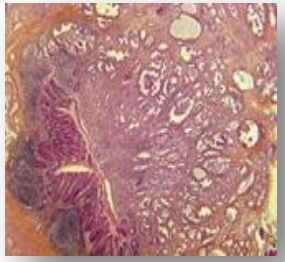
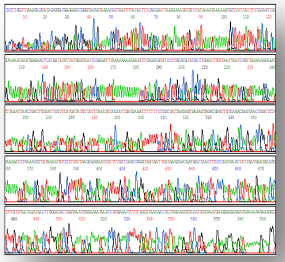
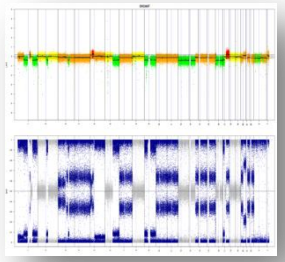
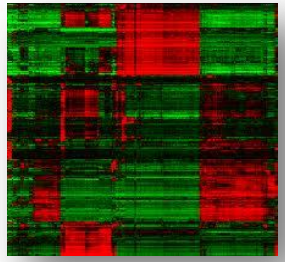
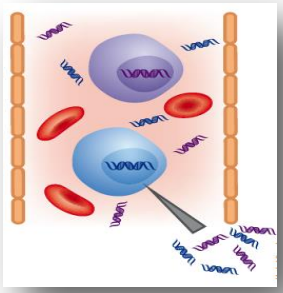
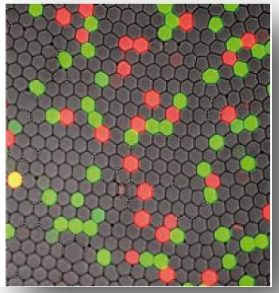
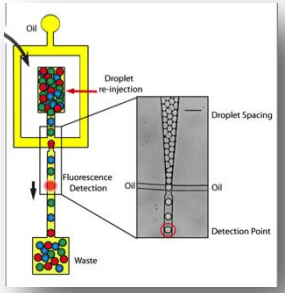
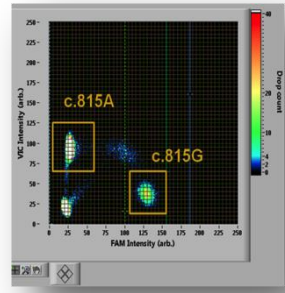





Biomarkers of primary resistance to targeted therapies

Pierre Laurent-Puig

UMR-S1147 Paris Descartes University

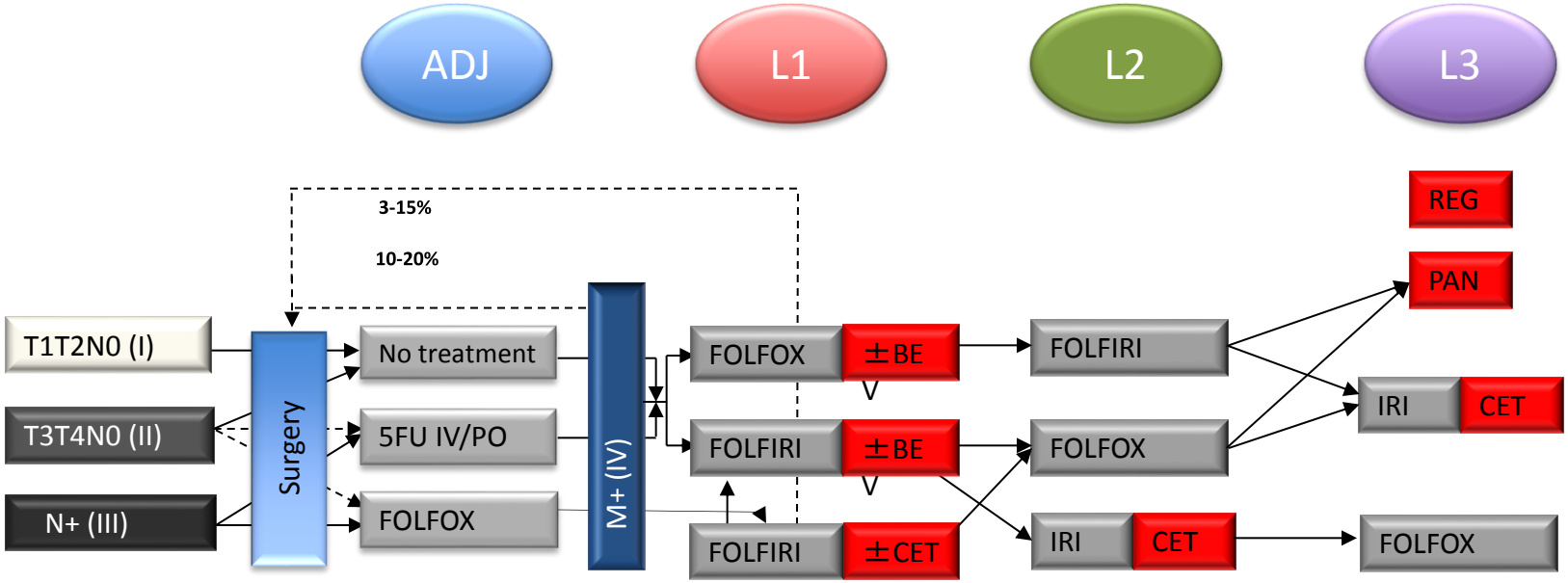






Link of interest

- AstraZeneca
- Boehringer-Ingelheim
- InteGragen
- Merck-Serono
- Sanofi

Landscape of colorectal cancer treatment



Twelves NEJM 2005
André NEJM 2004

Van Cutsem NEJM 2009
Saltz J Clin Oncol 2008
Tournigand JCO 2004
Hurwitz NEJM 2004
Adam Ann Surg 2004

Cunningham NEJM 2004

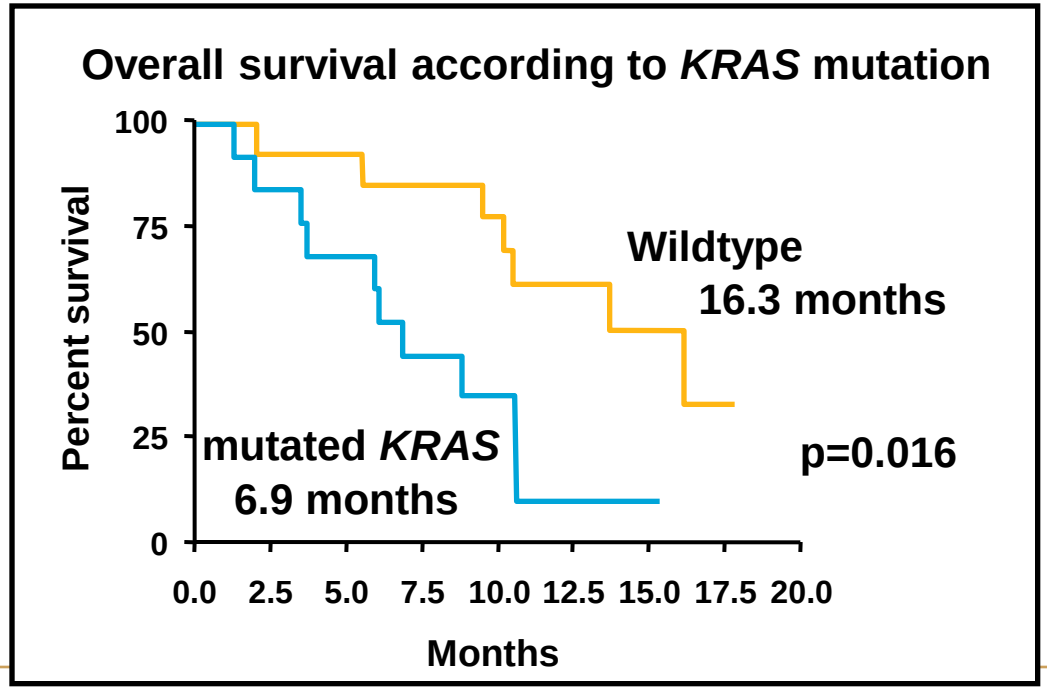
Van Custem NEJM 2009

BEV: Bevacizumab (Avastin Roche)
CET: Cetuximab (Erbitux. Merck KGaA/Imclone/BMS)
IRI: Irinotecan
PAN: Panitumumab
REGO Regorafenib

KRAS Mutation and Anti-EGFR therapy in advanced colorectal cancer

KRAS Status	Responders*	Non responders*	Total
KRAS mutation (%)	0 (0)	13 (100)	13
Wildtype (%)	11 (65)	6 (35)	17

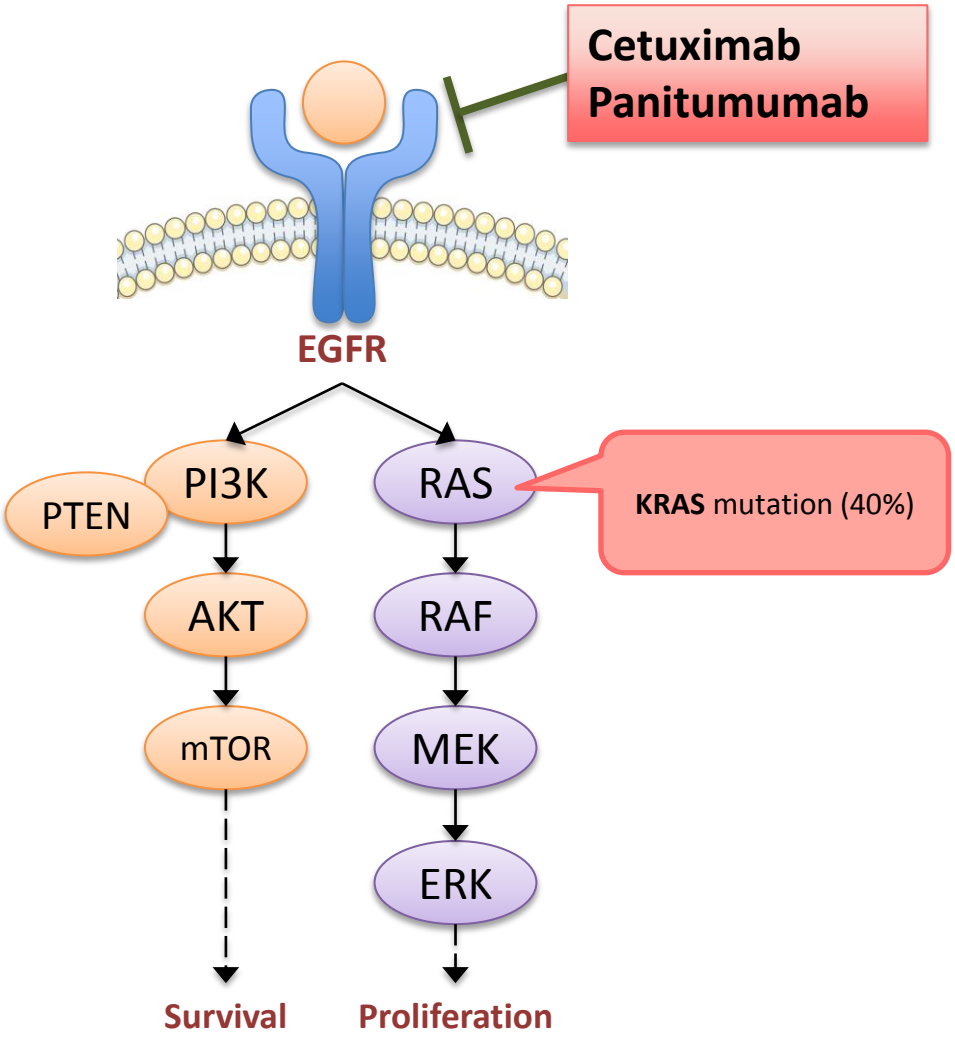
p=0.0003



Lièvre A et al. Cancer Res 2006.

