Biomarkers in lung cancer

Jean-Yves Pierga

Medical Oncology Department

Institut Curie & Université Paris Descartes FRANCE

Disclosure slide

- no Conflicts of Interest to declare

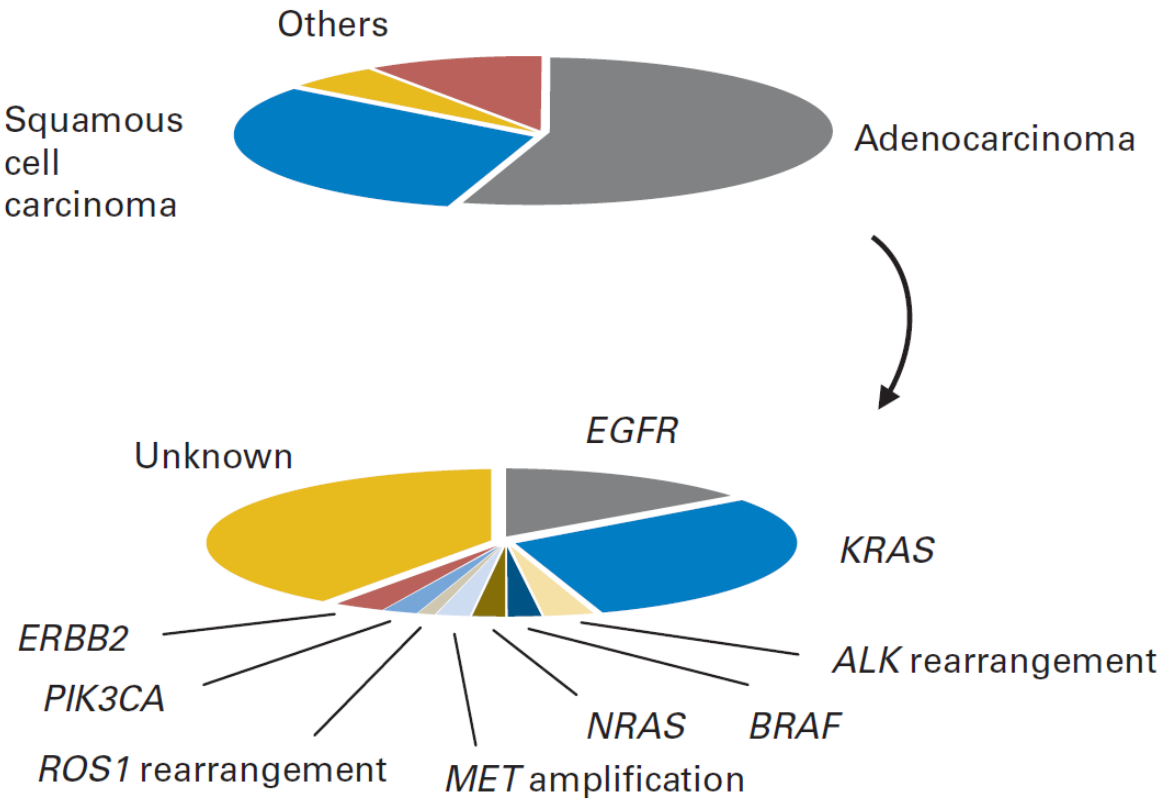
A 1670:

Prevalence and clinical outcomes for patients with ALK positive adenocarcinoma in Europe : preliminary results from the European Thoracic Oncology Platform Lungscape Project

F.H. Blackhall*, S. Peters*, K.M. Kerr, K.J. O'Byrne, H. Hager, A. Sejda, A. Soltermann, C. Dooms, E. Felip, A. Marchetti, E.J. Speel, N. Price, S. Savic, J. De Jong, M. Martorell, E. Thunnissen, L. Bubendorf, U. Dafni, R. Rosell, R.A. Stahel, on behalf of ETOP

