EUROPEAN LUNG CANCER CONFERENCE 2016

BIOMARKERS

An In depth look at trends in liquid biopsies

Invited Discussant for posters 2PD, 3PD and 61PD

Karachaliou Niki

Medical Oncology Department, University Hospital Sagrado Corazon
Translational Research Unit, University Hospital Quiron Dexeus
Barcelona, Spain

Friday, 04/15/2016, 02:45 - 03:45 PM, Room W

DISCLOSURE SLIDE

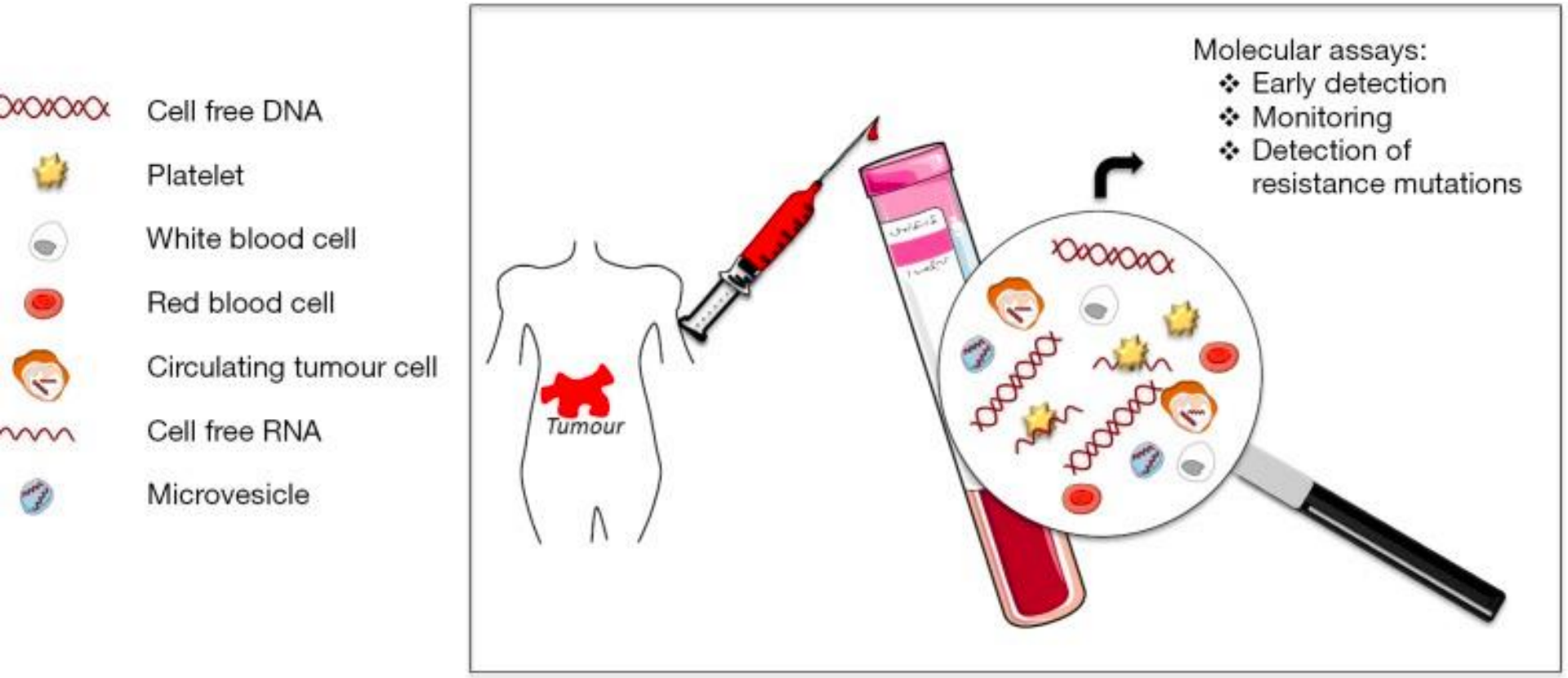
I have nothing to declare

2PD Monitoring of Secondary Drug Resistance Mutations in Circulating Tumor DNA of Patients with Advanced ALK Positive NSCLC. *Bordi Paola et al*

3PD Liquid biopsy in Patients with Adenocarcinoma of the Lung and its Correlation with their Tumor Tissue Genetic Profile. *Santos Edgardo et al*

61PD 24h-Blood profile Gene Expression Biomarkers of the Response to Targeted Therapy in Advanced Non-Squamous Non Small Cell Lung Cancer (NSCLC). *Baty Florent and Brutsche Martin*

Real-time liquid biopsies become a reality in cancer treatment



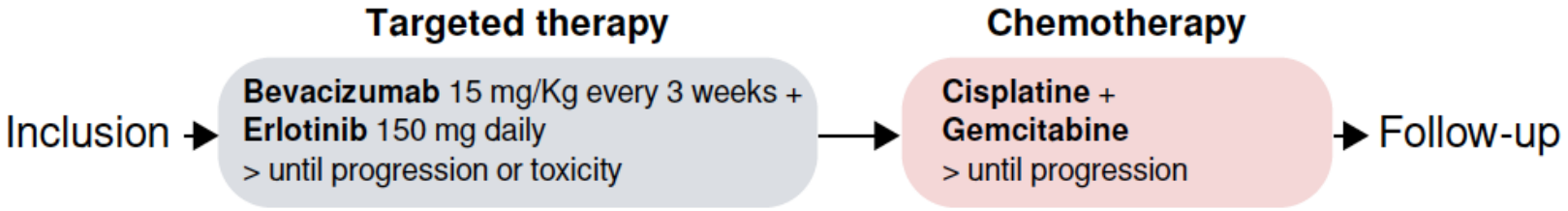
Karachaliou et al, ATM 2015

24h-blood profile gene expression biomarkers of the response to targeted therapy in advanced non-squamous non-small cell lung cancer (NSCLC)

Florent Baty, Martin Brutsche
Department of Pulmonary Medicine, Cantonal Hospital St. Gallen, Switzerland

