Deep DNA sequencing of tumor: ready for 'Prime Time' or not?

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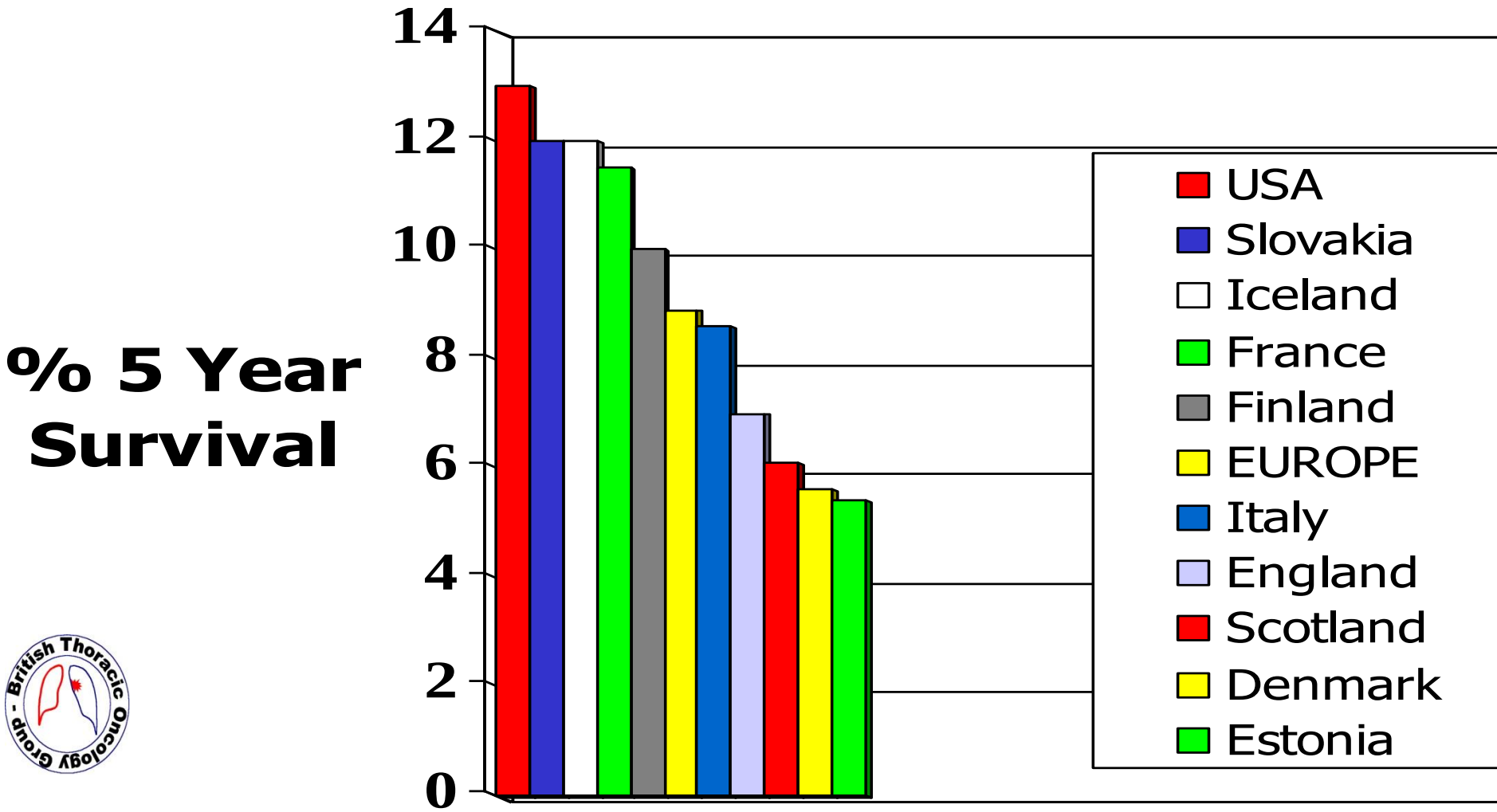
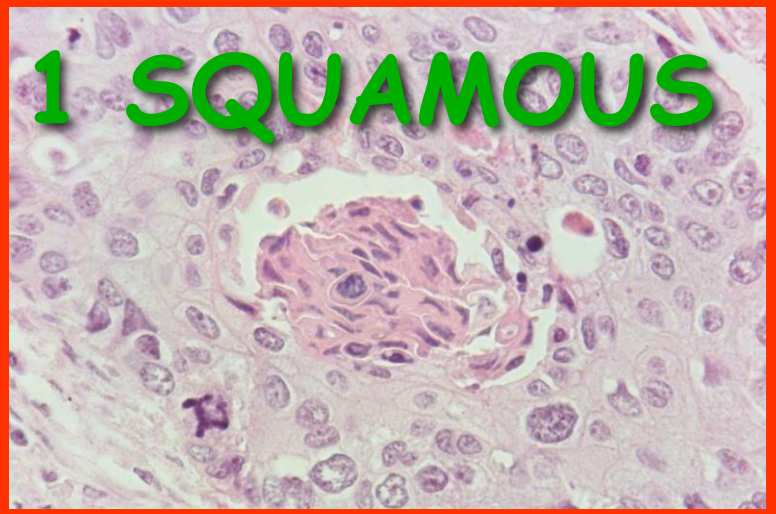
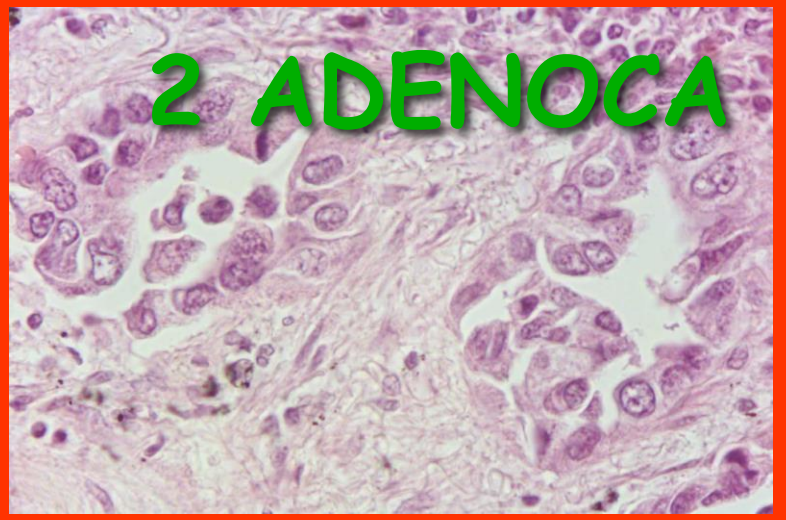
Disclosures

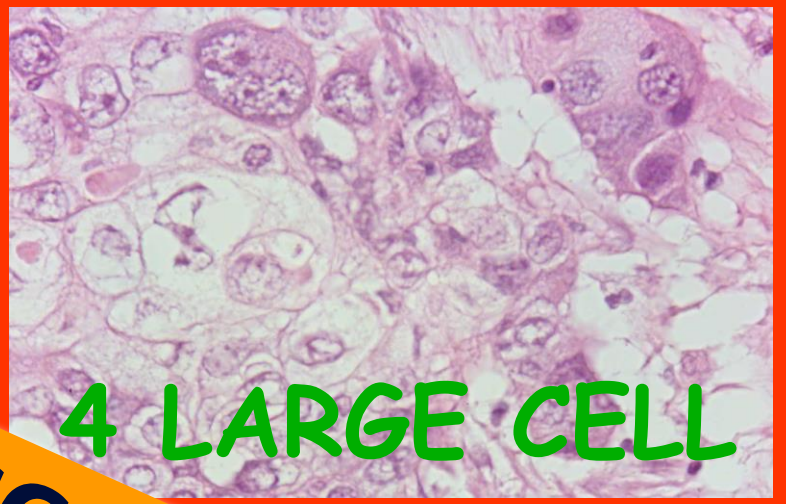
- I have worked as an advisor or speaker for the following companies
  - Pfizer
  - Boehringer Ingelheim
  - Lilly Oncology
  - Roche-Genentech
  - Merck Serono
  - Amgen
  - Clovis
  - Abbott Molecular
  - Novocure

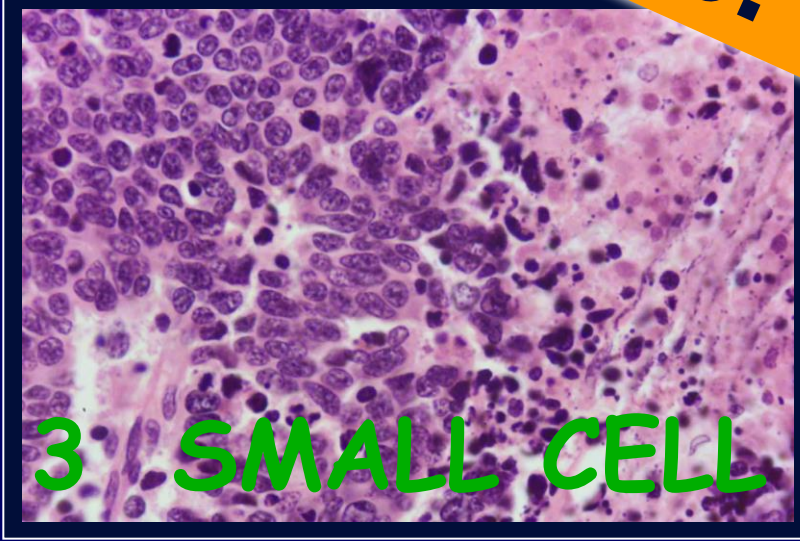
Lung Cancer: Selected Comparative 5 year Survival: males





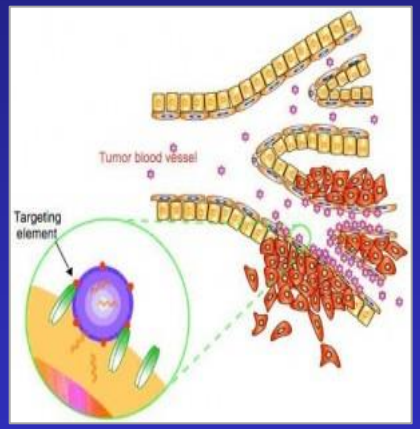
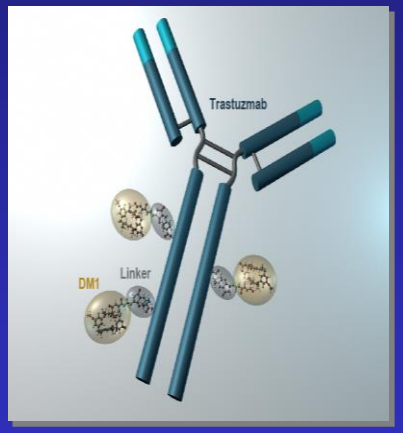






Few years ago: only SCLC vs NSCLC

How Does This Enable Personalized Medicine?

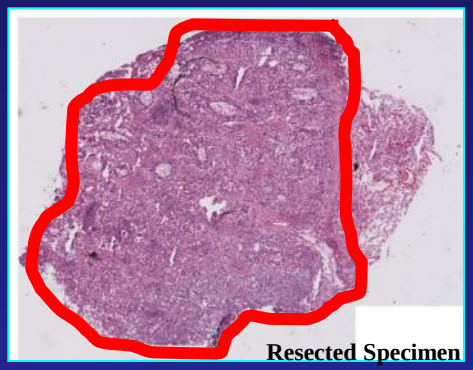
Right Target	Right Drug (or Combinations)	Right Patient
		
Genetic validation; Rare phenotypes	*Selective design and delivery; Combinations for complex diseases*	*Phenotyping and genotyping*

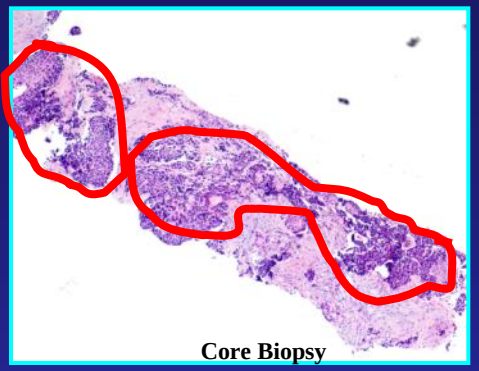
Sequencing technologies

- First generation sequencing technology
  - Sanger Sequencing
- Second generation sequencing technology
  - Roche - 454
  - Illumina – GA II
  - SOLiD
- Third gen sequencing technology
  - Helicose
  - PacBio
  - Illumina = HiSeQ, MiSeq
  - Ion Torrent
  - Oxford Nanopore

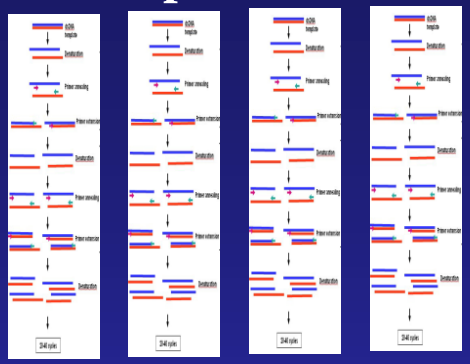
Multiplexed Mutation Assays

Tumor Tissue

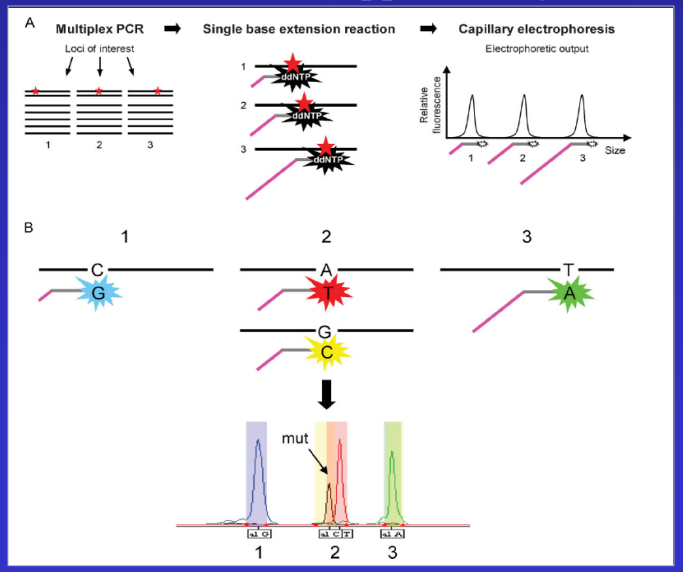




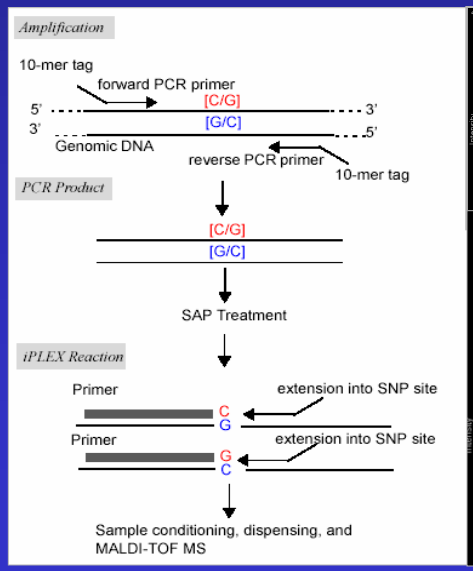
Multiplex PCR

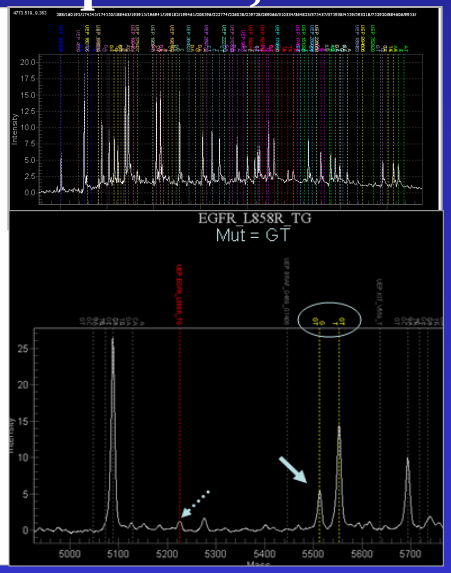


SNaPshot® (Applied Biosystem)