* Response according to RECIST criteria

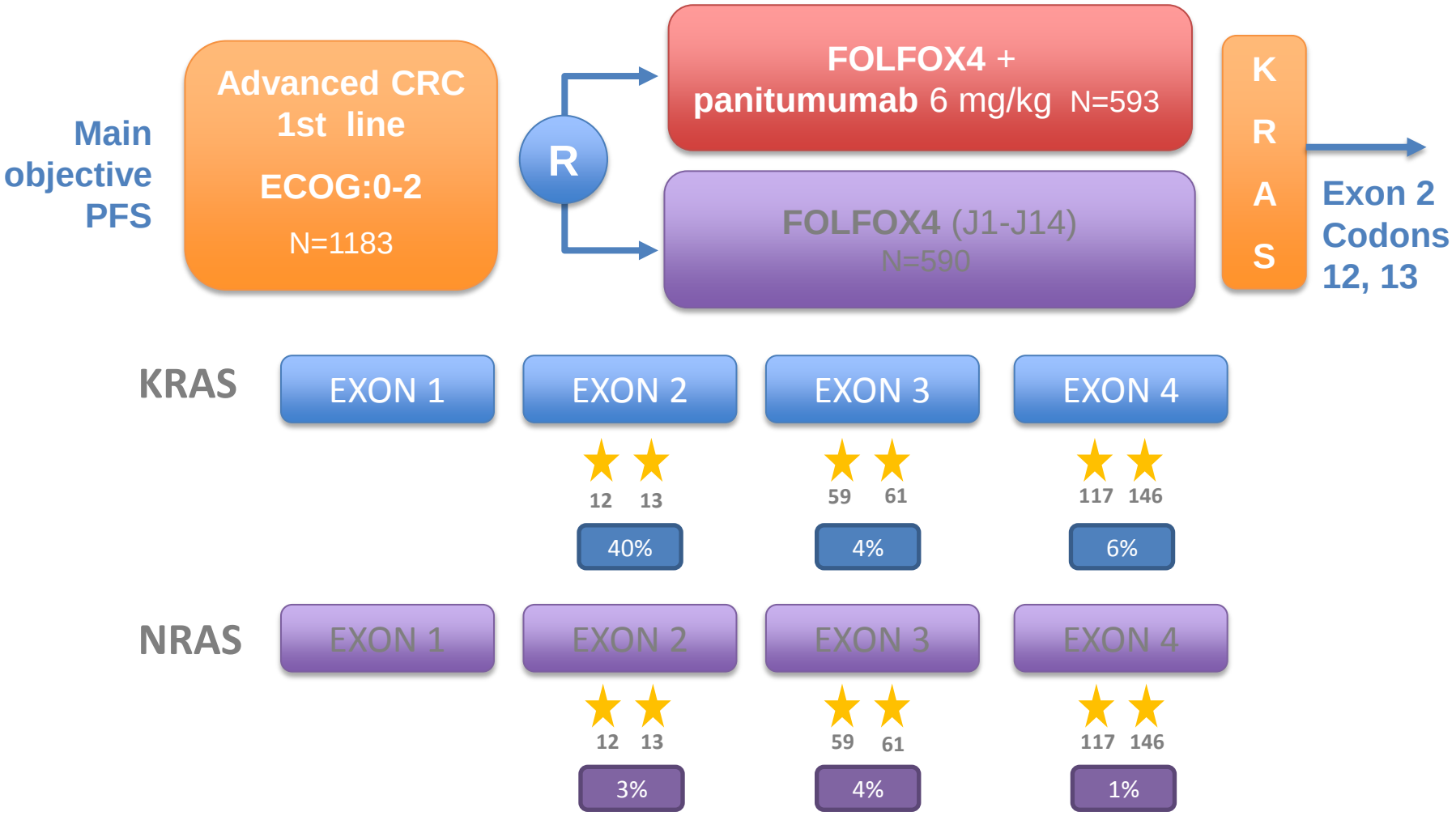
Target therapies



Lievre et al, JCO 2008
Douillard et al, NEJM 2013
Mekenkamp et al, BMC cancer 2012

- Candidate gene
  - BEYOND KRAS
    - RAS rare mutation
    - Amplification of KRAS
  - Role of minor KRAS mutant allele
- Without assumption
  - RNA
  - MirRNA
  - DNA

Impact of RAS mutation in PFS and OS in PRIME study



JY. Douillard *et al.*, N Engl J Med 2013;369:1023-34

Impact of RAS mutation in PFS and OS in PRIME study

		Panitumumab+FOLFOX	FOLFOX
No KRAS mutation in exon2			
	PFS	9.6 $CI_{95\%}$[9.2 – 11.1]	8 $CI_{95\%}$ [7.5 – 9.3]
	OS	23.8 $CI_{95\%}$[20.0-27.7]	19.4 $CI_{95\%}$ [17.4 – 22.6]
KRAS mutated in exon 2			
	PFS	7.3 $CI_{95\%}$[6.3 – 8]	8.8 $CI_{95\%}$[7.7 – 9.4]
	OS	15.5 $CI_{95\%}$[13.1 – 17.6]	19.2 $CI_{95\%}$[16.2 – 21.5]
No RAS mutation			
	PFS	10.1 $CI_{95\%}$[9.3 – 12.0]	7.9 $CI_{95\%}$[7.2 – 9.3]
	OS	25.8 $CI_{95\%}$[21.7-29.7]	20.2$CI_{95\%}$ [17.6 – 23.6]
No KRAS mutation in exon 2, other RAS mutation			
	PFS	7.3 $CI_{95\%}$[5.3 – 9.2]	8.0 $CI_{95\%}$[6.4 – 11.3]
	OS	17.1$CI_{95\%}$[10.8 – 19.4]	17.8 $CI_{95\%}$[13.0 – 23.2]

Douillard JY *et al.*, N Engl J Med 2013;369:1023-34

Impact of RAS mutation in PFS and OS in PRIME study

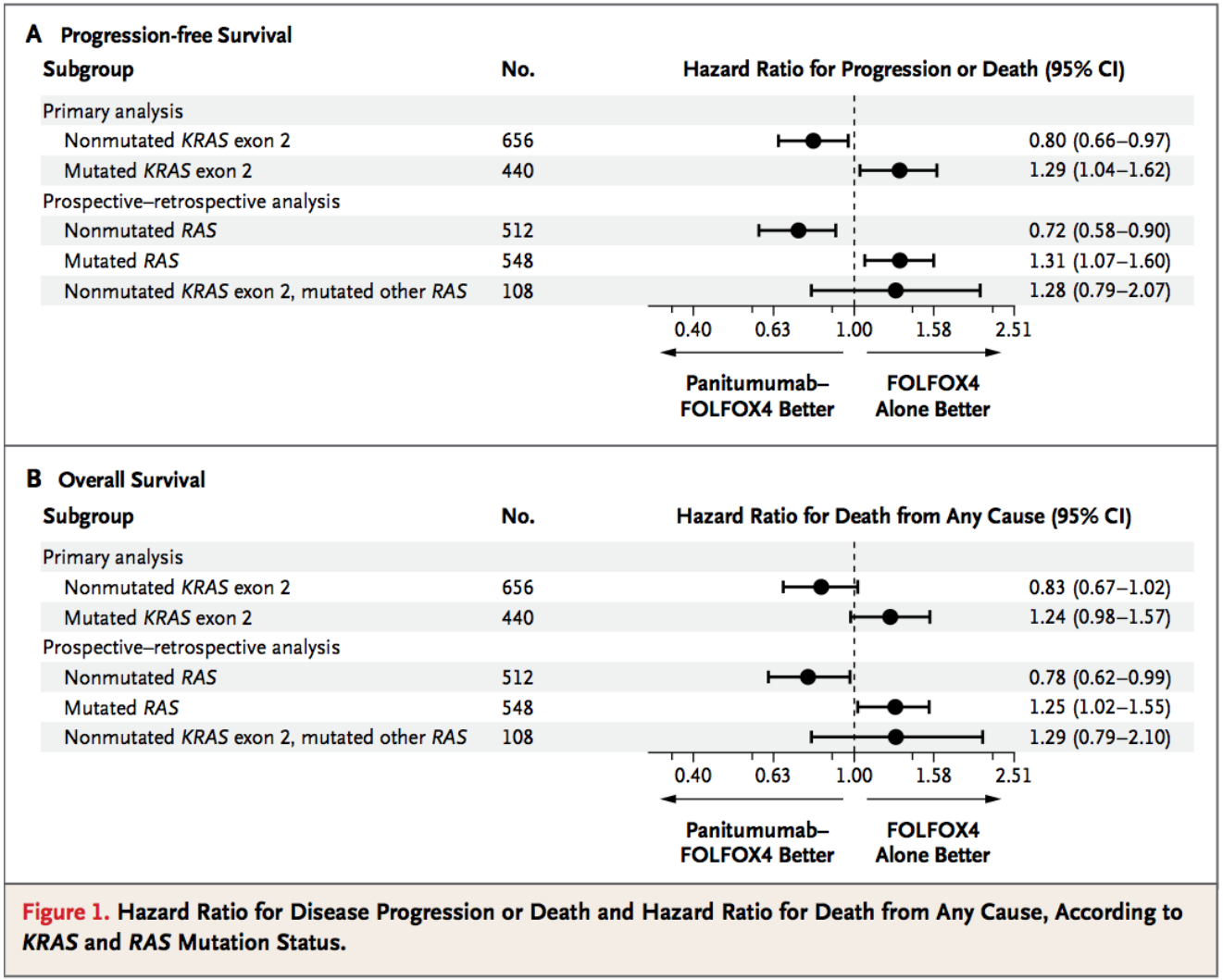


Figure 1. Hazard Ratio for Disease Progression or Death and Hazard Ratio for Death from Any Cause, According to *KRAS* and *RAS* Mutation Status.

Douillard JY *et al.*, N Engl J Med 2013;369:1023-34

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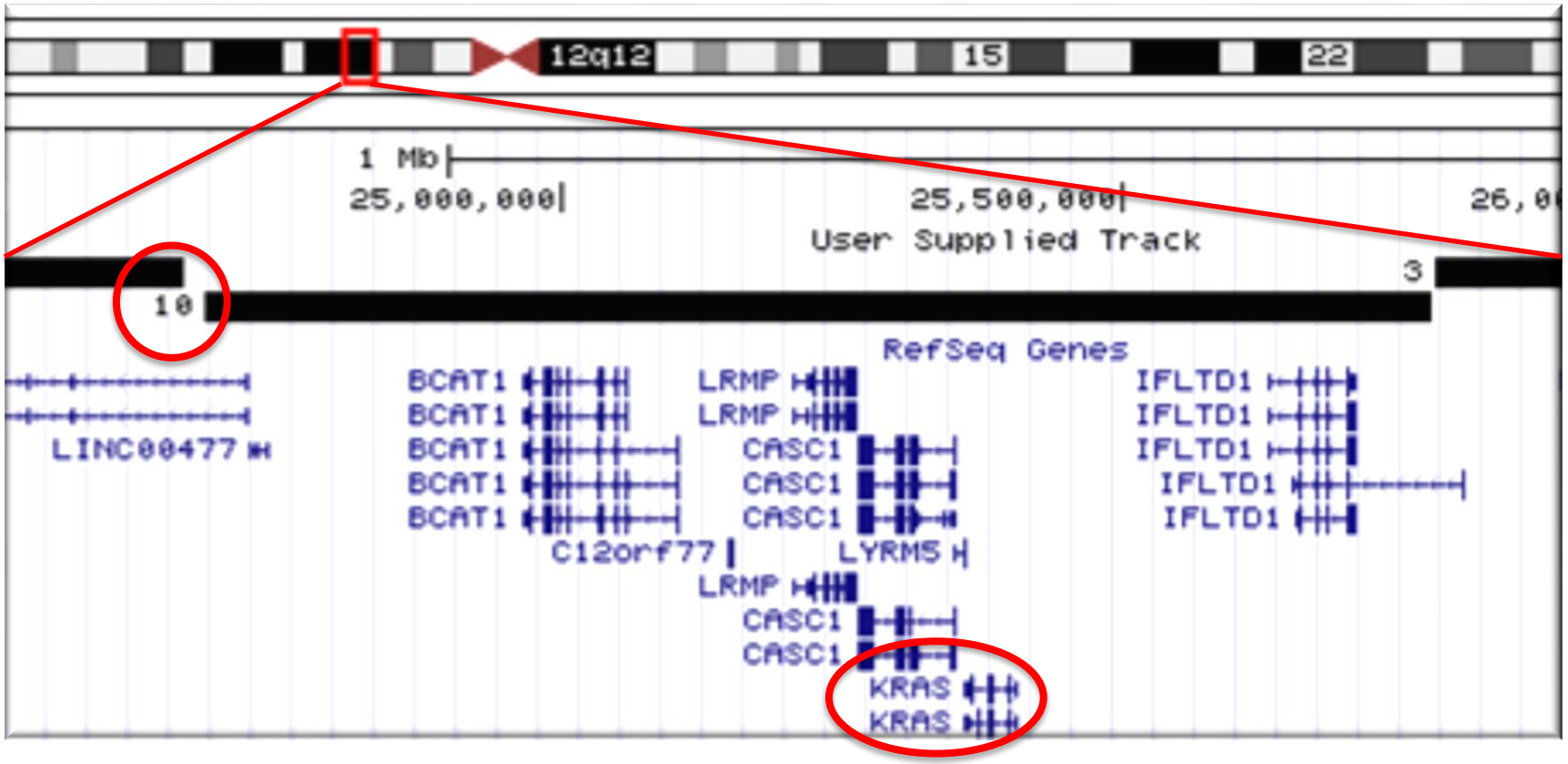
Other KRAS alterations

