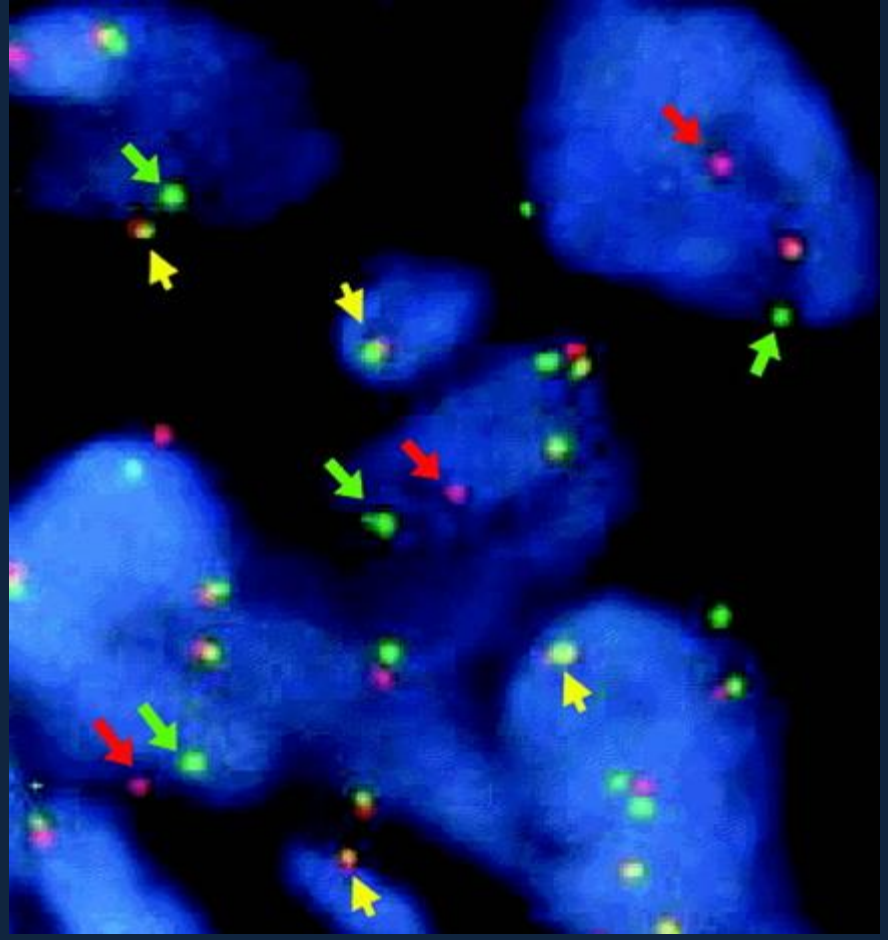
Prof. Solange Peters, MD-PhD
Oncology Department
Ludwig Institute
Lausanne
Switzerland



MOLECULAR CHARACTERIZATION AND SUBTYPING OF NSCLC

CANCER ETIOLOGY

Variation in cancer risk among tissues can be explained by the number of stem cell divisions

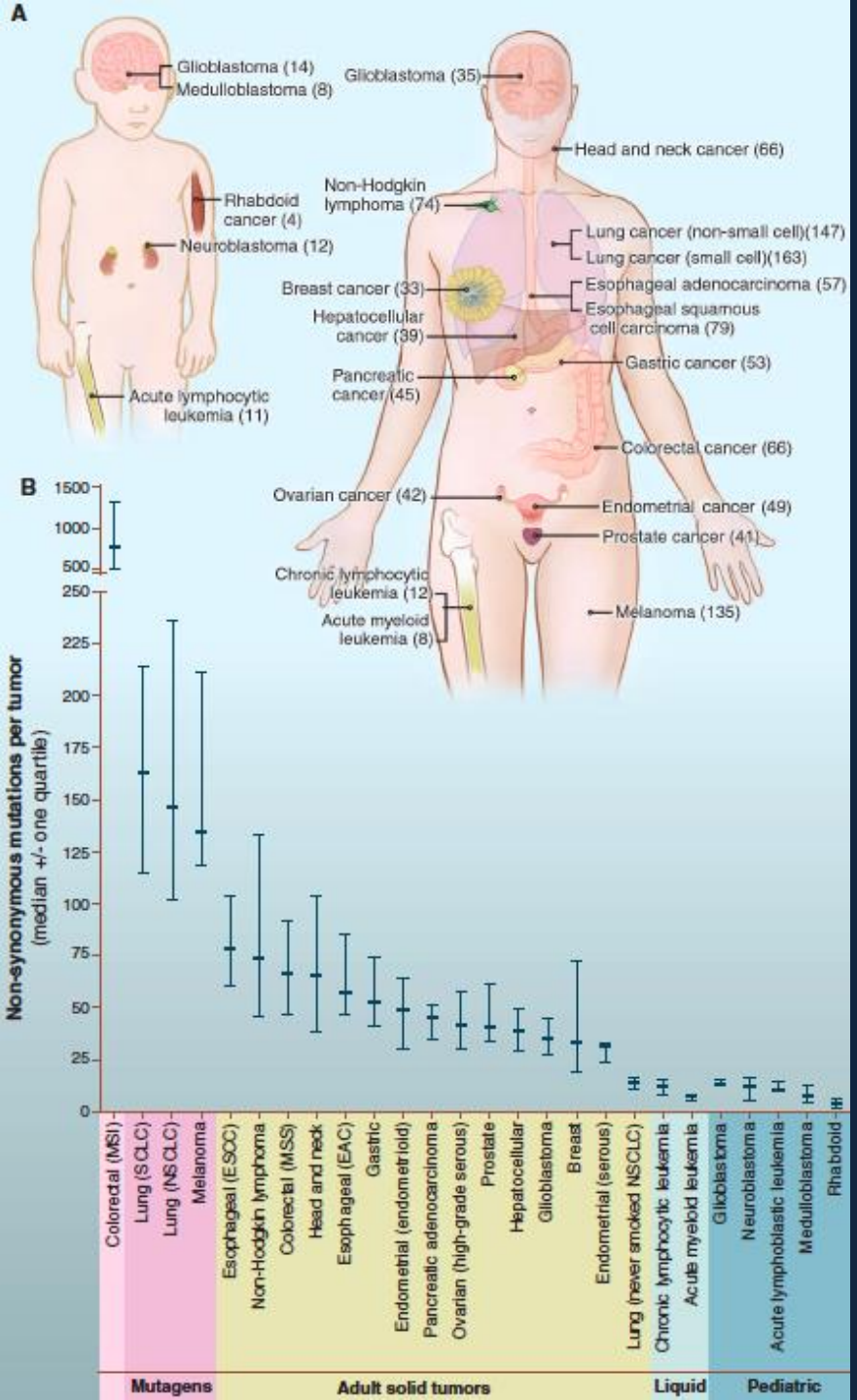
Cristian Tomasetti[1*] and Bert Vogelstein[2*]

- Lifetime risk of cancers is strongly correlated with the total number of divisions of the normal self-renewing cells maintaining that tissue's homeostasis.

- These results suggest that only a third of the variation in cancer risk is attributable to environmental factors or inherited predispositions.

The majority is due to bad luck (…).

Tomassetti & Vogelstein, Science 2015



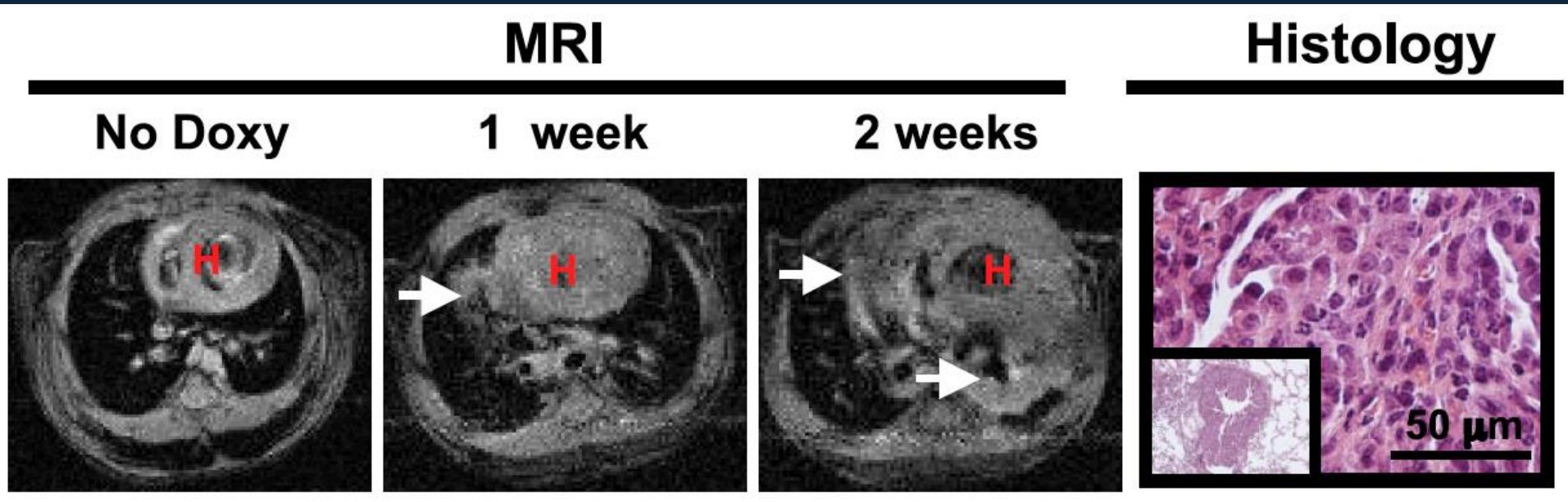
Lung tumors display many more mutations than average, with~200 nonsynonymous mutations per tumor.