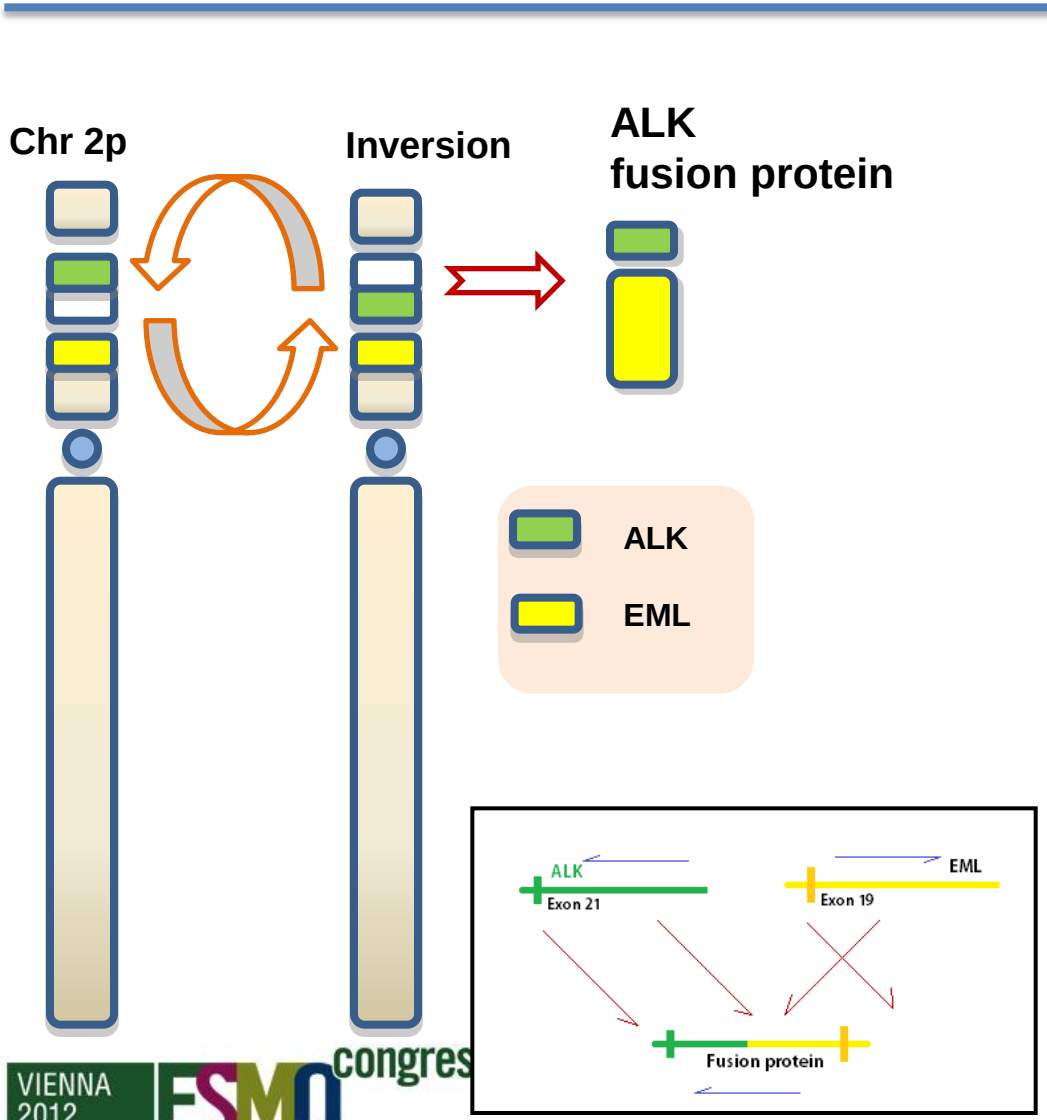
*equal contribution

In 2012: adenocarcinoma has been split in multiple molecular subtypes



Janne P et al , J Clin Oncol 2012

EML4–ALK rearrangement



Cell survival

PI3K–AKT–MTOR–S6K

|

BAD

STAT3/5

Proliferation

RAS–MEK–ERK

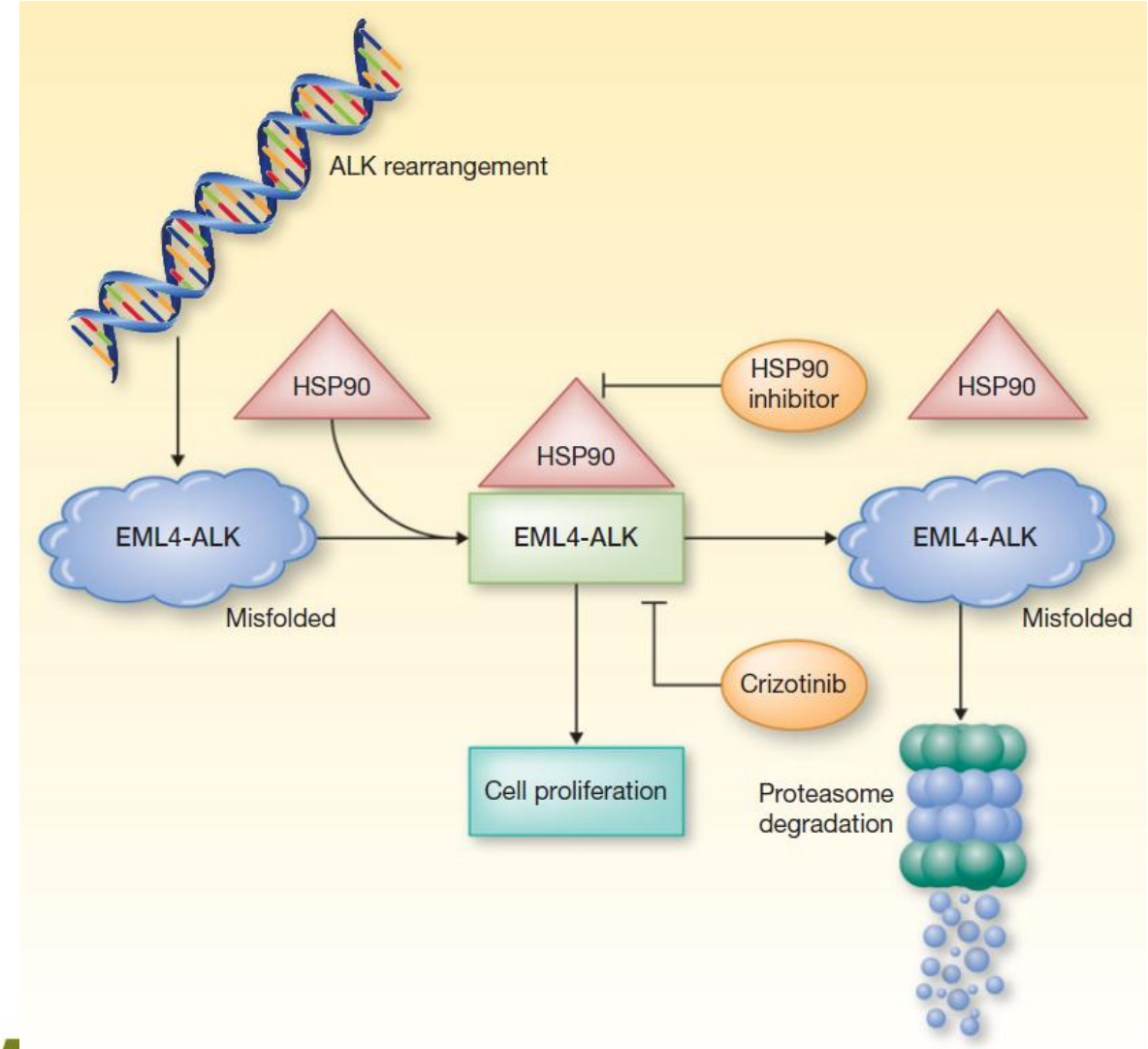
PLCγ–PIP_2–IP_3

VIENNA 2012 ESMO congres

www.esmo2012.org

Adapted from Shaw AT, et al. *Clin Cancer Res* 2011;17:2081–2086.

Targeting EML4-ALK



Crystal S; Shaw A et al, Clin Cancer Res 2012

ETOP Lungscape Project

- European Thoracic Oncology Platform (ETOP) has shown the feasibility of a non centralized review of IHC for ALK translocation in NSCLC from a large number of centers in Europe, building a comprehensive database
  - Large cohort of non metastatic patients
  - Assessing Prognostic value
  - Patients' characteristics

Peters S, J Thorac Oncol 2011;6:S994

Characteristics of"*EML4-ALK*" patients

ETOP Study

- 3-5% of NSCLC patients YES
- Male = Female YES
- Young YES
- non-smokers : never smokers , former light smokers YES
- In most cases mutually exclusive with EGFR and Kras mutations Don't know
- Refractory or poorly sensitive to EGFR TKI Don't know
- Same profile of response to chemotherapy (when compared to EGFR mutated patients)

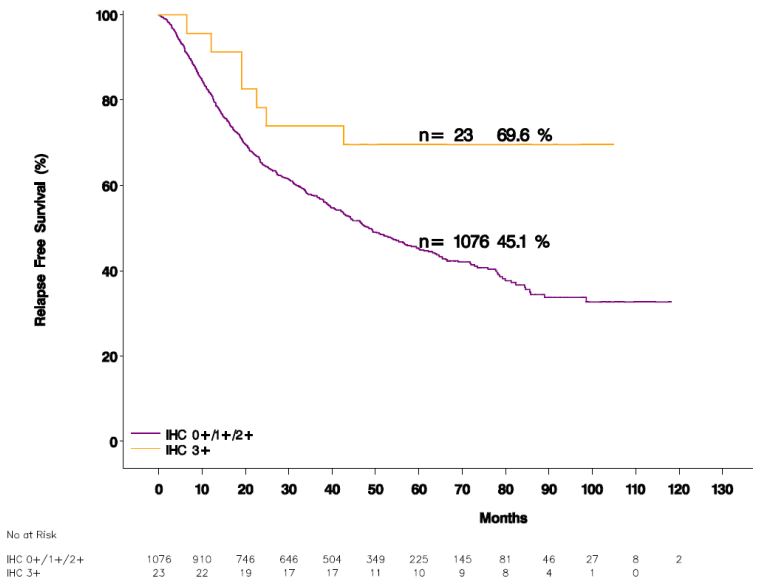
Shaw AT et al. J Clin Oncol 2009;27(26):4247-53.

Blackhall et et al, Prognostic value of ALK

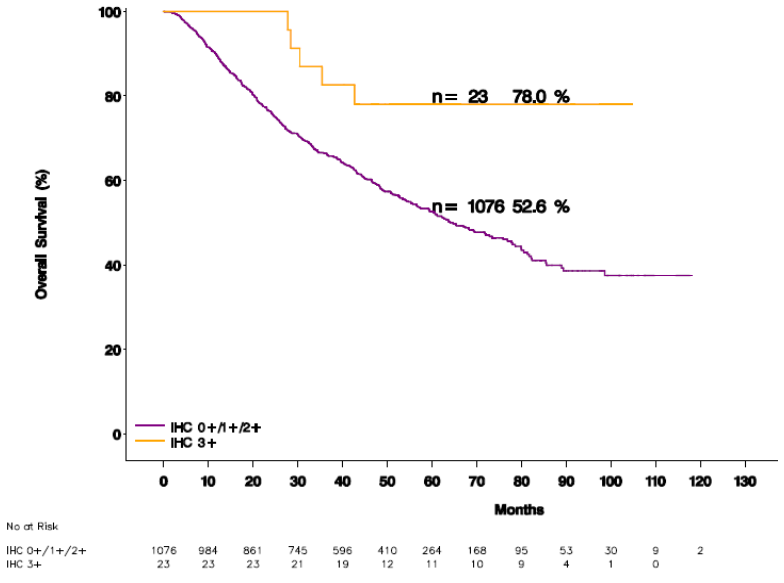
- The prognostic value of ALK remains controversial

ETOP RFS and OS for IHC 3+ vs IHC 0/1+/2+, N=1099

Log-rank test: p=0.0185



Log-rank test: p=0.0091



RFS Multivariate Cox Model:
N=1099; RFS events=591
HR IHC 3+ vs IHC 0+/1+/2+ =0.41
95% CI (0.19, 0.86), p=0.0189
Adjusted for Stage, Gender & PS

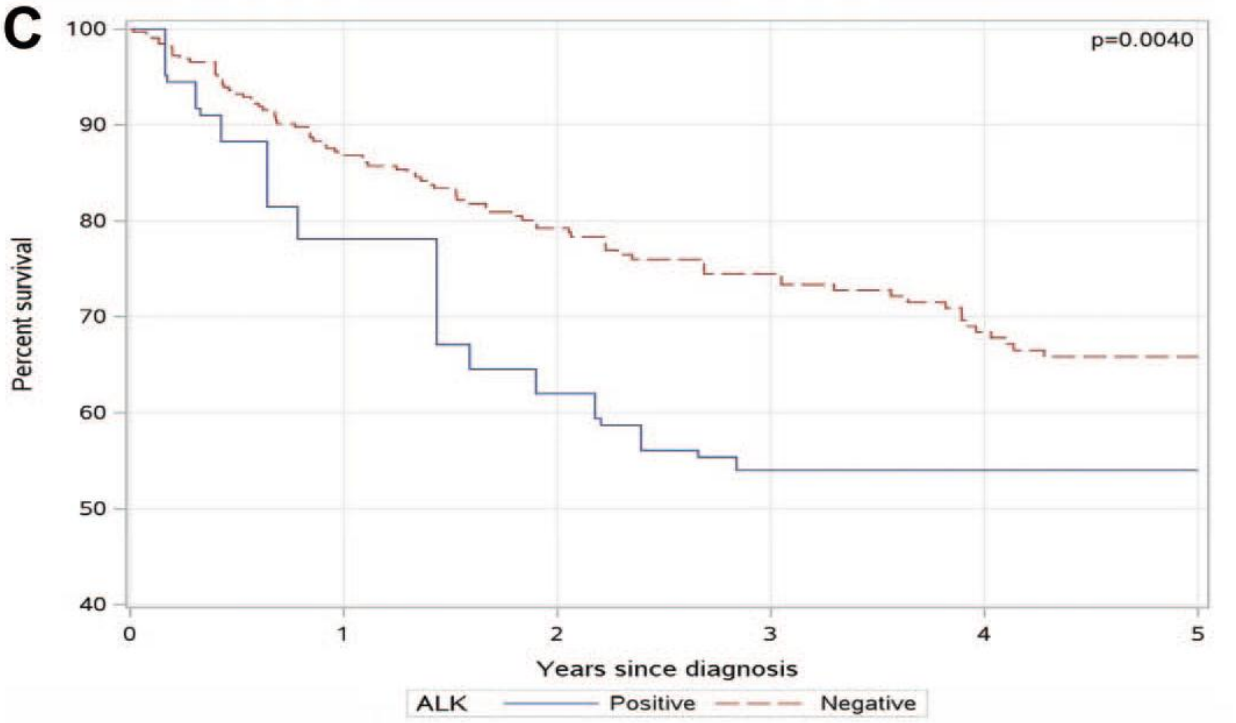
OS Multivariate Cox Model:
N=1099; Deaths=513
HR IHC 3+ vs IHC0+/1+/2+= 0.32
95% CI (0.13, 0.79), p=0.0127
Adjusted for Stage, Gender, PS & Age