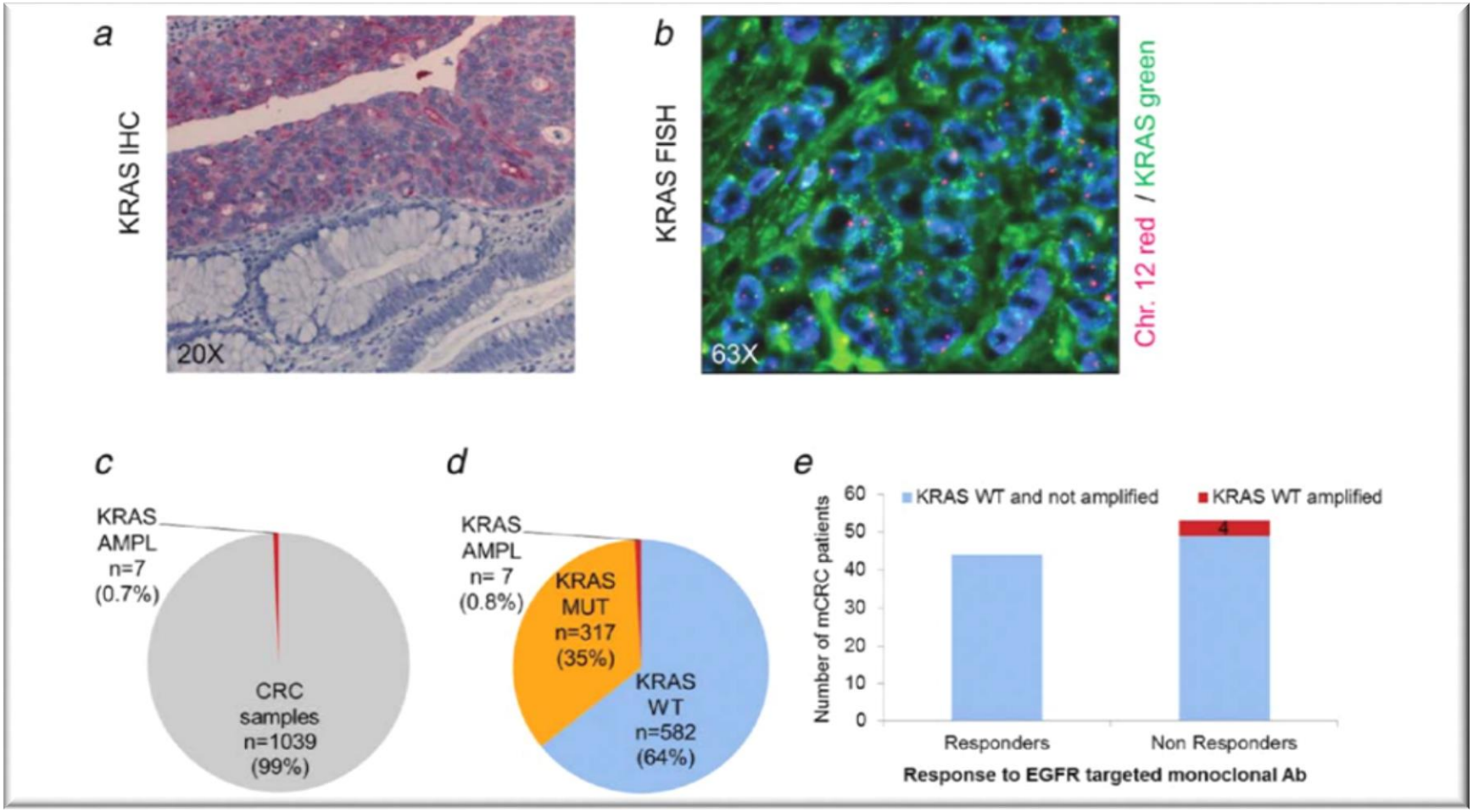


IJC
International Journal of Cancer

KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy

Emanuele Valtorta[1†], Sandra Misale[2,3†], Andrea Sartore-Bianchi[4], Iris D. Nagtegaal[5], François Paraf[6], Calogero Lauricella[1], Valentina Dimartino[7], Sebastijan Hobor[2], Bart Jacobs[8], Cristiana Ercolani[7], Simona Lamba[2,3], Elisa Scala[2,3], Silvio Veronese[1], Pierre Laurent-Puig[9], Salvatore Siena[4], Sabine Tejpar[8], Marcella Mottolese[7], Cornelis J.A. Punt[10], Marcello Gambacorta[1], Alberto Bardelli[2,3,11] and Federica Di Nicolantonio[2,3]

KRAS Amplification



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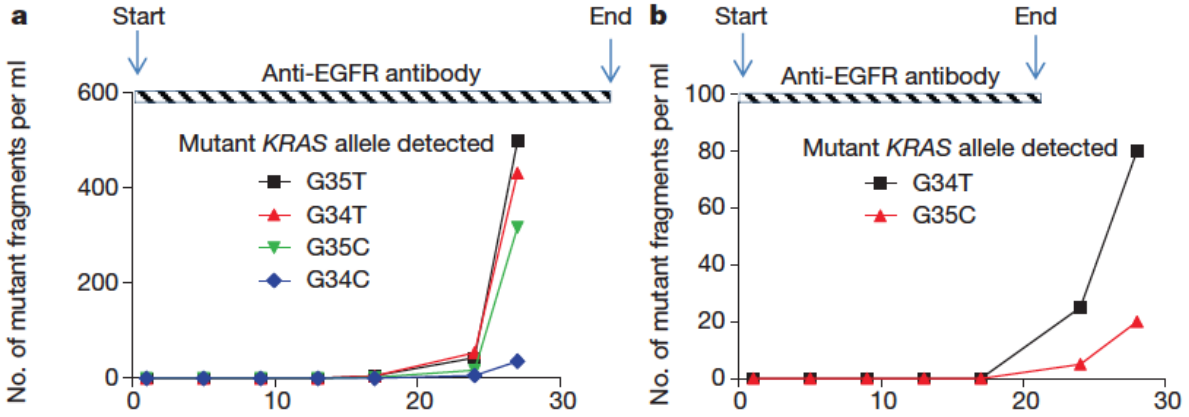
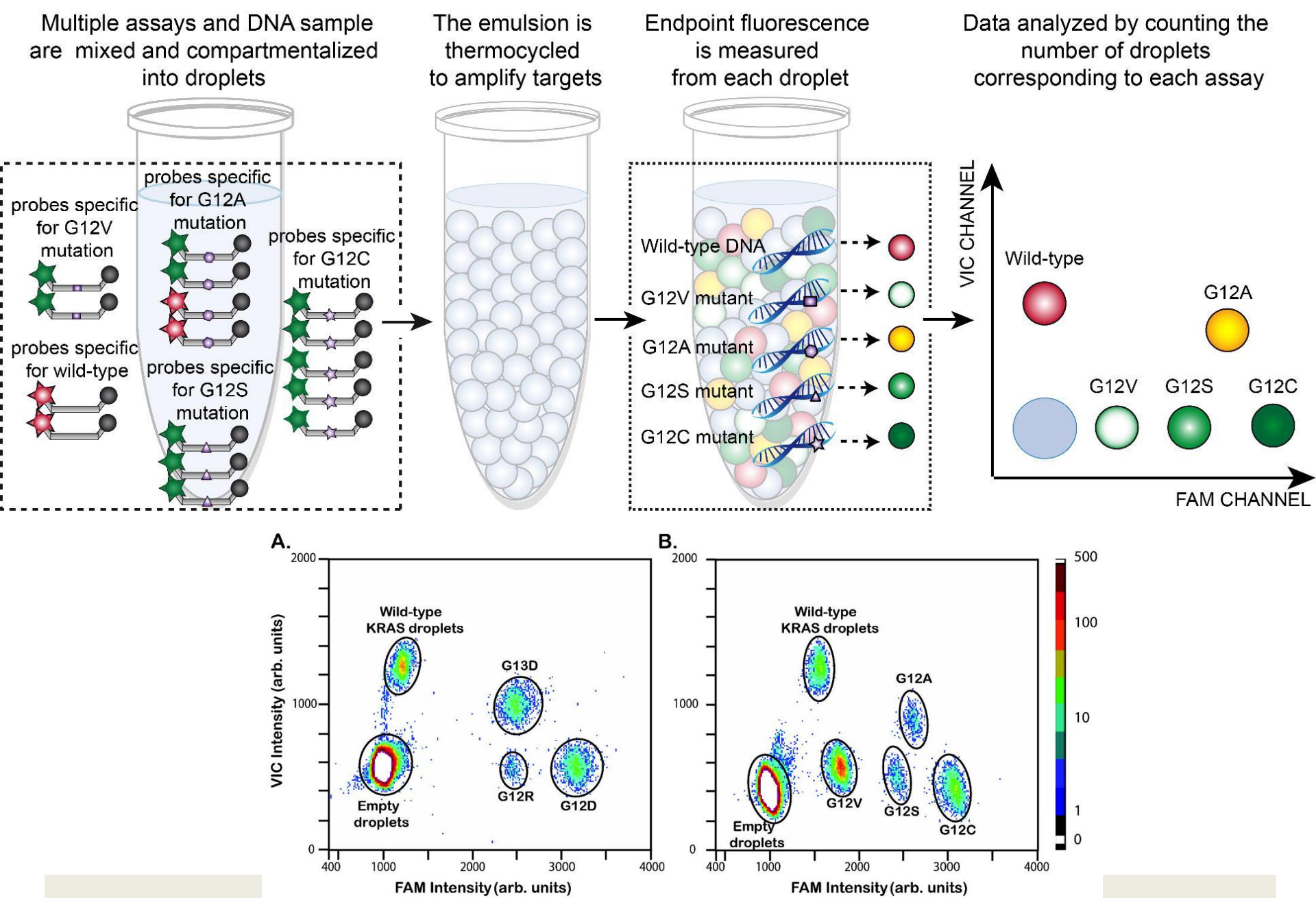
Role of intra tumor heterogeneity in response to antiEGFR therapy

- Can we go further in the prediction ?
- Recent papers showed
  - secondary resistance to anti EGFR is associated with
    - KRAS selected clones or
    - acquired KRAS mutant clones



- We decided to explore the intra tumor KRAS heterogeneity
  - By exploring the existence of KRAS minority sub clones
    - We used a digital droplet PCR (Raindance technologies)
  - By studying the clinical impact in response and in PFS and OS in advanced colorectal cancer patients treated by antiEGFR therapy.



2 Multiplex Panels developed covering KRAS Codon 12 &13[1]

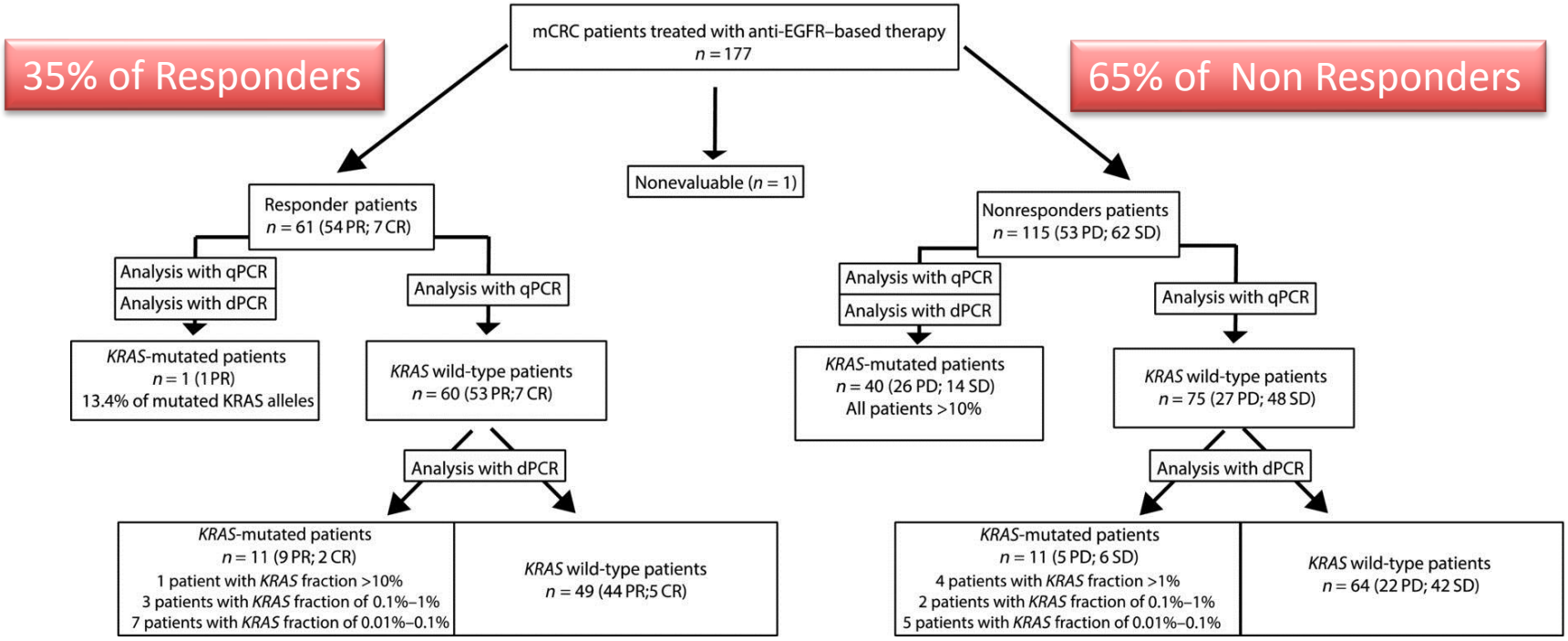
[1] *Laurent –Puig et al. J Clin Oncol 2009*