Mass ARRAY SNP - Sequenom, Inc



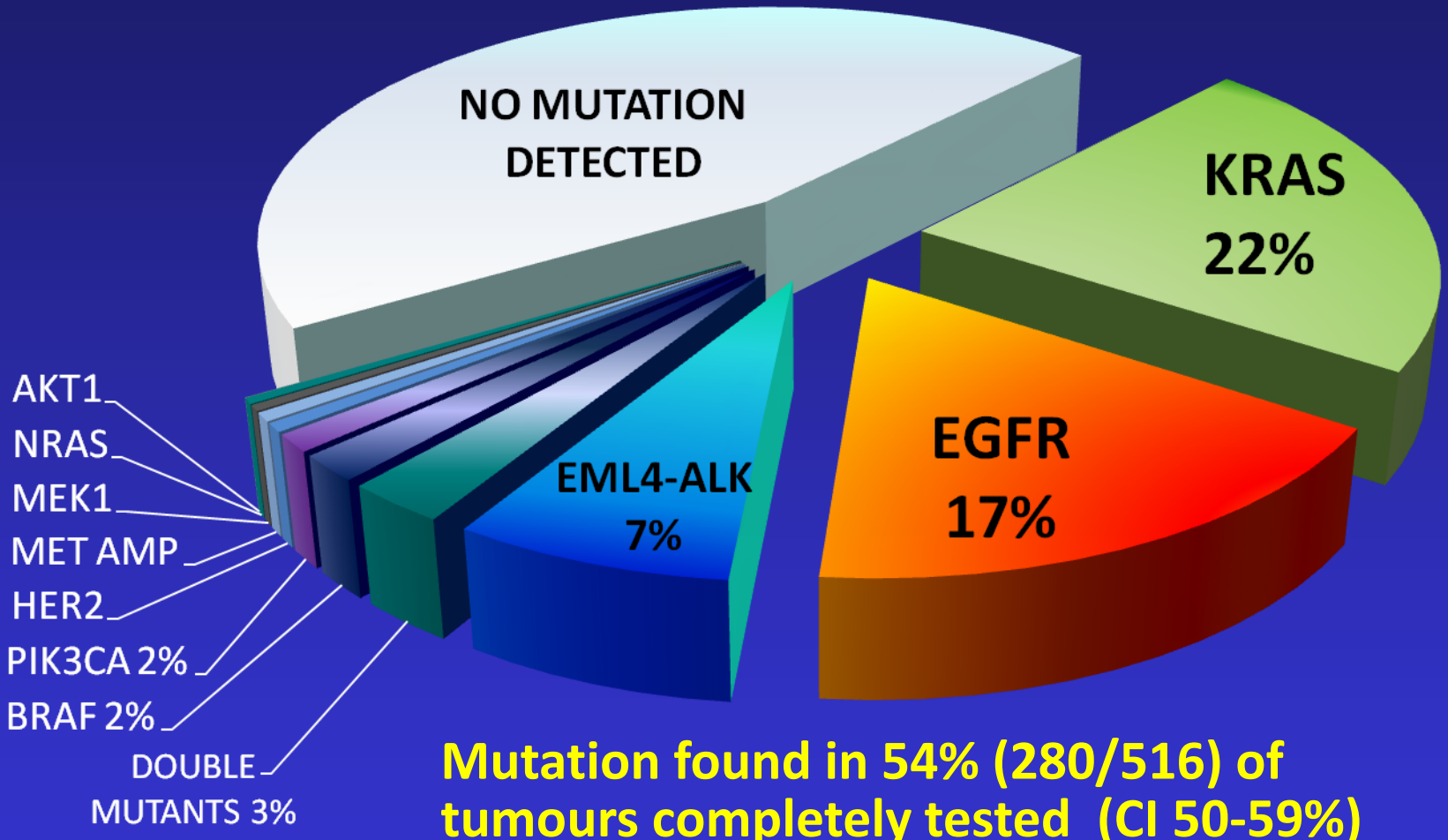


Dias-Santagata, EMBO Mol Med 2:146, 2010

10% Sensitivity and ~20ng DNA/multiplex reaction

Lung Cancer Mutation Consortium

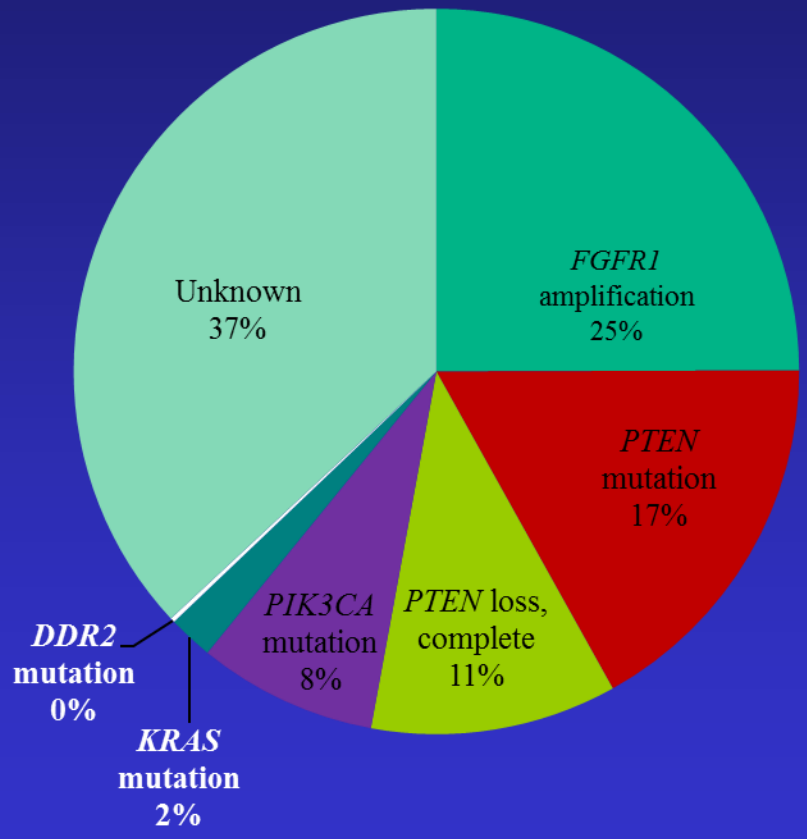
Incidence of Mutations Detected



Mutation found in 54% (280/516) of tumours completely tested (CI 50-59%)

Kris et al. ASCO 2011, Abstract 7506

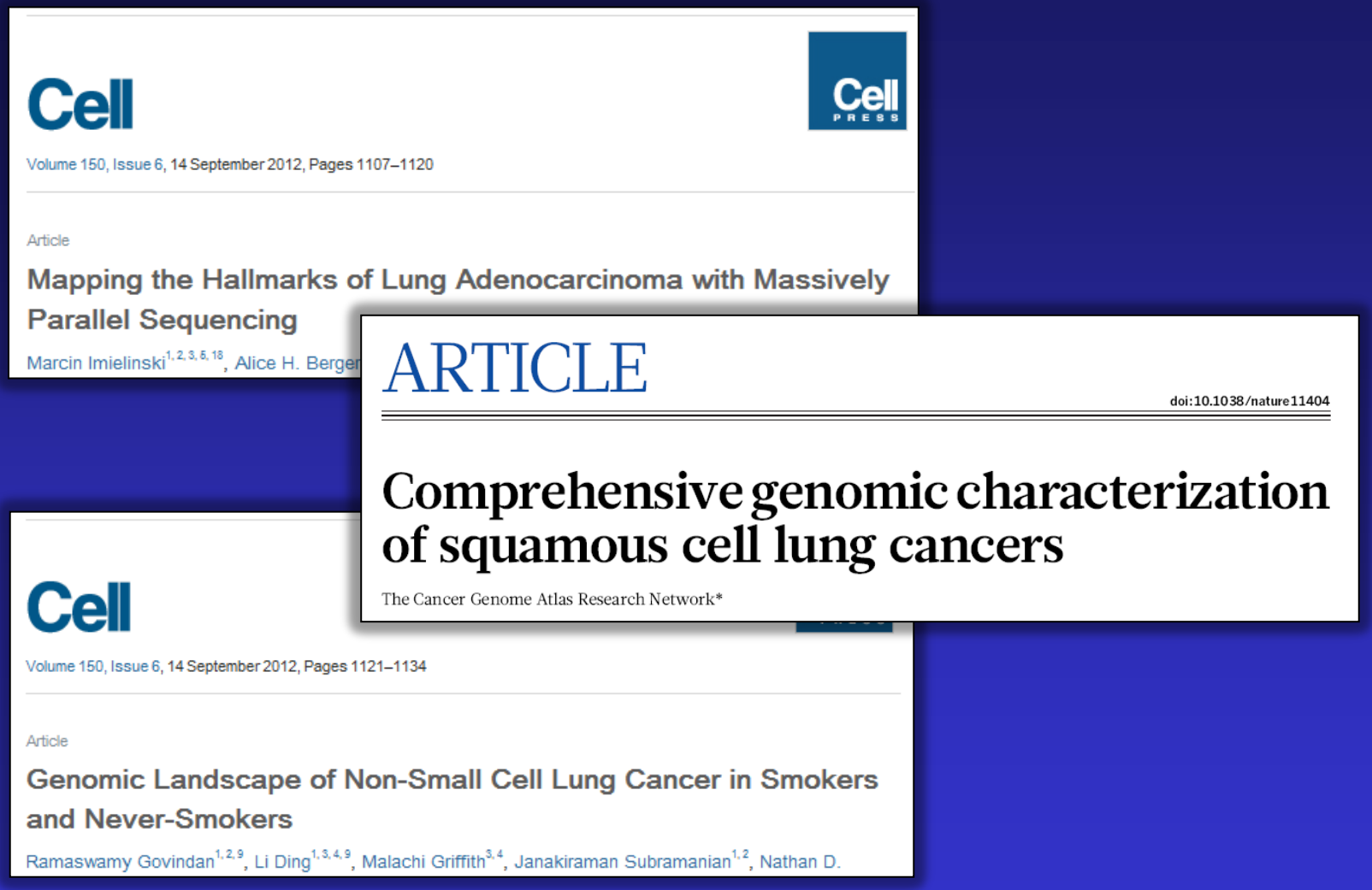
Multiplex Testing in Squamous Cell Lung Cancer: SQ-MAP integrated results



Target	N	Frequency	95% CI
FGFR1 amplification	13/52	25%	15–38%
PTEN mutation 17%	3/18	17%	5–37%
PTEN loss, complete	3/27	11%	3–26%
PIK3CA mutation	4/52	8%	2–17%
KRAS mutation	1/52	2%	1–9%
DDR2 mutation	0/18	0%	0–15%

Paik et al. J Clin Oncol 30: 2012 (suppl; abstr 7505)

The Evolving Molecular Landscape in Lung Cancer

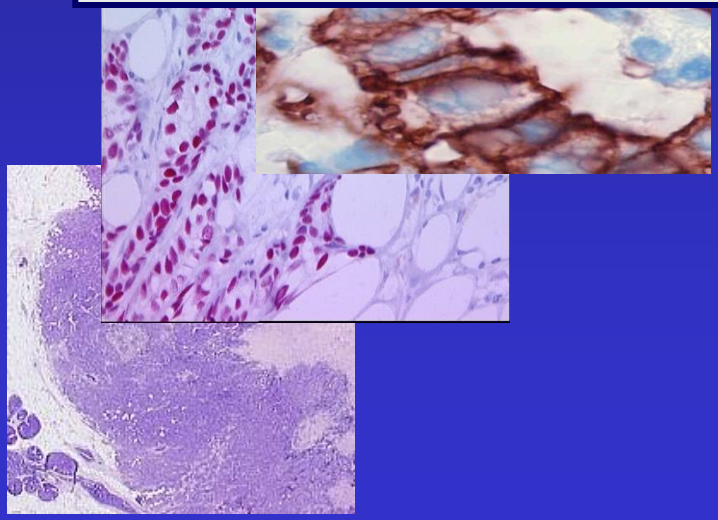


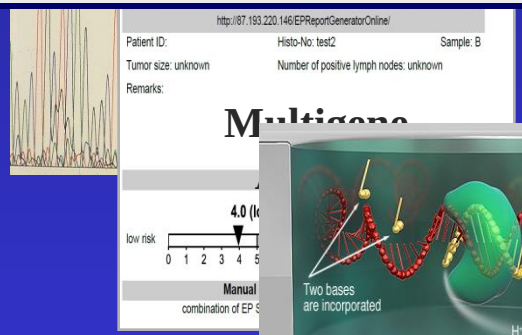
Imielinski M, et al. Cell 2012;150:1107–20; Govindan R, et al. Cell 2012;6:1121–34; The Cancer Genome Atlas Research Network. Nature 2012;Epub ahead of print

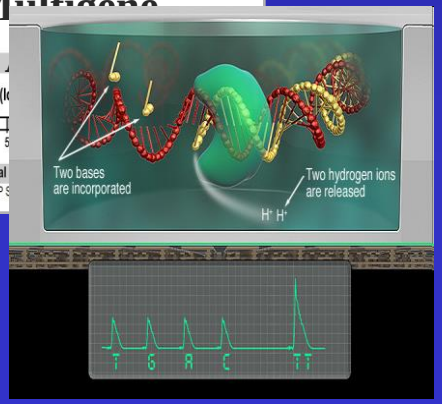
Clinical data tissue

All test are performed on formalin fixed paraffin embedded tissue

Individualised therapy will be based on the ‘Pathology Report of the Future’