These larger numbers reflect the involvement of potent mutagens. Accordingly, lung cancers from smokers have 10 times as many somatic mutations as those from nonsmokers.

Vogelstein, Science 2013

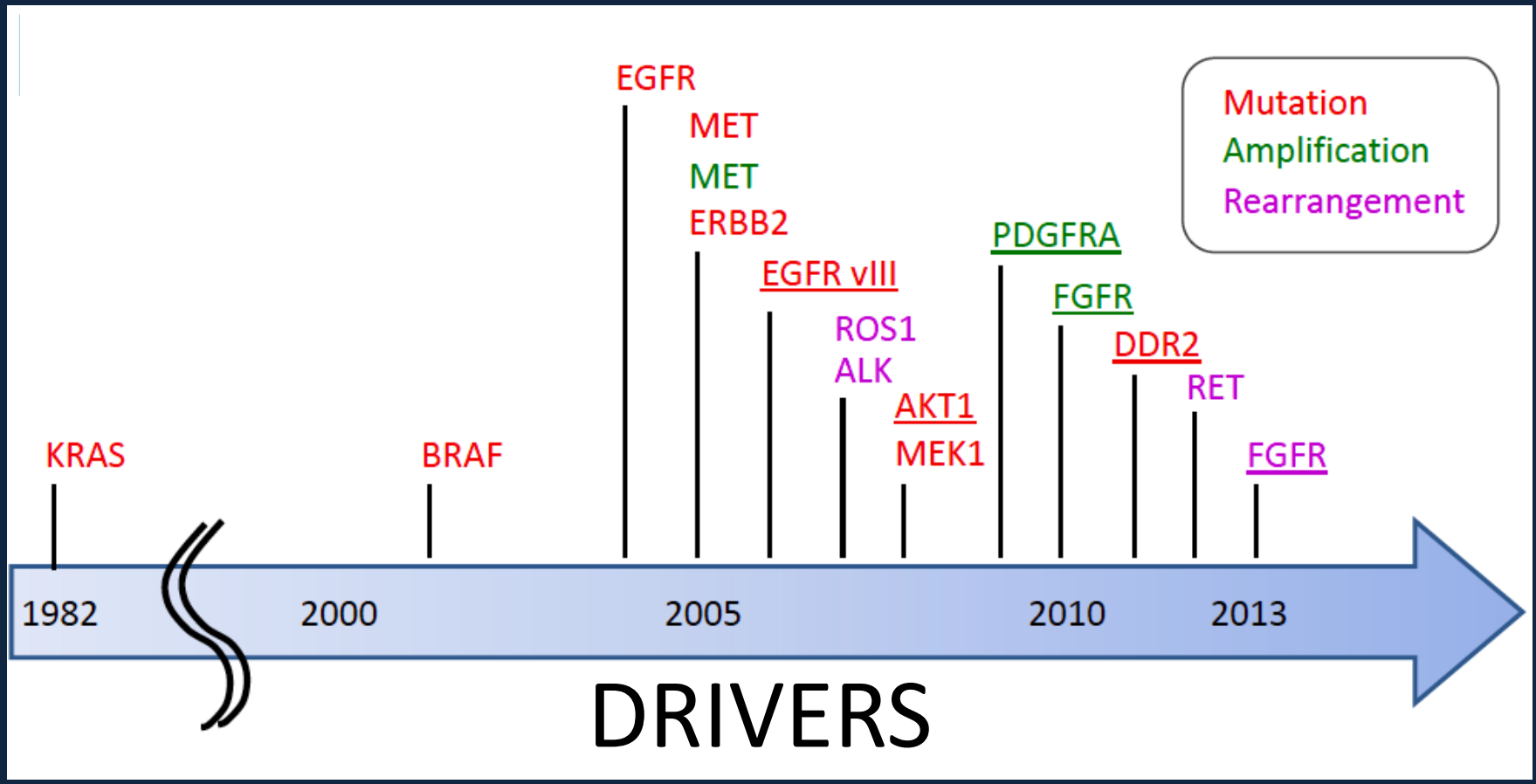
Definition of drivers & oncogene-addiction : HER2

Inducible expression of mutated HER2 (HER2YVMA):
Rapid development/maintenance of adenosquamous lung tumors in mice



Perera, PNAS 2009

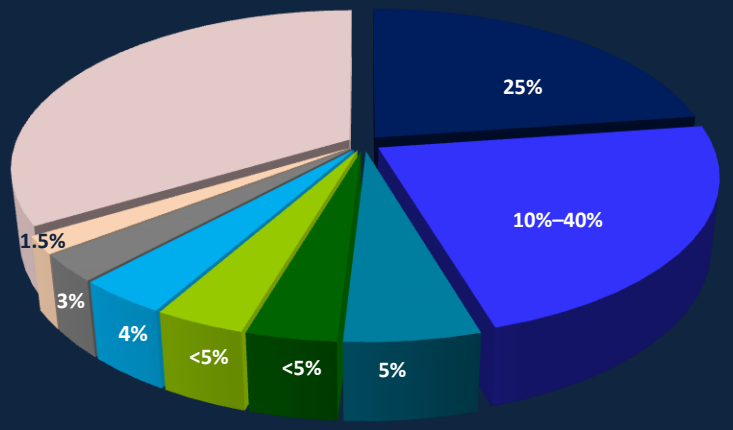
Oncogene-addicted NSCLC are often encountered in « never smokers »



Cortesy of Drs Mitsudomi and Suda

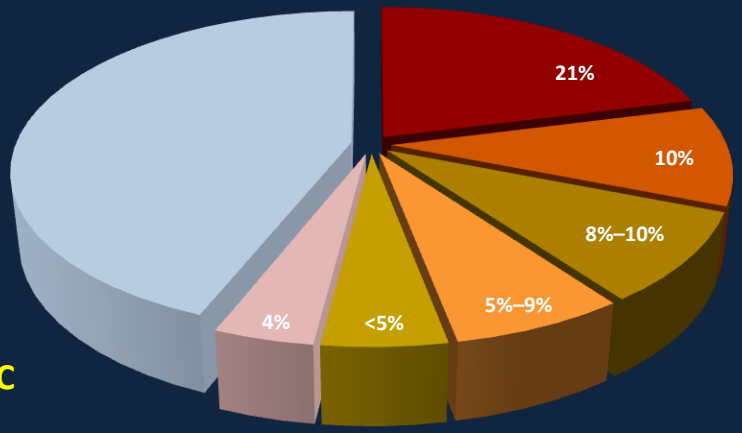
Actionable Molecular Alterations in NSCLC

Clinically relevant targetable genetic alterations vary with histologic classification



- KRAS mutation 20%–25%
- EGFR mutation 10%–15%
- ALK rearrangement 2%–8%
- HER2 mutation 4%
- PIK3CA mutation 4%
- MET amplification 4%
- BRAF mutation
- ROS1 rearrangement
- Others or unknown