Patients' characteristics

Patients' characteristics ($n = 177$)	Cases (n)	Percentages
Age, y		
≤65	104	58.7
>65	73	41.3
Gender		
Male	101	57.1
Female	76	42.9
Number of previous chemotherapy treatments		
None	1	0.6
1	22	12.4
2	86	48.6
3	44	24.9
4	16	9.0
5	5	2.8
6	3	1.7
EGFR-targeted therapies		
Cetuximab	2	1.1
Cetuximab + chemotherapy	144	81.4
Panitumumab	10	5.6
Panitumumab + chemotherapy	21	11.9
Response to EGFR-targeted therapies		
Complete response	7	4.0
Partial response	54	30.5
Stable disease	62	35.0
Progressive disease	53	29.9
Not evaluable	1	0.6

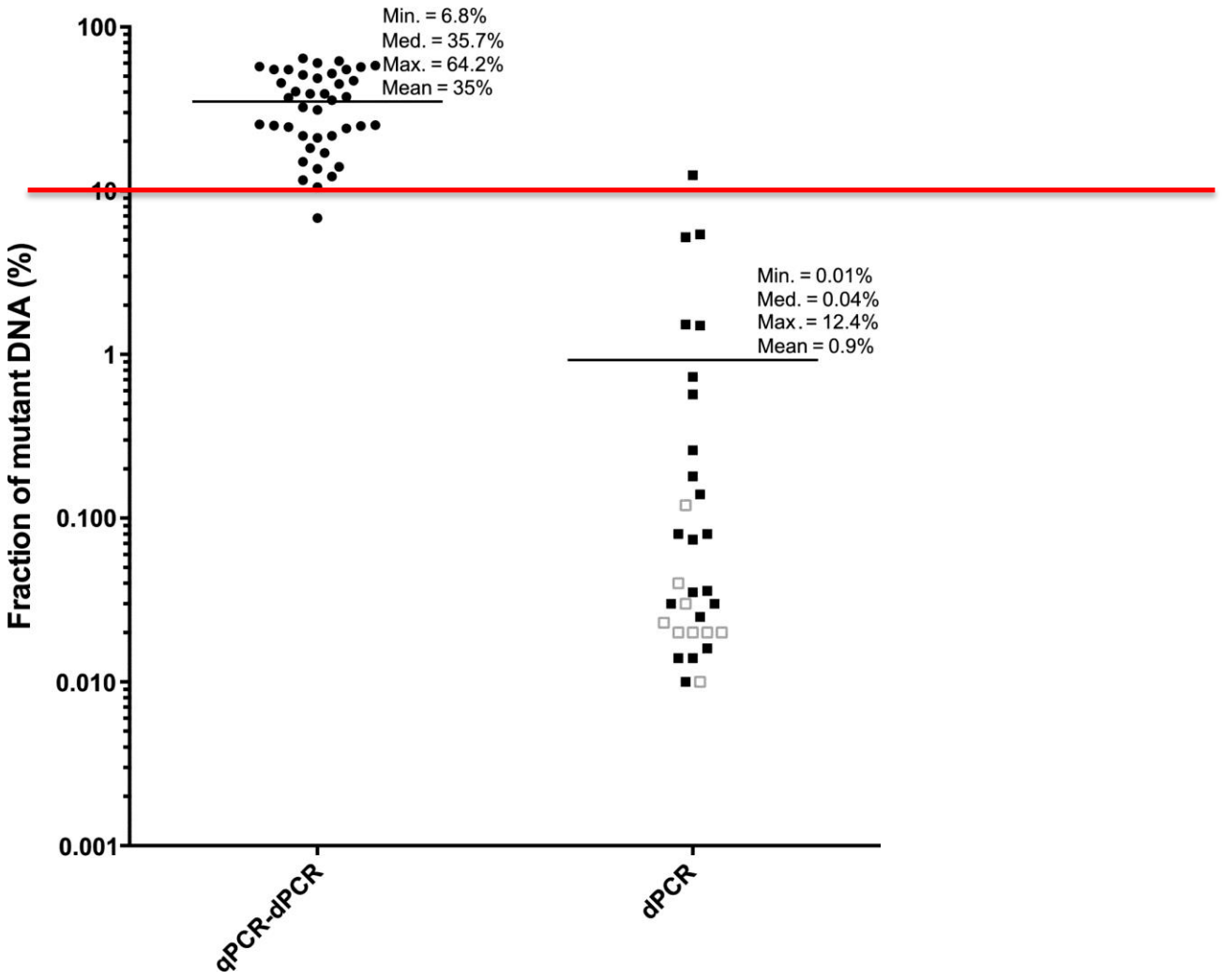
Laurent-Puig P et al. Clin Cancer Res 2015;21:1087-1097

Responses to anti-EGFR–based therapies and KRAS mutational status as determined by conventional procedures (qPCR) or droplet-based dPCR.



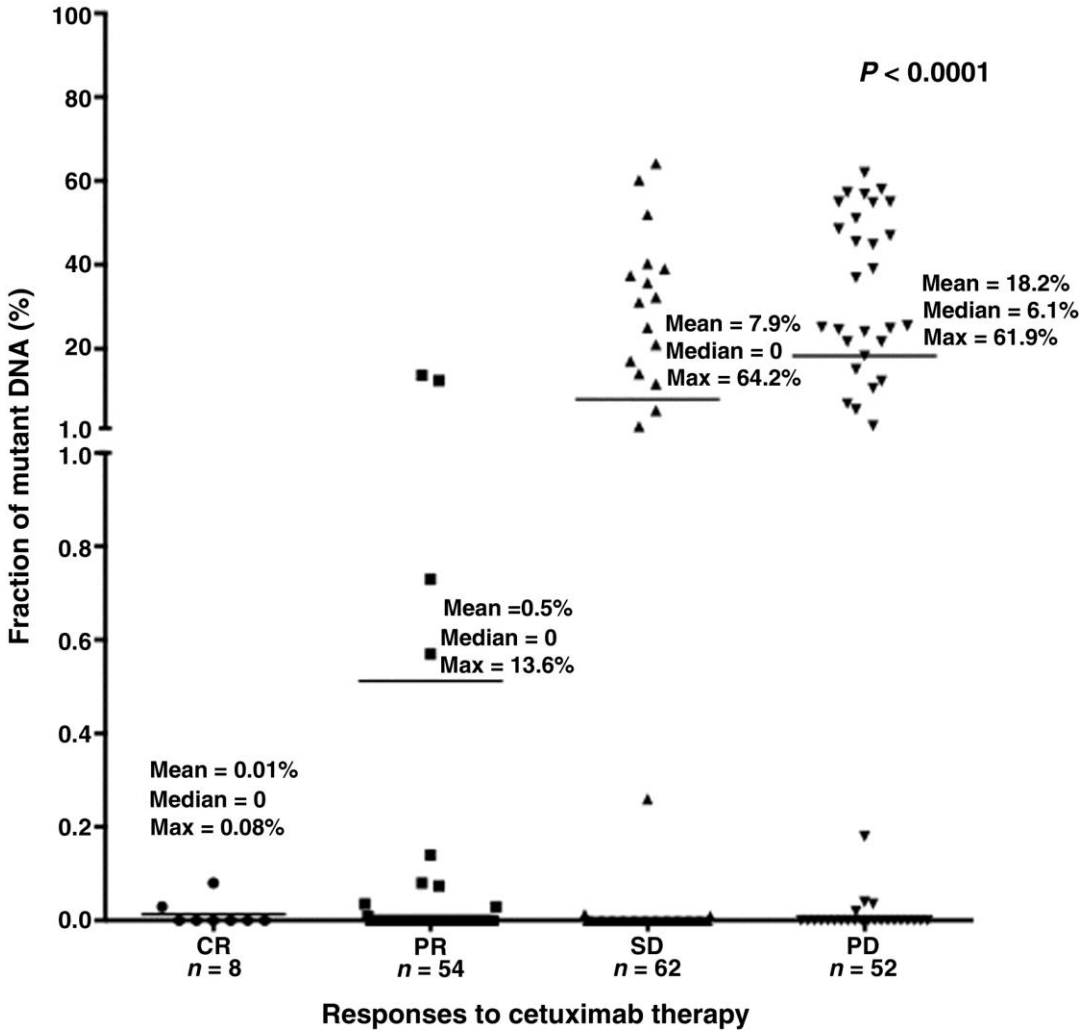
Laurent-Puig P et al. Clin Cancer Res 2015;21:1087-1097

Fraction of mutated KRAS alleles (including multiple subclones) in the patients detected by both conventional and droplet dPCR procedures.



Laurent-Puig P et al. Clin Cancer Res 2015;21:1087-1097

Patients response to cetuximab according to the fraction of KRAS-mutated alleles.



Laurent-Puig et al. P Clin Cancer Res 2015;21:1087-1097

Association between fraction of KRAS mutant allele and survival

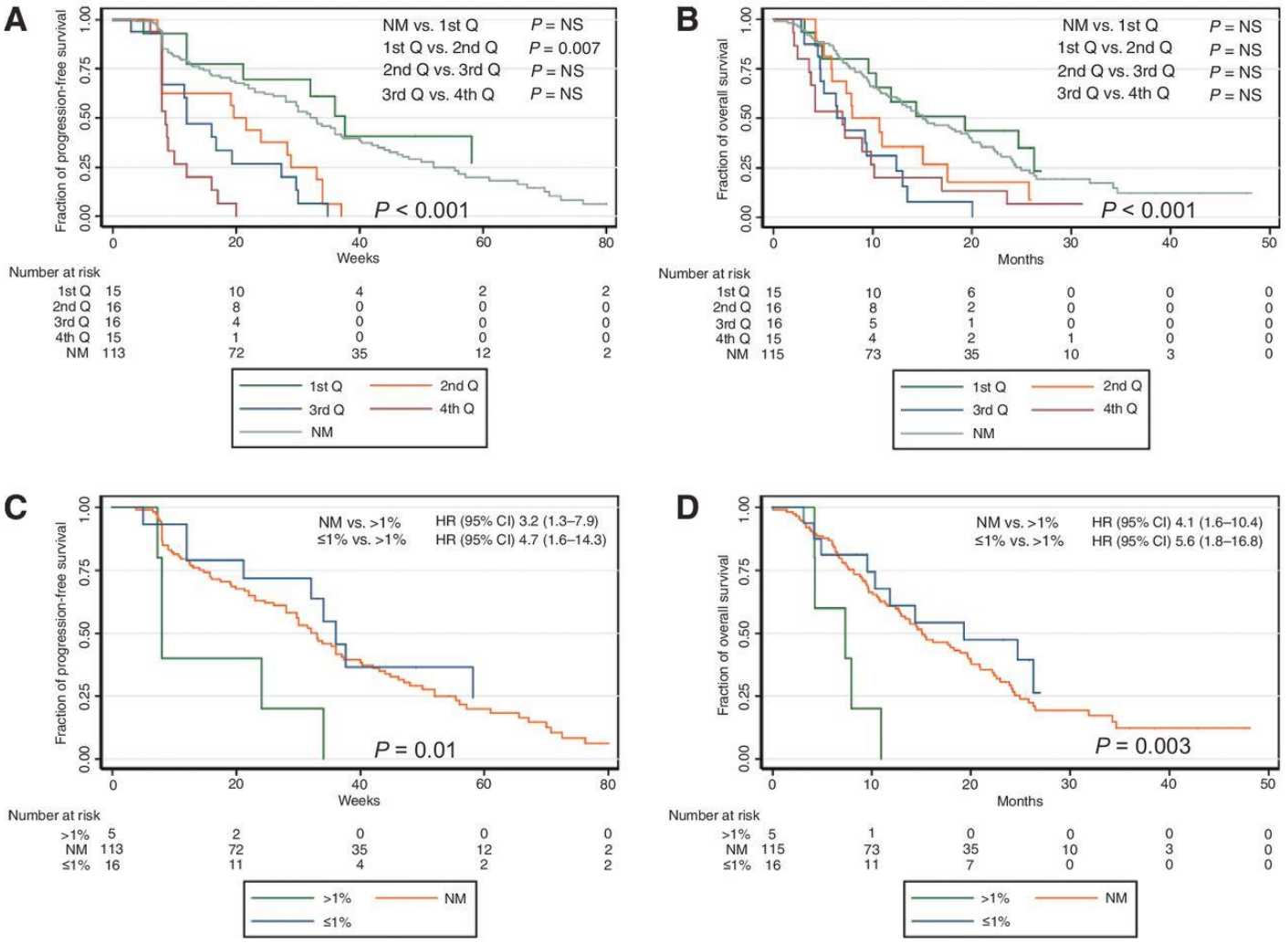
- In a Cox model :

the fraction of allelic KRAS mutant is associated with a shorter PFS and a shorter OS