Poster 61PD, Baty and Brutsche

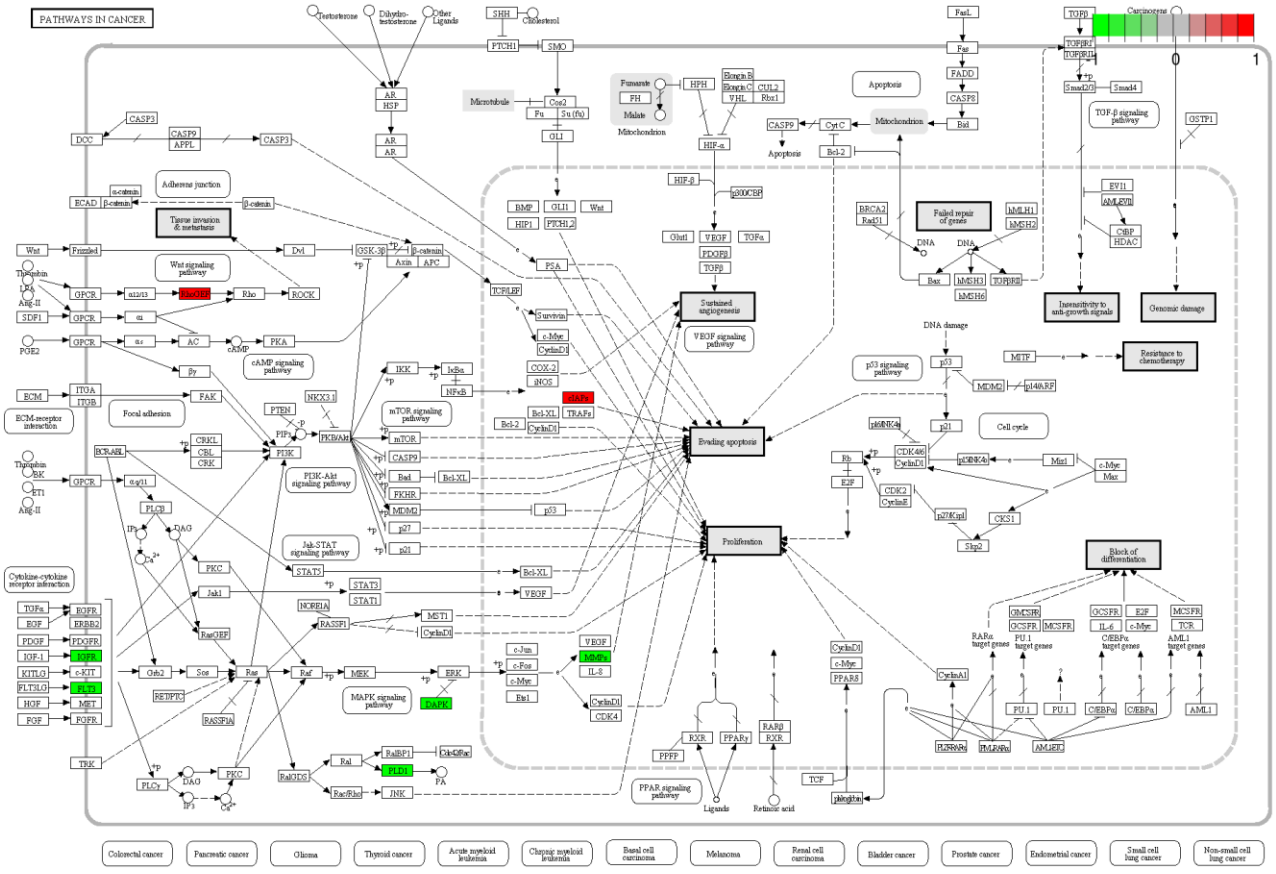
Bevacizumab and erlotinib (BE) first-line therapy in advanced non-squamous non-small-cell lung cancer (NSCLC) (stage IIIB/IV) followed by platinum-based chemotherapy (CT) at disease progression: a multicenter phase II trial (SAKK 19/05).



Zappa F, Droege C, Betticher D, von Moos R, Bubendorf L, Ochsenbein A, Gautschi O, Oppliger Leibundgut E, Froesch P, Stahel R, Hess T, Rauch D, Schmid P, Mayer M, Crowe S, Brauchli P, Ribi K, Pless M; Swiss Group for Clinical Cancer Research (SAKK).

- **Exon array analysis**
  - Blood samples taken from 43 non-squamous NSCLC patients at baseline and 24h after initiation of combined bevacizumab/erlotinib therapy.
  - mRNA extracted and hybridized on Affymetrix GeneChip Human Exon 1.0 ST arrays

Blood expression variation of genes from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway were measured at baseline and 24h after initiation of targeted therapy.



Poster 61PD, Baty and Brutsche

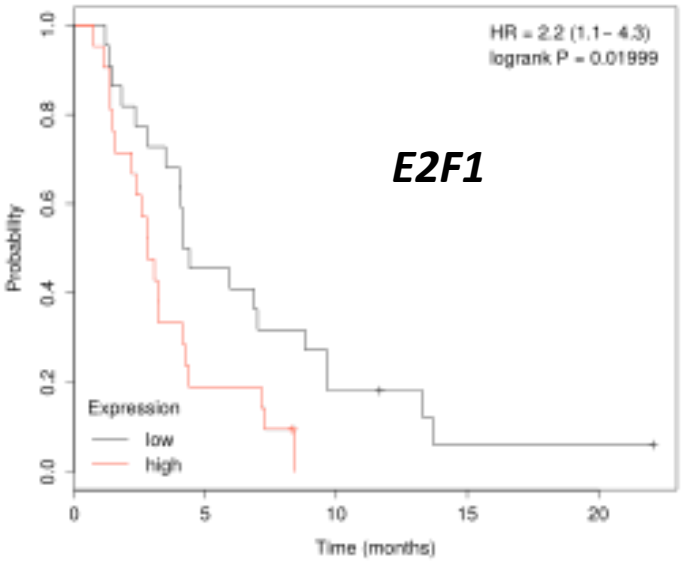
A significant down-regulation (p = 0.0204) was found in genes that are part of the KEGG pathway “Pathways in cancer”:

- **cytokine-cytokine receptor interaction** (**IGF1R, IGF2R**)
- ***MAPK*** signaling pathway (**DAPK2, PLD1, MMP9**)
- ***mTOR*** signaling pathway (**BIRC3**)

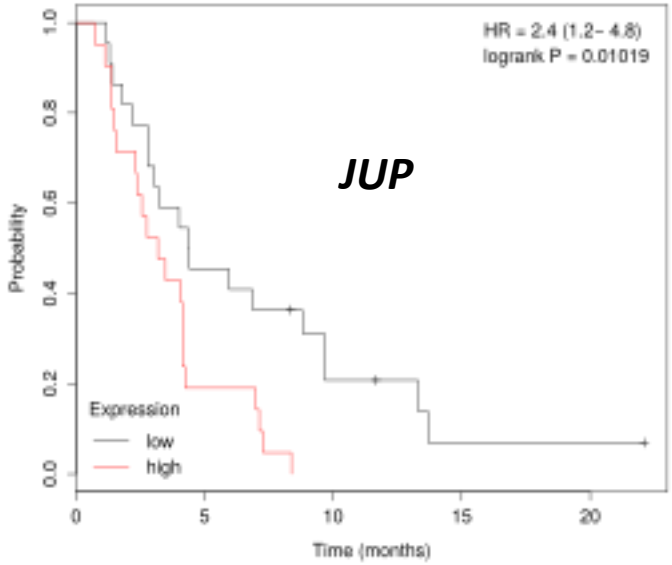
Other statistically significantly dysregulated pathways included the ***Hematopoietic cell lineage*** (KEGG hsa04640; p = 0.0085), and ***ABC transporters*** (KEGG hsa02010; p = 0.0094).