Adenocarcinoma

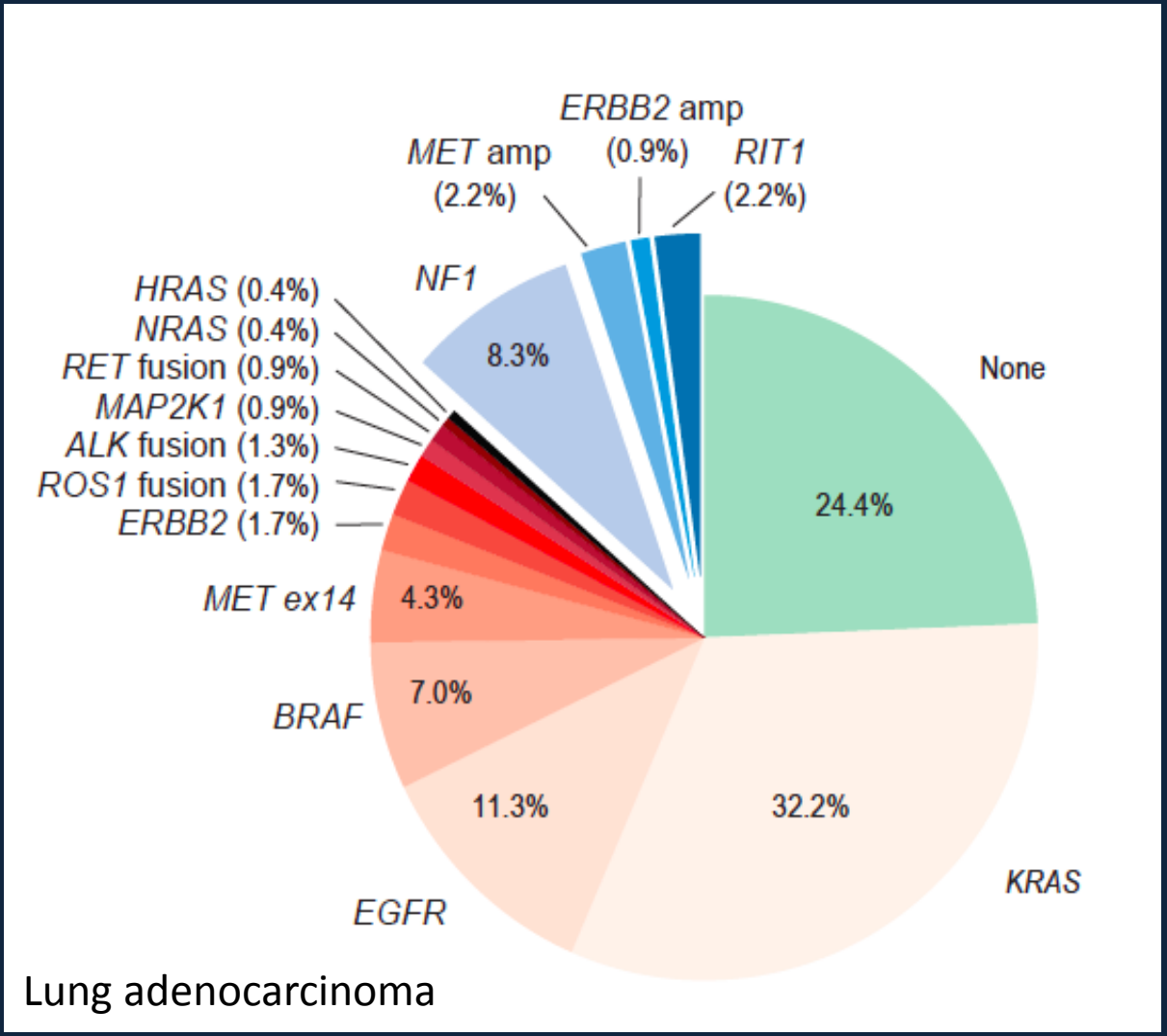


- FGFR1 amplification 21%
- PTEN mutation 10%
- PIK3CA mutation 5%–9%
- PDGFRA amplification 5%
- MET amplification
- DDR2 mutation 4%
- Others or unknown

Squamous NSCLC

1. Heist RS, Engelman JA. Cancer Cell 2012;21;448.e2; 2. Herbst RS, et al. N Engl J Med 2008;359:1367–1380; 3. Sholl LM, et al. J Thorac Oncol 2013;8:322–328; 4. Pao W, Girard N. Lancet Oncol 2011;12:175–180; 5. Paik PK, et al. J Clin Oncol 2011;29:2046–2051; 6. Dutt A, et al. PLoS One 2011;6:e20351; 7. Jin G, et al. Lung Cancer 2010;69:279–283; 8. Heist RS, et al. J Thorac Oncol 2012;7:924–933; 9. Spoerke JM, et al. Clin Cancer Res 2012;18:6771–6783; 10. Hammerman PS, et al. Cancer Discov 2011;1:78–89.

An evolving genetic map

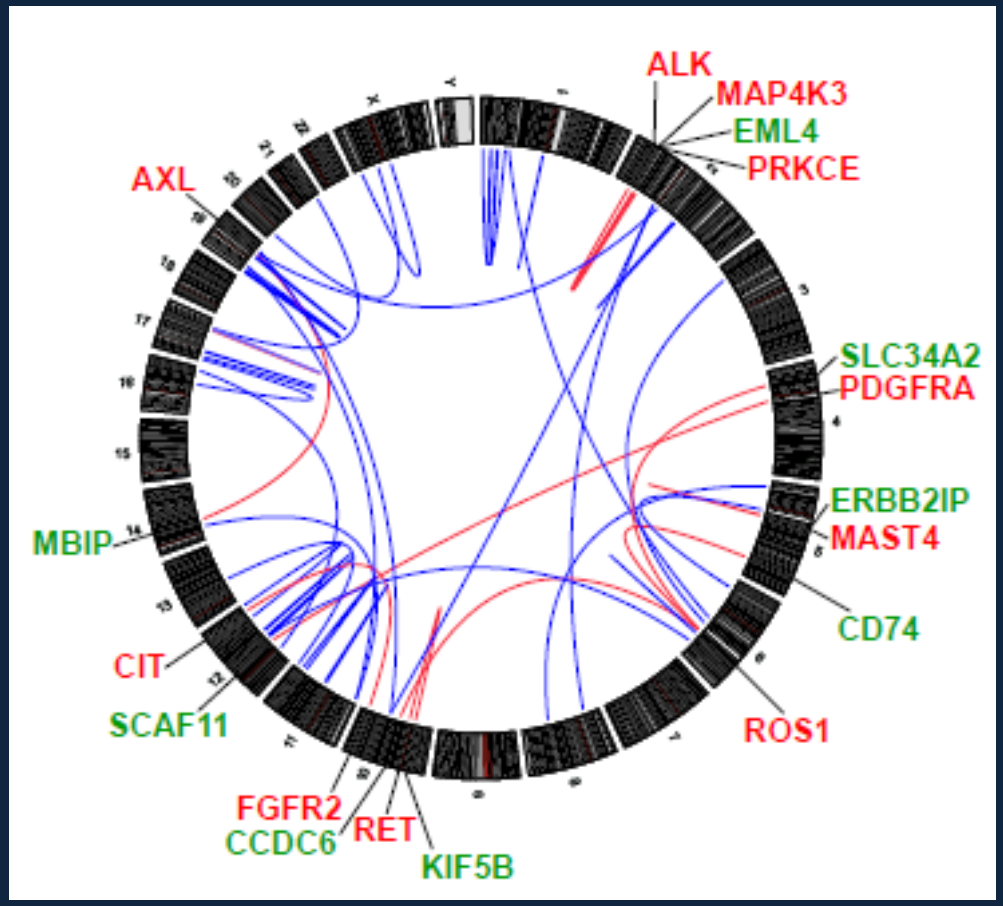


Lung adenocarcinoma

TCGA Genome Atlas, Nature 2014

The picture is possibly even more complex

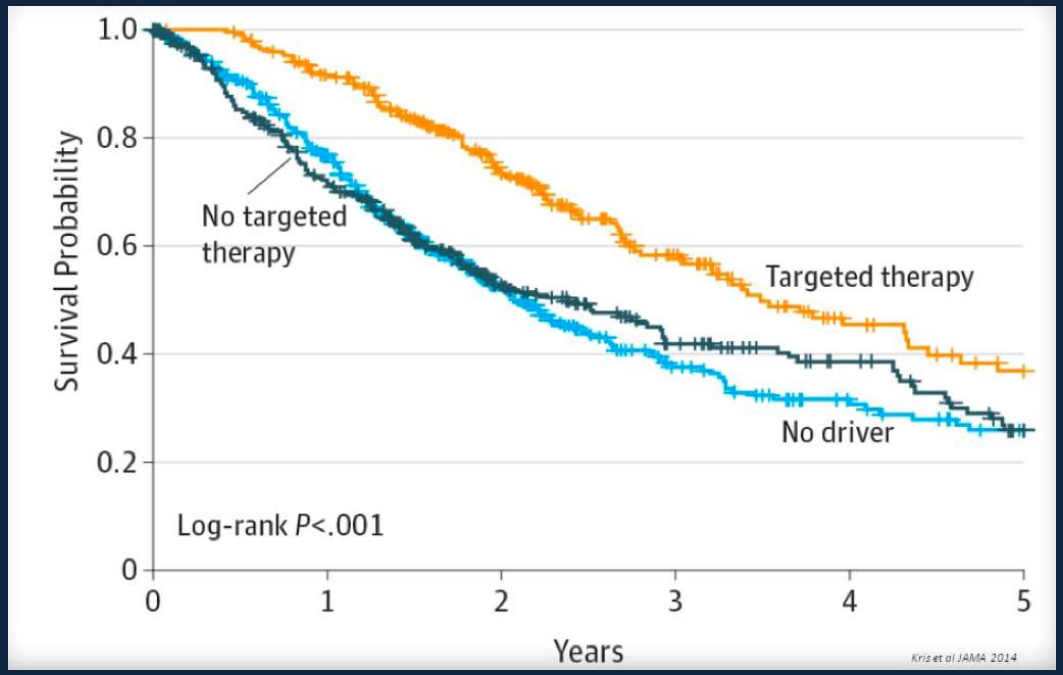
Graphical representation of 45 fusion genes from 87 adenocarcinomas



Seo, Genome Res 2012

Potential impact of personalized medicine

Only two alterations (EGFR/ALK) have been validated prospectively to date.