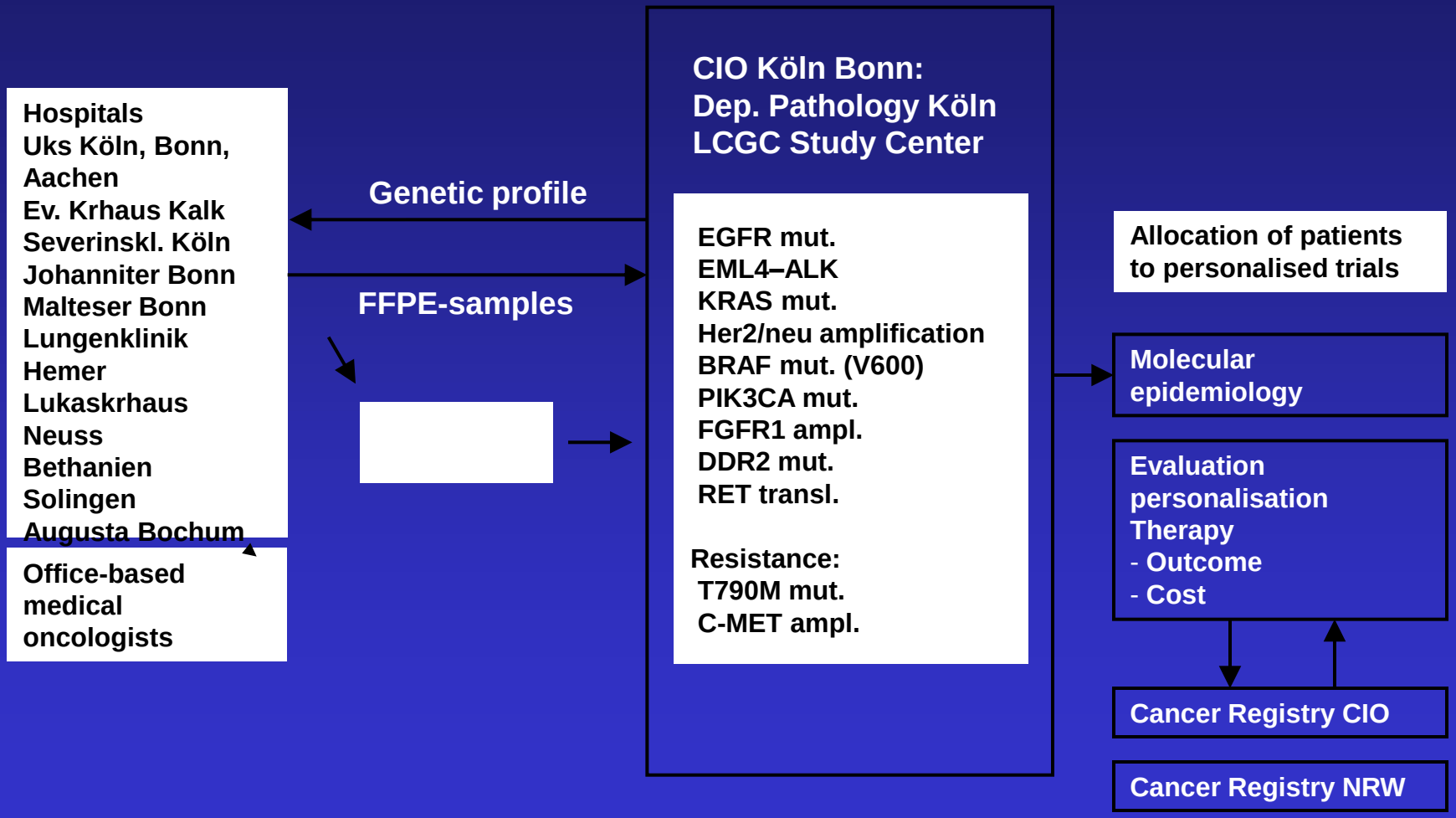






Regional Network Genomic Medicine Lung Cancer in the Catchment Area of the Center for Integrated Oncology Köln Bonn

March 2010–December 2011: 1,990 patients analysed; 81% evaluable for genotyping

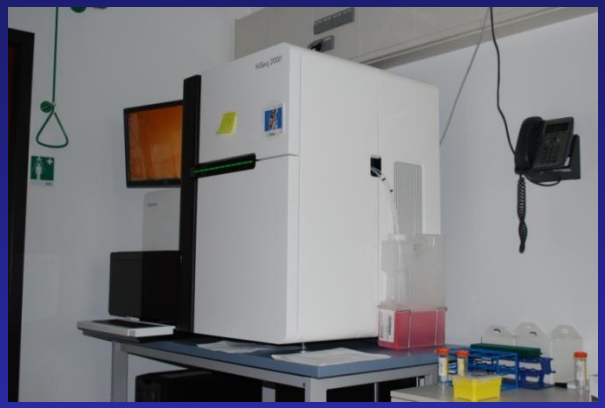


LTCG, Laboratory for Translational Cancer Genomics

Zander T, et al. Presented at ASCO 2012; Abstract CRA10529

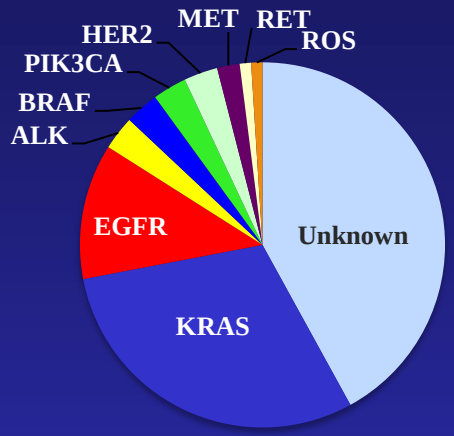
CIO Genome Scanner v2012.1

Next generation sequencing



Illumina HiSeq + MiSeq

Ion Torrent PGM



- **Cancer Hot Spot Primer Library**
- **42 genes**
- **5,271 known mutations**
- Pre-tailed Illumina amplicons

ABL1; CTNNB1; HRAS; MLH1; PTEN; TP53
APC; **EGFR**; IKBKB; MSH2; RB1; VHL
BRAF; **ERBB**2; JAK2; NF1; **RET**
BRCA1; FBXW7; JAK3; NF2; RUNX1
BRCA2; **FGFR1**; KIT; NOTCH1; SMAD4
CDH1; FGFR2; **KRAS**; NRAS; SMO
CDKN2A; FGFR3; MAP2K4; PDGFRA; SRC
CSF1R; FLT3; **MET**; **PIK3CA**; STK11

Active for lung in August 2012 at the CIO

Ensuring equity of access to innovation: France organisation of molecular centres for personalized medicine

Provides nationwide molecular diagnostic tests

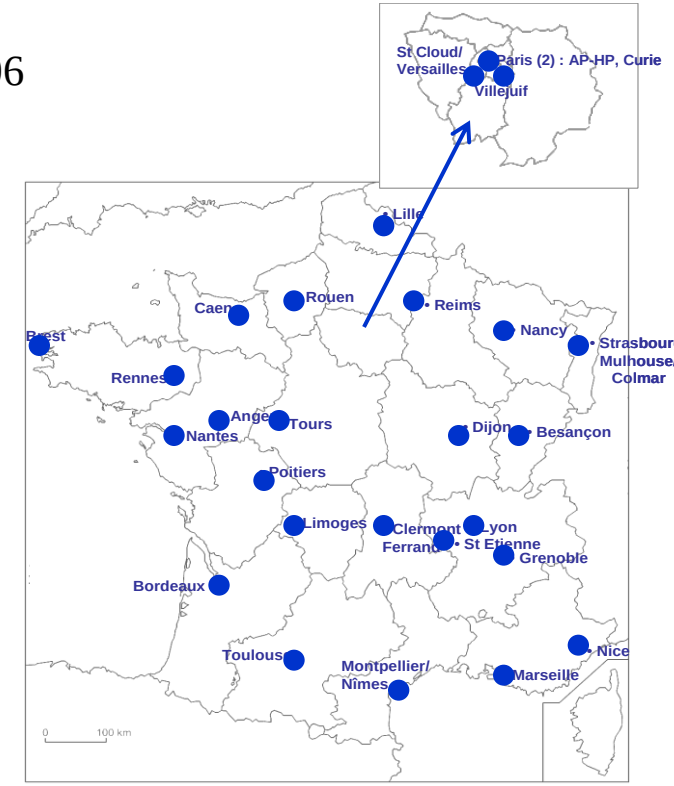
The programme is operated by the INCa/Ministry of Health since 2006

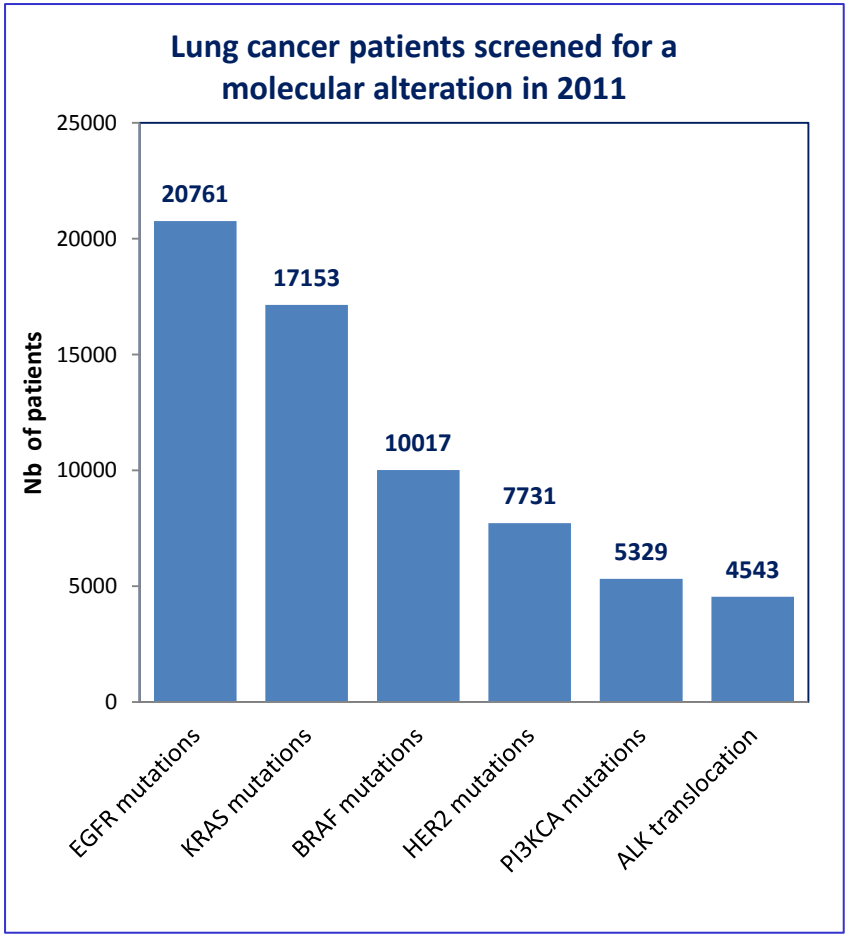
➢ Objectives

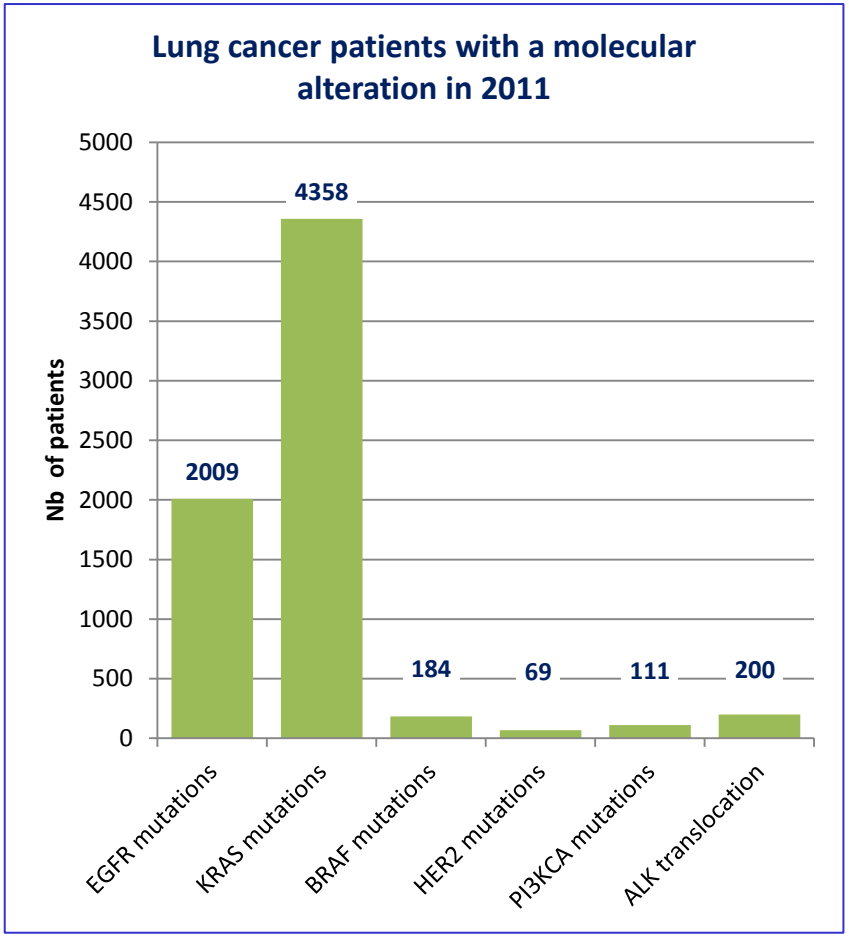
- **Perform molecular testing for all patients;**
- **Whatever the healthcare institution status (public hospitals, private hospitals…);**
- **Perform high quality tests;**
- **leukemia, solid tumours**

➢ 28 regional centres

- **Partnerships between several laboratories located in University hospitals and cancer centres**
- **Regional organization**
- **Cooperation between pathologists and biologists**