but….The magnitude of change over 24h was not predictive of patient outcome

Predictive markers at 24h for PFS and OS were genes that encode for transcription factors (**E2F1**, E2F8, MITF), for the DNA repair protein RAD51), for one tumor suppressor gene (**JUP** or γ-catenin or plakoglobin) and for immunoglobulins.

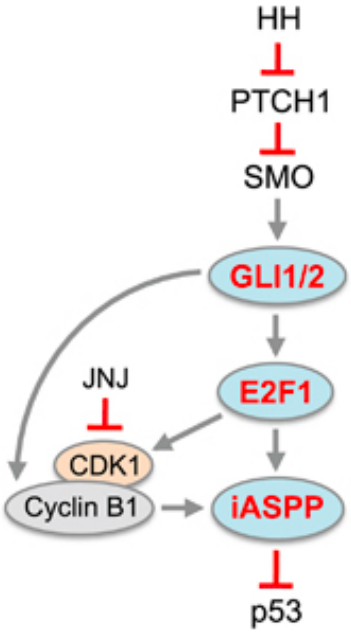


High level of E2F1 in blood at 24h was an indicator of poor prognosis (median PFS: 2.8 [2.2-4.4] months vs. 4.3 [4.0-9.7] months; log-rank p=0.020)



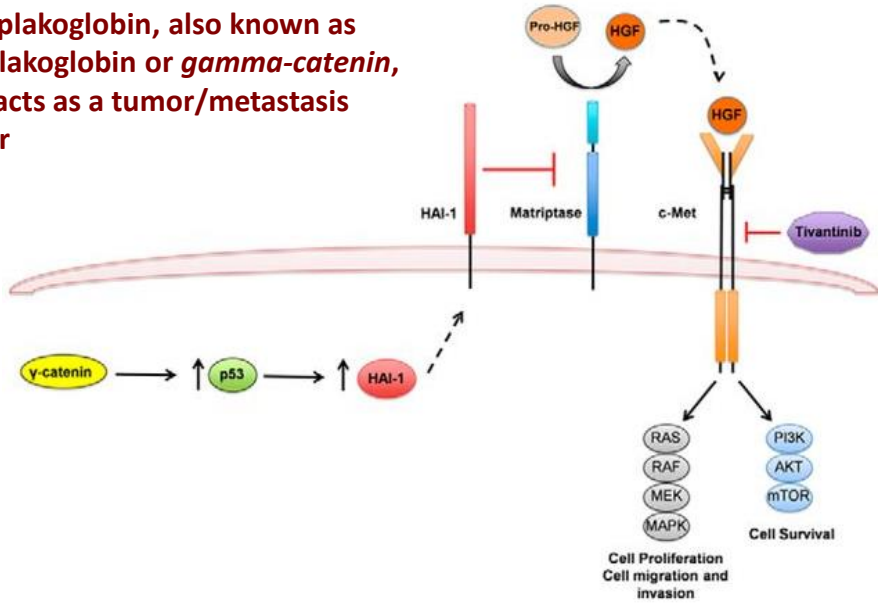
Similarly, high level of JUP in blood at 24h an indicator of poor prognosis (median PFS: 3.2 [3.1-9.7] months vs. 4.4 [3.1-9.7] months; log-rank p=0.010)

Poster 61PD, Baty and Brutsche



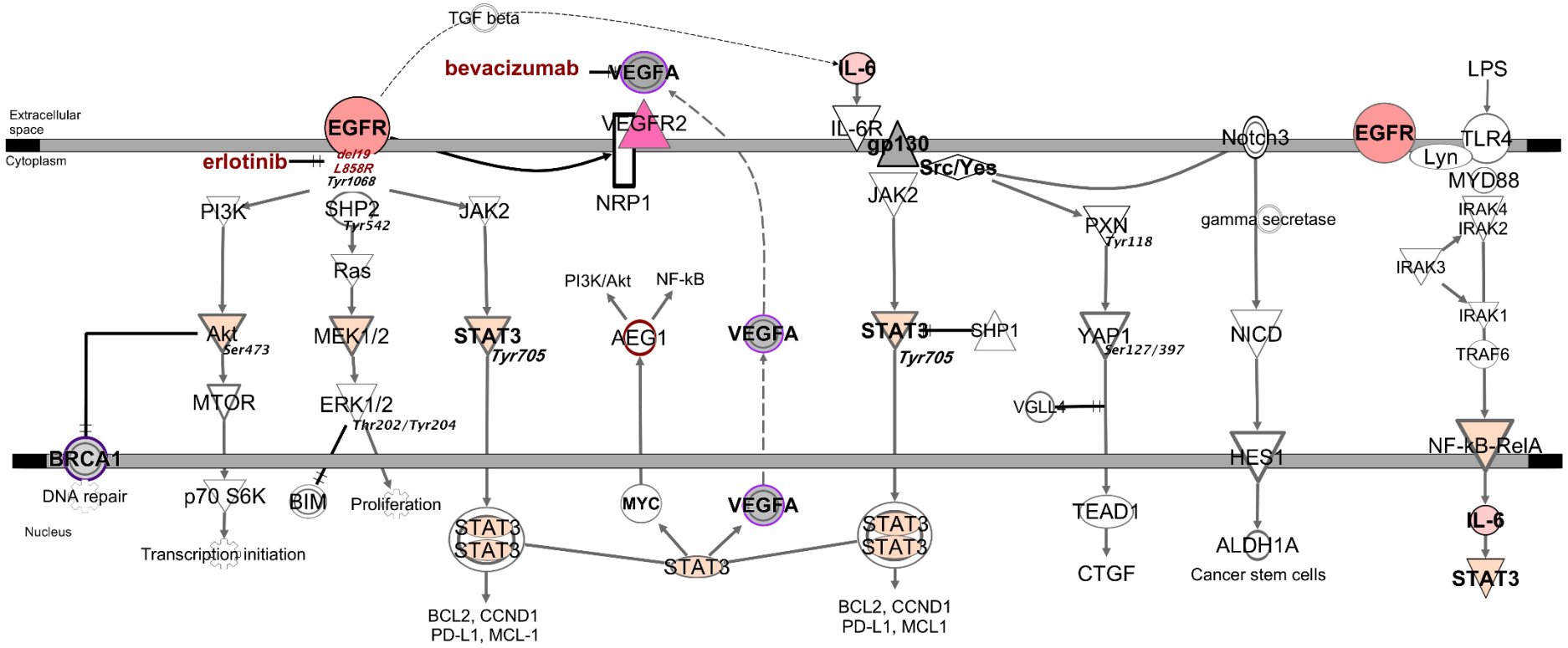
Pandolfi et al Cell Death Differ 2015

However, plakoglobin, also known as junction plakoglobin or *gamma-catenin*, generally acts as a tumor/metastasis suppressor