The prognostic significance of *ALK rearrangement in* NSCLC has not been settled

- Shaw et al. J Clin Oncol 2009, Lancet Oncol 2011
  - did not demonstrate any significant differences in overall survival (OS) for patients with NSCLC by *EML4-ALK* status in the era before crizotinib. ,
- Zhang et al.: AACR 2012
  - no survival difference according to *ALK status after adjusting* for disease stage, histology, and *EGFR/KRAS mutant* status .
- Lee et al. : Cancer 2012
  - patients with *ALK-rearranged* NSCLC had the shortest overall survival compared to wild-type patients, but the difference was not significant .
- Kim et al. Cancer 2012
  - able to demonstrate that patients with *ALK-rearranged NSCLC had a significant worse* OS outcomes after factoring in age, sex, histology, stage, and performance status .
- Yang et al. J Thorac Oncol 2012
  - *EML4-ALK status is a poor prognostic factor for relapse-free* survival after factoring in stage, sex, age, and treatment .

Worse Disease-Free Survival in Never Smokers with *ALK* Lung Adenocarcinoma

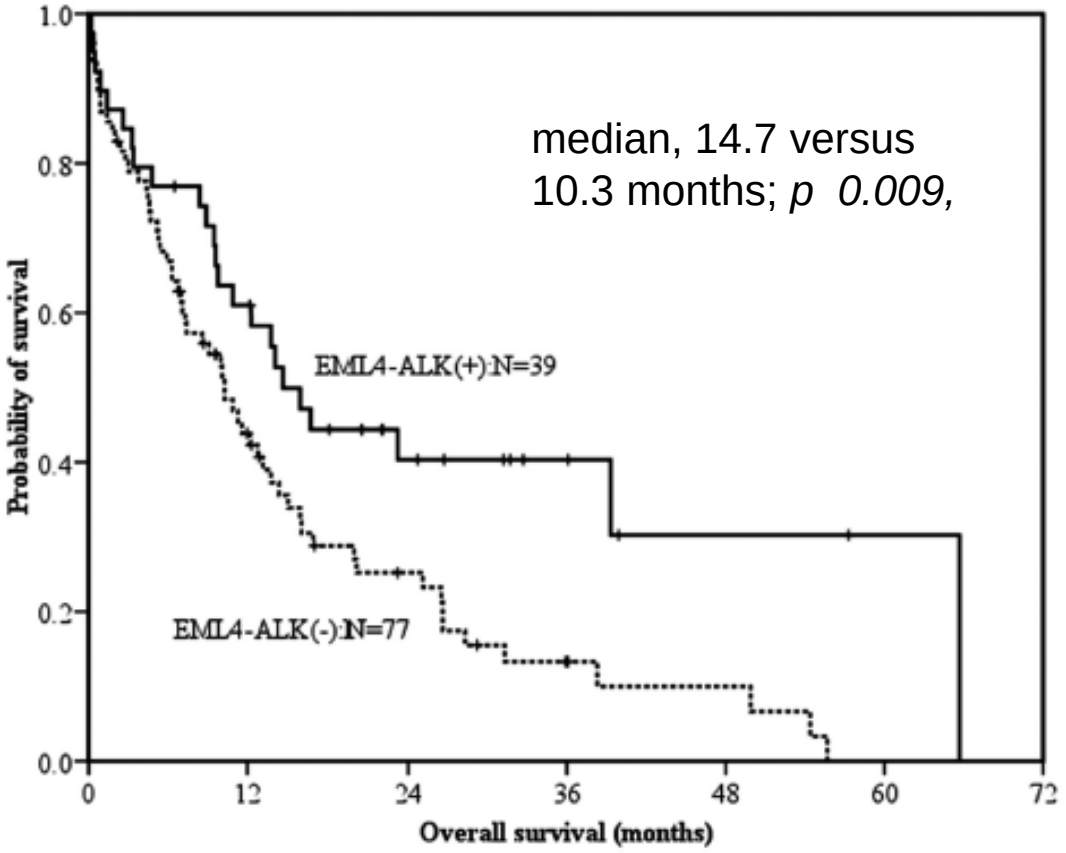
PFS/RFS survival curves for FISH/IHC3 (positive) and FISH-negative/ IHC0/1



300 never-smokers with lung adenocarcinoma from the observational Mayo Clinic Cohort *ALK positivity was 12.2% by IHC and confirmed at 8.2% of tumors* by FISH, with complete concordance between IHC 3+/0 andFISH+/-

P. Yang *J Thorac Oncol. 2012;7: 90–97*

Overall survival of lung adenocarcinoma patients with *EML4-ALK*



SG Wu *J Thorac Oncol. 2012;7: 98–104*

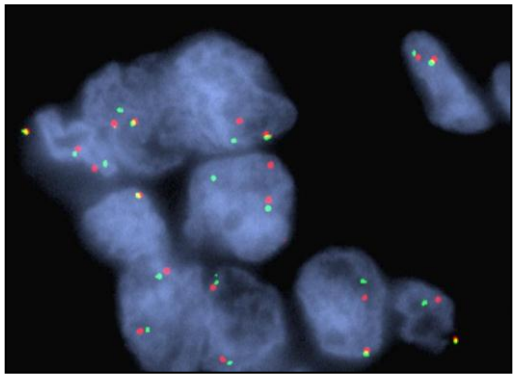
The prognostic significance of *ALK rearrangement in* NSCLC has not been settled

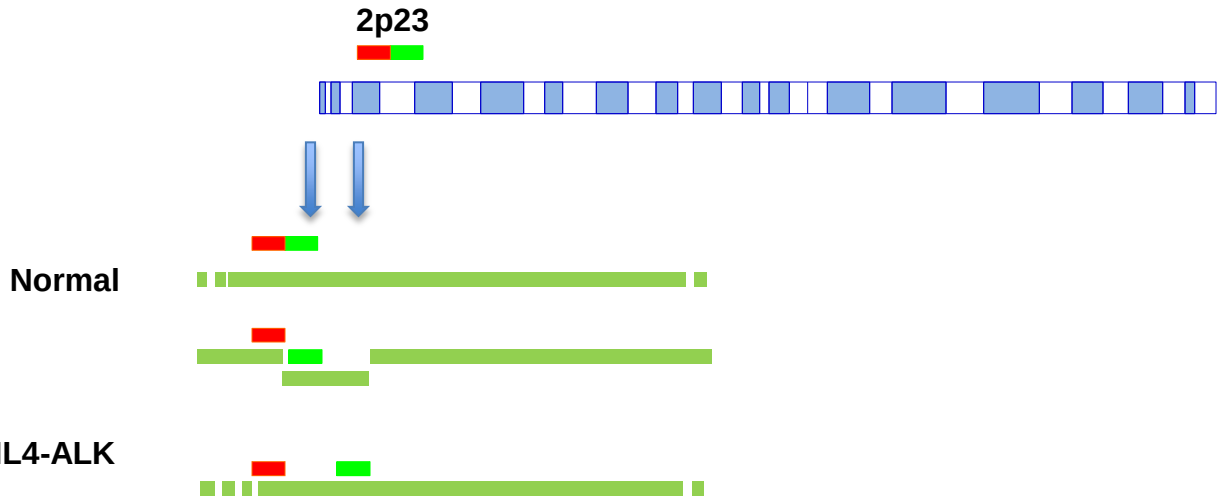
- Conversely, Wu et al. *J Thorac Oncol. 2012*
  - found that patients with *EML4-ALK* NSCLC identified from pleural effusion cytology had a significantly improved survival outcome compared with patients without *EML4-ALK NSCLC .*