- Considering an incremental of 1% mutant allele:
- The hazard ratio of PFS was 1.03 ($CI_{95\%}$[1.02 – 1.04], P<0.001)
- The hazard ratio of OS was 1.02 ($CI_{95\%}$[1.01 -1.03], P<0.001)

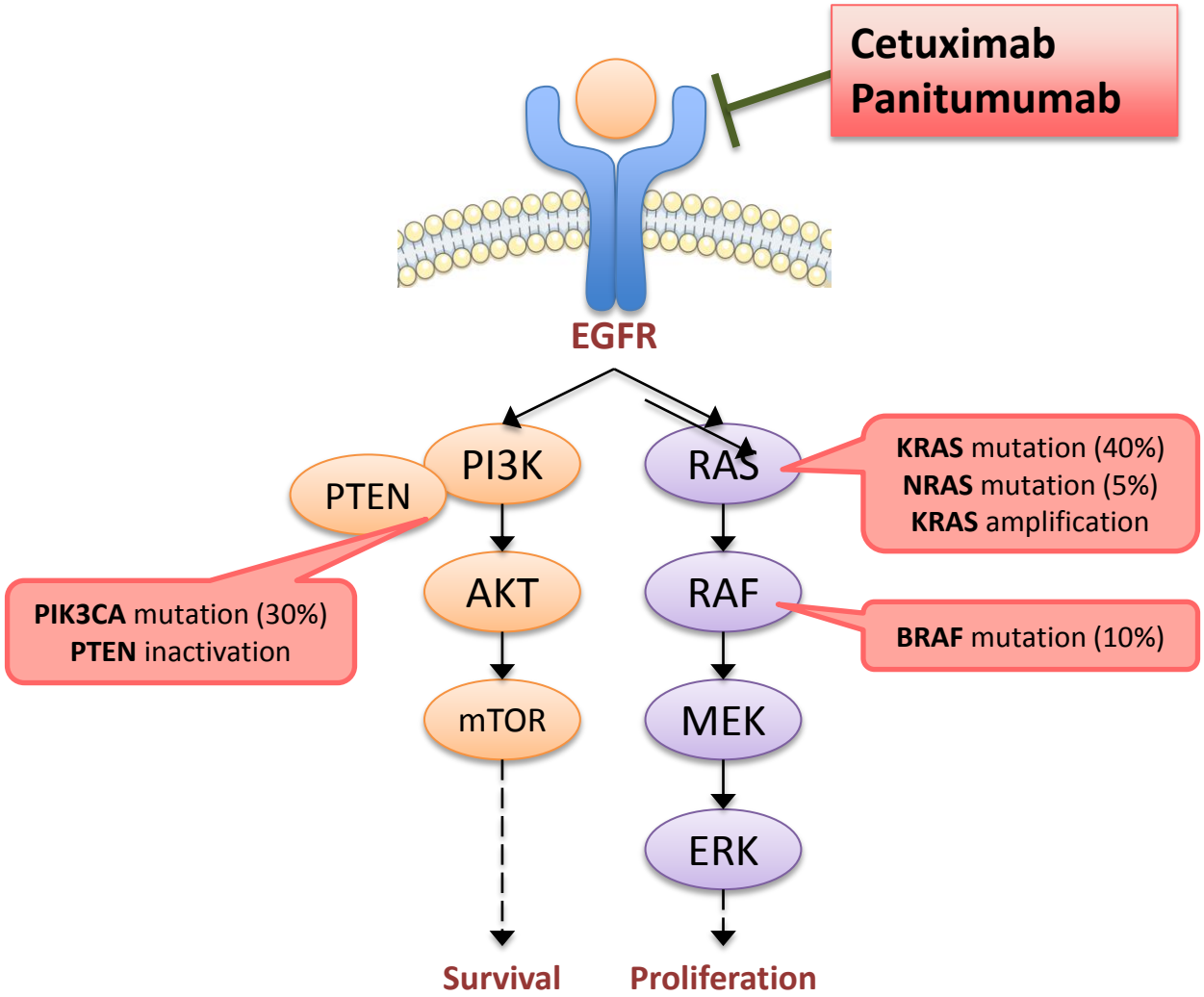
Laurent-Puig et al. P Clin Cancer Res 2015;21:1087-1097

Correlation between the fraction of mutated KRAS alleles in the tumor and patient survival.



Laurent-Puig P et al. Clin Cancer Res 2015;21:1087-1097

Target therapies



Lievre et al, JCO 2008
Douillard et al, NEJM 2013
Mekenkamp et al, BMC cancer 2012

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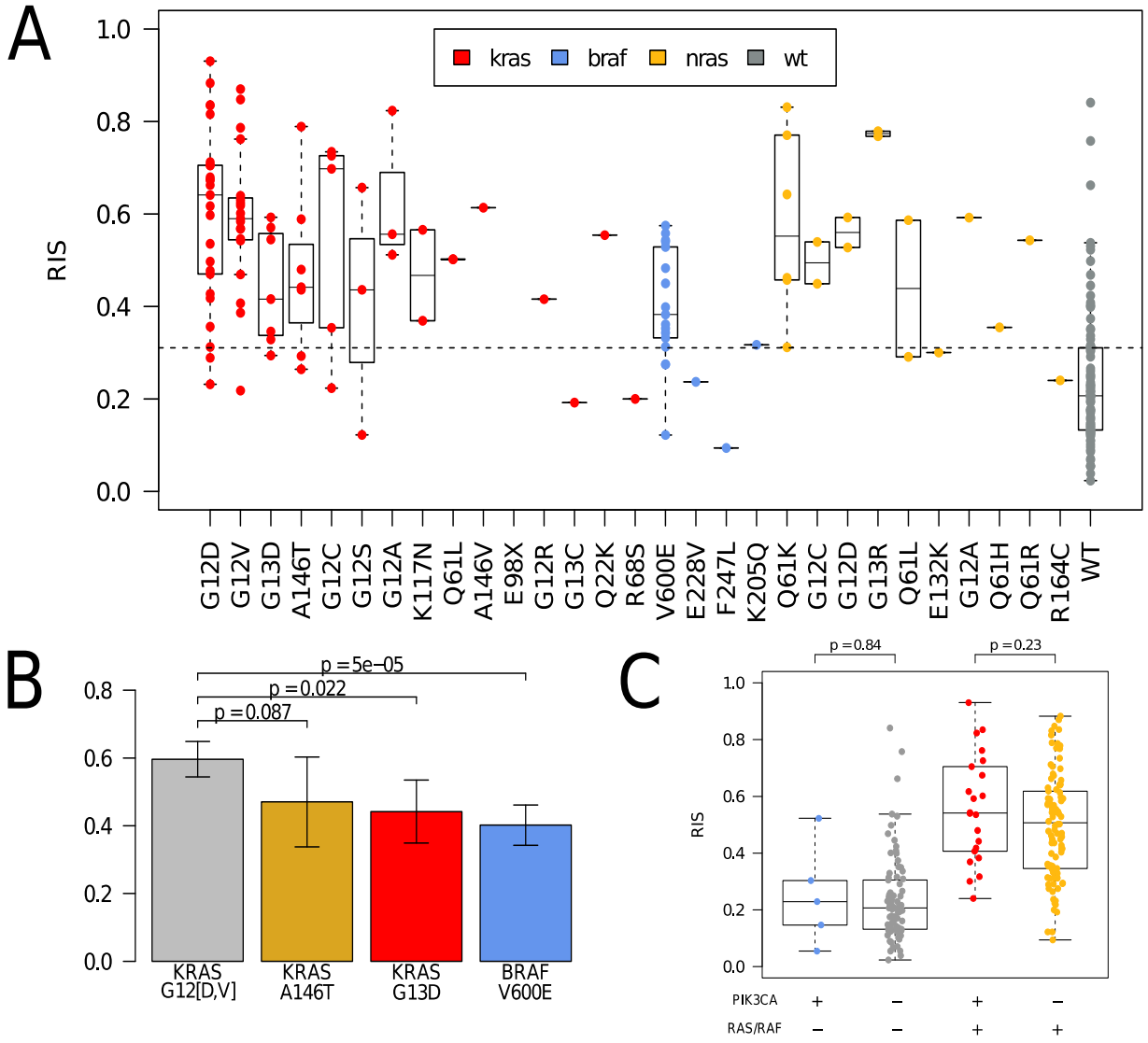
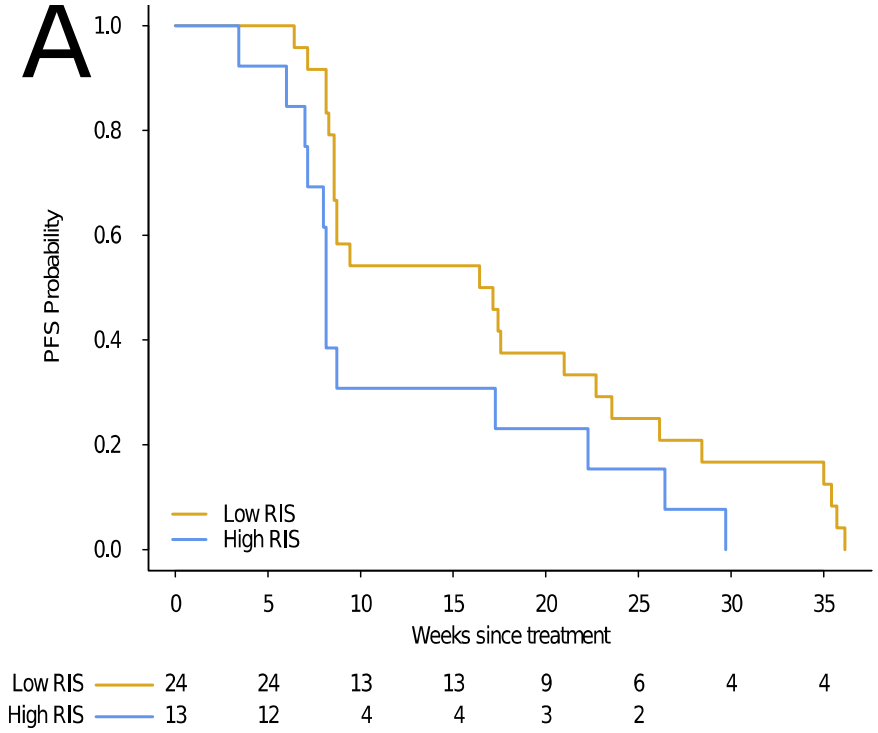
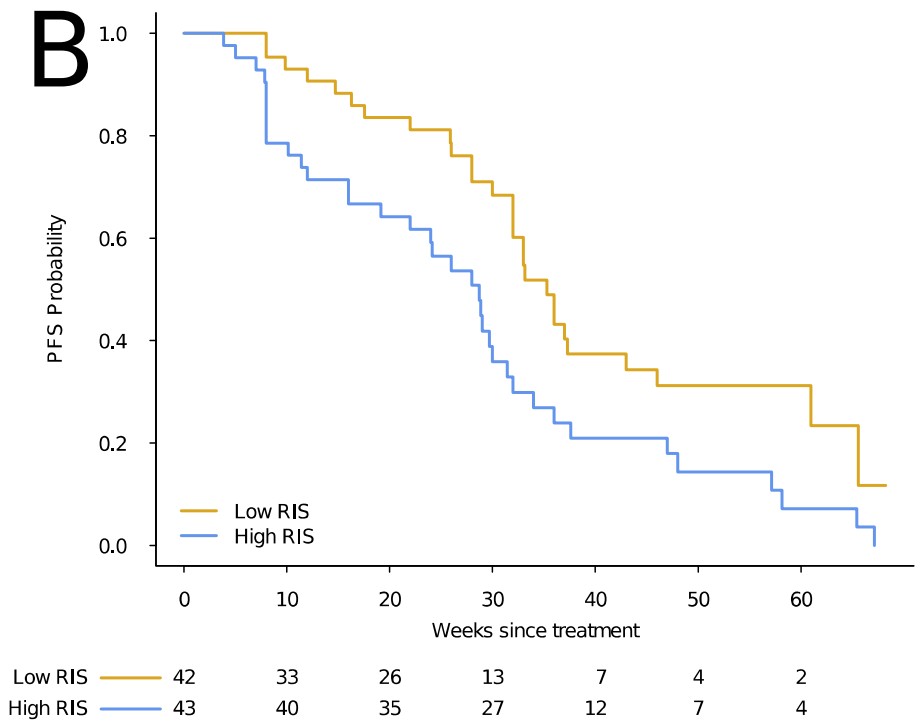
Modeling RAS phenotype in colorectal cancer