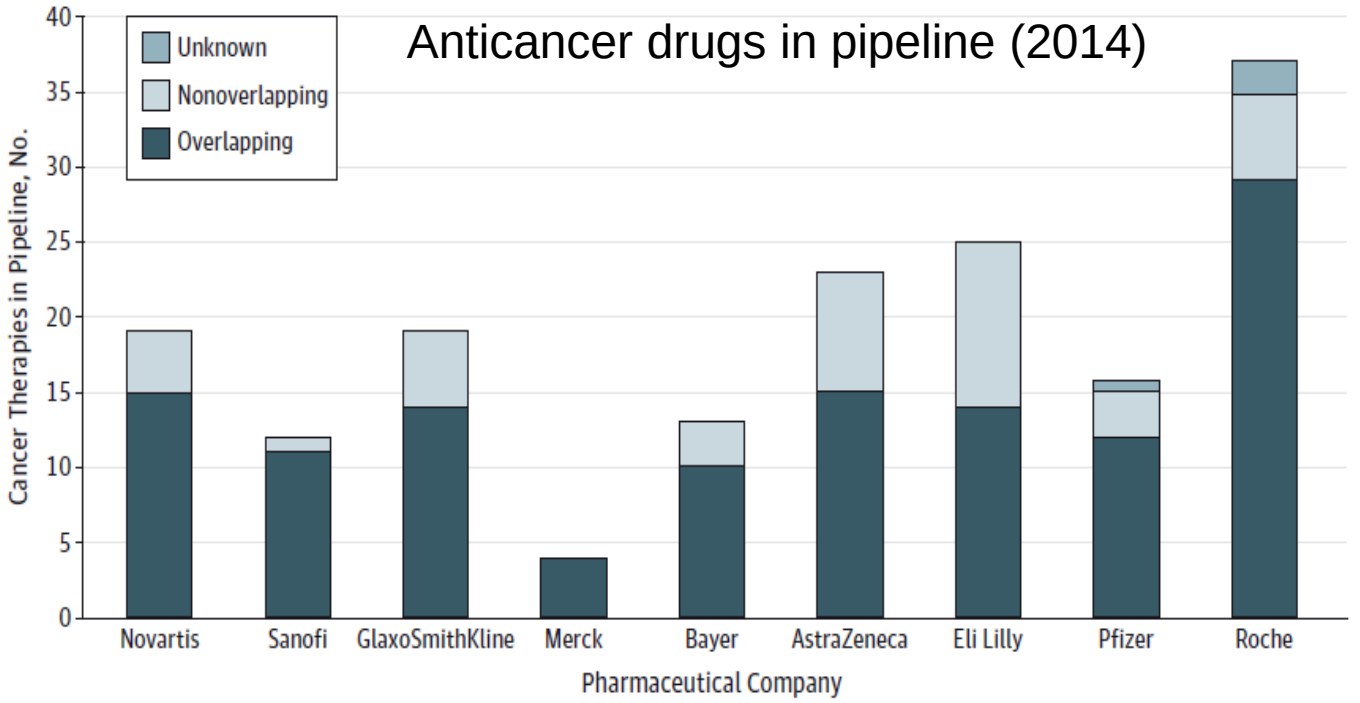


The opportunity of applying systemic molecular-based targeted approaches for other driver alterations is currently under evaluation

Reck and Peters, ESMO guidelines 2012 and 2014; Kris JAMA 2014

Key message 1: The number of actionable targets in evaluation remains limited

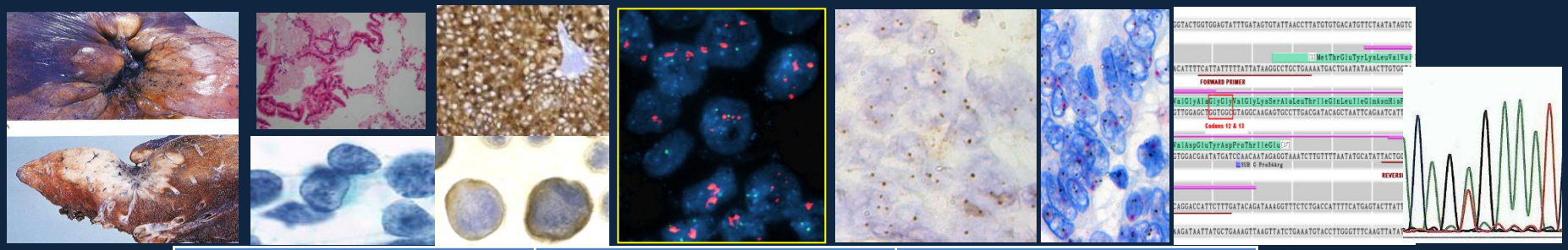
Comparison of Cancer Therapies in the Pipelines of Pharmaceutical Companies According to Their Putative Mechanisms of Action



Fojo, JAMA OtoHN 2014

Key message 2: An enormous effort in diagnostic tools development

MACRO | HISTO/ CYTO | IHC/ ICC | IN SITU HYBRIDIZATIONS | PCR



TEST	DETECTS	MISSES
IHC	PROTEIN EXPRESSION	ANY UNKNOWN ALTERATION
FISH	COPY NO. ALTERATIONS, REARRANGEMENTS	INDELS
HOT SPOT PANELS	SUBSTITUTIONS	CNVS, REARRANGEMENTS

Key message 2: An enormous effort in diagnostic tools development : NGS for all?

Goals

- High throughput, cost effective multiplexed sequencing assay with **deep coverage**
- Target clinically actionable regions in clinically relevant time

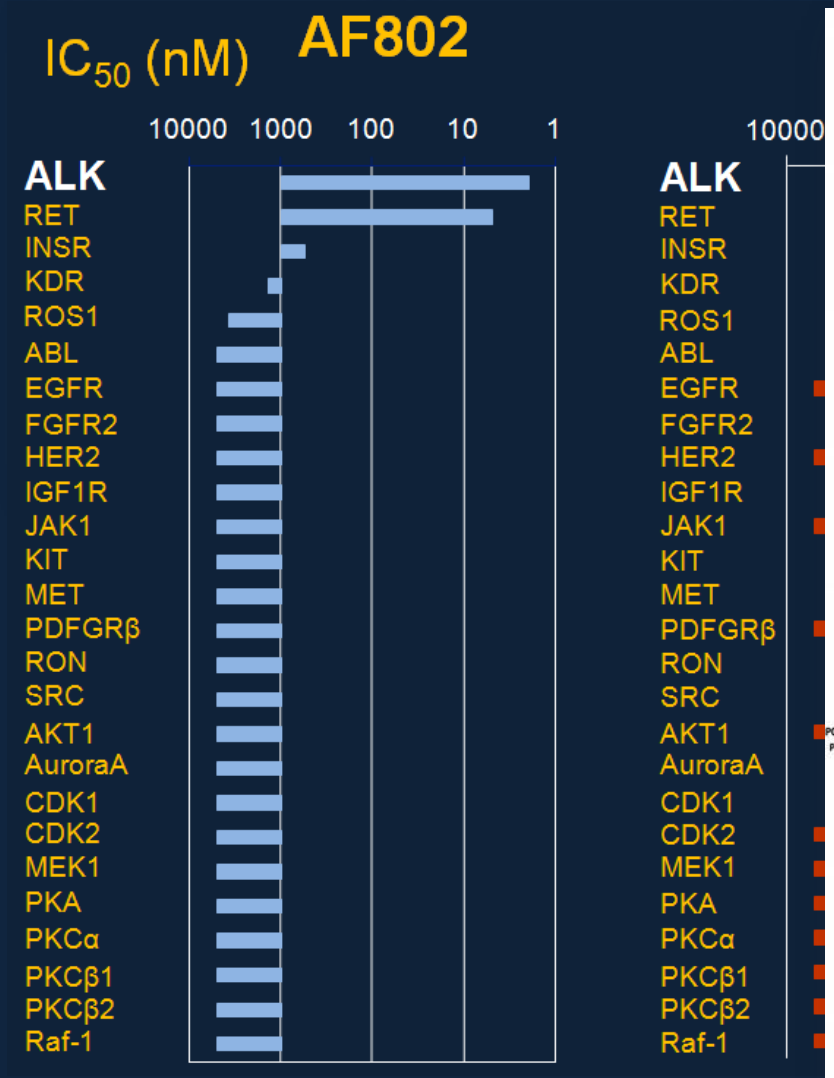
Challenges

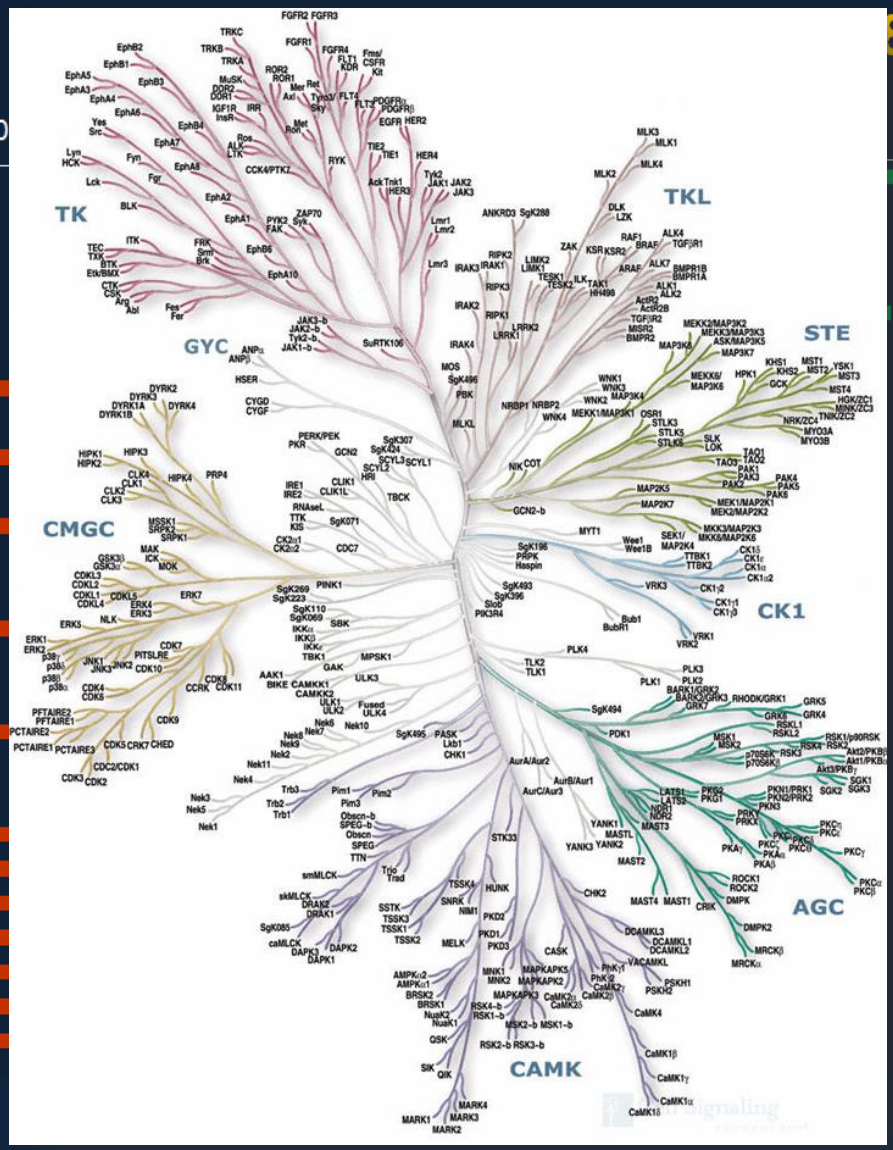
- Huge infrastructure costs
- Bioinformatic barriers
- Rearrangement/amplification assays validation