- *Takeuchi et* al. Nat Med 2012
  - receptor kinase fusion-positive NSCLC is an independent favorable prognostic factor after taking into consideration age, sex, stage, and smoking status .
- All of these studies are limited by the small number of *ALK-positive patients,* different comparison group of patients, the heterogeneous treatment patients received, and differences in the balance of prognostic factors (e.g., smoking status, surgical treatment, age) compared with *ALK-rearranged patients.*
- Blackhall ‘s results are based on a large series of non metastatic patients (Stade I to III), not treated with crizotinib

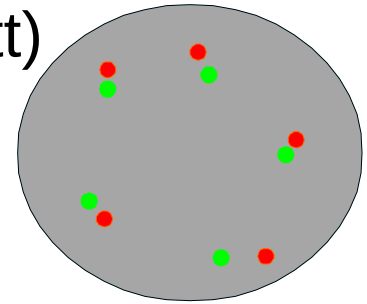
Several Methods of detection

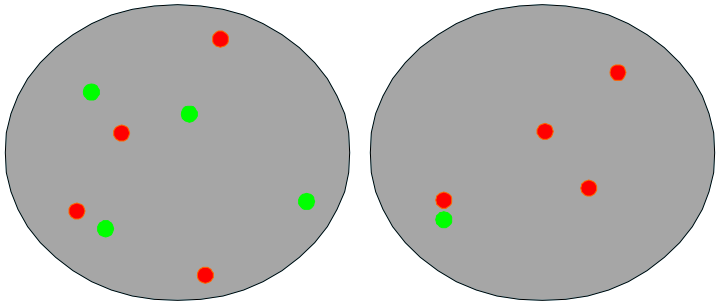
FISH ALK (Break apart probe)





Vysis (Abbott)





False positive risk: 5 %

Mok T et al., WLCC Amsterdam 2011 abstr. M12.2

Methods of detection

- Immunohistochemistry
  - Several antibodies
    - ALK1 clone, sensitivity of 90% & specificity 97.8%
    - 5A4 clone detecting EML4-ALK v1, v2, v3, v6 et v7 & KiF5B-ALK variant
    - D5F3 clone
- Multiplex RT-PCR

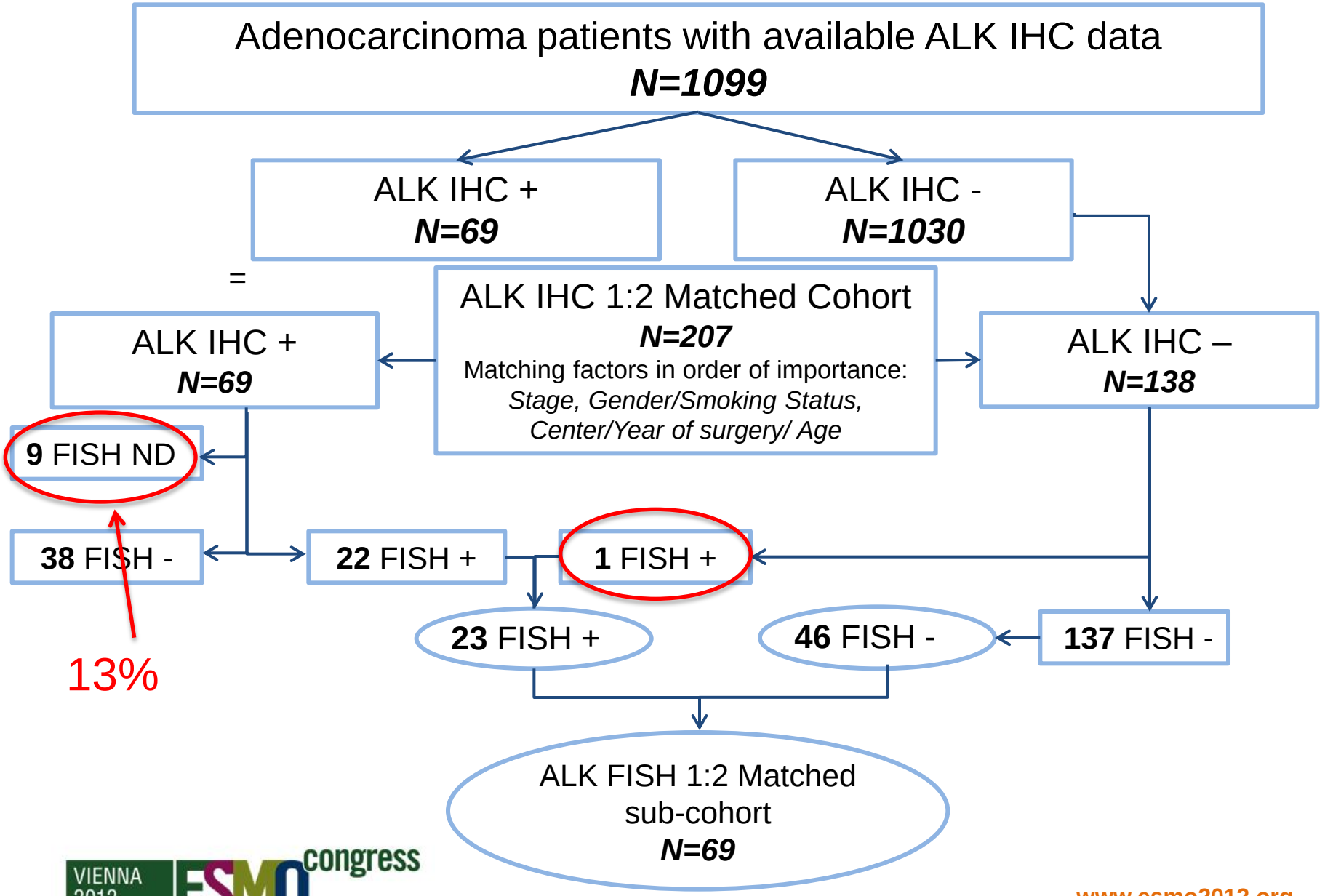
Correlation of ALK IHC and FISH

ALK IHC	ALK FISH		Total
	Positive	Negative	
0	0	234	234 (89.3%)
1+	5	1	6 (2.3%)
2+	11	2	13 (5.0%)
3+	9	0	9 (3.4%)
Total (%)	25 (9.5%)	237 (91.5%)	262 (100%)

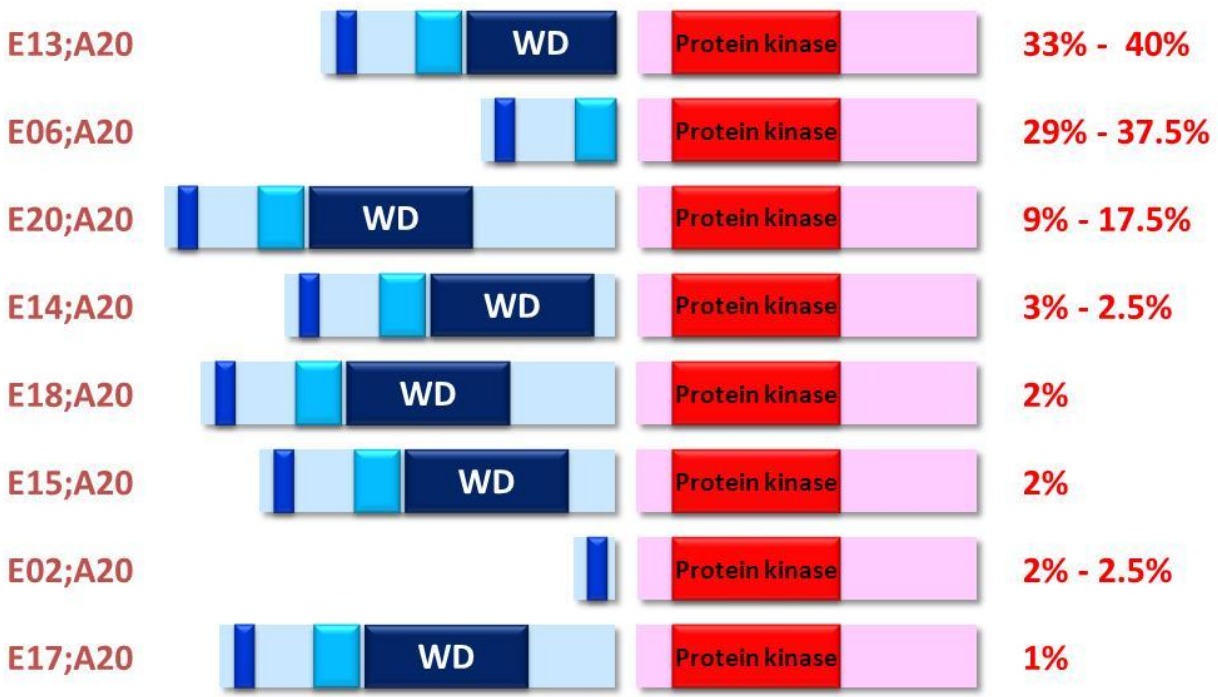
262 patients who were either EGFR wild-type or non-responders to previous EGFR tyrosine kinase inhibitors (TKI).

