disease control rate

SLD, sum of longest diameters

Best Response to Rociletinib (All Doses) in Plasma T790M+ Patients



	500mg	625mg	750mg	1000mg	Total
N	30	49	65	3	147
ORR (%)	57	55	49	[illegible]	53
DCR (%)	80	84	82	100	82

ORR, objective response rate; DCR, disease control rate

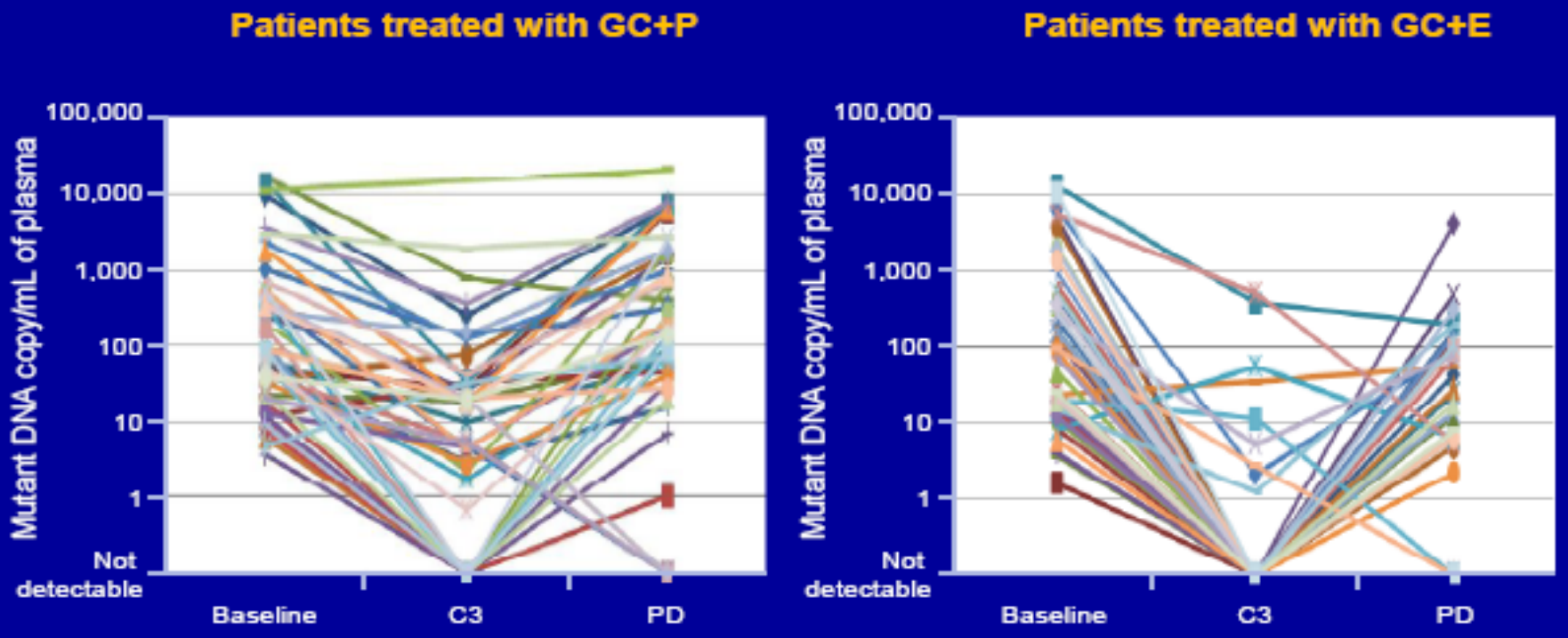
SLD, sum of longest diameters

Application 3

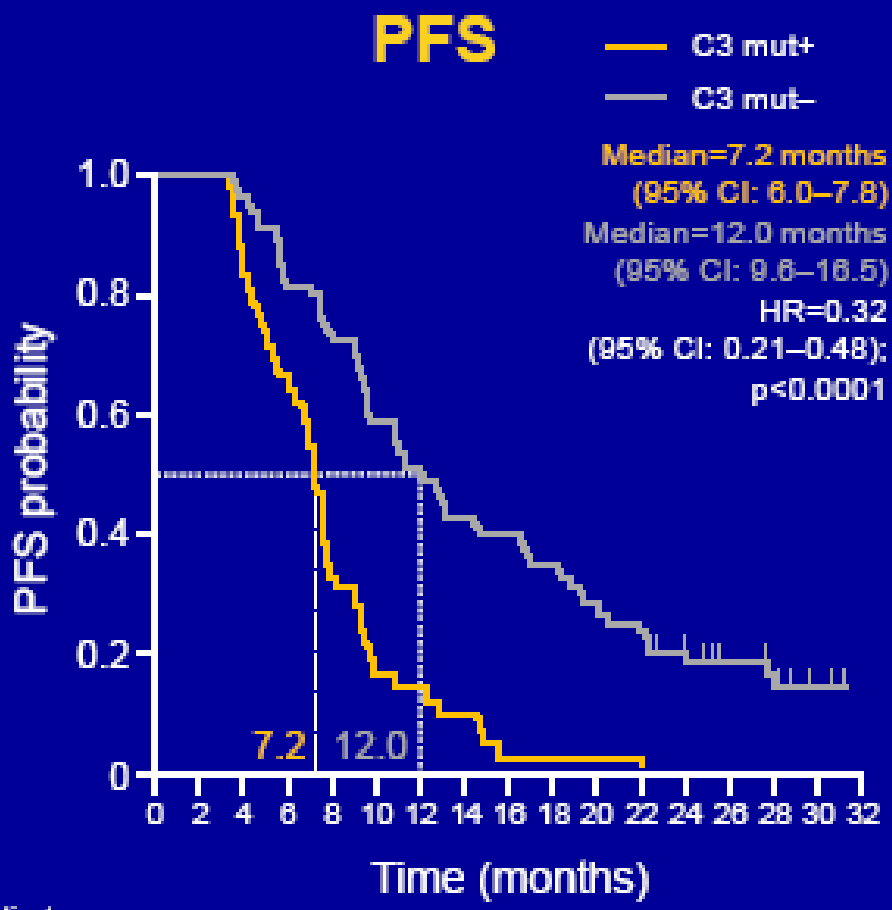
Dynamic monitoring both sensitive mutations and resistant biomarkers