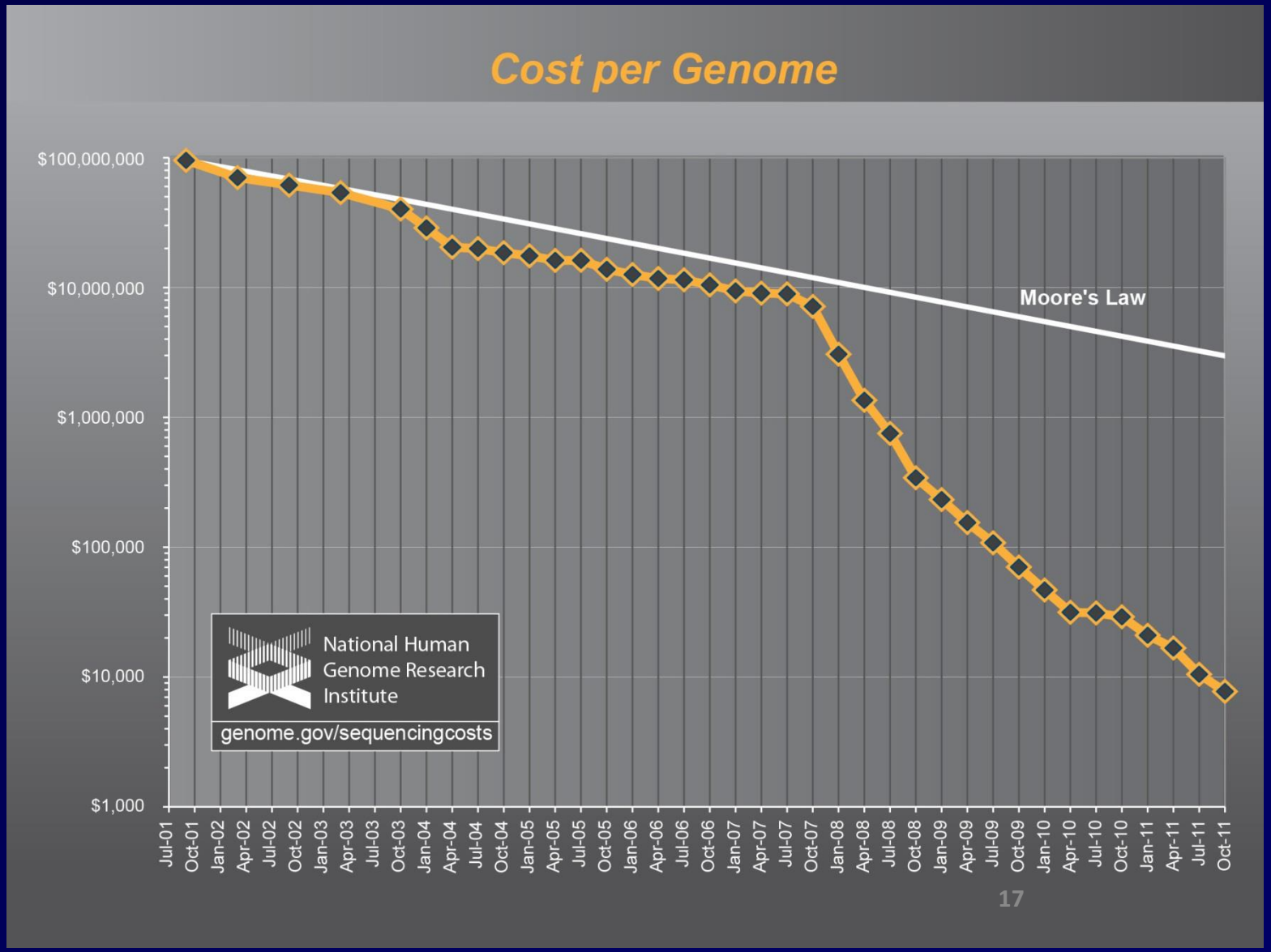

Lung cancer patients screened for a molecular alteration in 2011
Nb of patients
25000
20000
15000
10000
5000
0
20761
17153
10017
7731
5329
4543
EGFR mutations
KRAS mutations
BRAF mutations
HER2 mutations
PI3KCA mutations
ALK translocation


Lung cancer patients with a molecular alteration in 2011
Nb of patients
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
2009
4358
184
69
111
200
EGFR mutations
KRAS mutations
BRAF mutations
HER2 mutations
PI3KCA mutations
ALK translocation

Oxford Nanopore Technology

- Latest sequencing technology announced last month
- Size of USB drive
- May drive the next revolution in genomics
- Whole genome sequencing in 15 minutes for less than $1,000
- Commercially available by the end of this year

Cost Per Genome

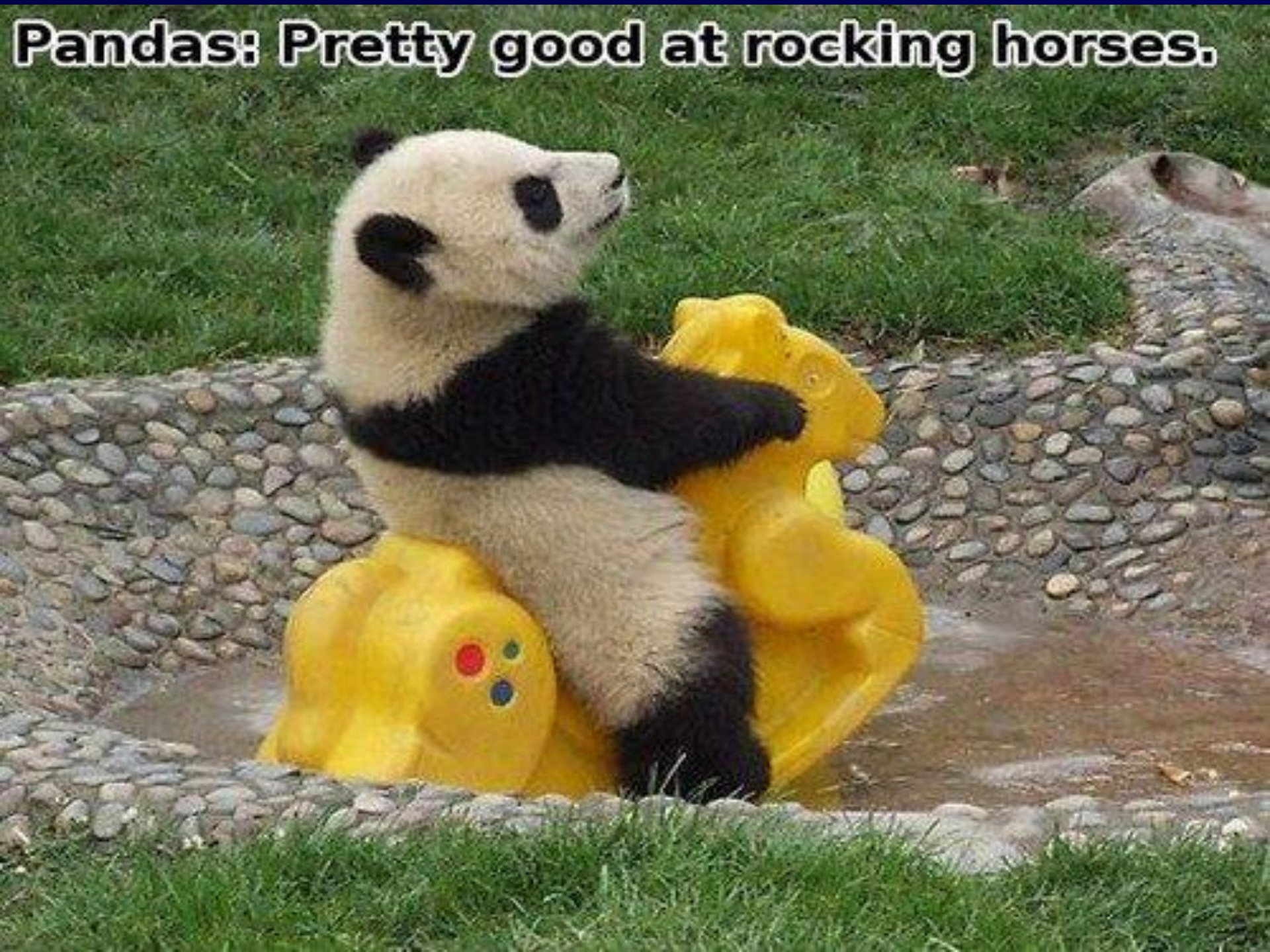


Summary
Personal genomics in medicine – The future

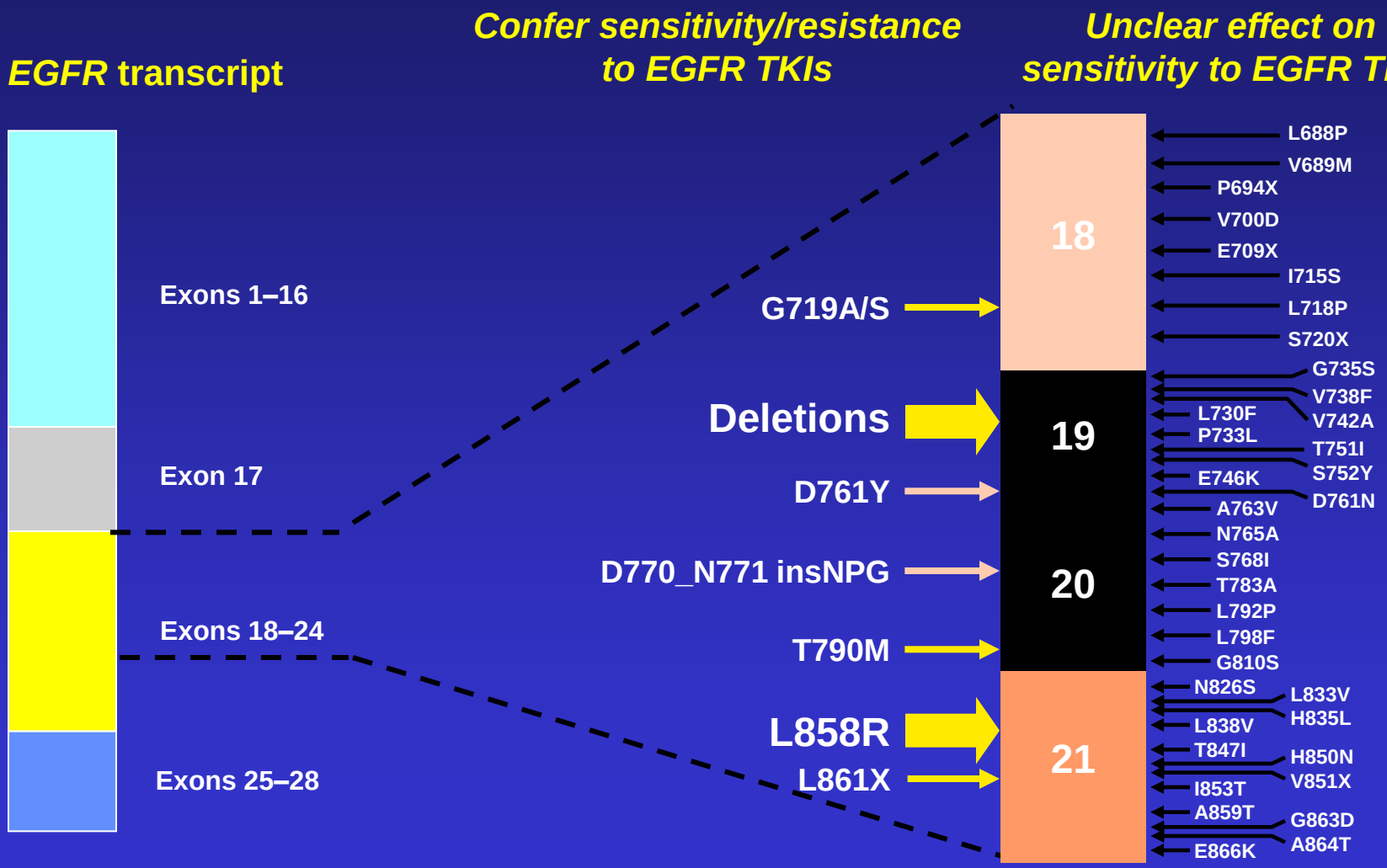
- Cost of sequencing genomes dropping - $1000 genome
  - Analysis and understanding will remain expensive
- Every child born or patient will likely have his or her genome sequenced fully
- This genome record should allow physicians to make treatment decisions based on patients genotypes
- Will allow individuals to make appropriate lifestyle choices
  - Food, exercise etc
- Genome data will allow rapid drug development

HOLD ON TO YOUR HORSES!

We Need a Reality Check!

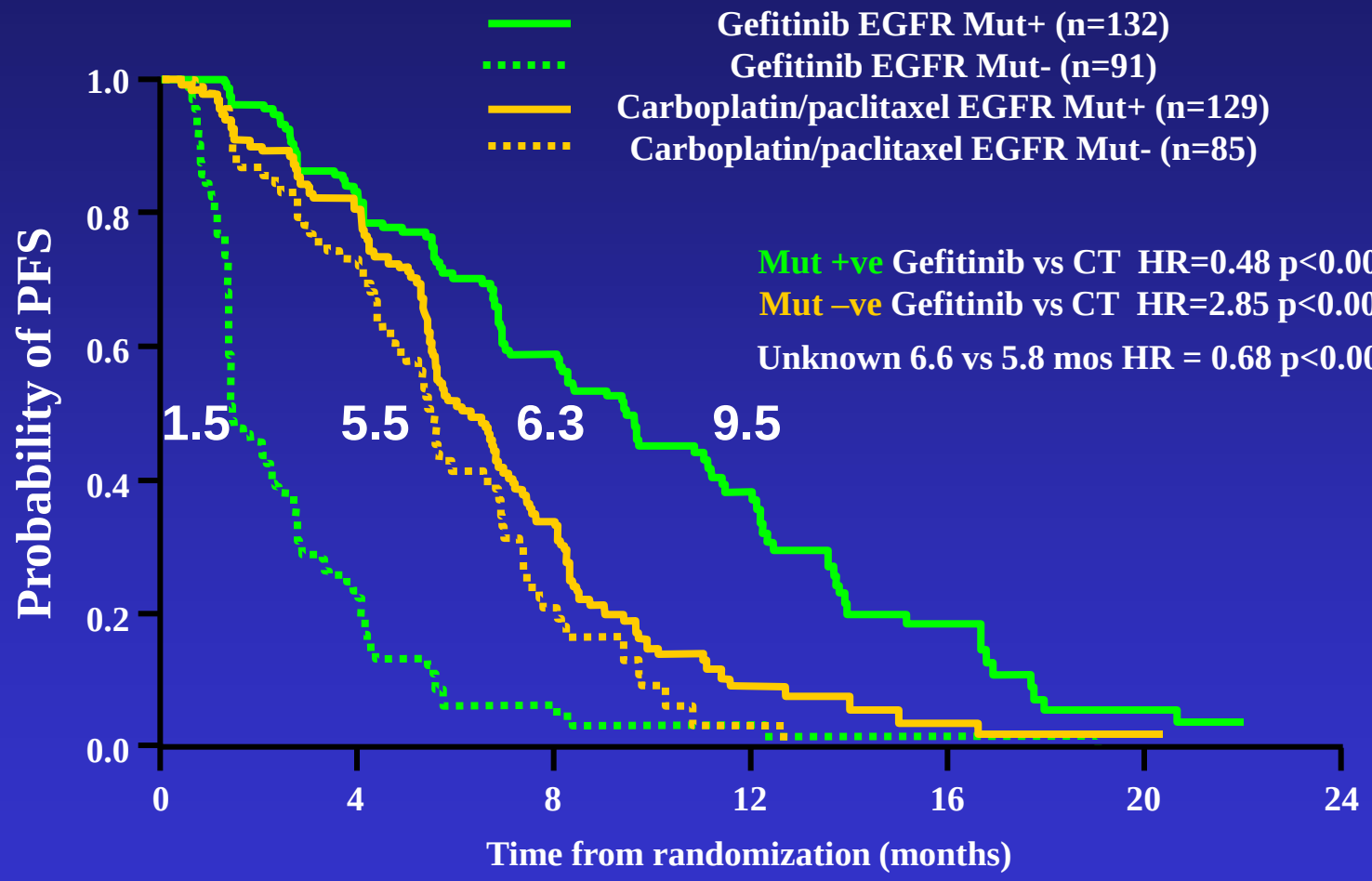

Pandas: Pretty good at rocking horses.