HS Park Lung Cancer 77 (2012) 288– 292

Flow chart


Adenocarcinoma patients with available ALK IHC data
N=1099
ALK IHC +
N=69
ALK IHC -
N=1030
=
ALK IHC 1:2 Matched Cohort
N=207
Matching factors in order of importance:
Stage, Gender/Smoking Status,
Center/Year of surgery/ Age
ALK IHC +
N=69
ALK IHC –
N=138
9 FISH ND
38 FISH -
22 FISH +
1 FISH +
23 FISH +
46 FISH -
137 FISH -
13%
ALK FISH 1:2 Matched
sub-cohort
N=69

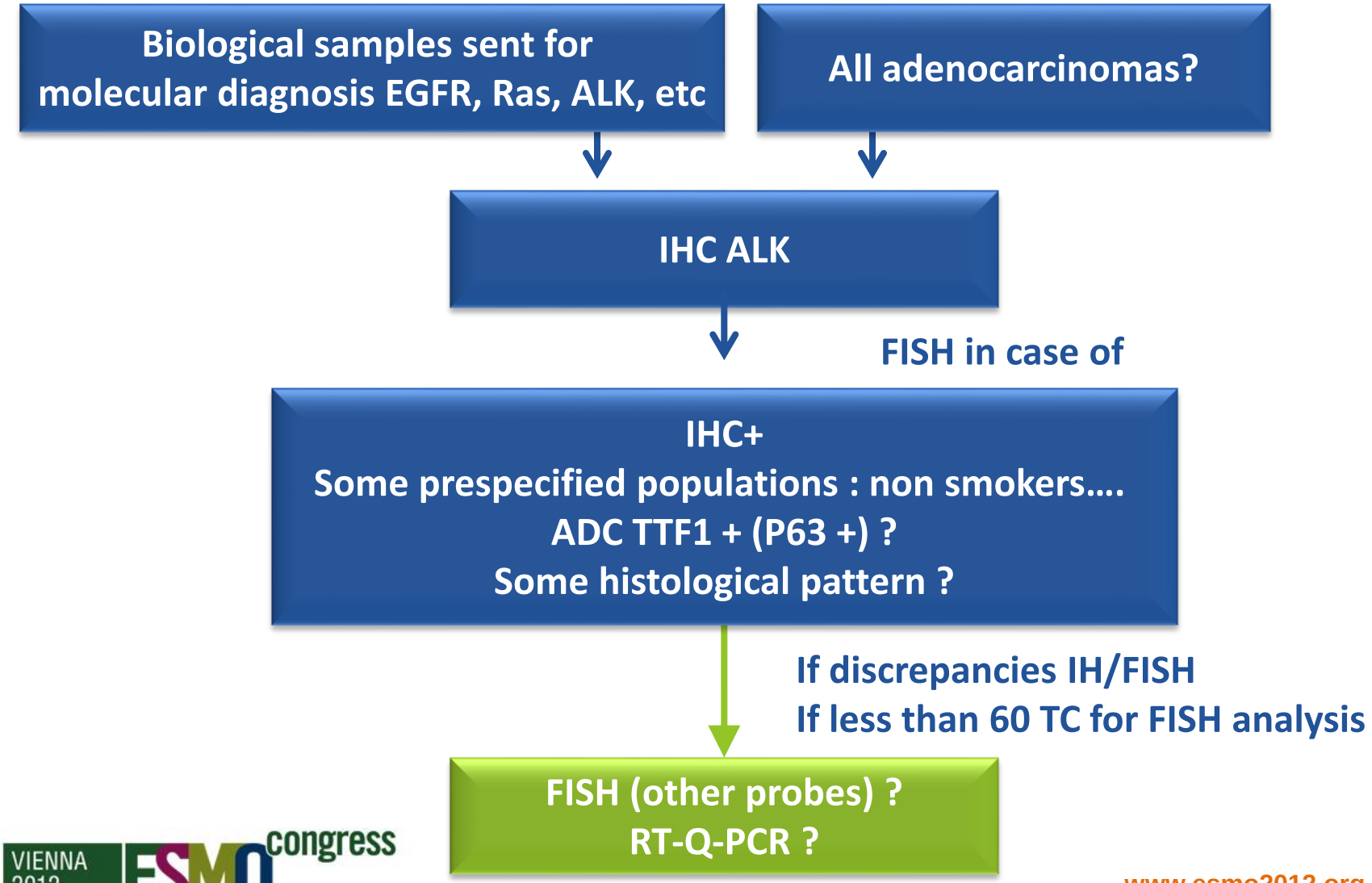
Different translocation variants



- FISH with Break Apart Rearrangement Probe confirms the presence of an ALK rearrangement but provides no information about the specific type of ALK fusion itself.
- RT-PCR can also identify the EML4-ALK variant

Takeuchi K *et al.* Nature Medicine 2012;18:378-81
Sasaki T *et al*. Eur J cancer 2010;46:1773-80
Crystal S; Shaw A et al, Cl Cancer Res 2012

How to screen ALK



Modified from S Lantuejoul

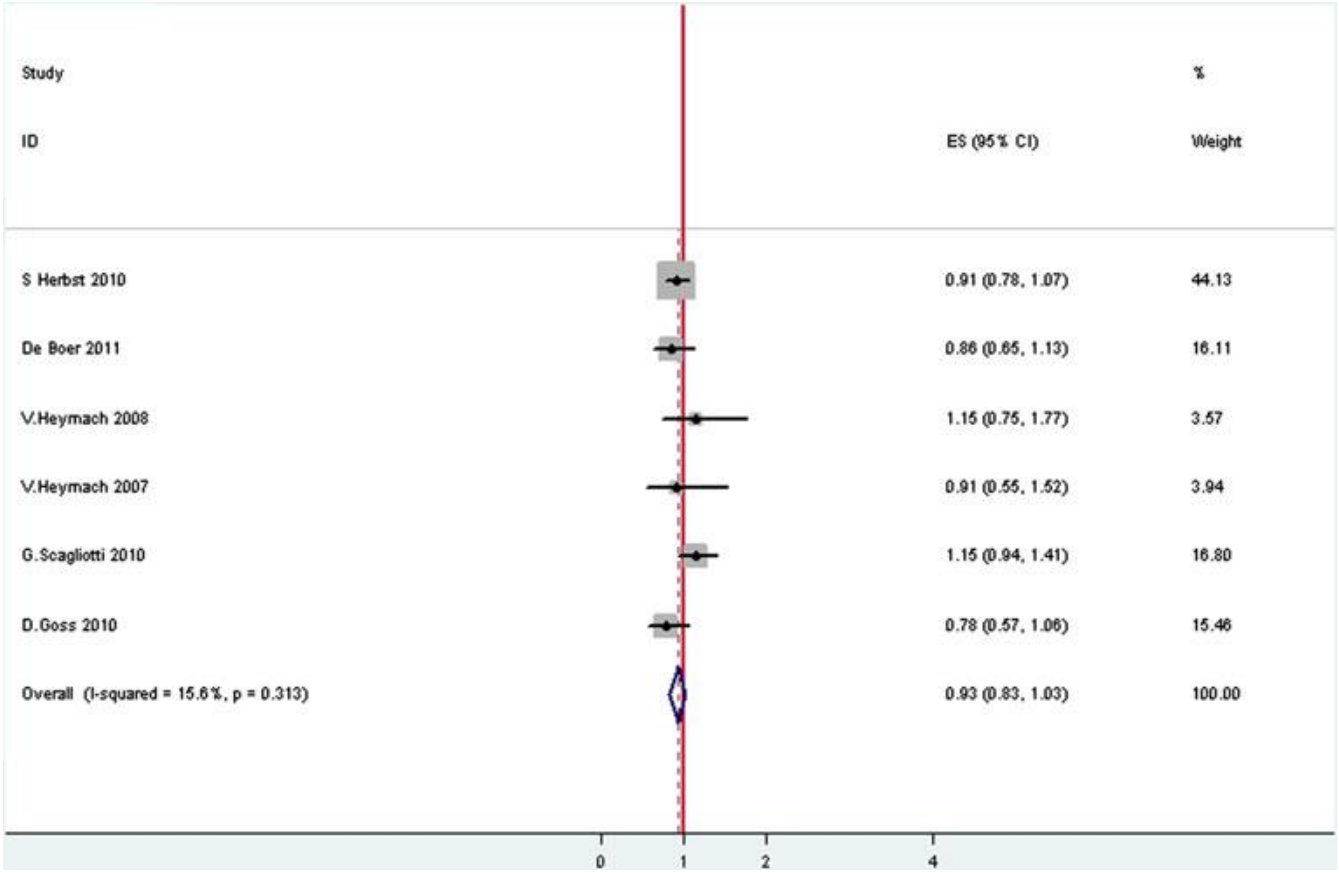
LBA 1304

Association between Tumor EGFR and KRAS Mutation Status and Clinical Outcomes in NSCLC Patients Randomized to Sorafenib plus Best Supportive Care (BSC) or BSC Alone: Subanalysis of the Phase III MISSION Trial

Tony S. Mok,[1] Luis Paz-Ares,[2] Yi-Long Wu,[3] Silvia Novello,[4] Erzsebet Juhasz,[5] Osvaldo Aren,[6] Yan Sun,[7] Vera Hirsh,[8] Egbert F. Smit,[9] Chetan Lathia,[10] Teng Jin Ong,[10] and Carol Peña[10]

[1]Chinese University of Hong Kong, Hong Kong, China; [2]Instituto de Investigaciones Biomédicas de Sevilla, Hospital Universitario Virgen del Rocío/Universidad de Sevilla/CSIC, Seville, Spain; [3]Guangdong General Hospital and Guangdong Academy of Medical Sciences, Guangzhou, China; [4]University of Turin, Turin, Italy; [5]Koranyi National Institute of TB and Pulmonology I and XIV, Budapest, Hungary; [6]Instituto Nacional de Cancer, Santiago, Chile; [7]Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China; [8]McGill University Health Centre, Montreal, Que., Canada; [9]Department of Pulmonary Diseases, VU University Medical Center, Amsterdam, The Netherlands; [10]Bayer HealthCare Pharmaceuticals, Montville, NJ, USA

Meta-analysis showingno significant difference bet ween the chemotherapy plus multitargeted antiangiogenic TKI and chemotherapy alone groups for overall survival in patients with advanced NSCLC (HR0.93, 95 % CI 0.83–1.03).