Solution

- Leverage expertise and resources across Pathology, Bioinformatics and Genetics

Key message 3: Making the best use of off–target activites of available TKIs



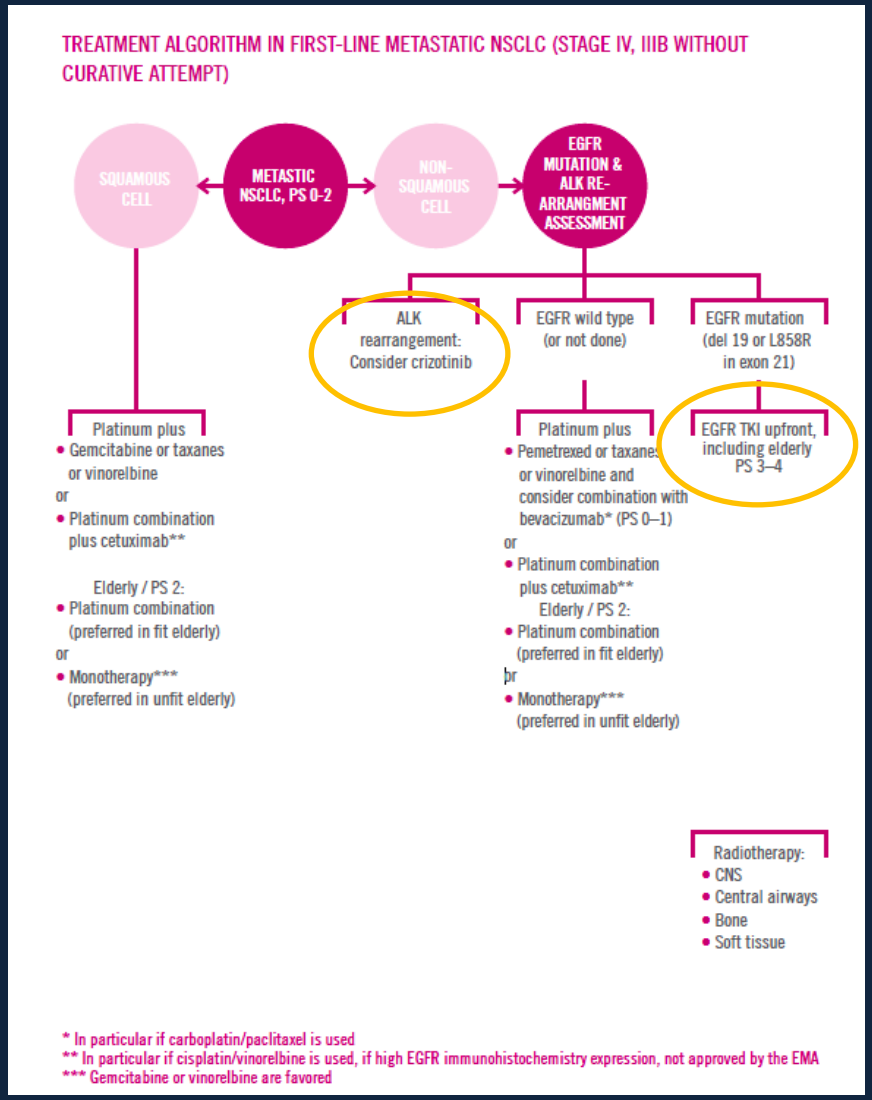


Karaman, Nature Biotech. 2008

Evidence-based strategy

UPFRONT TARGETED TREATMENT

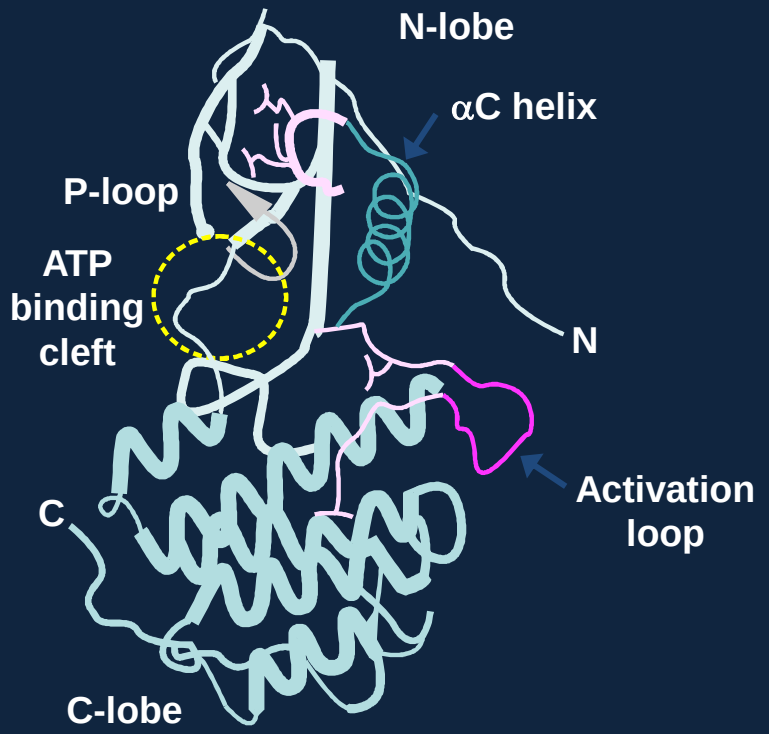
First line EGFR/ALK TKI? ESMO guidelines

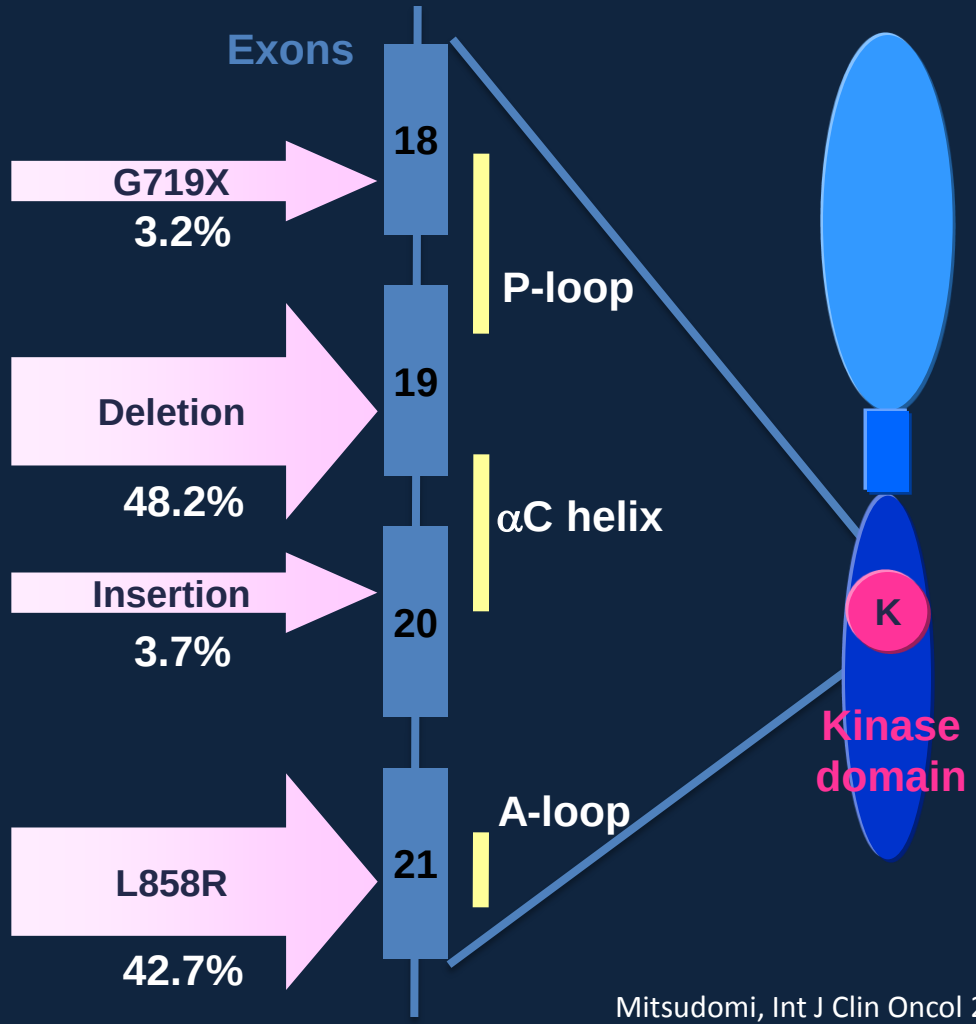


- EGFR mutation and ALK fusion gene testing is recommended (preferably in parallel) in all patients with advanced non-squamous NSCLC
- It is not recommended in those with a confident diagnosis of squamous cell carcinoma, except in never/former light smokers (<15 packs per year)