Mutations identified in *EGFR* gene



Riely, et al. Clin Cancer Res 2006

IPASS: EGFR mutation status defined population benefiting from treatment

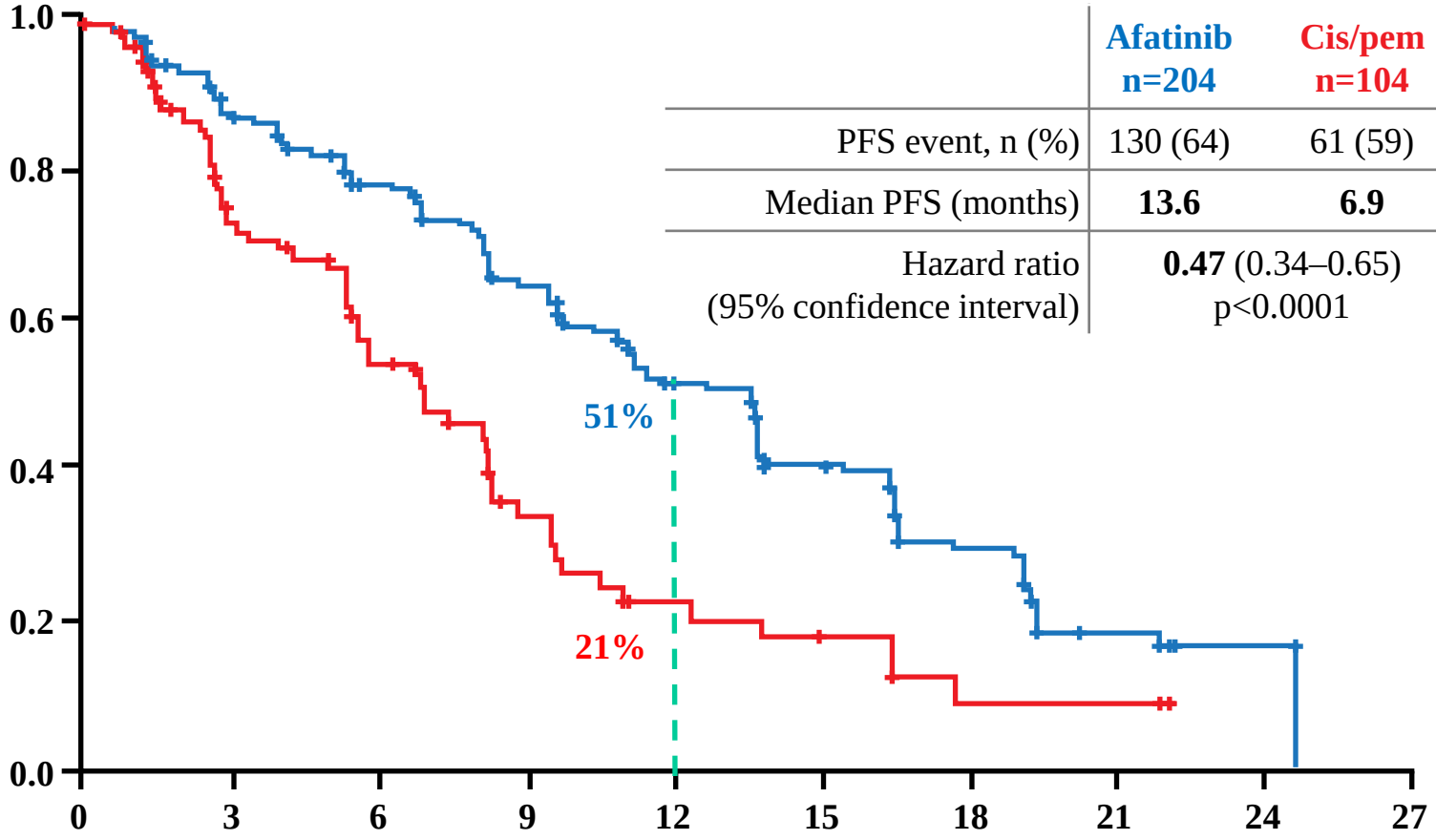


PFS treatment by EGFR mutation status interaction test: p<0.0001

Mok et al. NEJM 2009; Fukuoka et al. JCO 2009

LUX-Lung 3:

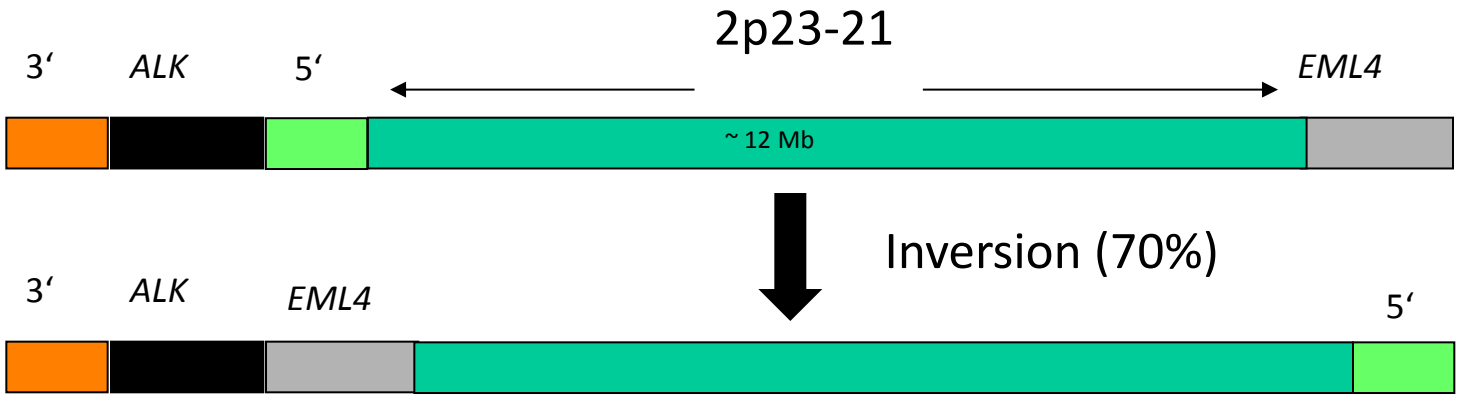
PFS common mutations (Del19/L858R)



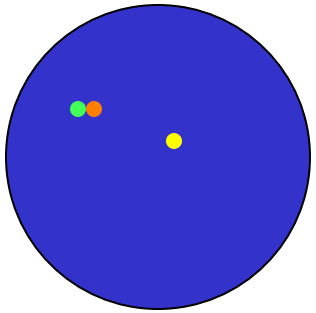
	Afatinib n=204	Cis/pem n=104
PFS event, n (%)	130 (64)	61 (59)
Median PFS (months)	**13.6**	**6.9**
Hazard ratio (95% confidence interval)	**0.47** (0.34–0.65) p<0.0001	

Afatinib	204	169	143	115	75	49	30	10	3	0
Cis/Pem	104	62	35	17	9	6	2	2	0	0

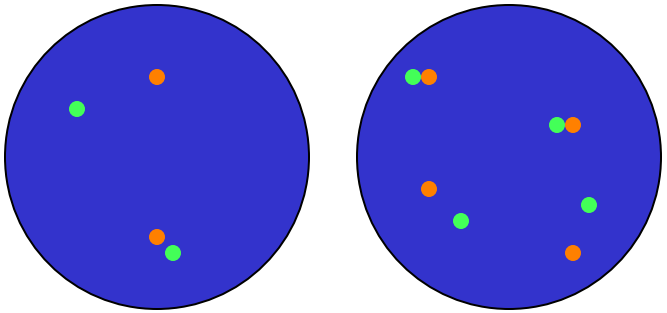
Fluorescence *in situ* Hybridisation

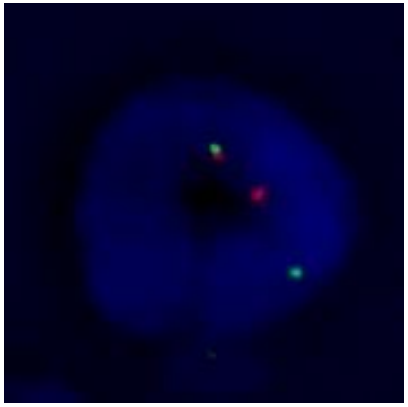


Gene Fusion



Negative

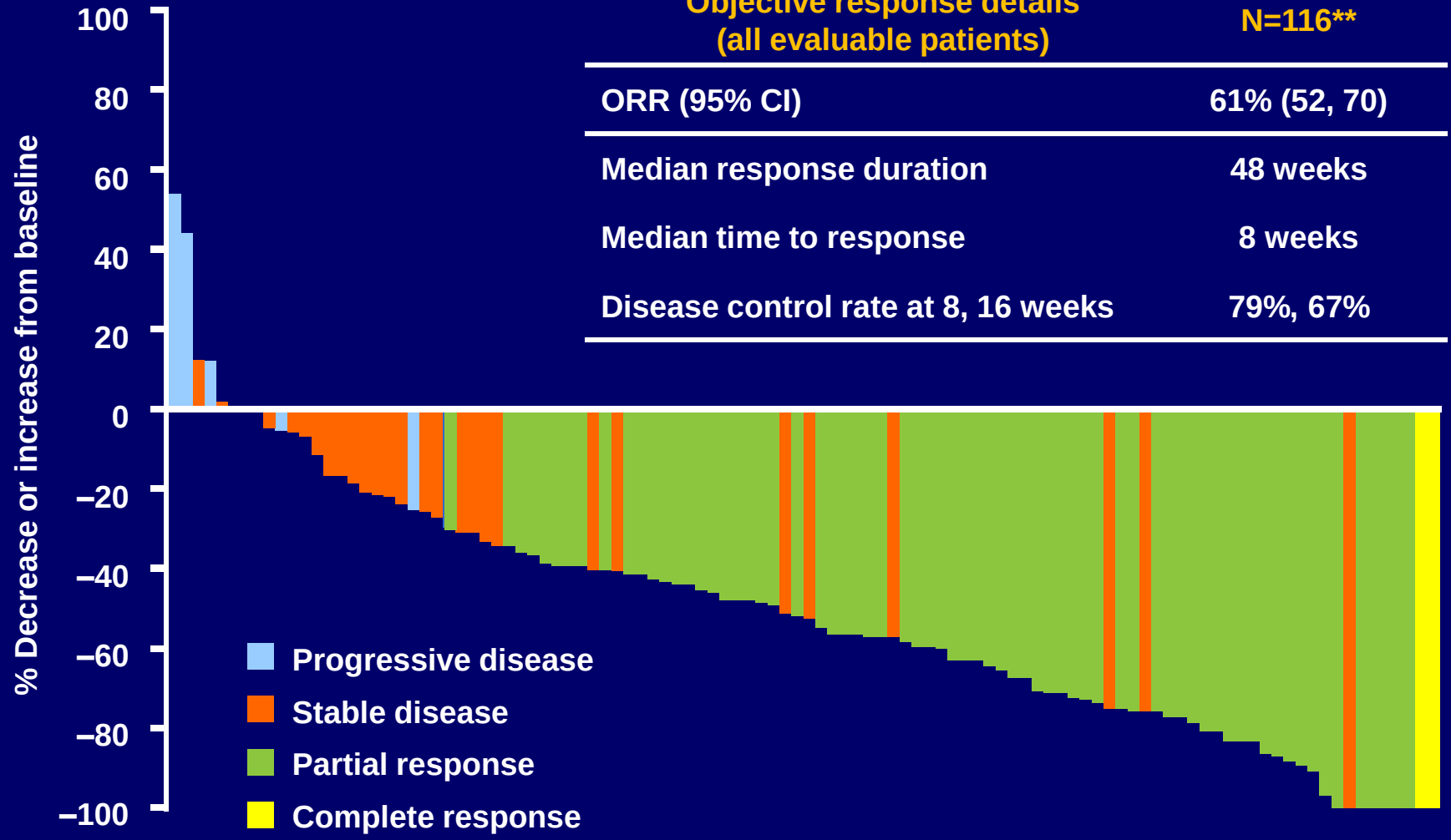




Positive (break-apart - inversion)

Best Percent Change from Baseline in Target Lesions*

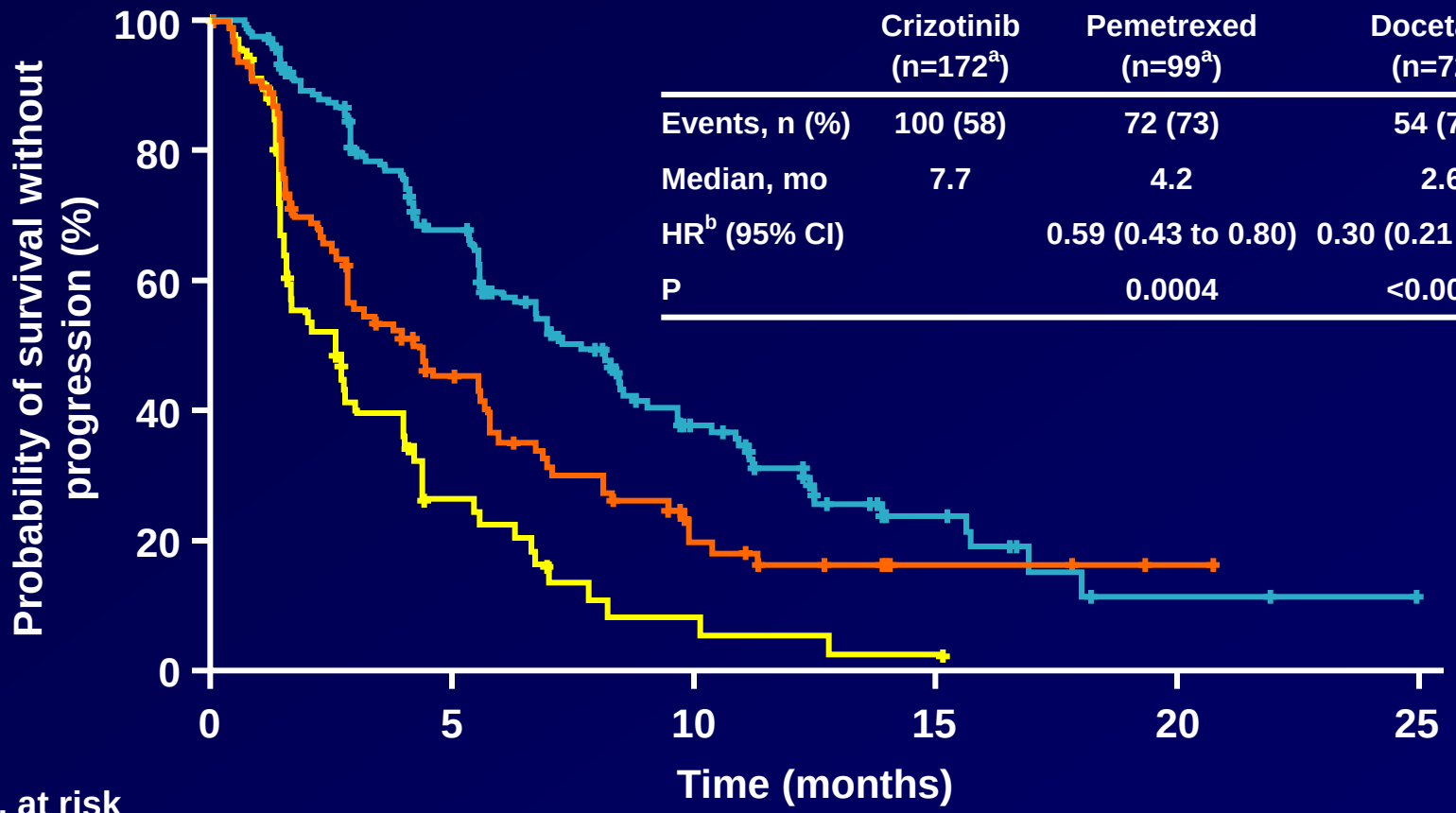
Objective response details (all evaluable patients)	N=116**
ORR (95% CI)	61% (52, 70)
Median response duration	48 weeks
Median time to response	8 weeks
Disease control rate at 8, 16 weeks	79%, 67%