Guinney, Ferté……. Laurent-Puig Clin Cancer Res 2014

Modeling RAS phenotype in colorectal cancer





HR=2.3, $CI_{95\%}$ [1.2-4.9], p=.016

HR =2.0 $CI_{95\%}$ [1.2-3.3] p=6.4e-03

Guinney, Ferté……. Laurent-Puig Clin Cancer Res 2014

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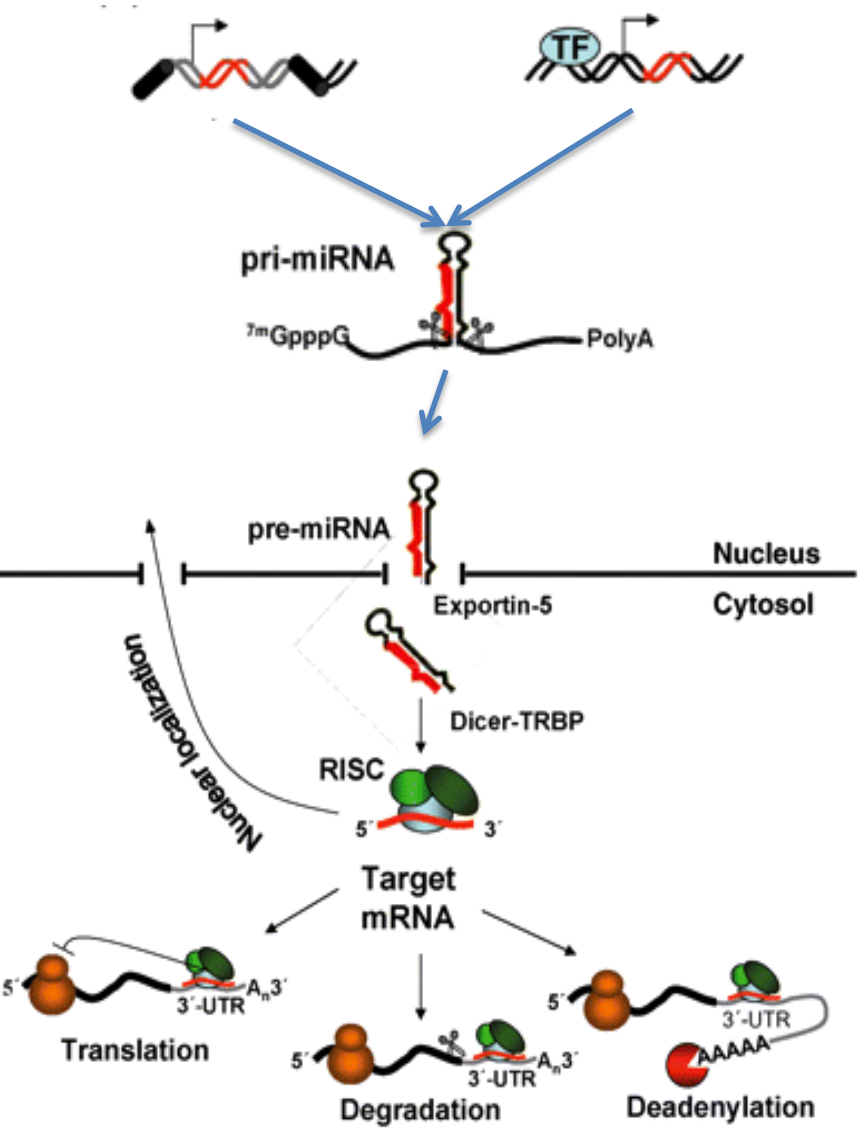
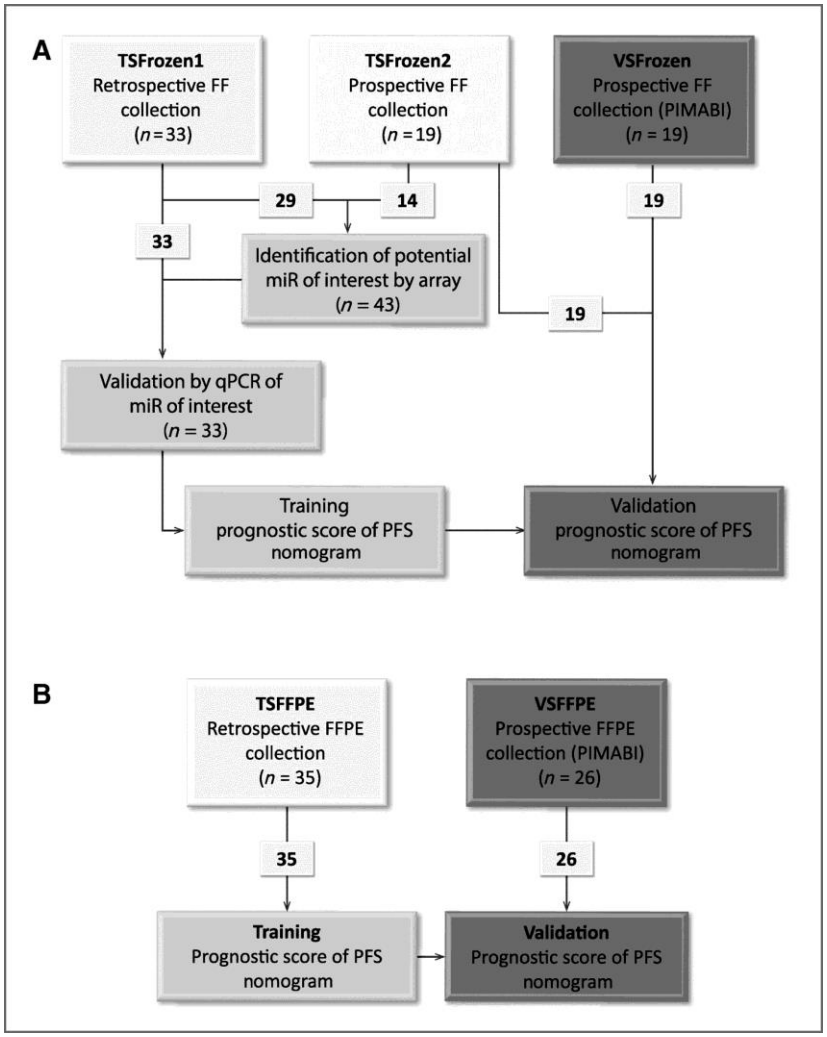
microRNAs



- microRNAs are non coding RNA of 22 nucleotides
- Around 1000 microRNAs have been identified in human genome
- These microRNA linked to transcribed RNA in their 3’ end non translated
- The role of these microRNA is to regulate gene expression mostly by inhibiting the RNA translation and/or by promoting the RNA degradation
- 60% of human genes are regulated by microRNA.

Fazi et al. Cardiovasc Res 2008;79:553-61

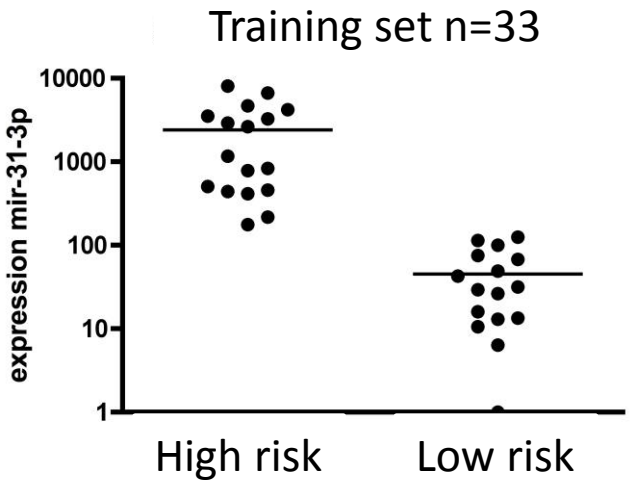
Flow chart.



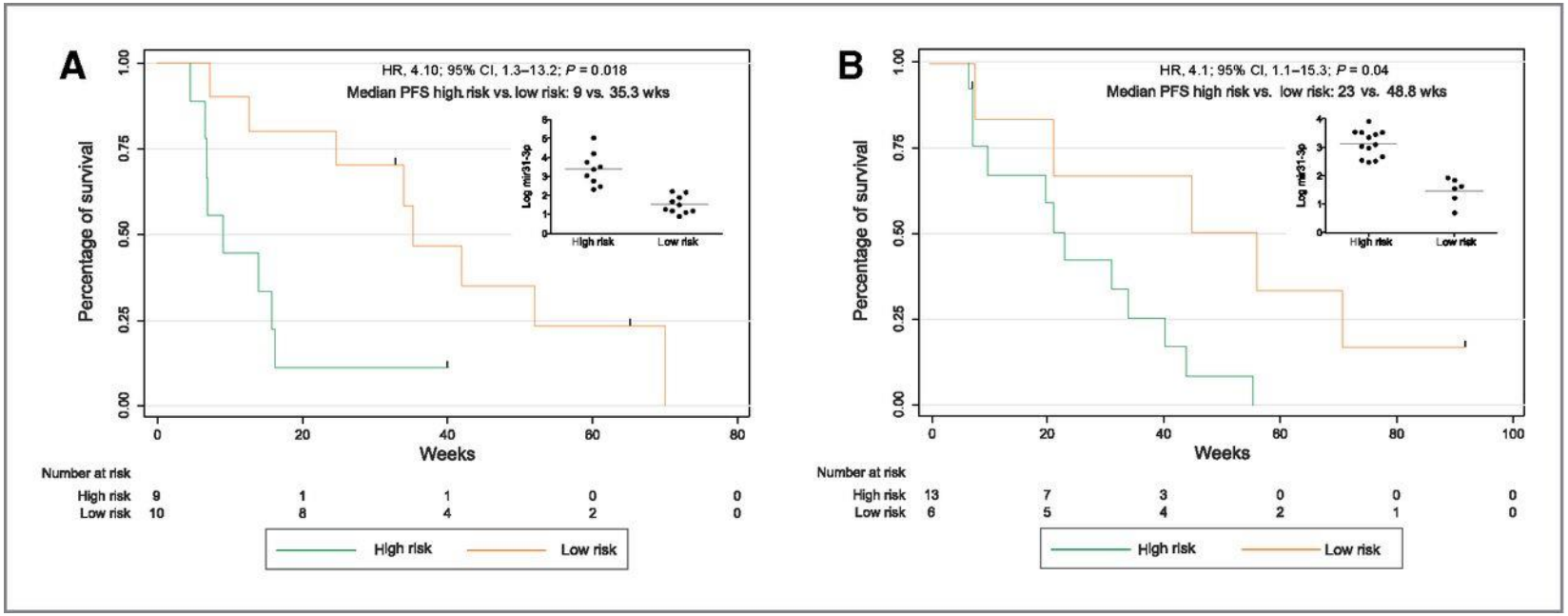
Manceau G et al. Clin Cancer Res 2014;20:3338-3347

Identification of microRNA associated with survival

- We analyzed 1154 microRNA on a Illumina microarray, 11 were associated with PFS according to a Cox model and principal component analysis allowing to calculate a progression score
- Among these 11 microRNA tested by qPCR, only one (hsa-mir31-3p) has a significantly different expression between patients with high and low risk ($p<0.0004$)