Sechler et al J Biol Chem 2015

Anti-VEGF antibody inhibits IL-6/IL-6R regulation of VEGF. Neither inhibition of PI3K or MAPK inhibits IL-6 mediated transcriptional up-regulation of VEGF.



Karachaliou et al (Rosell)

Blocking STAT3 pathway effectively abolishes IL-6 induced VEGF mRNA *(Wei et al Oncogene 2003)*

Discussion and conclusion

- The 24h effect of BE could be accurately (?) monitored in peripheral blood using the exon array technology

- Although the 24h changes had no predictive value with regard to the investigated endpoints, several markers were identified and validated from gene expression levels in blood at baseline and 24h.

- Blood gene expression levels at baseline and 24h after initiation of bevacizumab/erlotinib provide novel biomarkers (?) of the response to combined targeted therapy in unselected patients with advanced non-squamous NSCLC.

→ *A clinical trial is a unique opportunity for the development of novel biomarkers both in tissue and liquid biopsies.*

→ *This requires a rational and even pre-specified translational research design to avoid both wastage of biological material and generation of non-interpretable results.*

LIQUID BIOPSY IN PATIENTS WITH ADENOCARCINOMA OF THE LUNG AND ITS CORRELATION WITH THEIR TUMOR TISSUE GENETIC PROFILE

Edgardo S. Santos[1], Luis E. Raez[2], Lilibeth D.C. Castillero[3], Camila Marana[2], Brian Hunis[2]. [1]Thoracic Oncology Program, Eugene M. & Christine E. Lynn Cancer Institute, Boca Raton, FL, US; [2]Memorial Cancer Institute/Memorial Health Care System, Pembroke Pines, FL, US, [3]School of Medicine, Universidad de Panama, Panama, PA.

Poster 3D, Santos et al

Genomic alterations covered by the GH360 test

POINT MUTATIONS - ***Complete**** *or Critical Exon Coverage in 70 Genes*

AKT1	*ALK*	***APC***	***AR***	*ARAF*	***ARID1A***	*ATM*	***BRAF***	***BRCA1***	***BRCA2***
CCDN1	***CCND2***	***CCNE1***	*CDH1*	***CDK4***	***CDK6***	***CDKN2A***	***CDKN2B***	*CTNNB1*	***EGFR***
ERBB2	*ESR1*	*EZH2*	*FBXW7*	***FGFR1***	***FGFR2***	*FGFR3*	*GATA3*	*GNA11*	*GNAQ*
GNAS	*HNF1A*	***HRAS***	*IDH1*	*IDH2*	*JAK2*	*JAK3*	***KIT***	***KRAS***	*MAP2K1*
MAP2K2	***MET***	*MLH1*	*MPL*	***MYC***	***NF1***	*NFE2L2*	*NOTCH1*	*NPM1*	***NRAS***
NTRK1	***PDGFRA***	***PIK3CA***	***PTEN***	*PTPN11*	***RAF1***	***RB1***	*RET*	*RHEB*	*RHOA*
RIT1	*ROS1*	*SMAD4*	*SMO*	*SRC*	*STK11*	*TERT*	***TP53***	*TSC1*	*VHL*

AMPLIFICATIONS

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

FUSIONS

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

INDELS

EGFR exons 19/20	*ERBB2* exons 19/20	*MET* exon 14 skipping

81 consecutive patients having Guardant 360 test (liquid biopsy) only

Table 1. Most common gene alterations with its variants found in LBx.

Genomic Alterations	Variants
EGFR	Del 19, T790M, L858R, A118V, R531Q, G185D, Y1197H,V10971
TP53	Y234C, C238S, D259V, R158P, A161T, H179N, C141W, R273H
NF1	V17771, S348S, L1454L, V1308V, R873G
KRAS	V141, G12V, G12F, G12C, G12R
MET	E928E, E489K, T4741, L12L

Table 2. Incidence of the Most Common Gene Alterations found in Liquid Biopsy (LBx) using Guardant 360 Test.

Gene Alterations in LBx (n= 65)	%
TP53	49
EGFR	42
NF1	25
KRAS	17
MET	15
ARID1A	14
BRAF	11
AR	6

63 pts were used to compare TBx with LBx. 33 pts out of 63 (52%) had at least 1 similar genomic abnormality or MPRs found in both TBx and LBx.

Highest concordance in EGFR alterations (17/22; 77%).

173 Patients included in the EURTAC study

→ **50** Blood samples lost or damaged in shipment

123 Patients with blood samples for EGFR testing

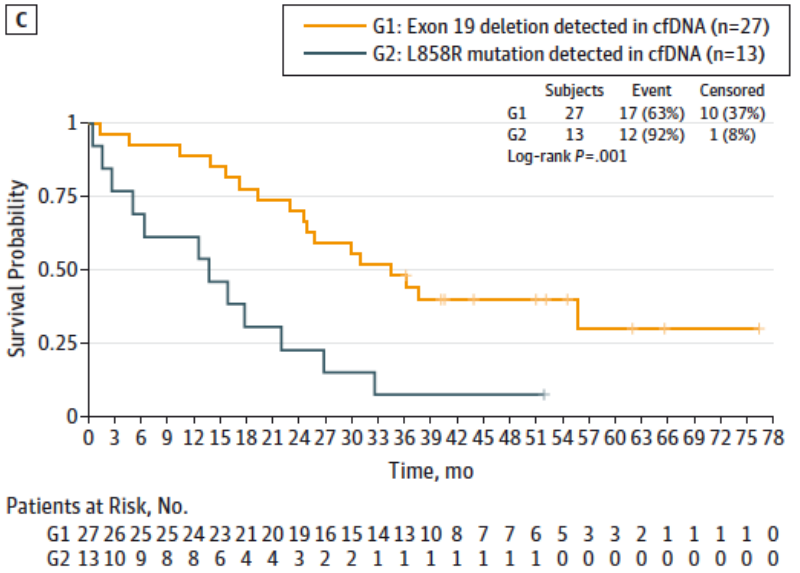
→ **26** Inadequate samples
14 Severely hemolyzed
12 Less than 0.5 mL