*excludes patients with early death and indeterminate response (n=106)
**includes patients with early death and indeterminate response (n=116)

Camidge *et al.* Oral abstract no. 2501 presented at ASCO 2011

PFS of Crizotinib vs Pemetrexed or Docetaxel



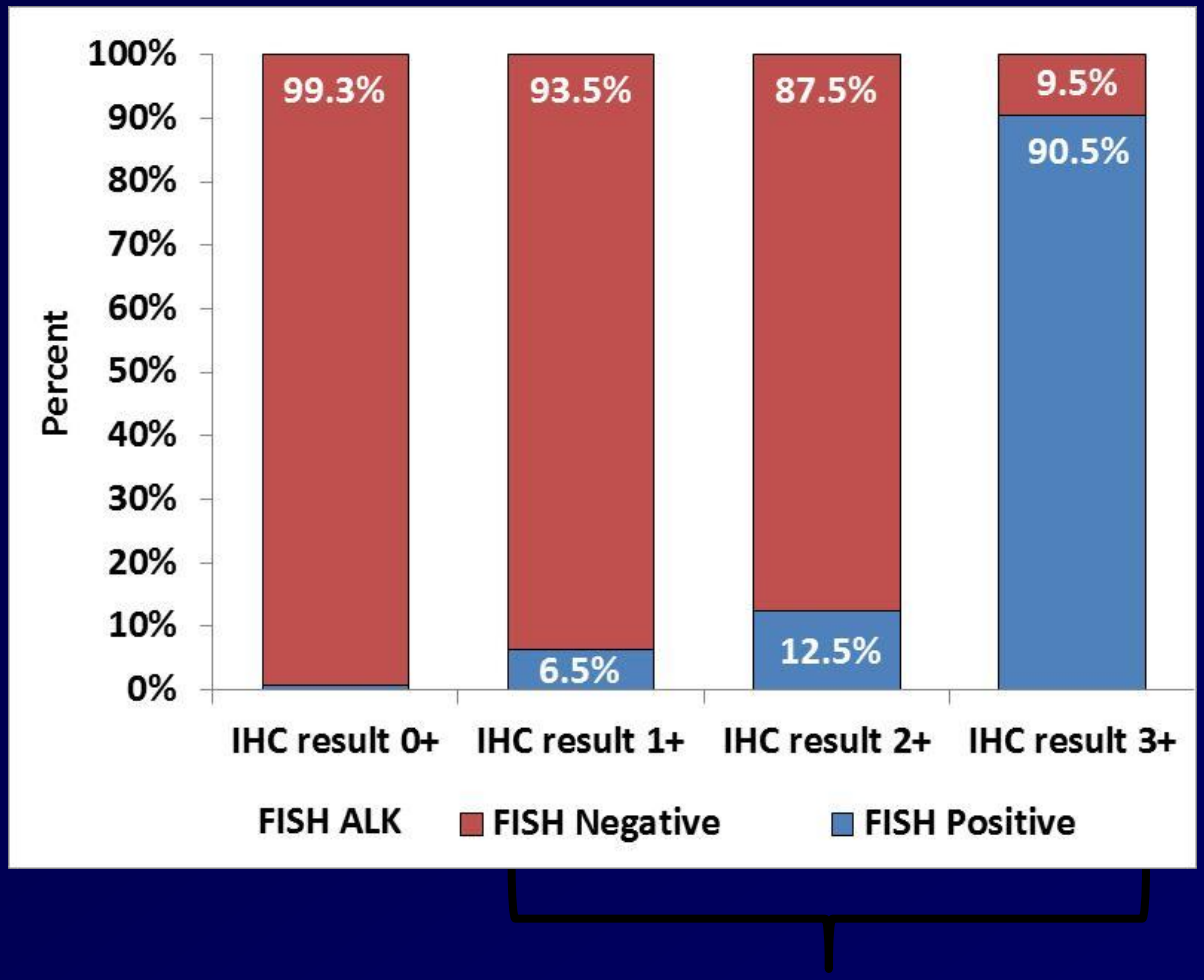
	Crizotinib (n=172[a])	Pemetrexed (n=99[a])	Docetaxel (n=72[a])
Events, n (%)	100 (58)	72 (73)	54 (75)
Median, mo	7.7	4.2	2.6
HR[b] (95% CI)		0.59 (0.43 to 0.80)	0.30 (0.21 to 0.43)
P		0.0004	<0.0001

No. at risk						
Crizotinib	172	93	38	11	2	0
Pemetrexed	99	36	12	3	1	0
Docetaxel	72	13	3	1	0	

[a]As-treated population: excludes 1 patient in crizotinib arm who did not receive study treatment and 3 patients in chemotherapy arm who did not receive study treatment; [b]vs crizotinib

Shaw et al, ESMO, 2012

Association of ALK IHC and FISH, N=198



36.7% of IHC+ are FISH+

For ALK IHC + vs –

(IHC 1+/2+/3+ vs IHC 0+)

FISH Sensitivity=36.7%
22 FISH + / 60 IHC +

FISH Specificity=99.3%
137 FISH - / 138 IHC –
p<0.001

For ALK IHC 3+ vs 0/1+/2+

FISH Sensitivity = 90.5%
19 FISH + / 21 IHC 3 +

FISH Specificity = 97.7%
173 FISH - / 177 IHC 0+/1+/2+
p<0.001

EGFR mutations and ALK rearrangements

- The only validated predictive biomarkers in NSCLC
- Quality assured in many centres
- Targeted by agents tested in randomised clinical trials that are available for our patients

Currently, Two Approved Personalised Treatment Options: Substantial Benefit for ~15% of Patients

Crizotinib in ALK-positive NSCLC

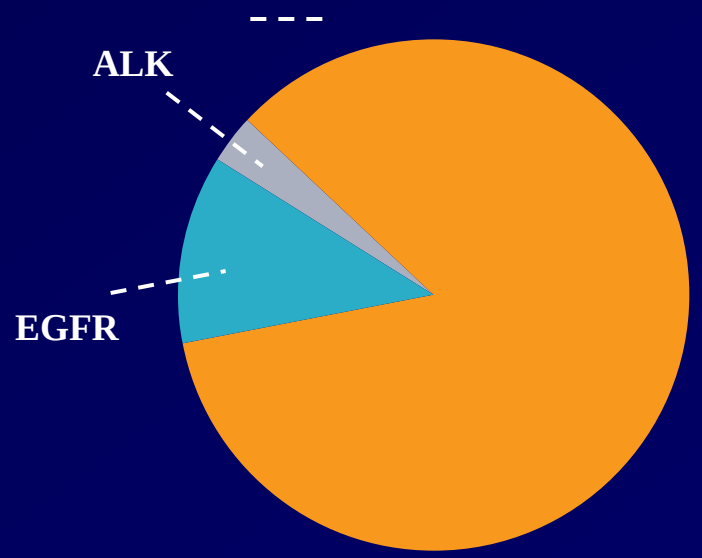
RR 60%, PFS 8 months

EGFR-TKIs in EGFR-mut NSCLC

Gefitinib, Erlotinib (US, EU)

RR 60–80%, PFS 10–13 months,

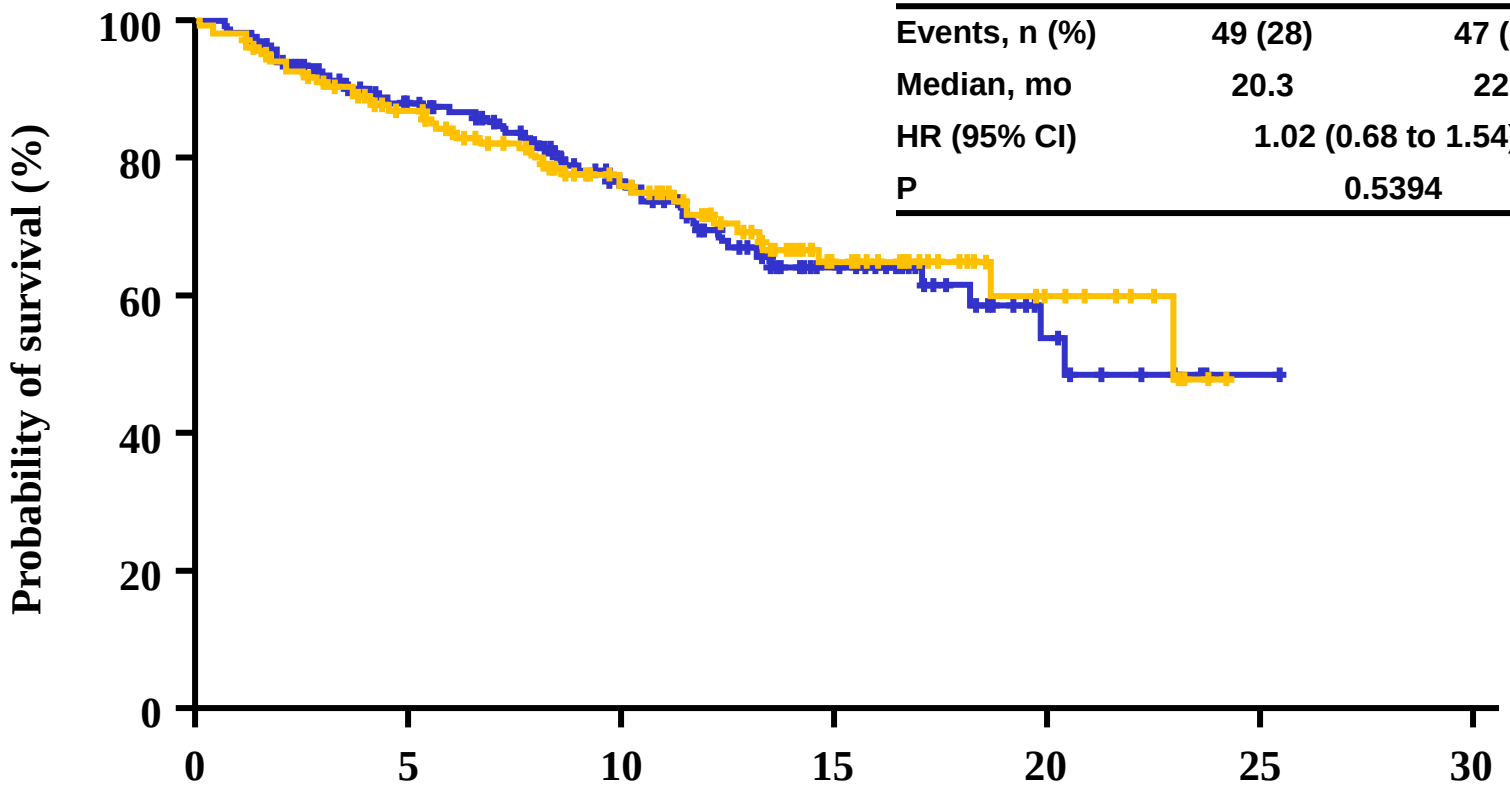
OS 19–30 months



……AND DO THESE TARGETED AGENTS IMPROVE SURVIVAL!

The Jury is Still Out!

PROFILE 007: Interim Analysis of OS



	Crizotinib (n=173)	Chemotherapy[a] (n=174)
Events, n (%)	49 (28)	47 (27)
Median, mo	20.3	22.8
HR (95% CI)	1.02 (0.68 to 1.54)[b]	
P	0.5394	

No. at risk							
Crizotinib	173	129	83	37	11	1	0
Chemotherapy	174	129	84	34	10	0	

[a]111 patients crossed over to crizotinib outside PROFILE 1007
[b]HR adjusted for crossover using rank-preserving structural failure time method: 0.83 (0.36 to 1.35)