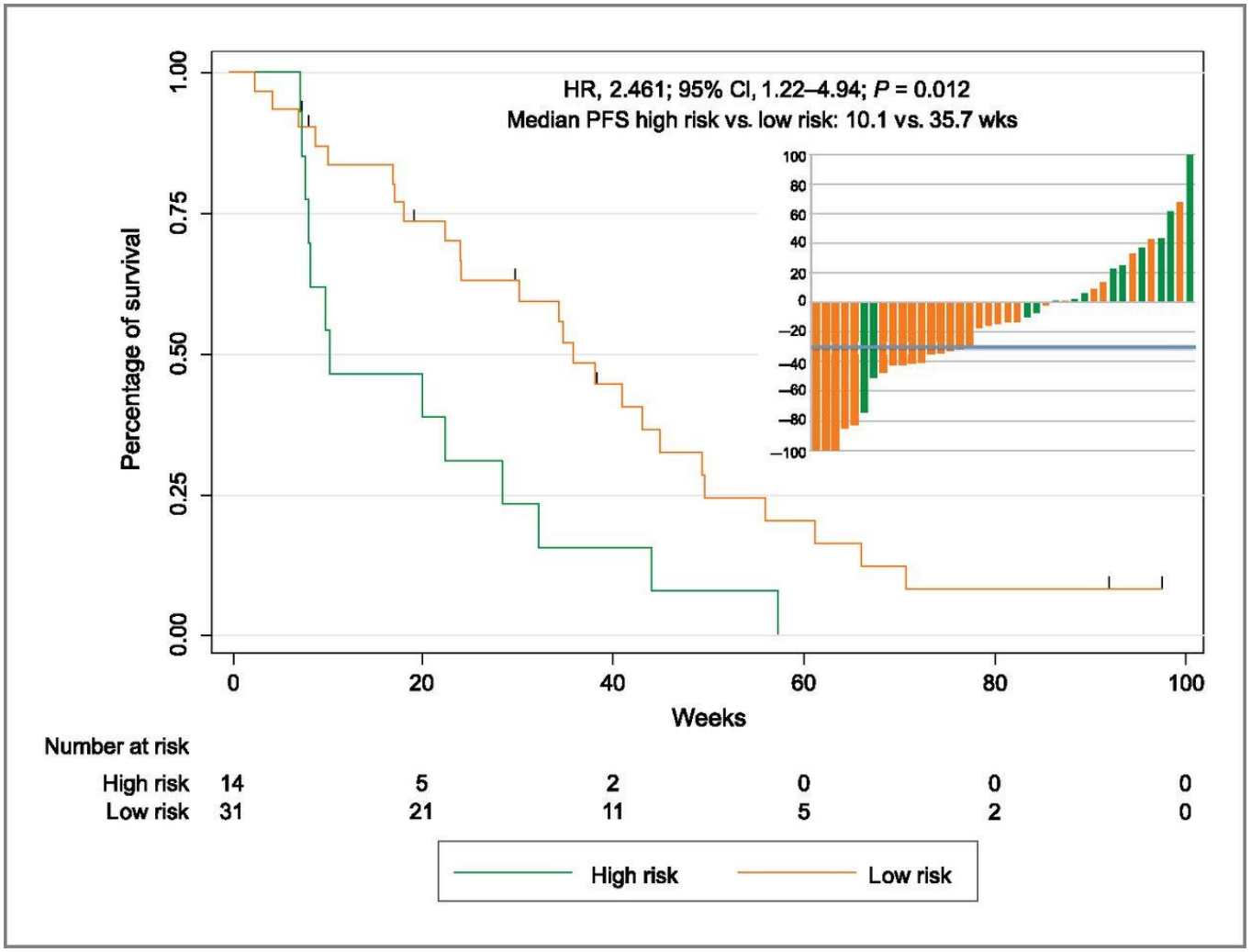
Kaplan–Meier curves for risk groups obtained from the second training set (A) and the validation set 1a (B).



Gilles Manceau et al. Clin Cancer Res 2014;20:3338-3347

Clinical Cancer Research
AACR American Association for Cancer Research

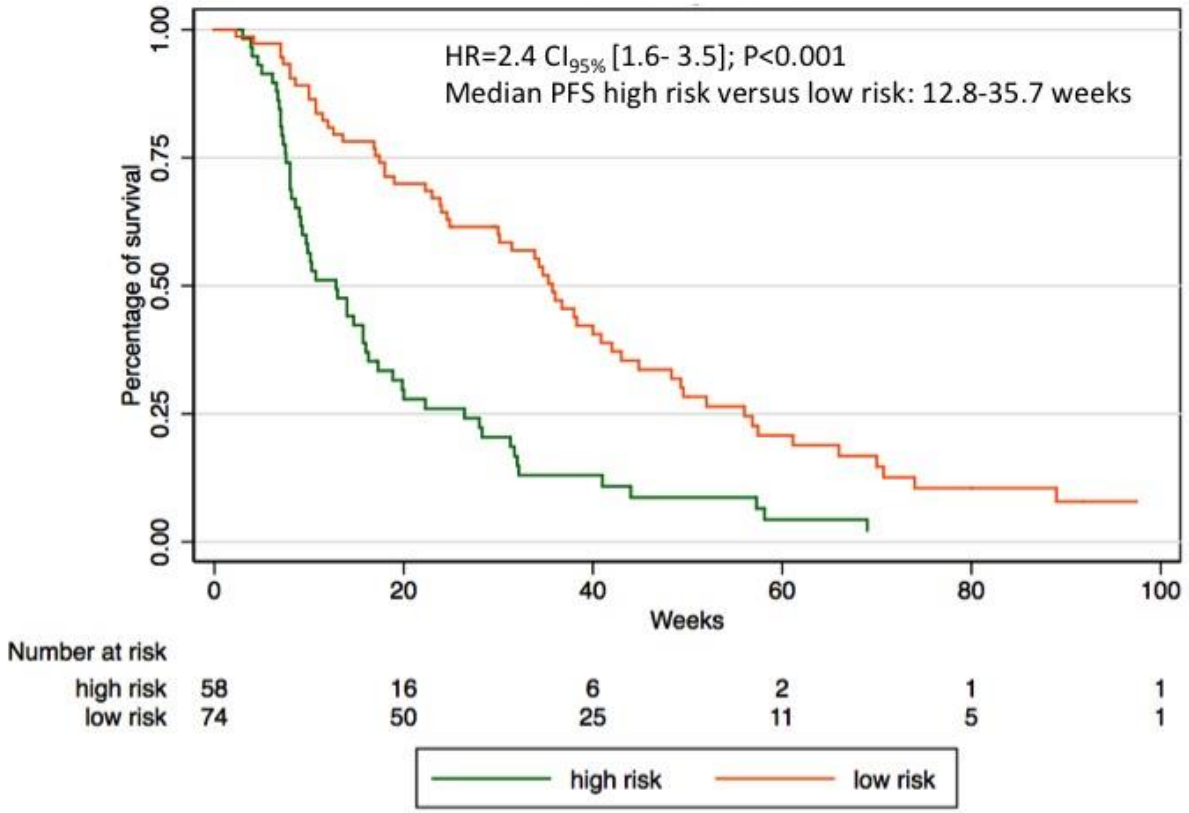
Kaplan–Meier PFS curves and the waterfall plot for RECIST criteria for the whole series of validation (PIMABI phase II; n = 45).



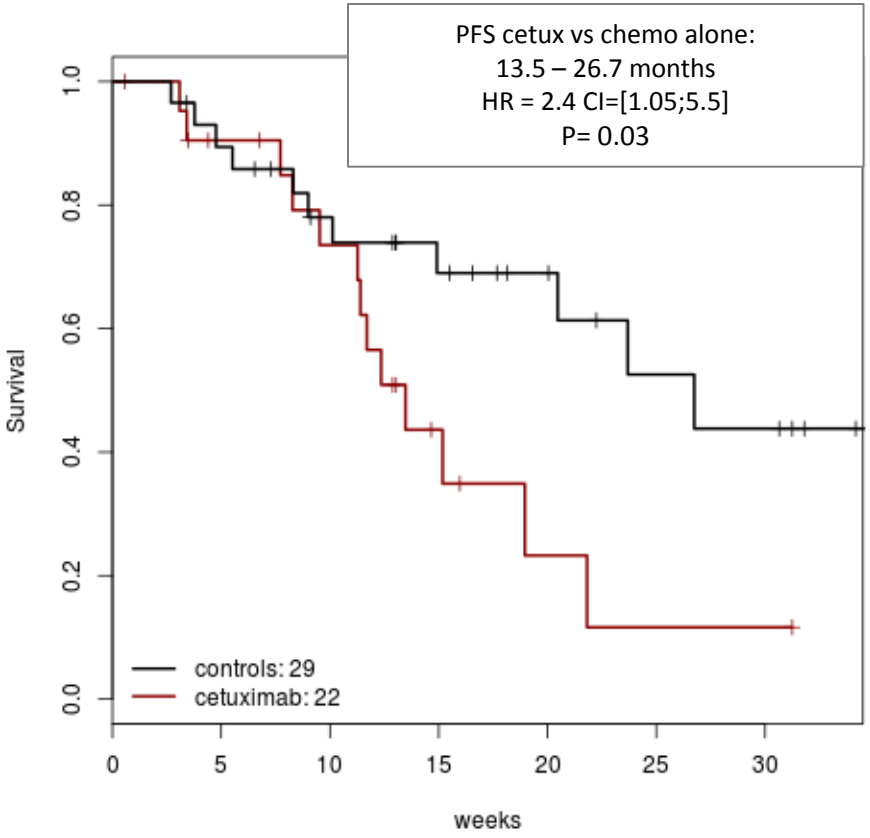
Gilles Manceau et al. Clin Cancer Res 2014;20:3338-3347

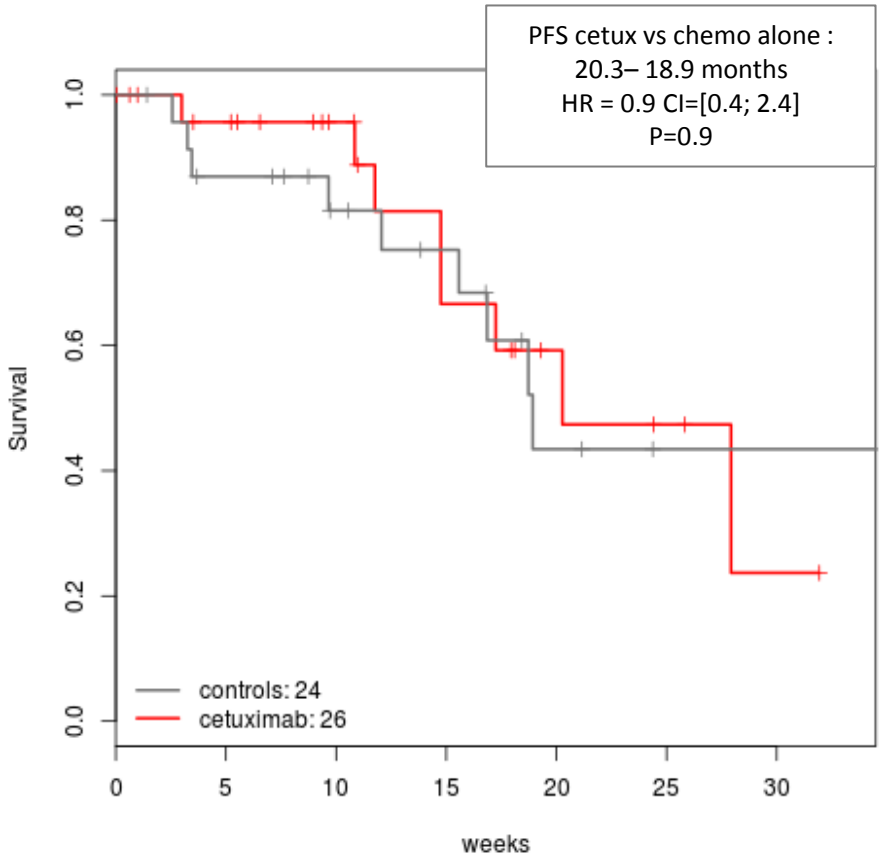
Clinical Cancer Research
AACR American Association for Cancer Research

All patients



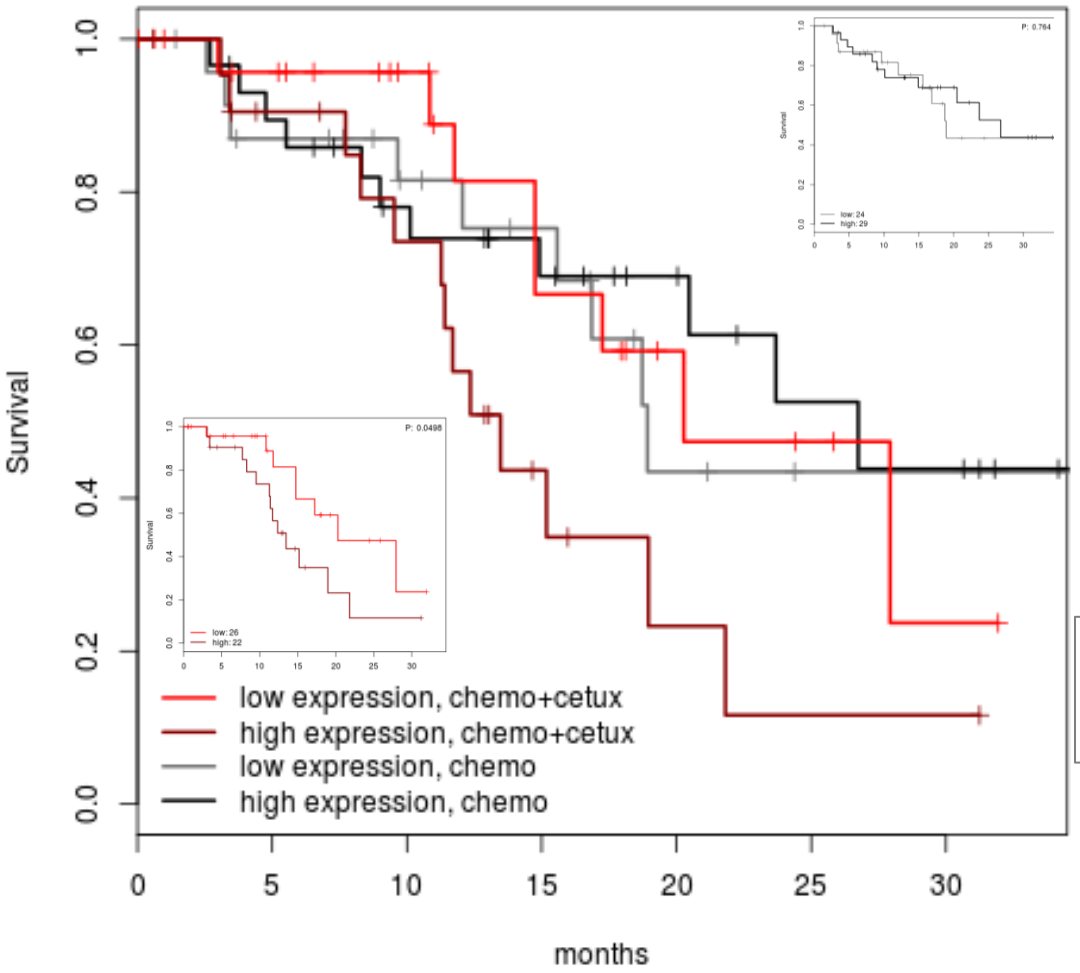
miR-31-3p is a predictive biomarker of cetuximab effects in a *post-hoc* analysis of New EPOC phase III trial