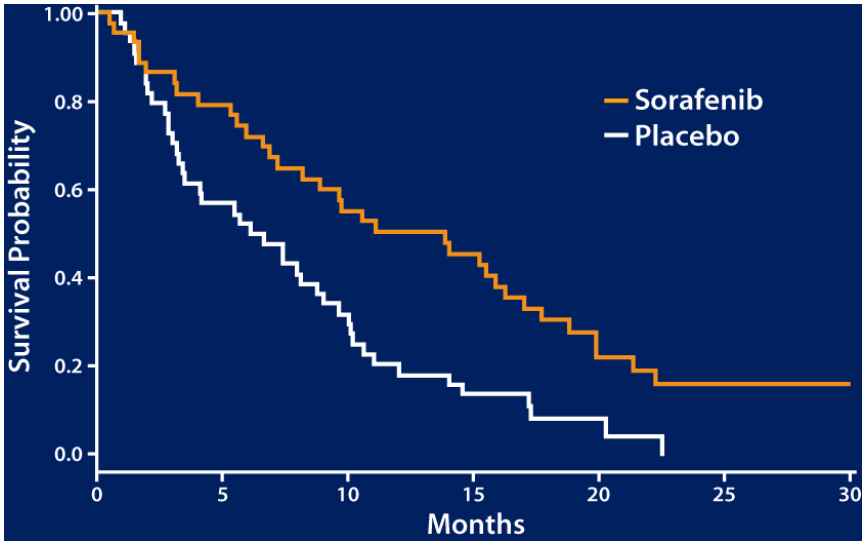


Xiao YY Eur J Clin Pharmacol 2012

Overall survival based on EGFR mutation status

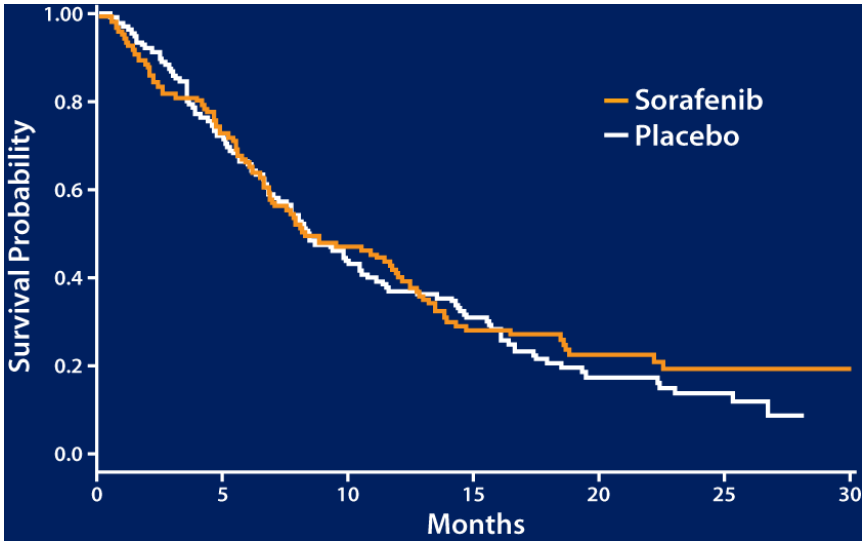
Pts with EGFR mut (in tumor or plasma)

- Sorafenib N=44; Placebo N=45
- HR=0.48 (95% CI 0.3,0.76)
- P-value=0.002
- Sorafenib median OS= 13.9 mo (423d)
- Placebo median OS= 6.5 mo (197d)



Pts with EGFR wt

- Sorafenib N=122; Placebo N=136
- HR=0.92 (95% CI 0.7,1.21)
- P-value=0.559
- Sorafenib median OS= 8.3 mo (253d)
- Placebo median OS= 8.4 mo (256d)



Biomarker*treatment interaction analysis: p-value=0.023

Results: biomarker analysis

	EGFR mutation	KRAS mutation
Tumor positive	12 / 90 (13%)	20 / 71 (28%)
Plasma positive	85 / 346 (25%)	62 / 346 (18%)
Either tumor or plasma positive	89 / 347 (26%)	68 / 347 (20%)

High rate of EGFR mutations in MISSION subgroup analysis

- EGFR and KRAS mutations are found in 5% to 15% and 15% to 20% of unselected whites with lung adenocarcinomas, respectively, to be mostly mutually exclusive.
- A rate of 26% of REGF mutation could be overestimated

Cadranel J, *Eur Respir J 2011;37:183–193.*

Detection of EGFR mutations in plasma cfDNA

- Various methods have been reported
  - Denaturing high-performance liquid chromatography (HPLC) by the TransgenomicWave Nucleic Acid 119 Fragment AnalysisSystem *(Bai et al, JCO 2009, Wang et al Clin Cancer Res, 2010)*
  - Plasma DNA analyzed by mass spectrometry-based genotyping (Sequenom) and mutant-enriched PCR (*Brevet et al, Lung Cancer 2011)*
  - Scorpions ARMS , could be more sensitive (L Qin, *Chin Med J 2011)*

Epidermal Growth Factor Receptor Mutations in Plasma DNA Samples Predict Tumor Response in Chinese Patients With Stages IIIB to IV Non–Small-Cell Lung Cancer

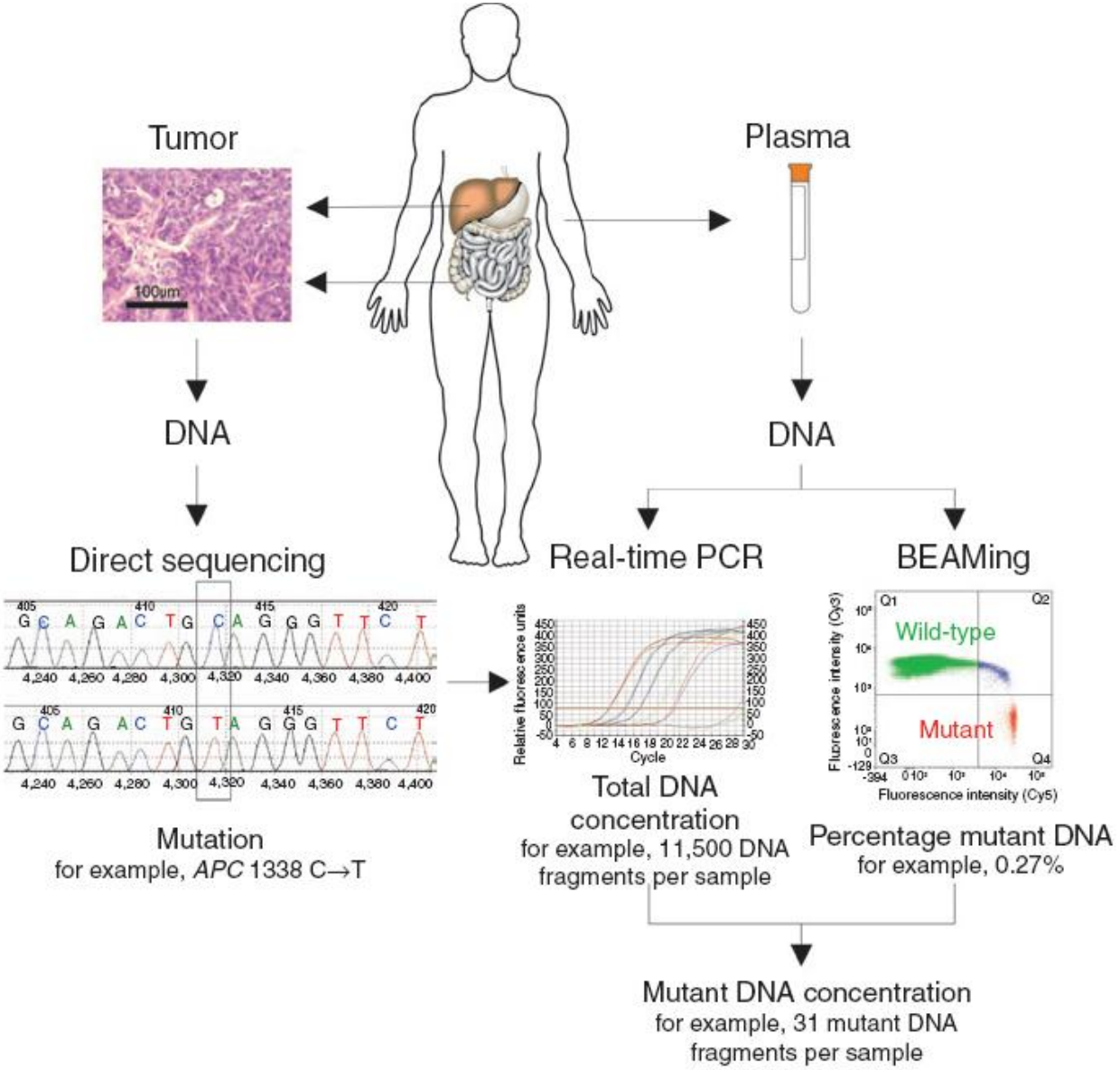
Table 2. Correlation of EGFR Mutations Between Plasma DNA and Primary Tumor DNA