97 Pretreatment blood samples with circulating free DNA extracted

EGFR mutation detected by PNA-mediated 5´ nuclease real-time polymerase chain reaction (TaqMan) assay

	Total	Erlotinib	Chemotherapy
EGFR mutations assessed	97	49	48
EGFR mutations detected	76 (78%)	40 (82%)	36 (75%)
EGFR mutations not detected	21 (22%)	9 (18%)	12 (25%)

Type of EGFR mutation detected
47/76 (62%) Exon 19 deletion
29/76 (38%) L858R deletion



A multiplex 5´nuclease real-time PCR (Taqman) assay to be used in the presence of a PNA clamp

Karachaliou et al (Rosell) JAMA Oncology 2015
Rosell et al Lancet Oncology 2012

DISCUSSION

Poster 3D, Santos et al

This study showed that LBx is an alternative for pts with lung cancer when TBx is not feasible or TBx has insufficient tumor cells.

➔ ***Only 33 pts out of 63 (52%) had at least 1 similar genomic abnormality or MPRs found in both TBx and LBx***

EGFR and its variant is the most common driver mutation found in LBx. It also has a high degree of correlation with TBx (77%).

➔ ***The correlation was not performed with samples collected at the same time (at diagnosis)***

Do we need an expensive test to detect/monitor EGFR mutations in the blood?

A well controlled study is required to define the usefulness of the test at time of diagnosis as well as at disease progression

MONITORING OF SECONDARY DRUG RESISTANCE MUTATIONS IN CIRCULATING TUMOR DNA OF PATIENTS WITH ADVANCED ALK POSITIVE NSCLC

Bordi P[1], Del Re M[2], Danesi R[2] and Tiseo M[1]
[1] Medical Oncology Unit, University Hospital of Parma, Parma [2] Clinical Pharmacology and Pharmacogenetics Unit, Department of Clinical and Experimental Medicine, University of Pisa

Poster 2D, Bordi et al

MATERIALS AND METHODS

- Patients progressing during ALK-TKI were enrolled
- Blood was collected after tumor progression
- Plasma was isolated by centrifugation
- DNA was extracted from plasma using QIAamp circulating nucleic acid kit (Qiagen®)
- **ALK secondary mutations and KRAS exon 2 (codon 12) mutations were tested using a Digital Droplet PCR (BioRad®)**
- Mutational analysis in plasma was repeated 2 months after PD.
- The study was approved by local ethic committee

No. Of Patients	16
Age	53 [40-81]
Females	11 (68.6%)
Never smokers	9 (56.3%)
Line of therapy 3	4 (25%)
2	10 (62.5%)
1	2 (12.5%)
Brain PD	12 (75%)

- Best response were: 12 PR, 3 SD, 1 PD
- Brain was a site of PD in 12 patients, the only site of PD in 9 patients
- Median PFS was 8 months (range 4-29)

Poster 2D, Bordi et al

RESULTS

Patient No.	sex	age	Smoking habit	TKI-therapy	Line of treatment	Best response	PFS (mo)	Site of progression	Resistance mutation	Treatment after progression
1	F	52	former	ceritinib	3	PR	8.6	Brain	-	SRT+ceritinib
2	F	81	never	crizotinib	2	PR	13	brain, liver	ALK L1196M G1269A	brigatinib
3	M	44	never	crizotinib	3	SD	29	Brain	KRAS G12V	brigatinib
4	M	73	former	crizotinib	2	PR	7.4	lung,pleura	KRAS G12D	brigatinib
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9	M	44	former	crizotinib	1	PR	5.8	lung, brain	-	brigatinib
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13	F	57	never	crizotinib	3	PD	10	Brain	-	brigatinib
14	M	53	Never	crizotinib	2	PR	5	Lung, node	-	SRT+crizotinib
15	F	72	Never	crizotinib	2	SD	10	Lung, node	-	Alectinib
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Poster 2D, Bordi et al