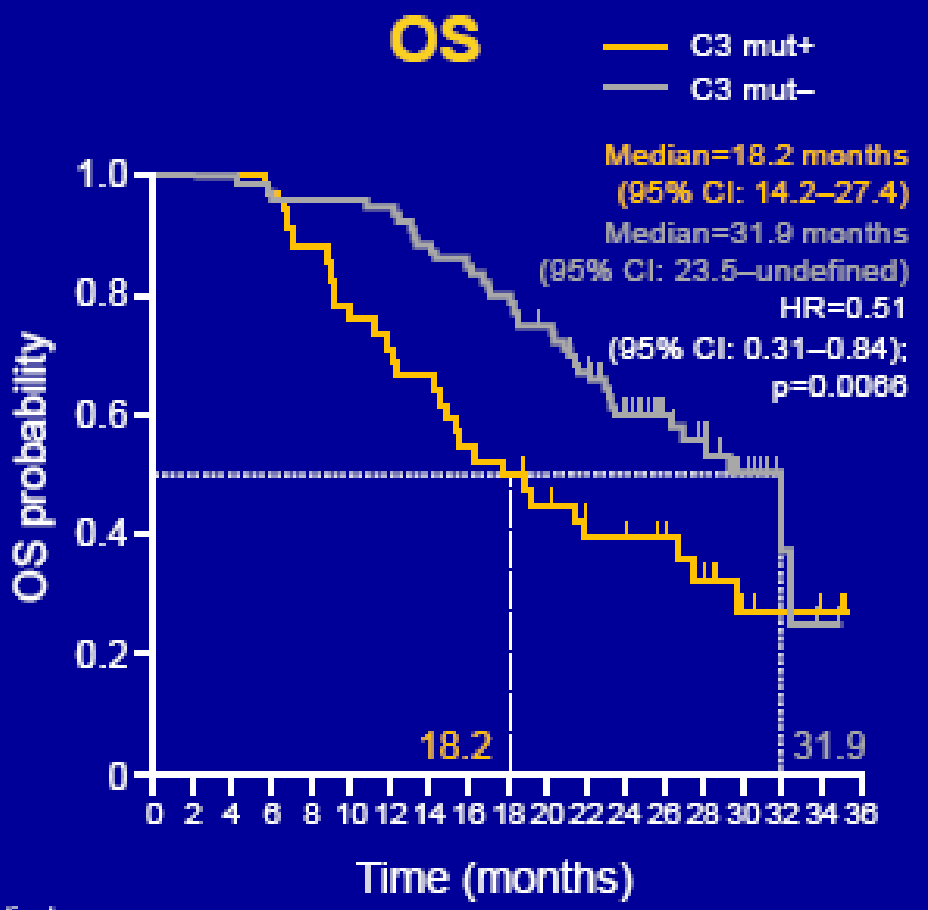
FASTACT-2 study

Dynamic mutant DNA change during therapy



Association between p*EGFR* mut+ at C3 and PFS/OS (both treatment arms combined)





Patients, n																	
C3 mut+	42	42	35	28	14	7	6	4	1	1	1	1	0	0	0	0	0
C3 mut–	80	80	77	65	59	47	40	34	32	28	23	19	13	10	7	3	0

Patients, n																								
C3 mut+	42	42	42	41	37	32	30	28	23	21	18	14	14	12	9	4	3	2	0
C3 mut–	80	80	80	77	77	77	76	71	68	64	59	52	38	29	22	12	3	1	0

OS = overall survival

Positive p*EGFR* at baseline followed by negative p*EGFR* at C3 is associated with improved outcomes; patients positive at baseline and still positive at C3 experienced worse outcomes