Correlate	Tumor		Case Number
	EGFR-Positive	EGFR-Negative	
Plasma			
EGFR-positive	63	16	79
EGFR-negative	14	137	151
Case number	77	153	230*

Abbreviation: EGFR, epidermal growth factor receptor.
*Correlation index = 0.74.

Bai H *J Clin Oncol 27:2653-2659 , 2009*

Detection of specific mutation in circulating DNA



Diehl F Nature Medicine 2008

Results: biomarker analysis

	EGFR mutation	KRAS mutation
Tumor positive	12 / 90 (13%)	20 / 71 (28%)
Plasma positive	85 / 346 (25%)	62 / 346 (18%)
Either tumor or plasma positive	89 / 347 (26%)	68 / 347 (20%)

EGFR		Tumor	
		Wild-type	Mutant
Plasma	Wild-type	75	2
	Mutant	4	8
Total number		89	
Concordance (%)		93.3*	

KRAS		Tumor	
		Wild-type	Mutant
Plasma	Wild-type	45	5
	Mut	6	14
Total number		70	
Concordance (%)		84.3*	

EGFR mutational status discrepancies in non small cell lung cancer (primary tumor versus distant metastasis).

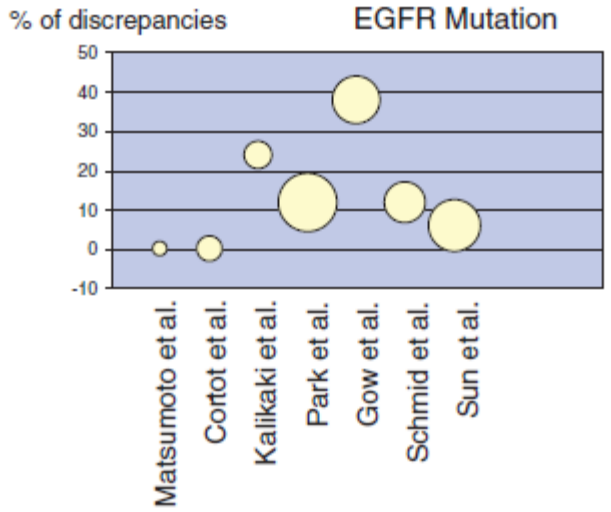
Published data on EGFR mutational status discrepancies in non small cell lung cancer (primary tumor versus distant metastasis). Discrepancies are reported in %.

Reference	Year	*N*	Main site	EGFR all mutations				EGFR activating mutation[b]			
				Δ (%)	+/− (%)	−/+ (%)	Different Mut. (%)	Δ (%)	+/− (%)	−/+ (%)	Different mut. (%)
Matsumoto	2006	8	Brain 100%	0	0	0	0	0	0	0	0
Cortot	2010	21	Brain 62%	0	0	0	0	0	0	0	0
Kalikaki	2008	25	Lung 36%	24	16	8	4	8	8	0	4
Park[a]	2008	101	Node 100%	12	11	1	0	11	11	0	0
Gow[a]	2009	67	Brain 38%	38	13	25	0	34	10	24	0
Schmid	2009	48	Node 100%	12	6	6	0	12	6	6	0
Sun	2011	80	Node 100%	6	0	6	0	6	0	6	0
Han	2011	37	Pleura 57%	16	11	5	3				

Δ: discrepancies; +/−: samples being positive in the primary tumor and negative in the metastatic setting; −/+: samples being negative in primary tumor and positive in the metastatic setting.

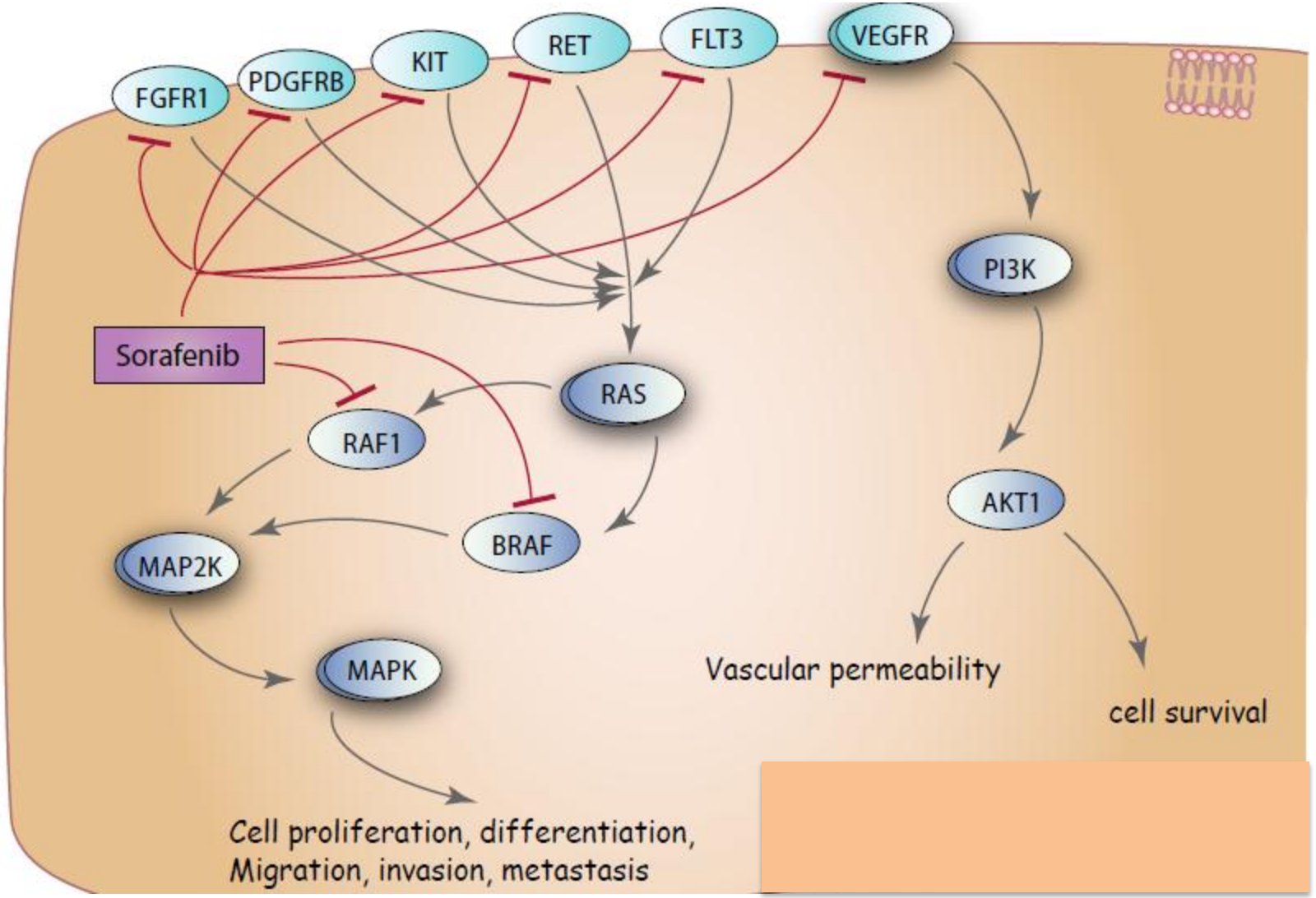
[a] By direct sequencing.

[b] Activating EGFR mutation on exons 19 and 21.