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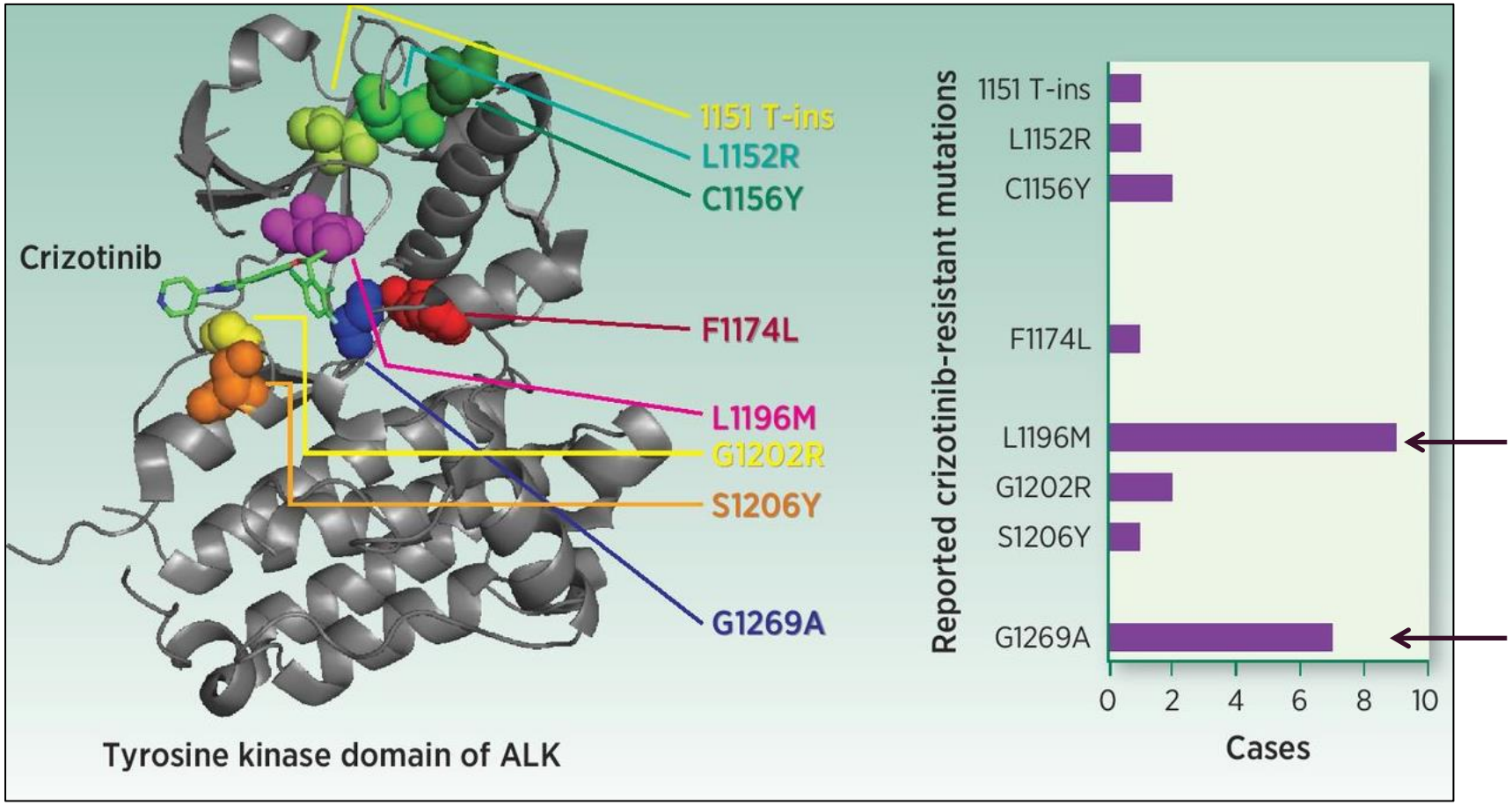
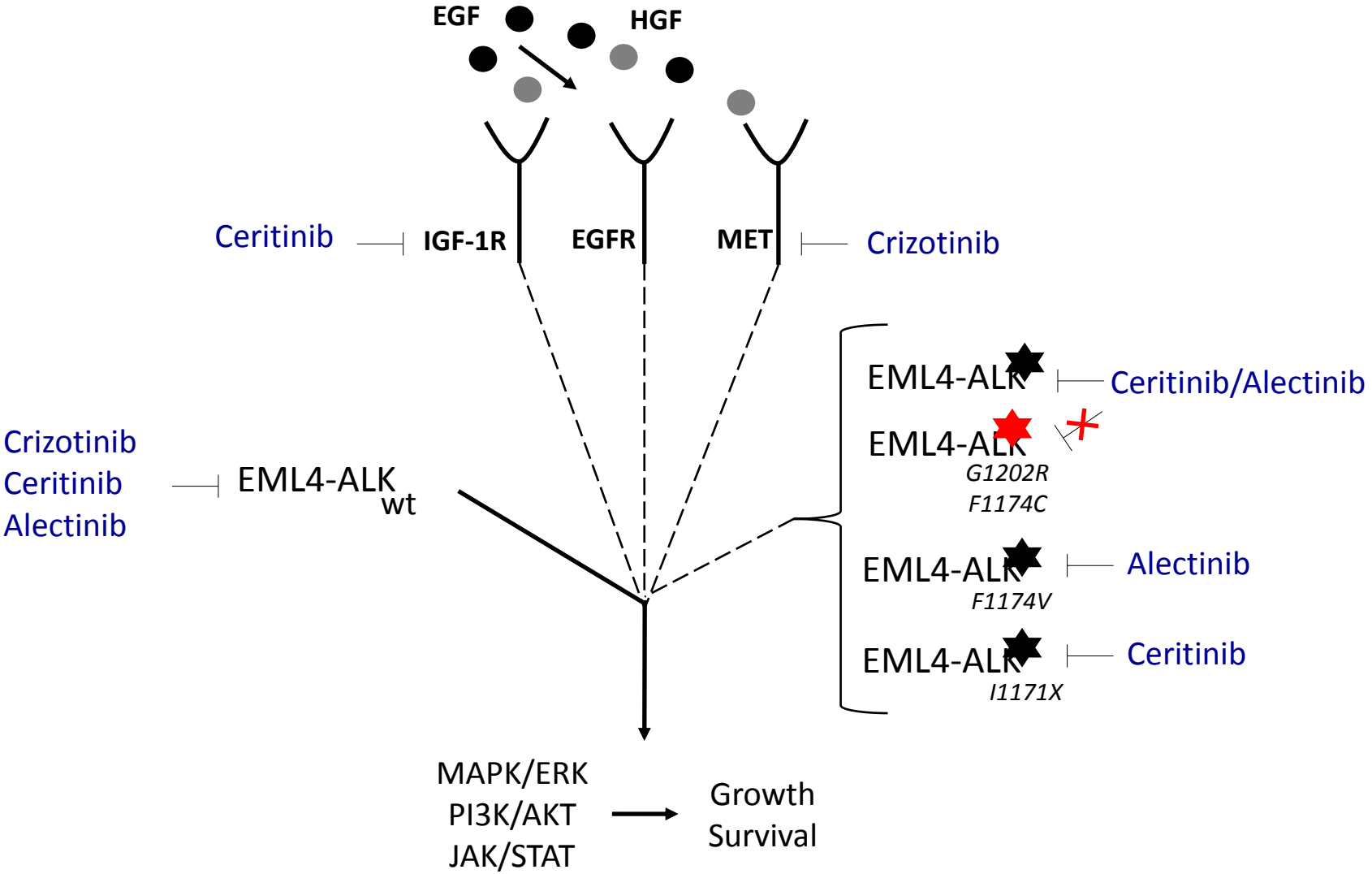