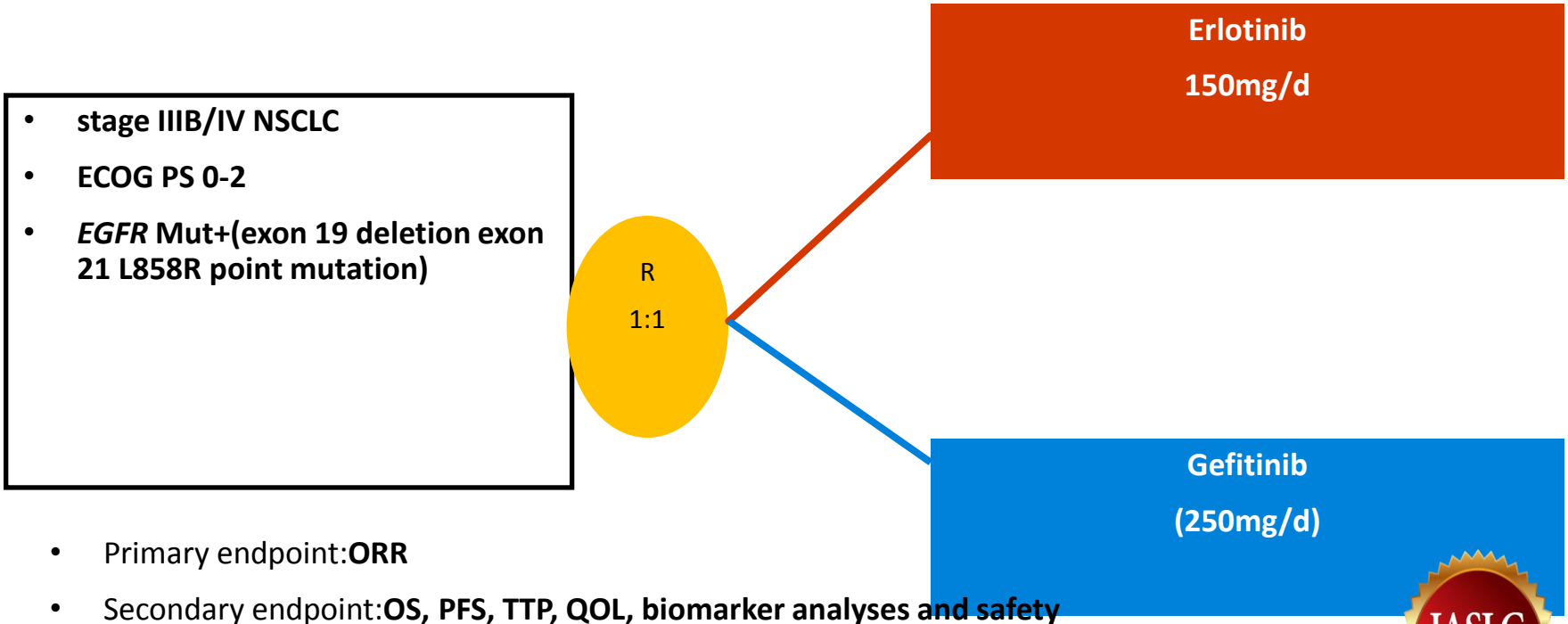
Serial assessment of response and resistance for patients with *EGFR-L858R* mutant lung cancer on a prospective trial

•Qing Zhou, Jin-Ji Yang, Zhi-Hong Chen, Xu-Chao Zhang, Hong-Hong Yan, Jian Su, Hua-Jun Chen, Chong-Rui Xu, Hai-Yan Tu, Wen-Zhao Zhong, Xue-Ning Yang, Yi-Long Wu*