Peters, Ann Oncol 2013
Reck, Ann Oncol 2014

EGFR mutations are located predominantly on exons 19 and 21





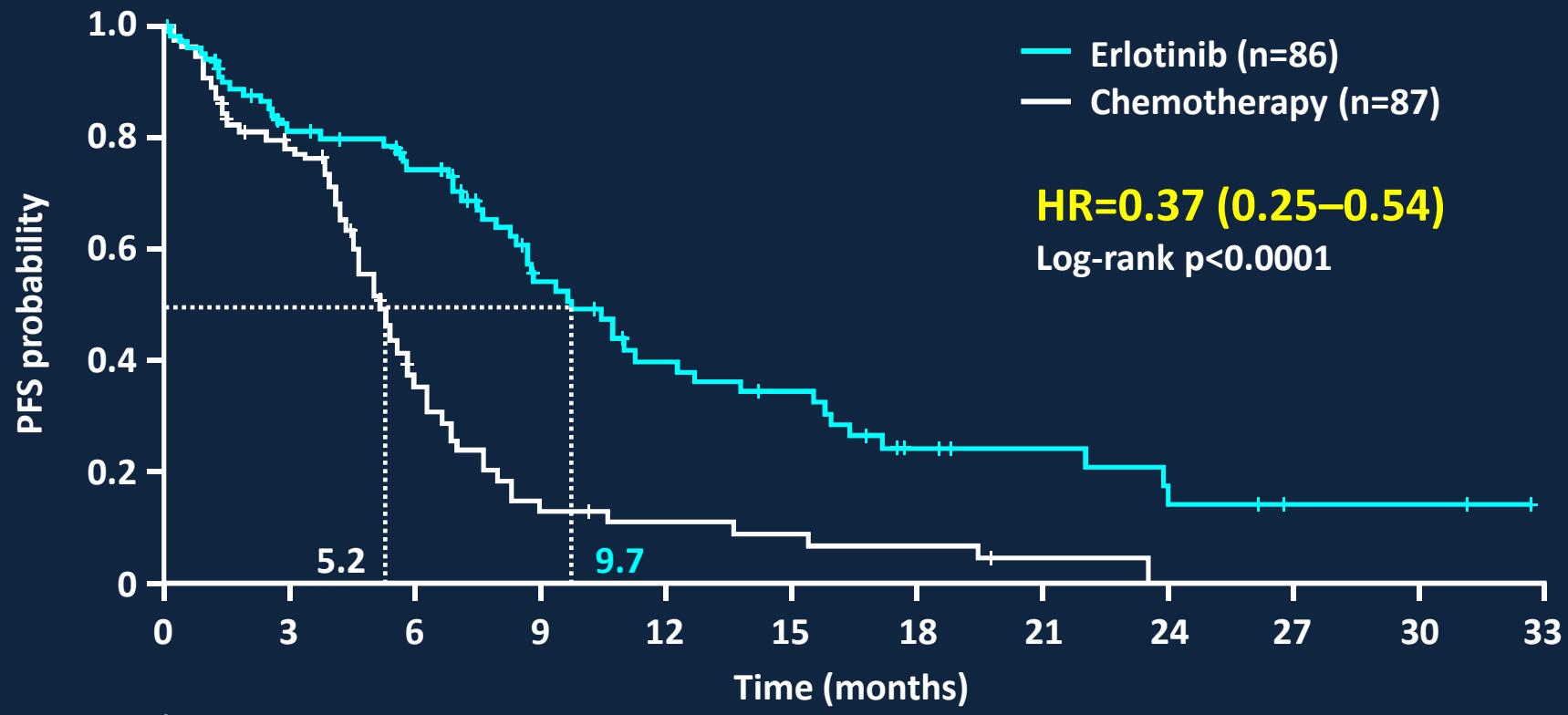
Approximately 10-15% of all NSCLC Patients have EGFR mutated disease

Mitsudomi, Int J Clin Oncol 2006
Rosell, NEJM 2009
Riely, CCR 2006

Frontline EGFR TKIs versus Chemotherapy PFS

Study	EGFR TKI	n	Median PFS in TKI arm (months)	P value	HR
OPTIMAL	Erlotinib	154	13.1	<0.0001	0.16
First Signal	Gefitinib	42	8.4	0.084	0.61
IPASS	Gefitinib	261	9.5	<0.0001	0.48
WJTOG 3405	Gefitinib	177	9.2	<0.001	0.48
NEJSG 002	Gefitinib	200	10.8	<0.001	0.36
Ensure	Erlotinib	217	11	<0.0001	0.34
EURTAC	Erlotinib	174	9.4	<0.0001	0.42
LUX-3	Afatinib	308	13.6	<0.0001	0.47
LUX-6	Afatinib	364	11.0	<0.0001	0.28