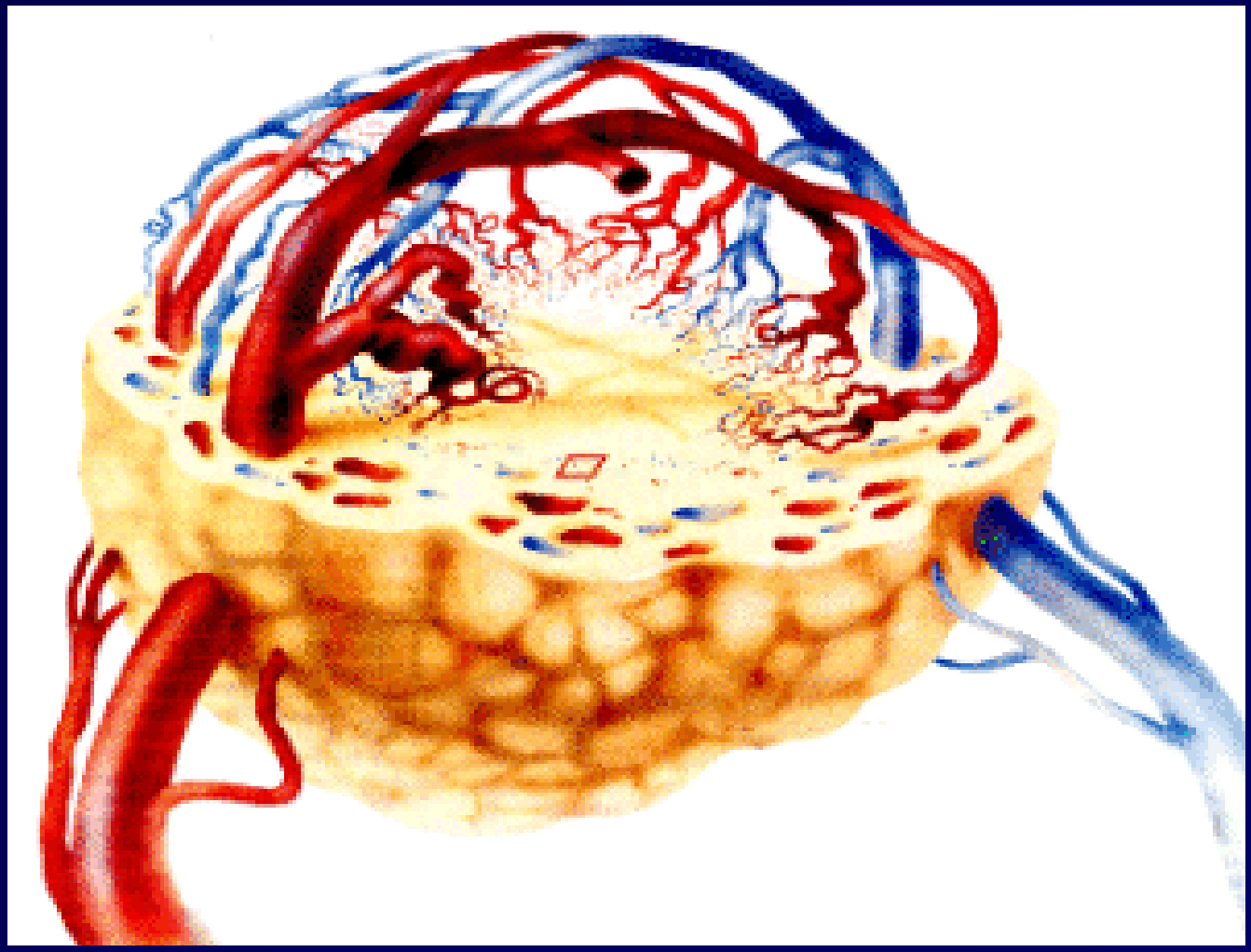
Crossover vs Tumour Biology……

LESSONS LEARNT THE HARD…

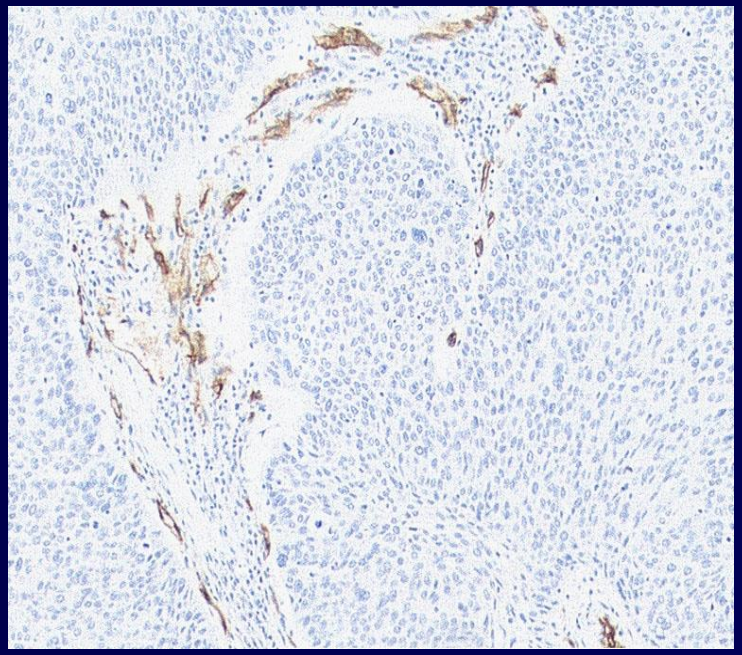
AND VERY EXPENSIVE…

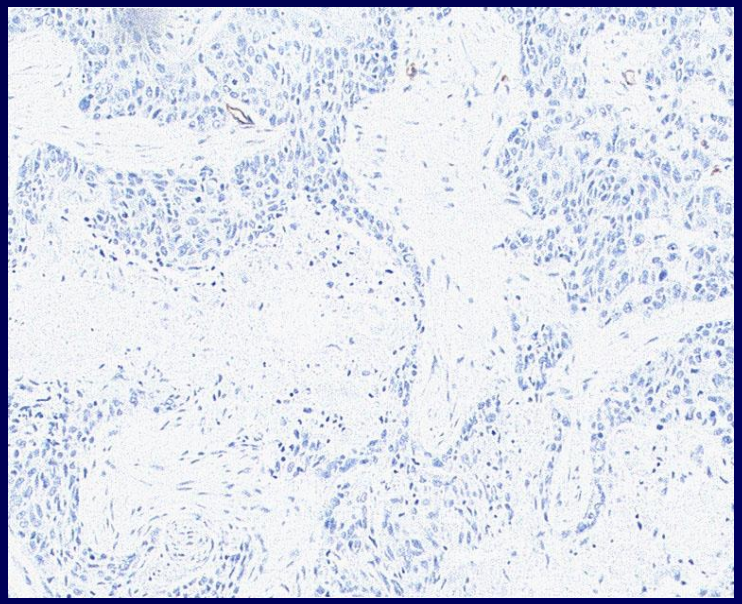
WAYS!

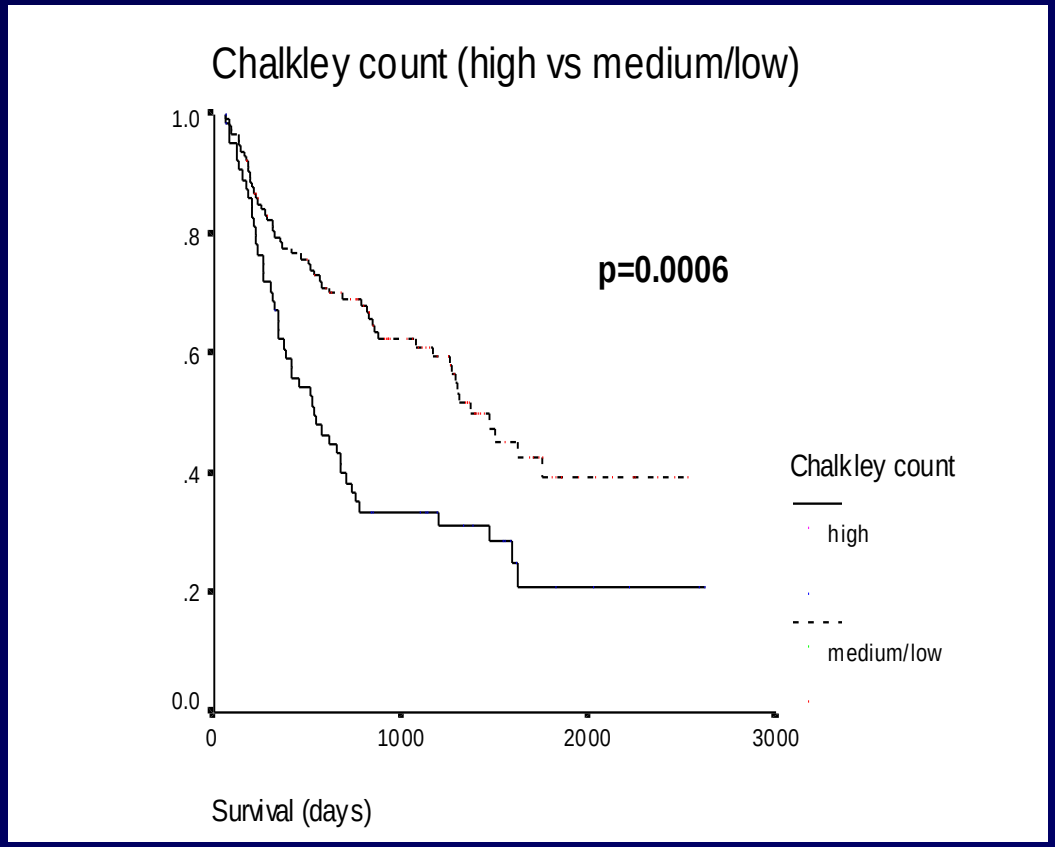
Tumour Angiogenesis



Prognostic Significance of MVD in NSCLC





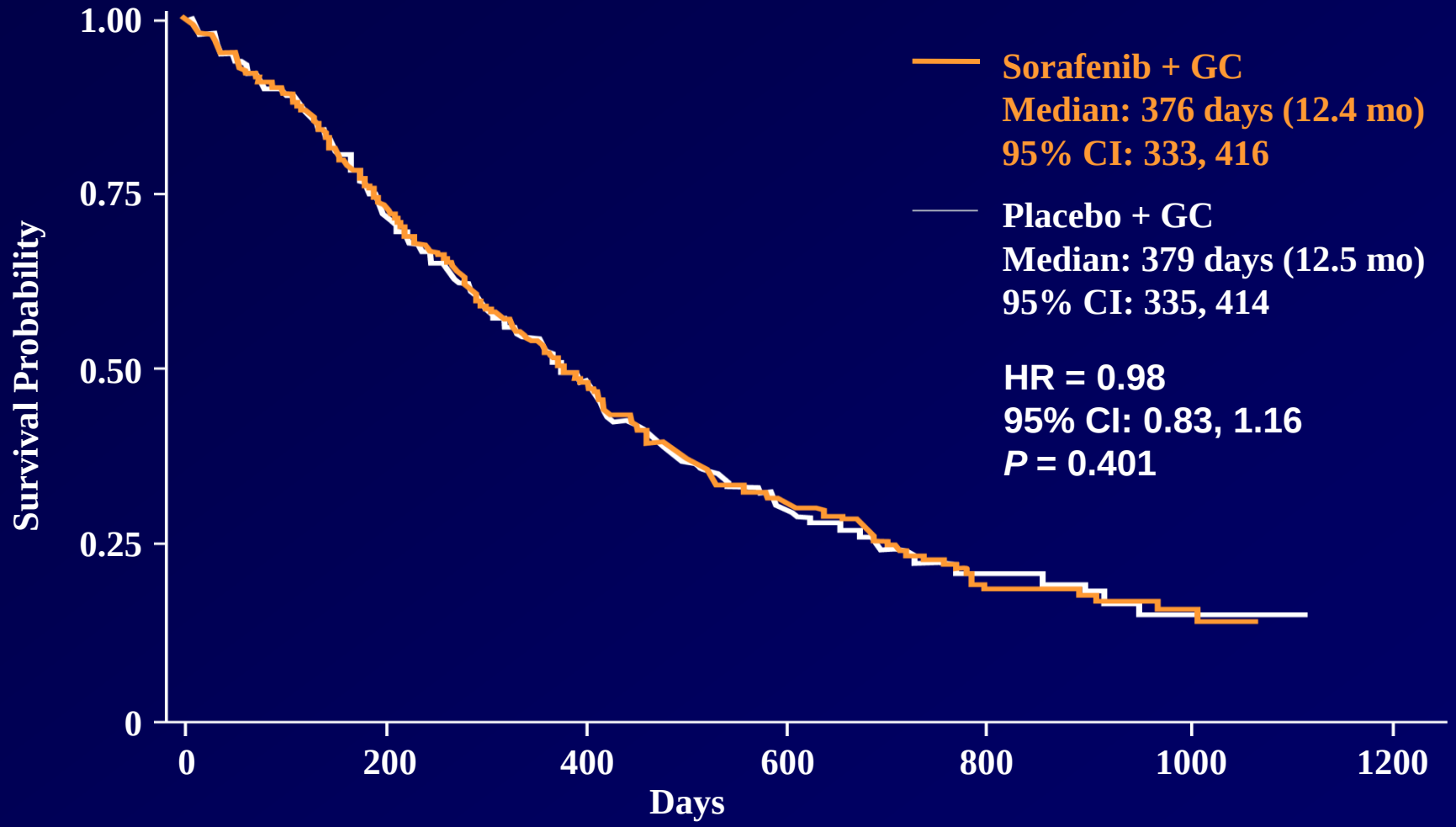


Anti-angiogenic TKI in mNSCLC

TKI	VEGFR1-3	PDGFR	c-KIT	BRAF	RAF-1	RET	FLT-3
Apatinib	√						
Axitinib	√	√	√				
BIBF1120*	√	√					
Cediranib	√	√	√				
Motesanib	√	√	√				
Pazopanib	√	√	√				
Sorafenib	√	√	√	√	√		√
Sunitinib	√	√	√			√	√
Vandetanib**	√					√	

*Binds also to FGFR
**Binds also to EGFR

NEXUS Overall Survival
Non-Squamous Population (ITT)



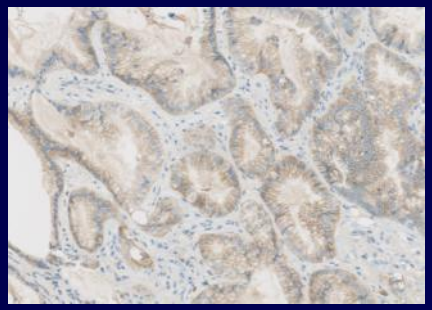
Gatzemeier U et al ESMO 2010 Ann Oncol 2010 S8 LBA 16

……AND WHAT ABOUT THE PROTEINS!

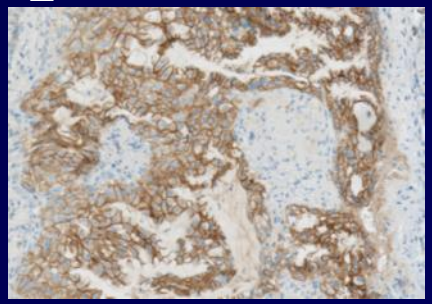
Development of Met IHC as a Diagnostic

- **Intensity of Met staining on tumor cells scored on 0–3+ scale**

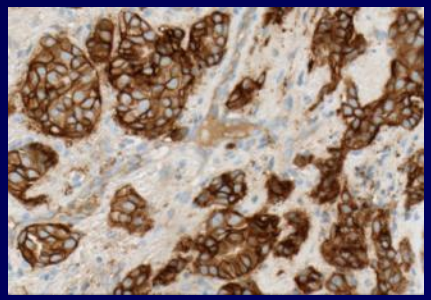
1+



2+



3+



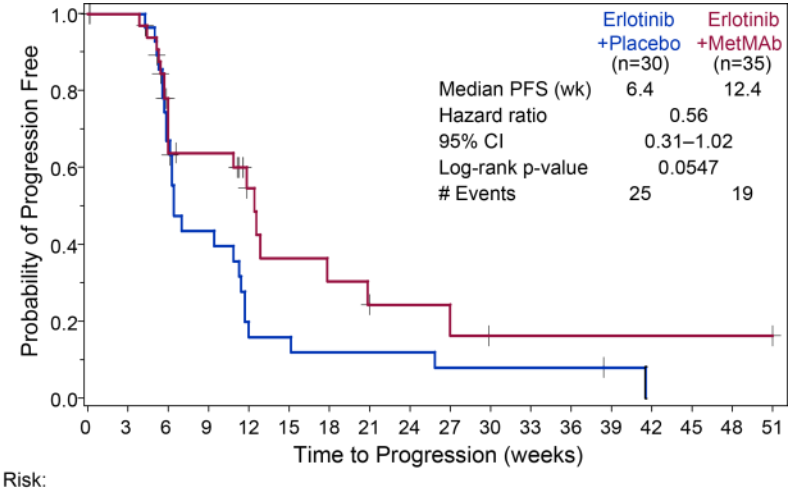
- **Estimated that ~50% of patients would have 'Met High' tumors**
- **Met by IHC was assessed after randomization**

'Met High' was defined prior to unblinding as:
≥50% tumor cells with a staining intensity of 2^+ or 3^+

- Tissue was obtained from **100%** of patients.
- **95%** of patients had adequate tissue for evaluation of Met by IHC.
- **54%** patients had 'Met High' NSCLC.