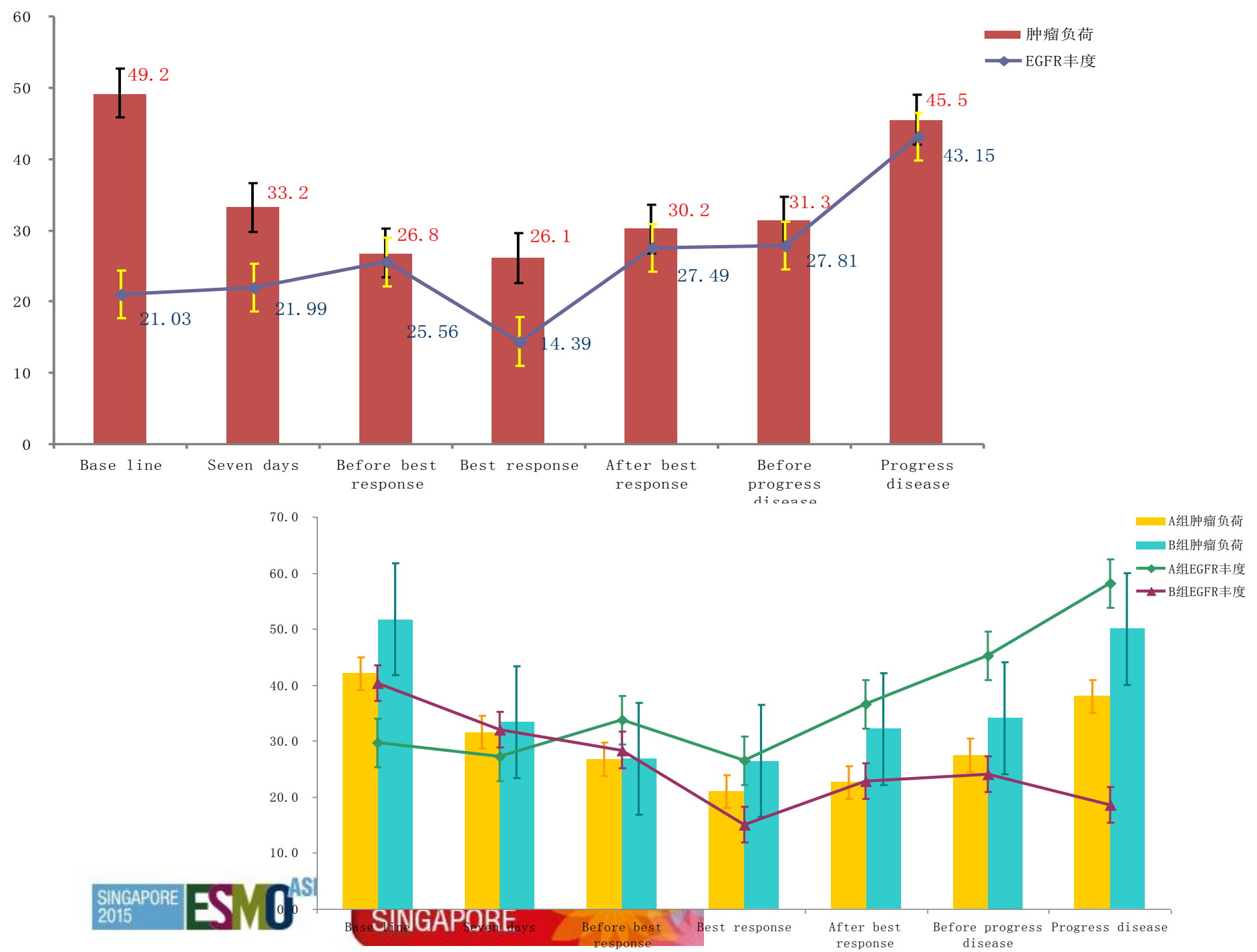
•Guangdong Lung Cancer Institute
•Guangdong General Hospital&
•Guangdong Academy of Medical Sciences

CTONG0901

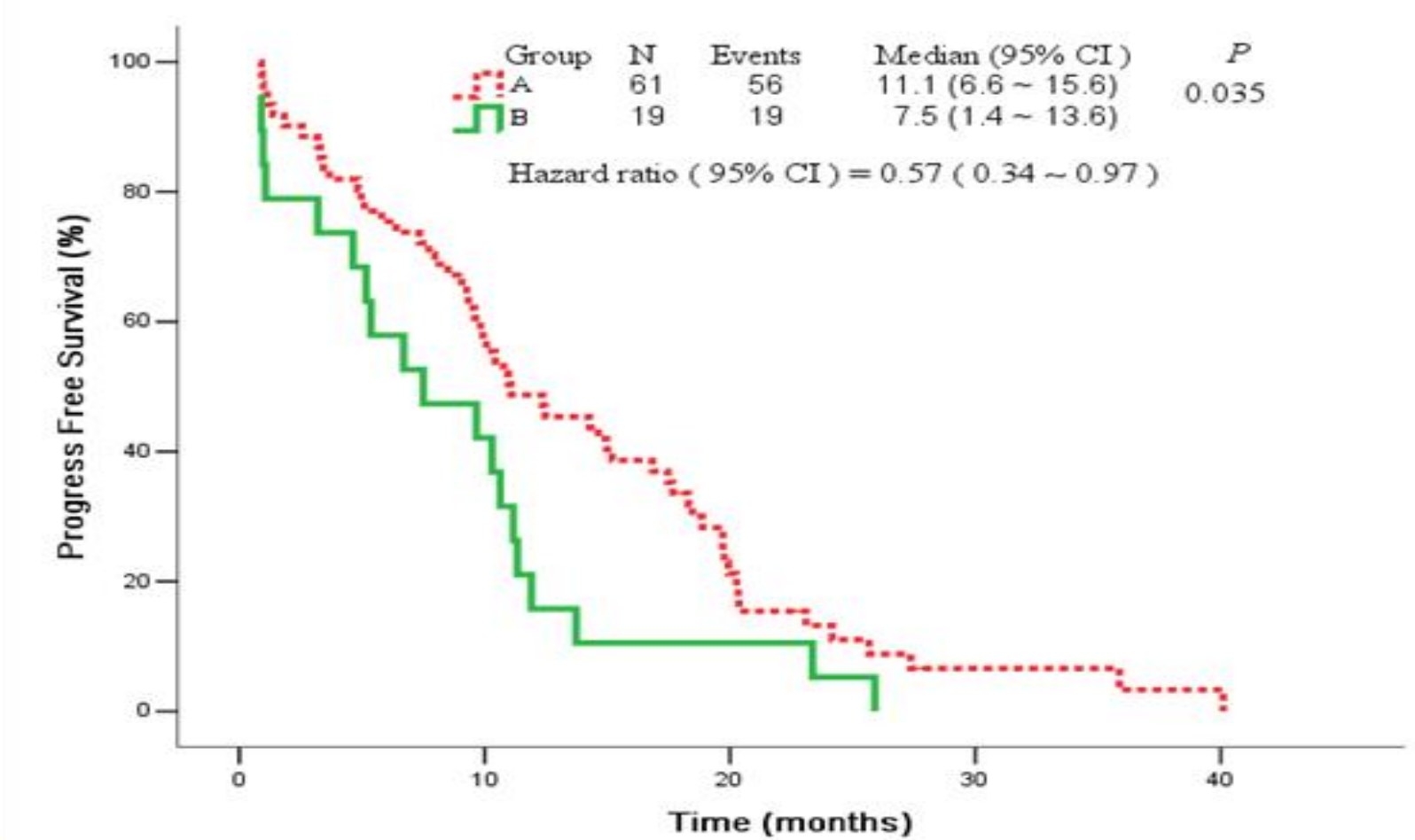
- Based on a randomized trial (CTONG0901, NCT01024413)