EURTAC: first-line erlotinib versus chemotherapy in Europe

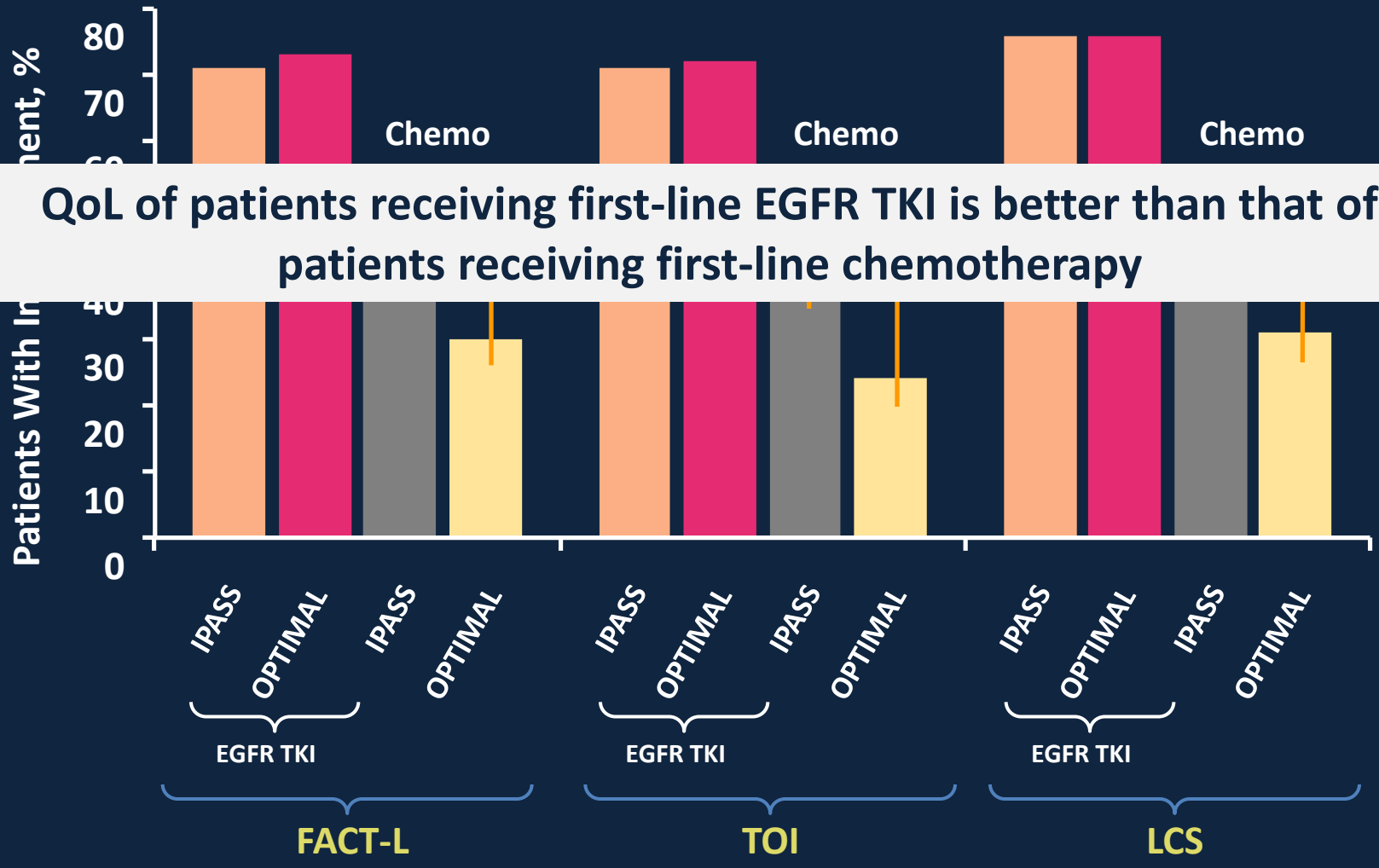


Patients at risk

Erlotinib	86	63	54	32	21	17	9	7	4	2	2	0
Chemo	87	49	20	8	5	4	3	1	0	0	0	0

Rosell, Lancet Oncol 2012

Better QoL With First-Line EGFR/ALK TKIs



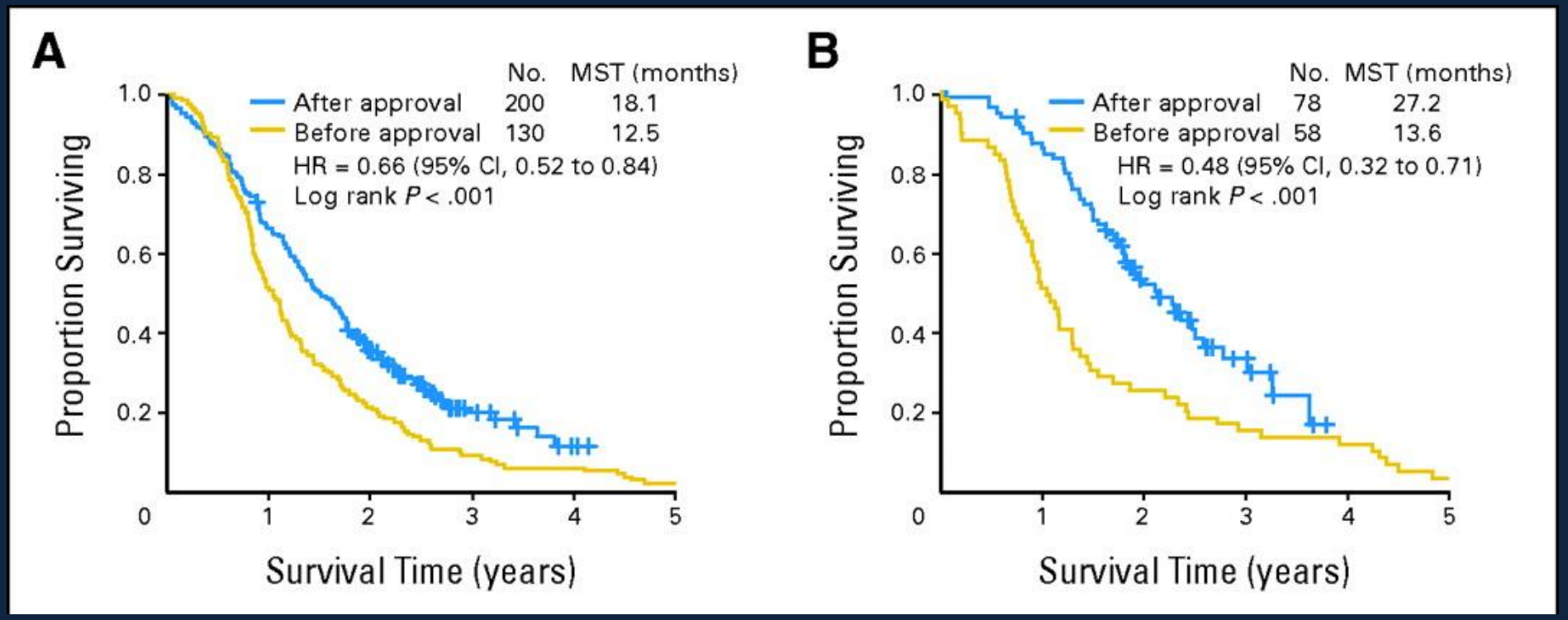
QoL of patients receiving first-line EGFR TKI is better than that of patients receiving first-line chemotherapy

Mok, N Engl J Med. 2009; Zhou C, ASCO 2011; Solomon NEJM 2014

Comparison of survival for patients with lung adenocarcinoma in Japan before and after gefitinib approval

All patients

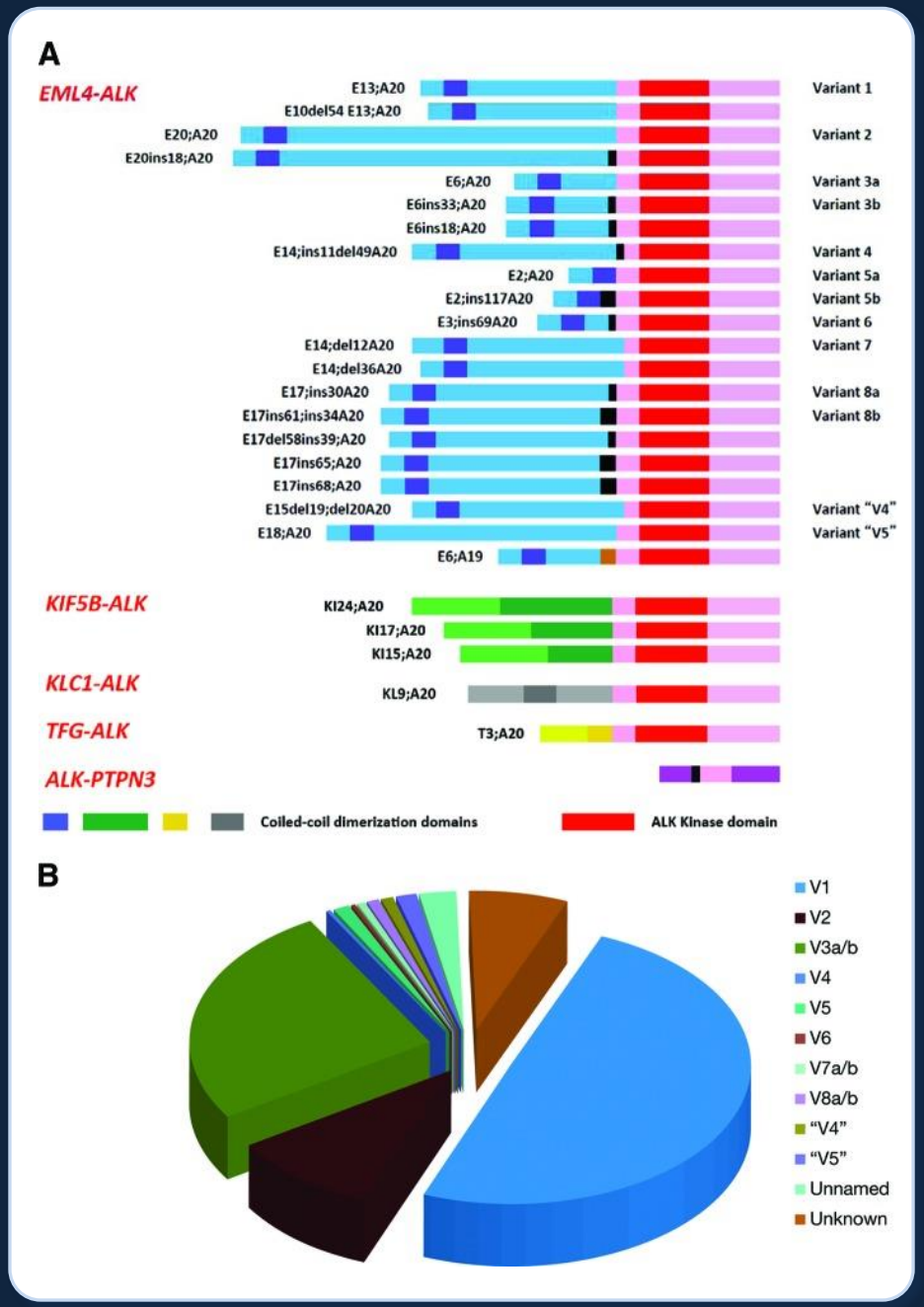
EGFR mut+ patients



Takano, JCO 2008

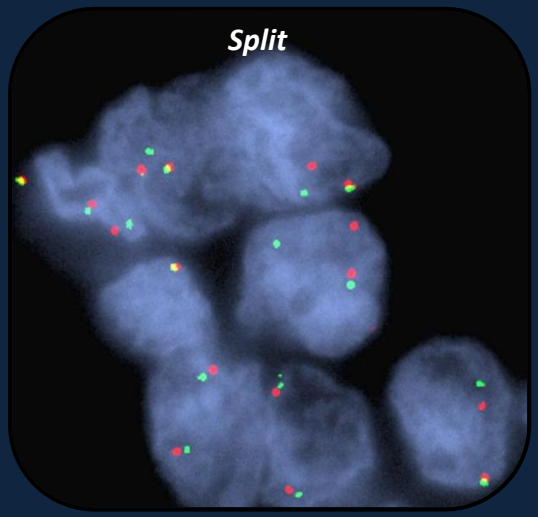
ALK Rearrangements

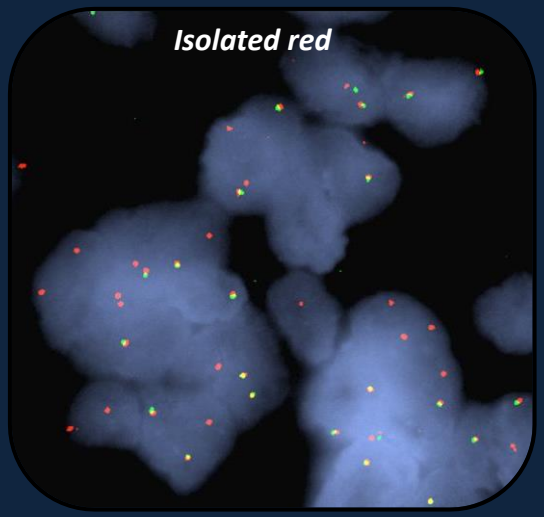
- First discovered in Anaplastic Large Cell Lymphoma (ALCL), in NSCLC in 2007
- At least 28 different ALK gene rearrangement variants have been described
- Rearrangement leads to kinase expression, activation and oncogene addiction in 1-7% of NSCLC