Katayama, Lovly and Shaw. Clin Cancer Res 2015



Rosell et al Lancet Respiratory Medicine 2014

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Poster 2D, Bordi et al

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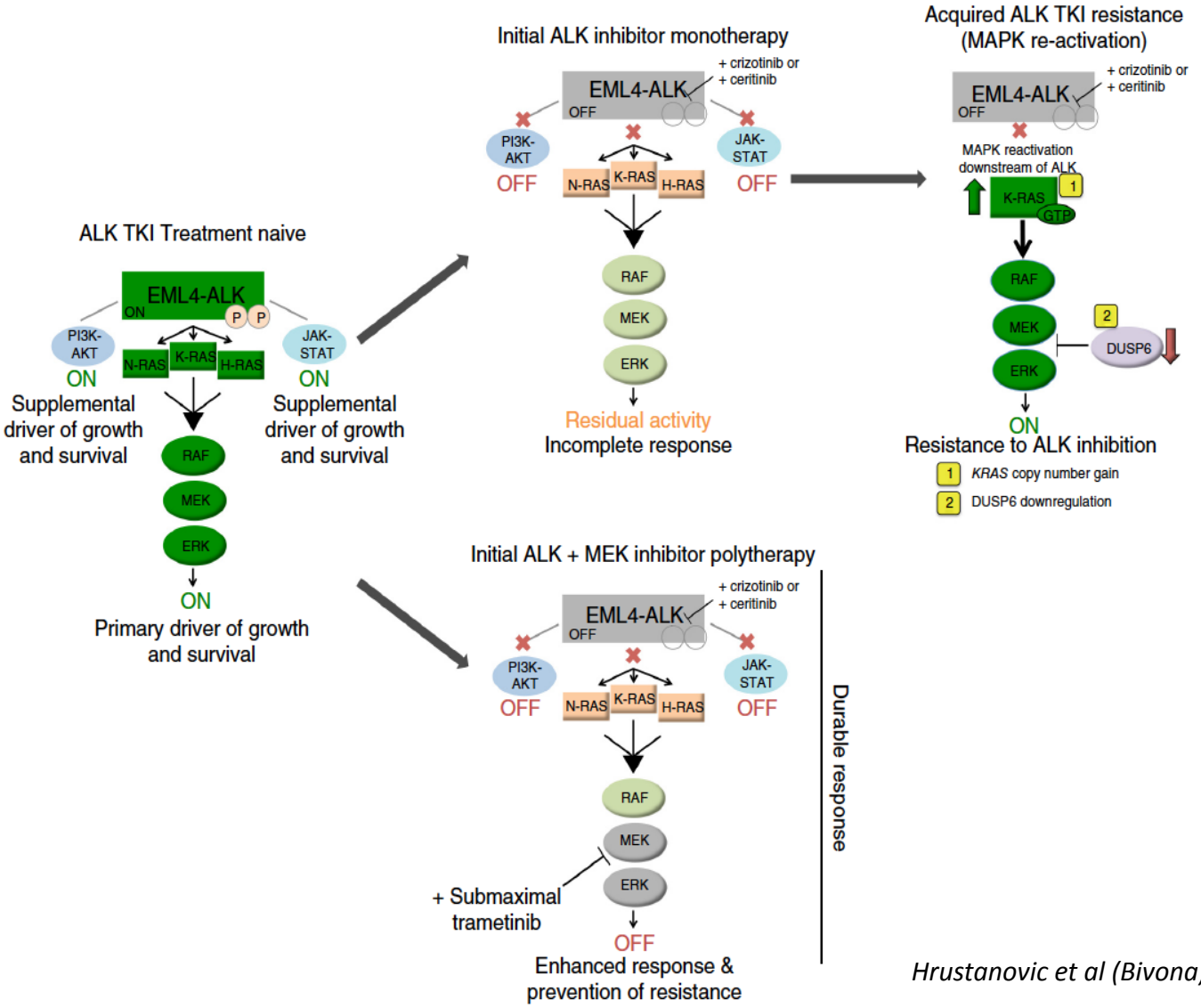
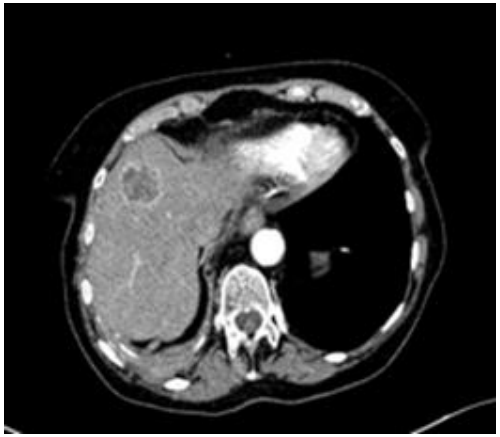
Poster 2D, Bordi et al

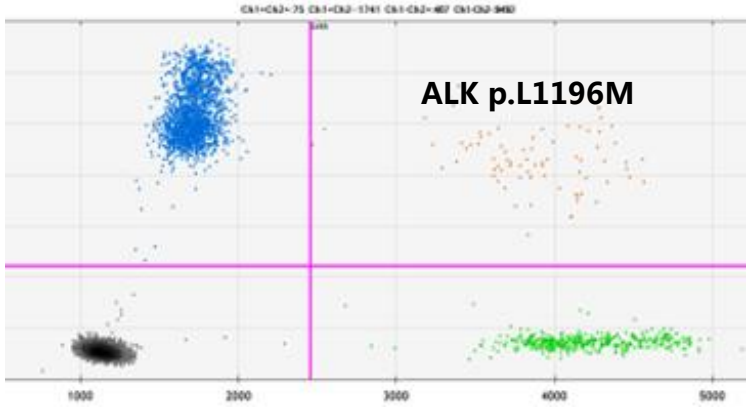


Hrustanovic et al (Bivona) Nature Medicine 2015

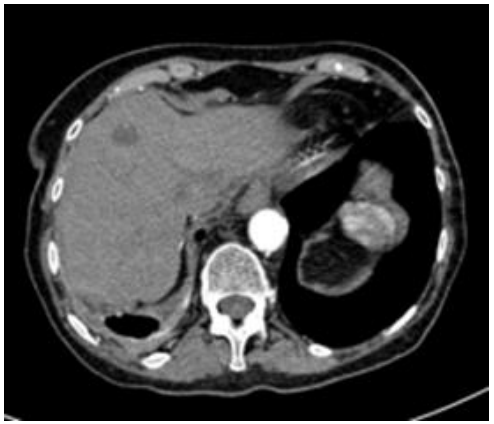
The concomitant occurrence of KRAS mutations and ALK rearrangement has been described in a subset of adenocarcinomas, generally leading to primary resistance to crizotinib

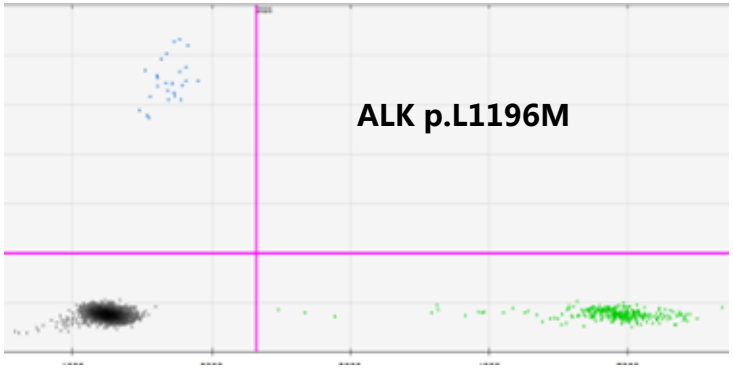
Doebele et al Clin Cancer Res 2014; Mengoli et al Lung Cancer 2016