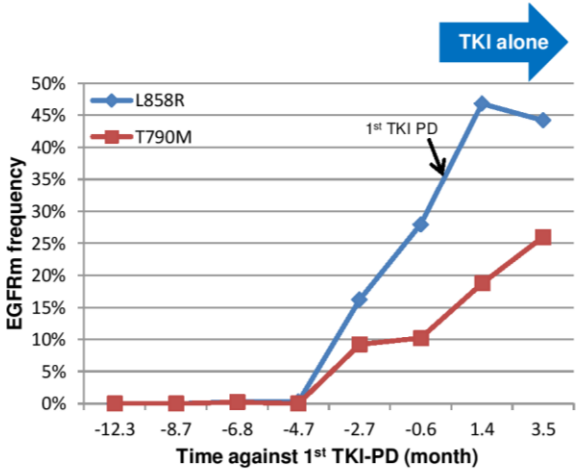
- Primary endpoint:**ORR**
- Secondary endpoint:**OS, PFS, TTP, QOL, biomarker analyses and safety**

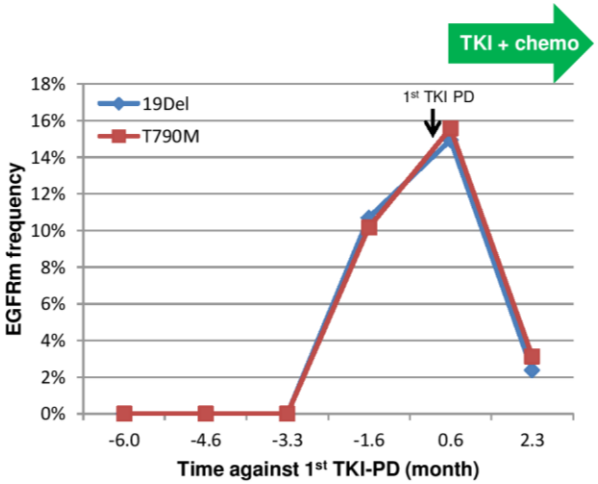

肿瘤负荷
EGFR丰度
60
50
40
30
20
10
0
49.2
33.2
26.8
26.1
30.2
31.3
45.5
21.03
21.99
25.56
14.39
27.49
27.81
43.15
Base line
Seven days
Before best response
Best response
After best response
Before progress disease
Progress disease
A组肿瘤负荷
B组肿瘤负荷
A组EGFR丰度
B组EGFR丰度
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Base line
Seven days
Before best response
Best response
After best response
Before progress disease
Progress disease