Laurent-Puig P et al, ESMO 2014

miR-31-3p is a predictive biomarker of cetuximab effects in a *post-hoc* analysis of New EPOC phase III trial



P = 0.12
HR = 1.7 CI=[0.88- 3.08]

Laurent-Puig P et al ESMO 2014

hsa-mir31-3p maturation regulated by AGO2

MicroRNAs (miRNAs) are generated by two-step processing to yield small RNAs that negatively regulate target gene expression at the post-transcriptional level

Among those some have large loop for which the maturation is negatively controlled by AGO2 which is phosphorylated by EGFR in hypoxia condition

hsa-mir31-3p is one of these microRNA

High level of these microRNA suggested the absence of EGFR response to hypoxia in tumors

It should be linked to the absence of effect to anti-EGFR therapy

EGFR modulates microRNA maturation in response to hypoxia through phosphorylation of AGO2 Nature Med 2013

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Exome Sequencing
37 tumours and normal DNA

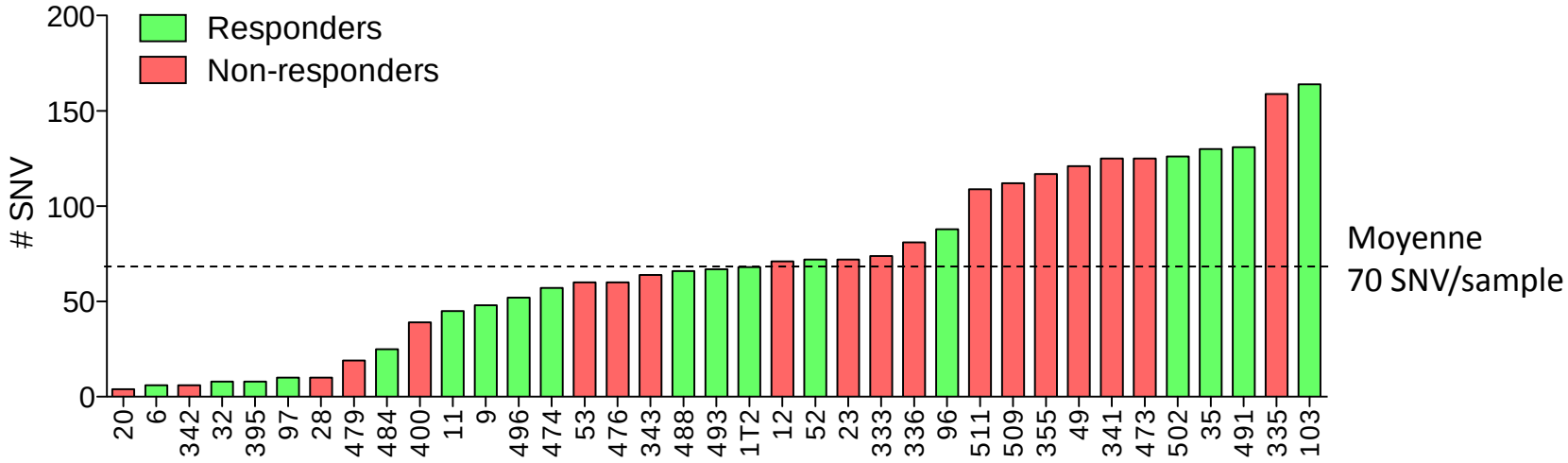
All treated by cetuximab

All *KRAS, NRAS, BRAF* WT

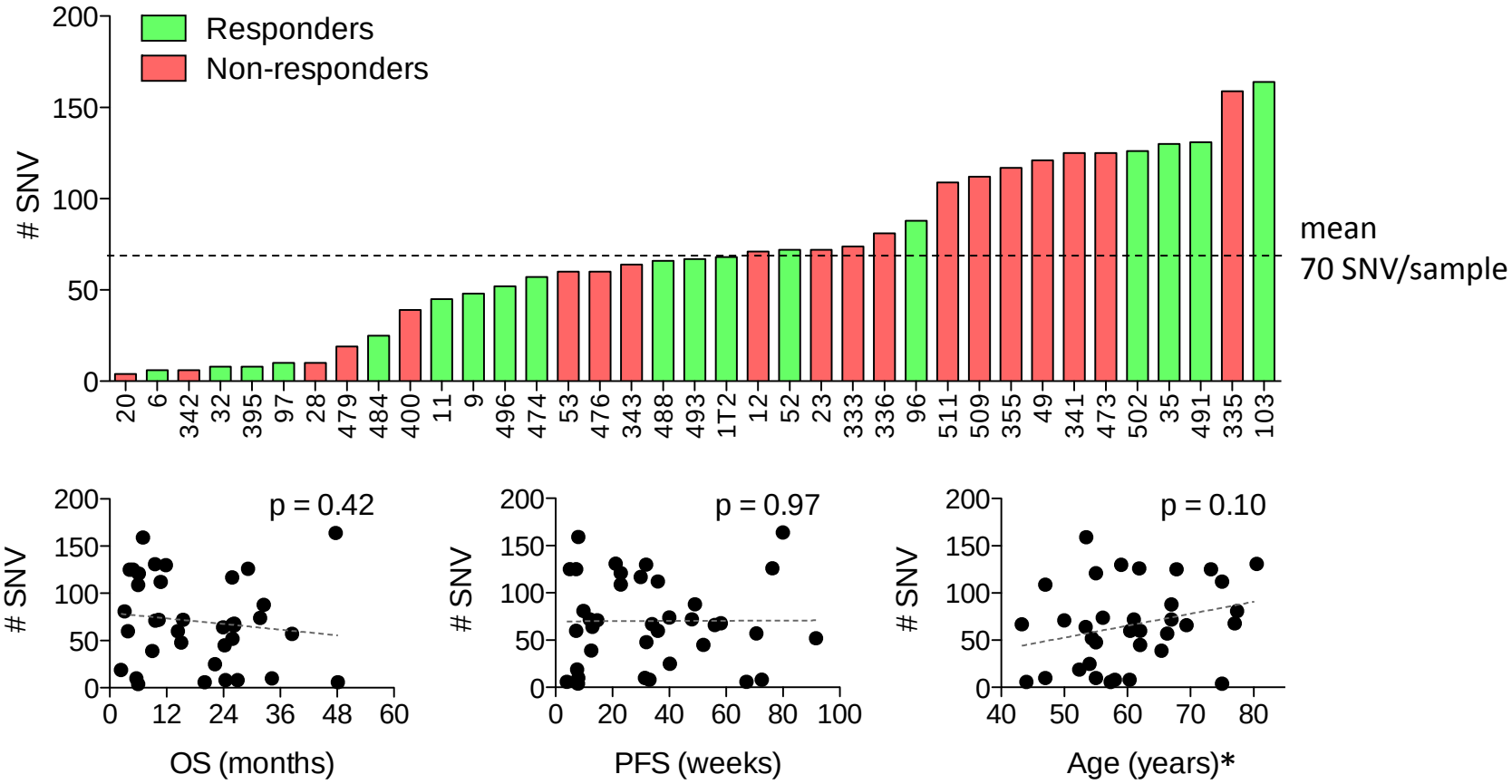
- 18 responders
- 19 non responders

INTEGRAGEN
Clinical Genomics Experts

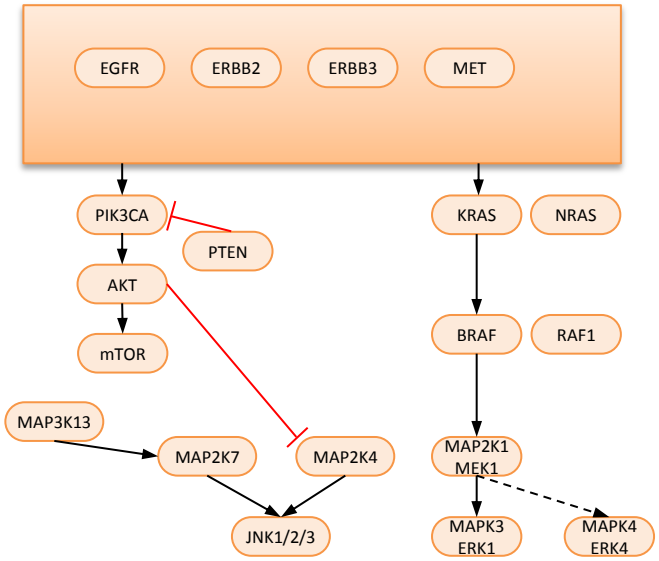
Correlations #SNV



Correlations #SNV

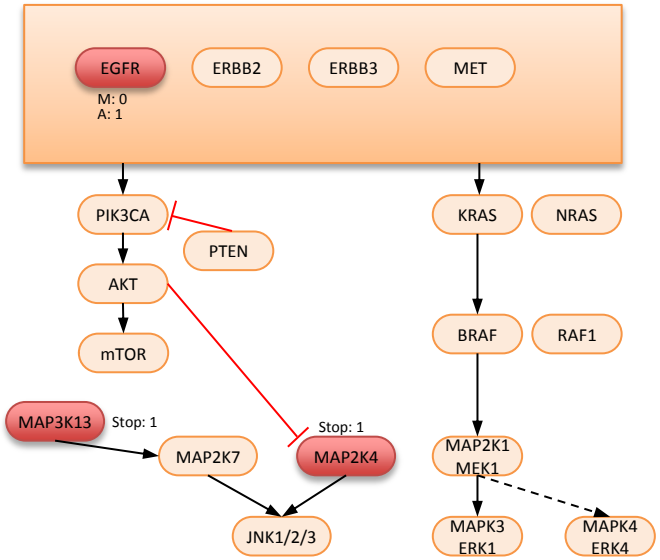


The mapkinase pathway

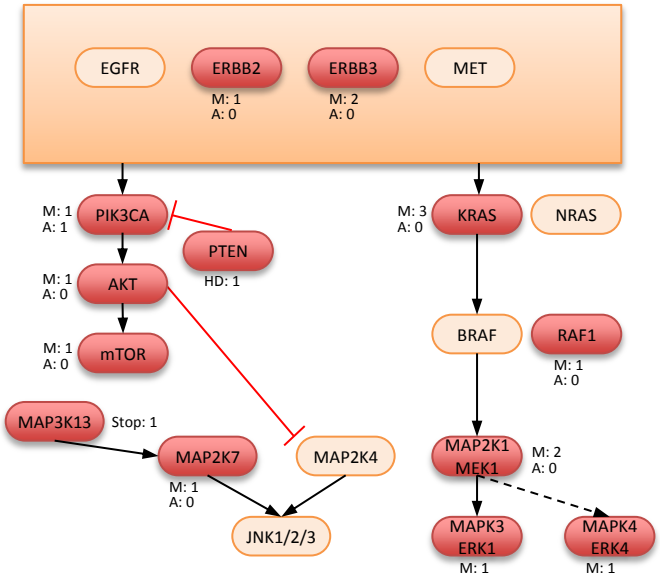


The mapkinase pathway mutations in responders and non responders patients

Responders n=18



Non-Responders n=19



Conclusions

- Need of **INTEGRATION**
- **The unique marker for a clinical problem is probably an illusion**
- **Different faces of the same god**

Sanjusangendo temple Kyoto
1001 God of Mercy