Patient 2: Serum and radiological PD after crizotinib

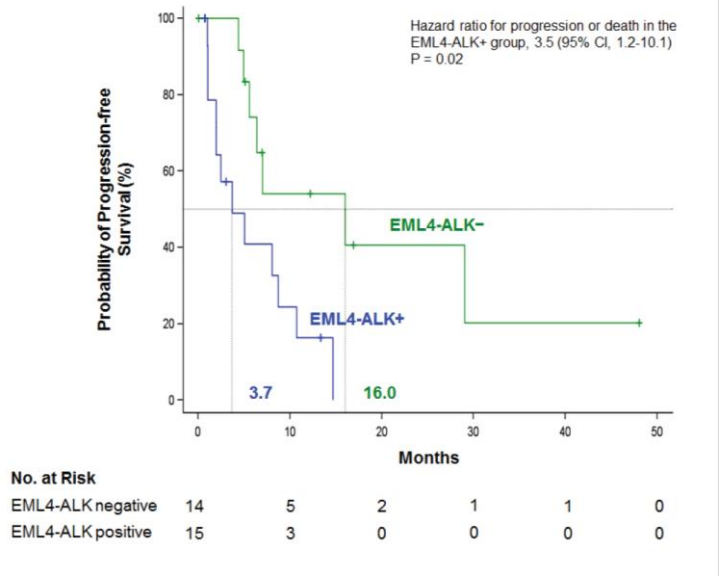




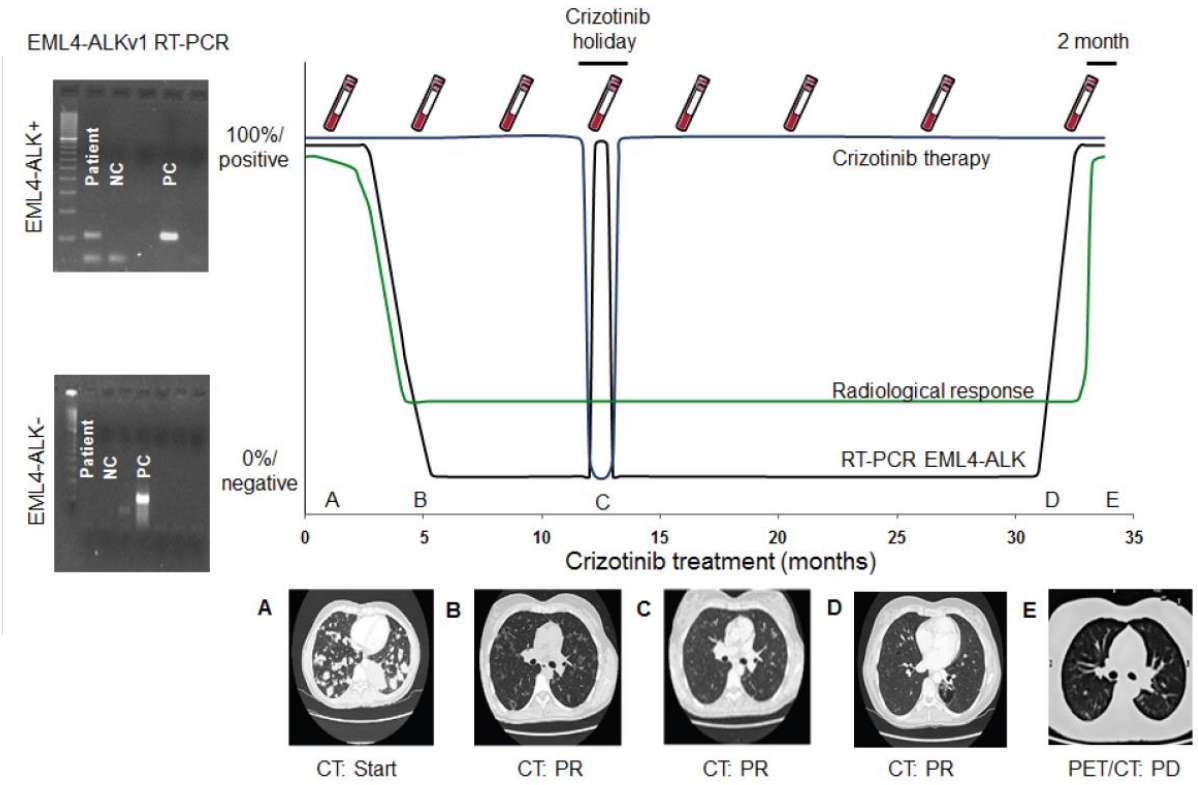
Patient 2: Serum and radiological response to brigatinib

Poster 2D, Bordi et al

Platelet EML4-ALK status by RT-PCR



Longitudinal monitoring of crizotinib response



Nilsson et al Oncotarget 2015

CONCLUSIONS

- ddPCR can detect resistance mutations in cfDNA of ALK+ NSCLC and may represent an effective alternative to re-biopsy. √
- The assessment of mutated allele burden could be used for response monitoring during treatment. √
- The percentage of patients with resistant ALK mutations is slightly inferior to that reported in the literature. This may be explained by the fact that some patients had progression in the brain, which could be considered a pharmacokinetic progression rather than mediated by clone selection.
- Our results are limited by the absence of tissue re-biopsy. √
- We observed a high incidence of KRAS mutations in blood of resistant patients. KRAS mutations have occasionally been reported as a resistance mechanism and their role in acquired resistance should be further investigated.

  Only exon 2, codon 12 mutations were examined. It would be interesting to explore the role of KRAS mutations as a mechanism of intrinsic resistance.

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3PD **Liquid biopsy in Patients with Adenocarcinoma of the Lung and its Correlation with their Tumor Tissue Genetic Profile.** ***Santos Edgardo et al***

61PD **24h-Blood profile Gene Expression Biomarkers of the Response to Targeted Therapy in Advanced Non-Squamous Non Small Cell Lung Cancer (NSCLC).** ***Baty Florent and Brutsche Martin***

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- *ALK secondary and KRAS mutations can be accurately measured by digital droplet PCR.*
- *KRAS mutations can be present pretreatment highlighting the need for combinatorial treatment.*
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- *The GH360 test covers a wide range of genomic alterations in blood.*
- *A better designed study with concomitant tissue and blood samples and information on treatment responses should be performed.*

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- *It is difficult to identify biomarkers of response to erlotinib/bevacizumab in an unselected population of NSCLC patients.*
- *The results of the exon array technology regarding expression changes at 24h of erlotinib/bevacizumab treatment are not convincing.*

Thank you