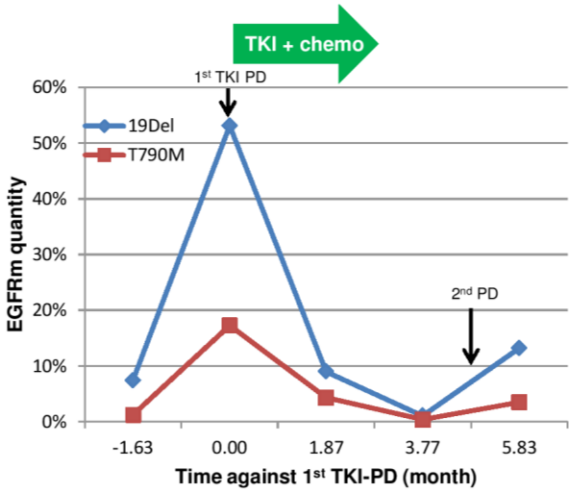
PFS between two groups

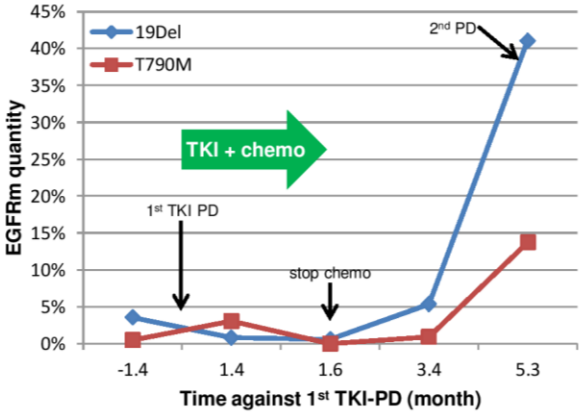


Dynamic monitoring of T790M in ctDNA









Zheng D, et al. 2015 ASCO Abstract 8080.

Application 4

Exploring Novel biomarkers

Resistance mechanism for AZD9291

Nat Med 2015 Jun;21 (6): 560-2.

全文索取

Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M.

Thress KS , Paweletz CP , Felip E , Cho BC , Stetson D , Dougherty B , Lai Z , Markovets A , Vivancos A , Kuang Y , Ercan D , Matthews SE , Cantarini M , Barrett JC , Jänne PA , Oxnard GR .

Abstract

Here we studied cell-free plasma DNA (cfDNA) collecte **C797S mutation** tumors had developed resistance to the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) A cing of cfDNA from seven subjects and detected an acquired EGFR C797S mutation in one; expression of this muta to AZD9291. We then performed droplet digital PCR on serial cfDNA specimens collected from 15 AZD9291-treated efore treatment, but upon developing AZD9291 resistance three molecular subtypes emerged: six cases acquired the C797S mutation, five cases maintained the T790M mutation but did not acquire the C797S mutation and four cases lost the T790M mutation despite the presence of the underlying EGFR activating mutation. Our findings provide insight into the diversity of mechanisms through which tumors acquire resistance to AZD9291 and highlight the need for therapies that are able to overcome resistance mediated by the EGFR C797S mutation.

IASLC
INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER
16TH WORLD CONFERENCE ON LUNG CANCER
SEPTEMBER 6–9, 2015 → DENVER, COLORADO, USA

Mechanisms of acquired resistance to AZD9291 in EGFR T790M positive lung cancer

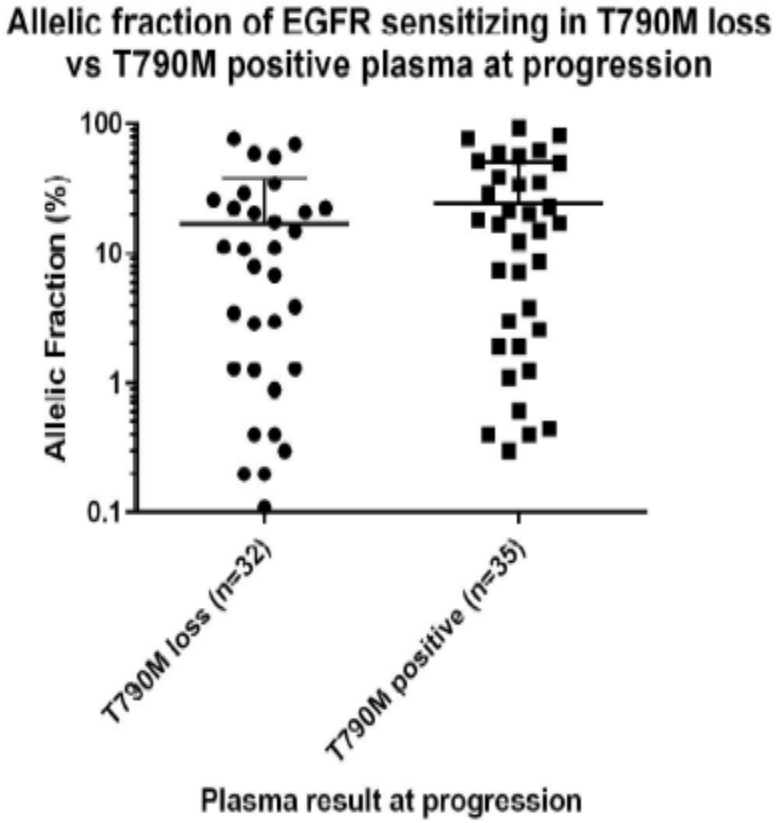
Geoffrey R. Oxnard[1], Kenneth S. Thress[2], Cloud P. Paweletz[1], Daniel Stetson[2], Brian Dougherty[2], Zhongwu Lai[2], Aleksandra Markovets[2], Enriqueta Felip[3], Ana Vivancos[3], Yanan Kuang[1], Lynette Sholl[4], Amanda J. Redig[1], Mireille Cantarini[5], J. Carl Barrett[2], Rathi N. Pillai[6], Byoung Chul Cho[7], David Planchard[8], Jean-Charles Soria[8], Pasi A. Jänne[1]