PFS and OS: Met High Population

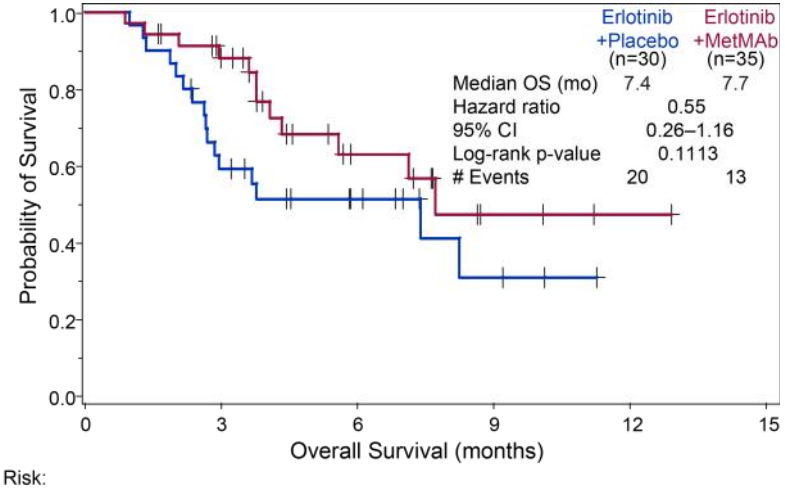
PFS, HR=0.56



Number at Risk:

Erlotinib+Placebo	30	18	5	3	3	2	2	0	0
Erlotinib+MetMAb	35	22	9	5	3	1	1	1	1

OS, HR=0.55

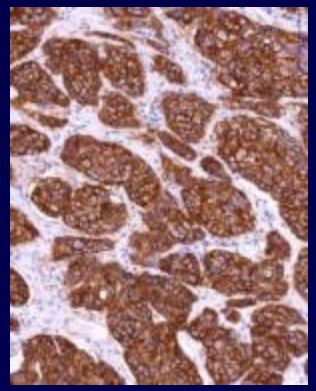


Number at Risk:

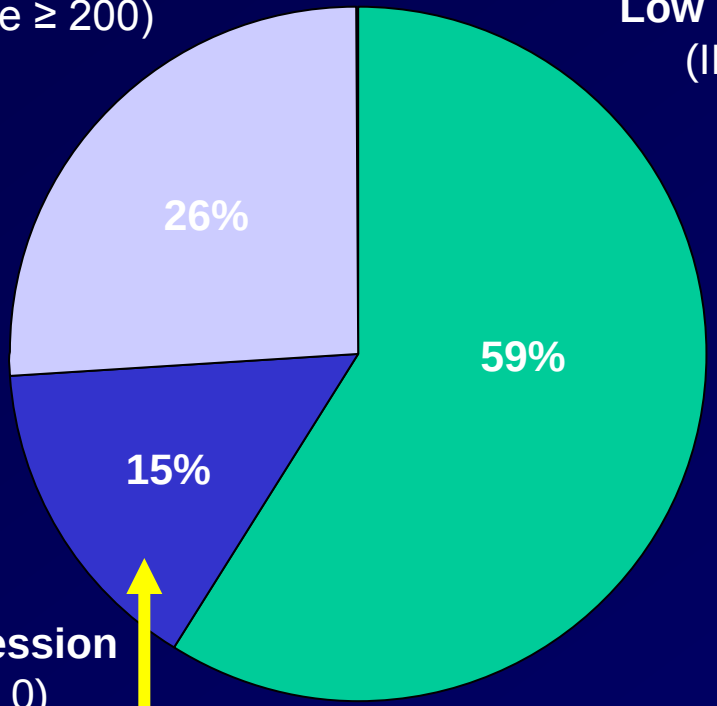
Erlotinib+Placebo	30	17	9	3	0	0
Erlotinib+MetMAb	35	26	10	3	1	0

MetMAb+Erlotinib improves both PFS and OS in Met High NSCLC patients

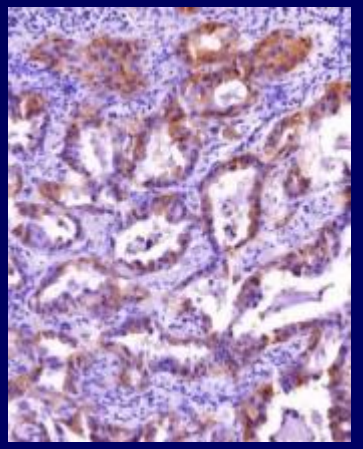
EGFR expression in FLEX patients

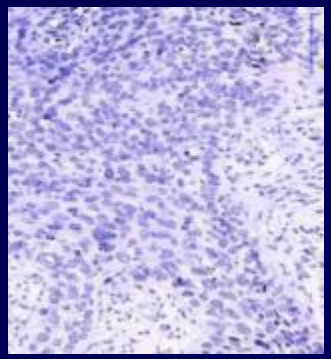


High EGFR Expression
(IHC score ≥ 200)



Low EGFR Expression
(IHC score <200)





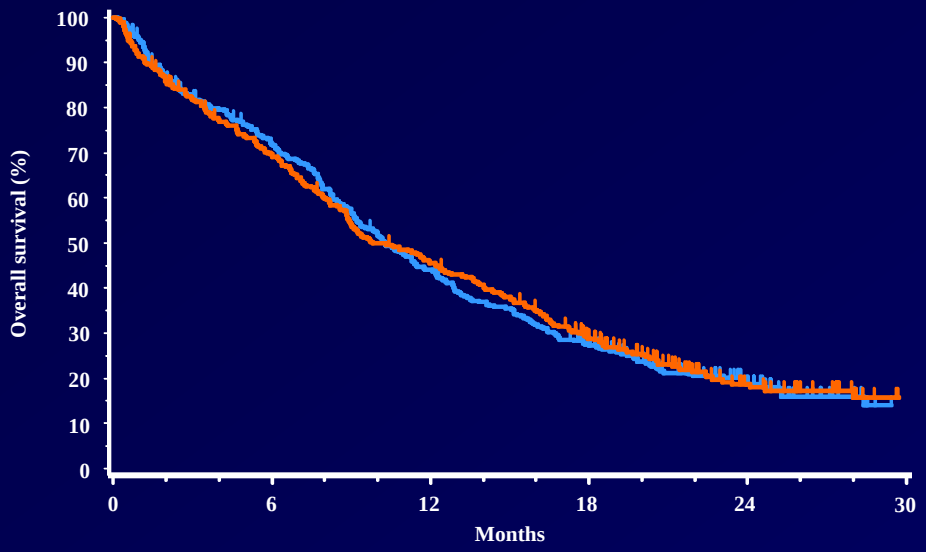
No EGFR Expression
(IHC score = 0)

Not included in FLEX

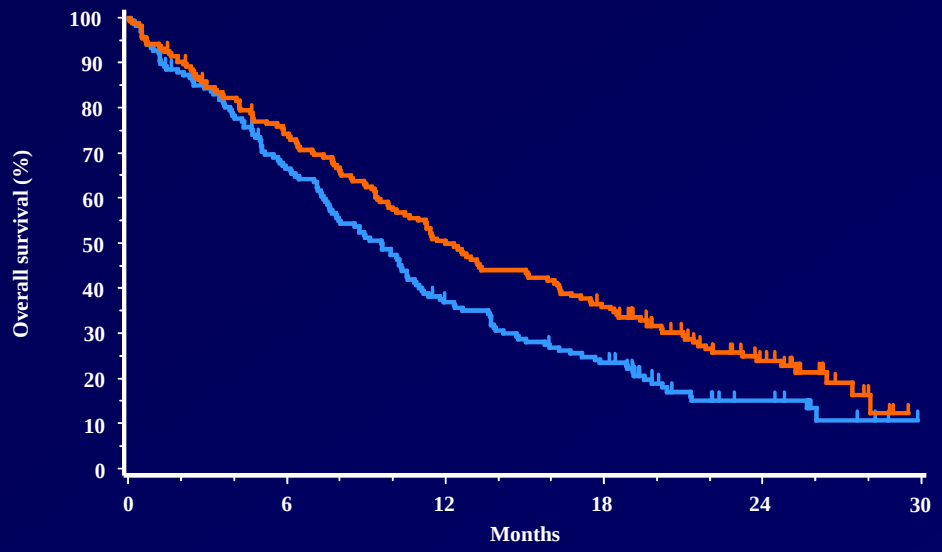
Rüschoff et al, WCLC, 2011

High EGFR is predictive for survival benefit with CT + cetuximab

Low EGFR



High EGFR



CT
CT + cetuximab

Interaction p-value=0.044

CT, chemotherapy

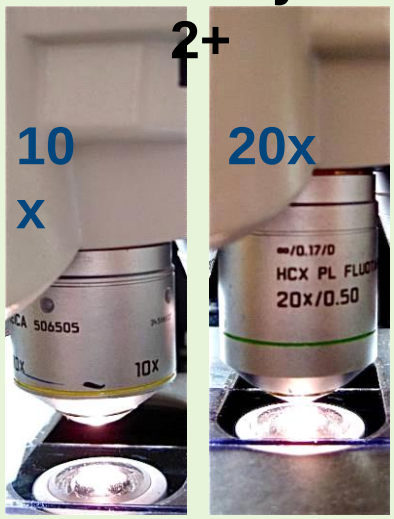
EGFR IHC scoring instructions

- Four intensities: 0 = no staining,1+ = weak, 2+ = moderate, 3+ = strong staining
- Intensities defined by “magnification rule” (Rüschoff et al. 2010):

Staining intensity: 3+

Visible by eye or under low power examination: 4x or 5x

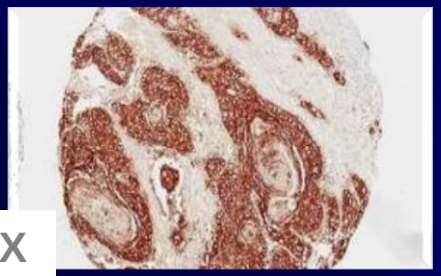
Staining intensity: 2+

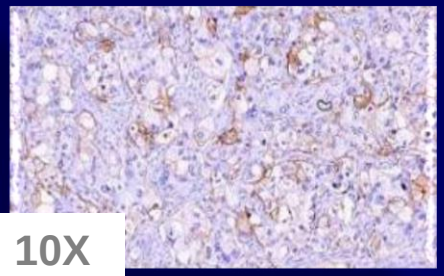


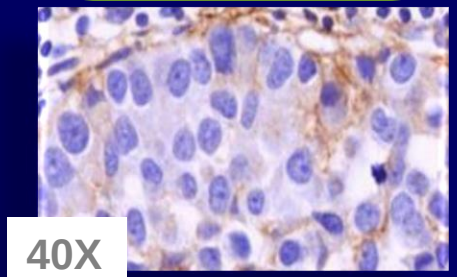
Needs a more detailed magnification : 10x – 20x

Staining intensity: 1+ or 0

Needs high magnification: 40x







Summary

- Predictive biomarker tests must undergo
  - Validation
  - Quality Assurance
- Deep DNA sequencing is a research tool
- Generates clinically irrelevant data
  - May confuse clinician
  - May confuse the patient

Conclusion

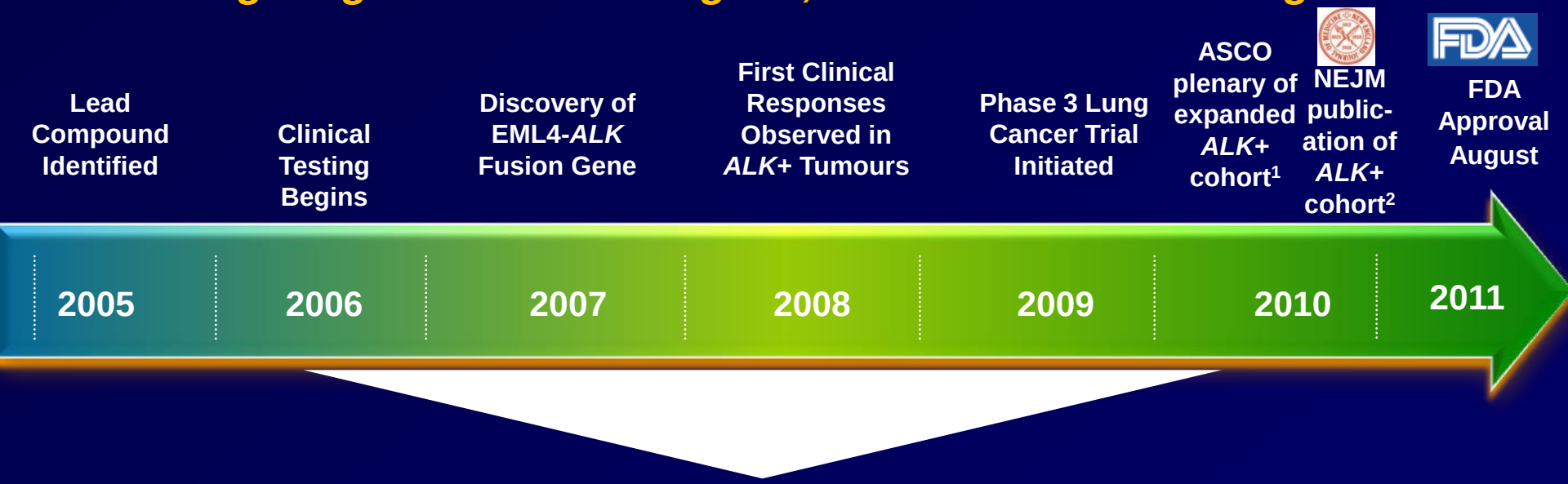
- Deep DNA sequencing is very nice and intellectually very stimulating!

BUT

- Is not yet ready for routine use in the clinic
- Needs to be **controlled** and **utilised** appropriately
  - **As a research tool**

Crizotinib: Pathway from Compound Identification to Discovery of *ALK* Target and Clinical Results

Crizotinib (PF-02341066) scientific breakthrough: Targeting the ALK fusion gene, a direct driver of oncogenesis



Rapid Timeline from Compound Identification, Target Discovery and Clinical Results

Clinical Results to Date

- **Objective response rate = 61%[3]**
- **Disease control rate – Wk 8 (CR+PR+SD) = 79%[3]**
- Median duration of response = 48 weeks*[3]
- Median PFS = 10 months†[3]

*in responding patients

†Fifty-nine (50%) patients remain in follow-up for PFS

1. Bang *et al*. Oral presentation at ASCO, 2010
2. Kwak *et al. New Engl J Med*. 2010;363:1693−03
3. Camidge *et al.* Oral 2501 presented at ASCO, 2011

The Promise of Higher Responses in a Targeted Populations