Vignot S et al Critical Reviews in Oncology/Hematology (2012)

Sorafenib mechanisms of action



OPEN ACCESS Freely available online 2011 PLoS one

Antitumor Activity of Sorafenib in Human Cancer Cell Lines with Acquired Resistance to EGFR and VEGFR Tyrosine Kinase Inhibitors

Floriana Morgillo*, Erika Martinelli, Teresa Troiani, Michele Orditura, Ferdinando De Vita, Fortunato Ciardiello

Division of Medical Oncology, Department of Clinical and Experimental Medicine and Surgery "F. Magrassi e A. Lanzara" Second University of Naples, Naples, Italy

- Sorafenib reduced the activation of MEK and MAPK and caused an inhibition of cell proliferation, invasion, migration, anchorage-independent growth in vitro and of tumor growth in vivo of all TKI-resistant CALU-3 and HCT116 cell lines.
- These data suggest that resistance to EGFR inhibitors is predominantly driven by the RAS/RAF/MAPK pathway and can be overcame by treatment with sorafenib

EGFR pathway and angiogenesis

- **The BATTLE trial :**
  - Sorafenib had a higher disease control rate in EGFR--wild-type patients ($P < 0.001$), but a worse disease control rate in patients with EGFR mutation ($P = 0.01$) or high EGFR polysomy ($P = 0.05$) compared with other agents *Kim ES. Cancer Discov 2011;1:44-53*
- **Randomized, Phase II trial sorafenib + erlotinib versus erlotinib +placebo.**
  - 67 patients with tumors bearing wild-type EGFR, sorafenib/erlotinib group showed a superior median PFS (3.38 months in sorafenib/ erlotinib group versus 1.77 months, $P = 0.018$) and a superior mean overall survival (8 months for sorafenib/erlotinib versus 4.5 months, $P = 0.019$) *Spigel DR J Clin Oncol 2011;29(18):2582-9*

EGFR pathway and angiogenesis

- Despite the importance of both the EGFR pathway and angiogenesis in lung cancer, inhibition of both these pathways using the combination of sorafenib plus erlotinib, does not appear to improve the therapeutic ratio.

PM Ellis Critical Reviews in Oncology/Hematology 84 (2012) 47–58

Treatment summary

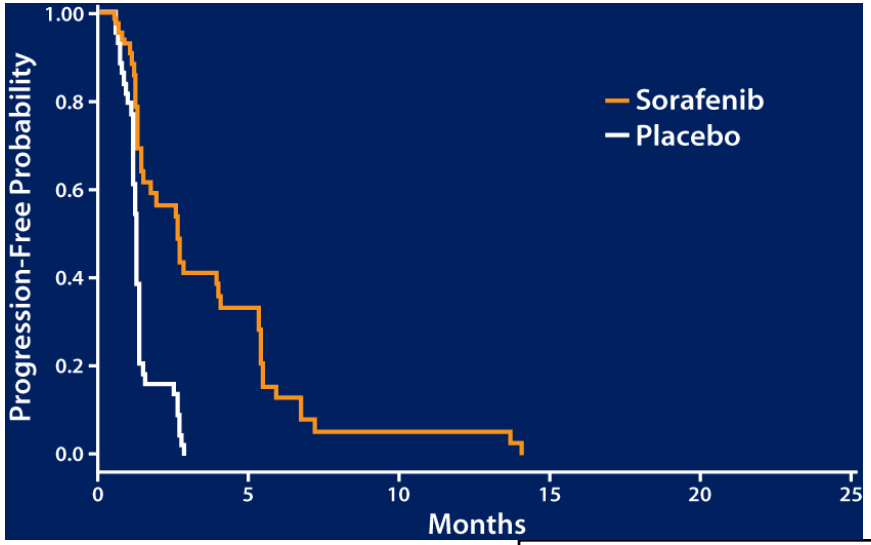
	EGFR mutation		EGFR wild type	
	Sorafenib n=44 (%)	**Placebo n=45 (%)**	**Sorafenib n=122 (%)**	**Placebo n=136 (%)**
On-study				
Duration of therapy Mean (weeks)	16.6	6.1	19.6	12.4
Dose interruption, n (%)	13 (30)	6 (13)	60 (49)	27 (20)
Dose reduction, n (%)	8 (18)	1 (2)	47 (39)	8 (6)
Any	26 (59)	25 (56)	54 (44)	84 (62)
2+	16 (36)	9 (20)	22 (18)	33 (24)
Anti-EGFR	19 (43)	8 (18)	18 (15)	37 (27)

P=0.01

Progression-free survival based on EGFR mutation status

Pts with EGFR mut (in tumor or plasma)

- Sorafenib N=44; Placebo N=45
- HR=0.27 (95% CI 0.16,0.46)
- P-value<0.001
- Sorafenib median PFS= 2.7 mo (83d)
- Placebo median PFS= 1.4 mo (42d)



As the PFS was prolonged in patient receiving sorafenib, in patients in 3d to 4th line of treatment, did it gave time to have access to anti EGFR targeted treatment

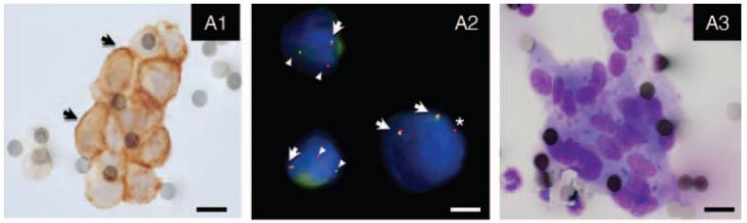
Biomarker*treatment interaction analysis: p-value=0.015

Sorafenib benefit in EGFR mutated subgroup

- OS outcome may be biased by the unbalanced use of post-study EGFR TKI (sorafenib arm 43%; placebo arm 18%
- The patient subgroup with available samples for biomarker analysis is not representative of the overall population
- Limited sample size (47% of overall population)
- Biomarker analyses in MISSION were retrospective

Perspectives

- Liquid biopsy with blood sample reflecting metastatic or micrometastatic disease
  - Could be repeated during treatment
  - Emergence of treatment resistance
- Circulating cell free DNA for KRAS and EGFR
- Circulating tumor cell for ALK translocation
  - (Ilie et al Ann Oncol2012)

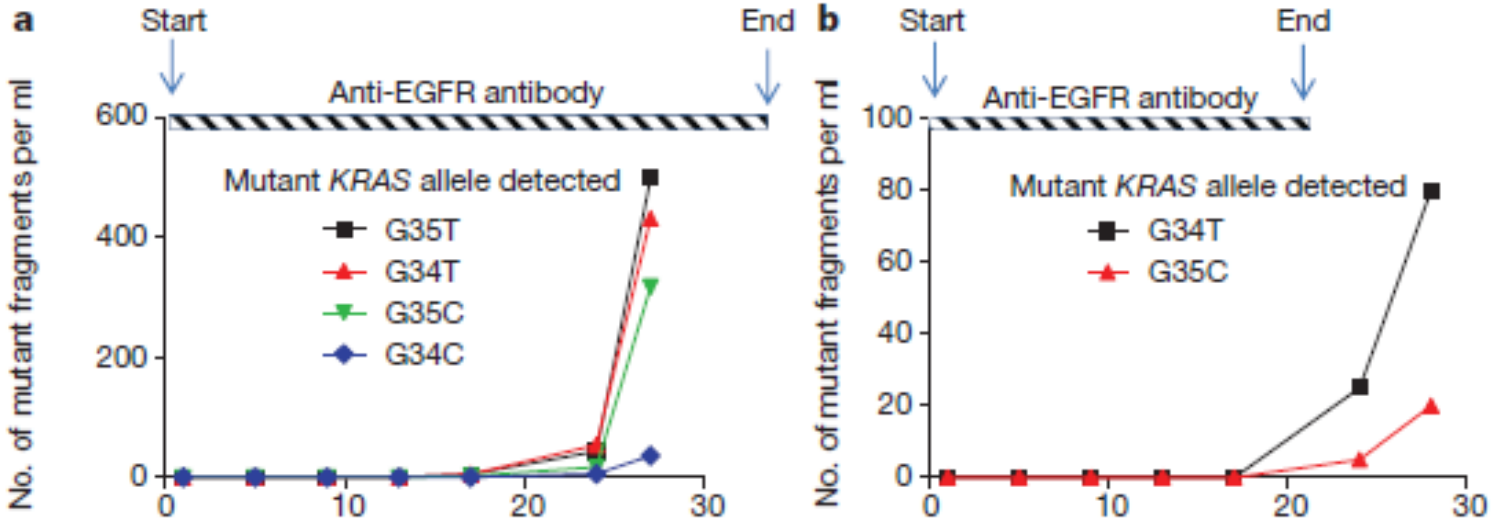


LETTER

doi:10.1038/nature11219

The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers

Luis A. Diaz Jr[1,2], Richard T. Williams[3], Jian Wu[1,4], Isaac Kinde[1], J. Randolph Hecht[5], Jordan Berlin[6], Benjamin Allen[7], Ivana Bozic[7], Johannes G. Reiter[7,8], Martin A. Nowak[7], Kenneth W. Kinzler[1], Kelly S. Oliner[3] & Bert Vogelstein[1]



Acknowlegments

- Pr Denis Moro-Sibilot
- Dr Anne Vincent-Salomon
- To Drs Blackhall and Mok for their great job and providing me the slides