[1]Dana-Farber Cancer Institute, Boston, MA, USA; [2]AstraZeneca, Gatehouse Park, Waltham, MA, USA; [3]Vall d'Hebron University Hospital, Vall d'Hebron Institute of Oncology, Barcelona, Spain; [4]Brigham and Women's Hospital, Boston, MA, USA; [5]AstraZeneca, Alderley Park, Macclesfield, UK; [6]Winship Cancer Institute, Emory University, Atlanta, GA, USA; [7]Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea; [8]Gustave Roussy, Paris, France

IASLC 40 YEARS

Results: T790M loss

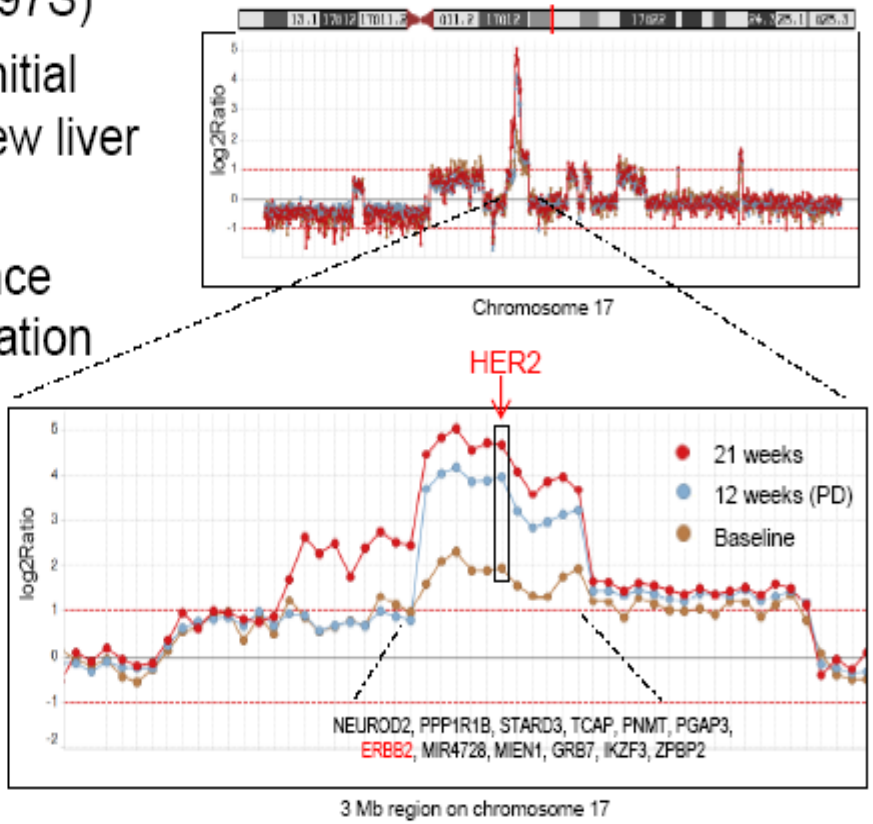
- 32 of 67 (48%) had no detectable T790M in plasma despite presence of the EGFR-TKI-sensitizing mutation, suggesting overgrowth of an alternate resistance mechanism
- A few patients with loss of T790M had a very low allelic fraction of sensitizing mutation, such that a missed low level T790M cannot be ruled out



Data source: G. Oxnard, C. Paweletz, R. Alden, K. Thress

Results: HER2 amplification

- 15 cases completed plasma NGS after resistance to AZD9291 (4 showing C797S)
- One patient treated at 80 mg had an initial unconfirmed PR (-38%) followed by new liver metastasis
- Whole genome sequencing of resistance cfDNA found high level HER2 amplification



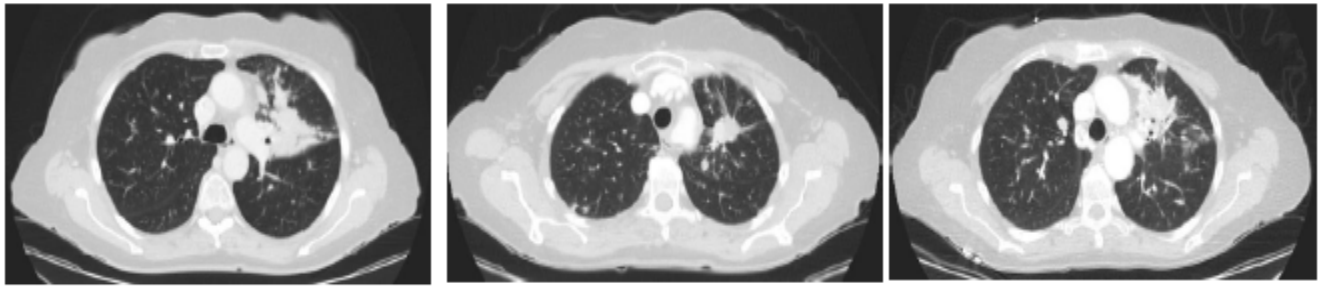
	Baseline	12 weeks (PD)	21 weeks (off tx)
L858R	85%	79%	82%
T790M	42%	0%	1%
EGFR CNV	6	5	6
ERBB2 CNV	6	11	32

Data source: D. Stetson, A. Markovets, B. Dougherty, Z. Lai, C. Barrett, K. Thress
CNV, copy number variation; PD, progressive disease; PR, partial response; tx, treatment

Results: MET amplification

- 69-year-old female with EGFR-mutant NSCLC metastatic to liver, adrenal, bones who had progression after first-line chemotherapy and subsequent erlotinib
- Resistance biopsy was inadequate for genotyping, but plasma genotyping positive for L858R (26%) and T790M (4%)
- Initiated AZD9291 and responded on the first scan (-40%) but progressed after 24 weeks
- Resistance biopsy undergone for targeted NGS:
  - Positive for L858R, negative for T790M, positive for MET amplification
  - MET protein overexpression also seen on IHC

Pre-AZD9291 plasma genotype:
L858R (26%)
T790M (4%)



Baseline | 4 months | 6 months

Progression tumor genotype:
L858R
T790M negative
MET amplified

Data source: R. Pillai; S. Ramalingam
IHC, immunohistochemistry; NSCLC, non-small cell lung cancer

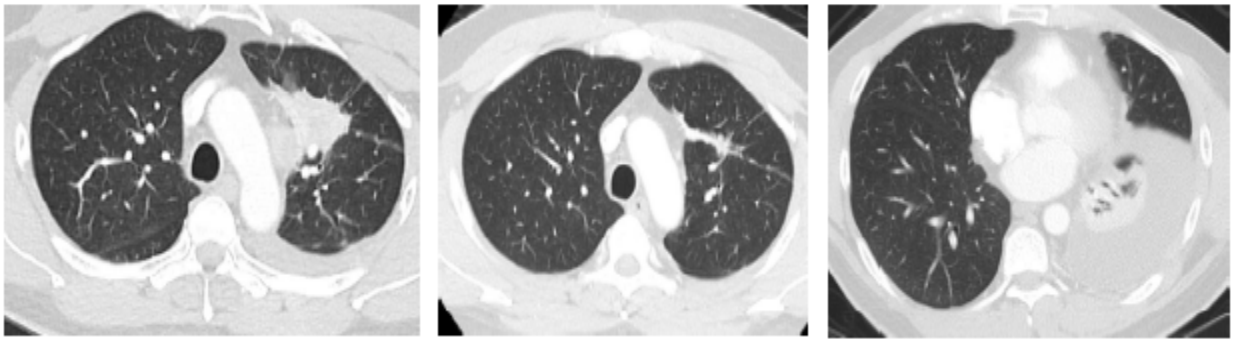
Results: BRAF V600E

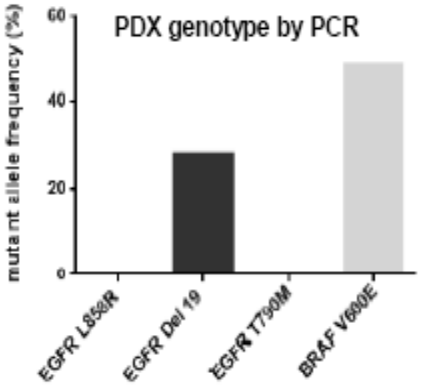
- 49-year-old male with metastatic NSCLC positive for EGFR exon 19 deletion
- Developed resistance to first-line erlotinib after 11 months, T790M positive biopsy
- Had a confirmed PR to AZD9291 but growth of lung mass, effusion after 5 months
- Targeted NGS of progression biopsy shows exon 19 deletion (8% of reads), no T790M, BRAF V600E (6% of reads)
  - A patient-derived xenograft is in development

Pre-AZD9291
Ex19del/T790M

2 months

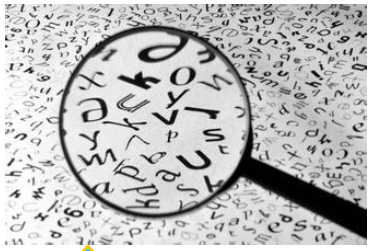
6 months
Ex19del/BRAF V600E

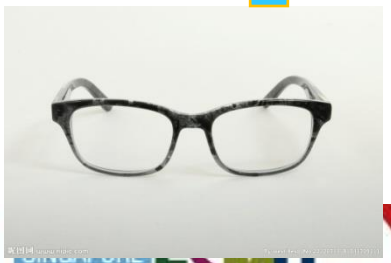




Data source: P.A. Jänne, A.J. Redig

ctDNA guides us to see the fact more clearly and further!





Summary

Applications

- Molecular diagnosis & predicting efficacy
- Detect resistance biomarkers
- Dynamic monitoring both sensitive mutation and resistant biomarkers
- Exploring novel biomarkers

Challenges

- More precise techniques false-negative or false-positive results
- Clinical meaning of dynamic monitoring
- Reasonable explanation of results (NGS)

Thanks!