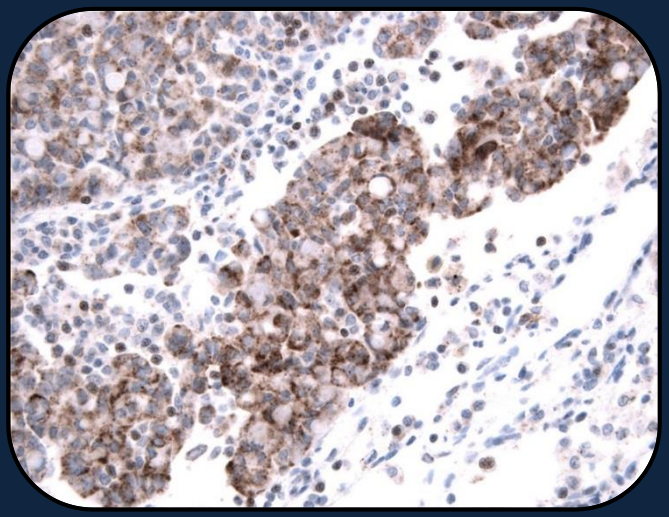
Peters, Lung Cancer 2013;
Kwak, N Engl J Med. 2010

Diagnosing ALK rearrangement





ALK break-apart FISH assay

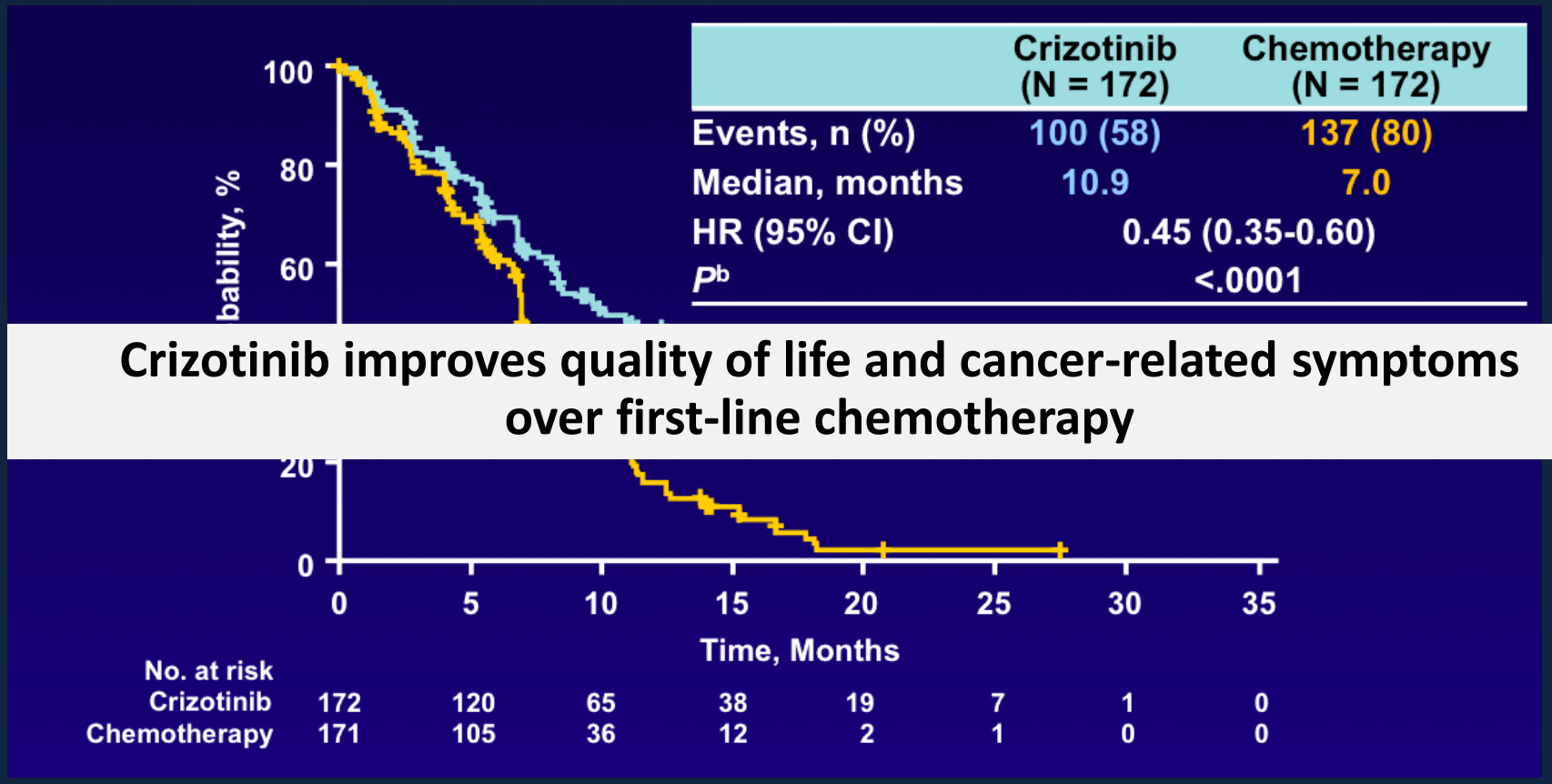


ALK immunohistochemistry

FISH, fluorescence *in-situ* hybridization

Crizotinib Superior to 1L Pemetrexed-Based Chemotherapy in Prolonging PFS

	Crizotinib (N=172)	Chemo[a] (N=171)
ORR, % (n)	74 (128)	45 (77)

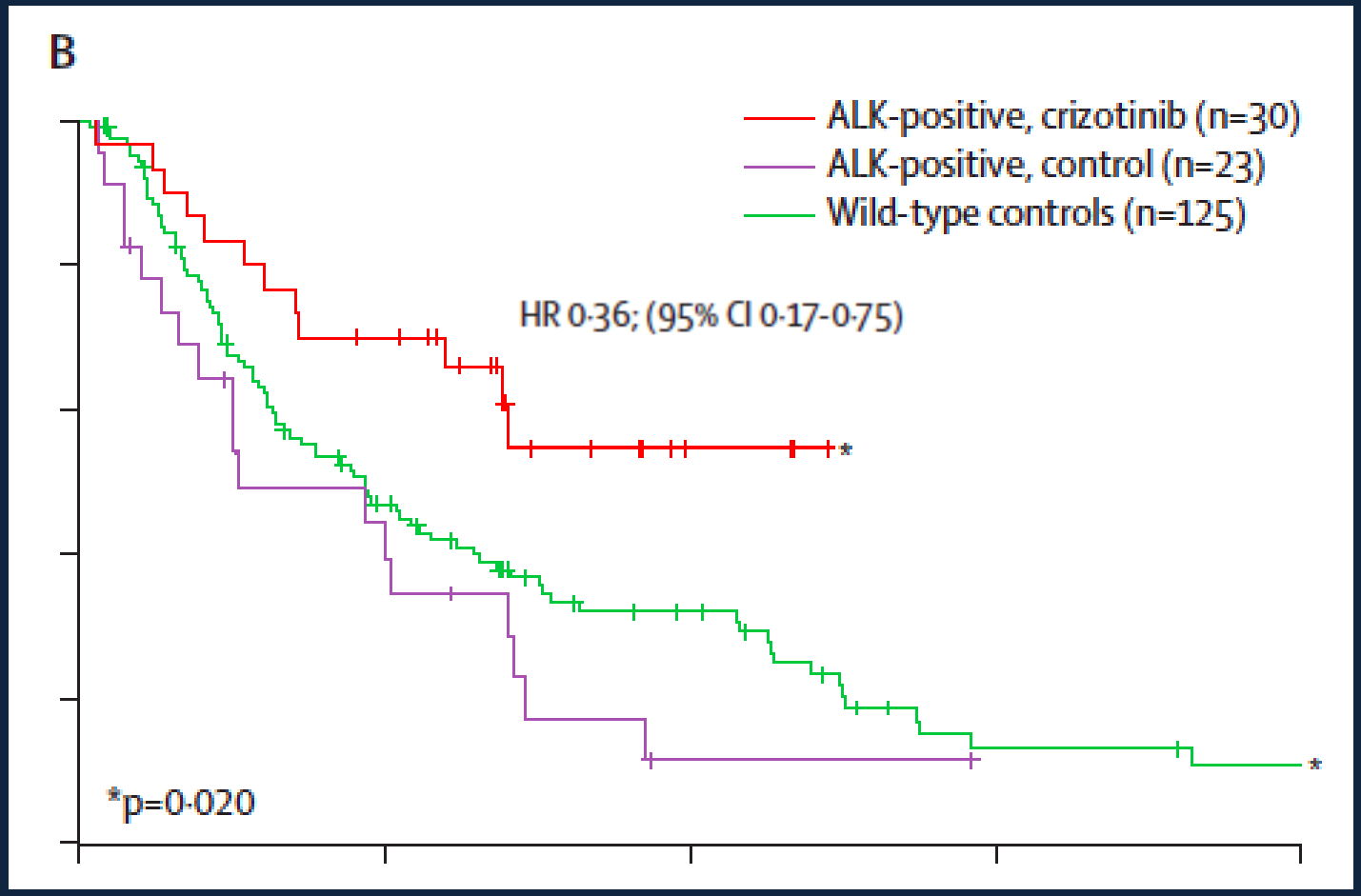


	Crizotinib (N = 172)	Chemotherapy (N = 172)
Events, n (%)	100 (58)	137 (80)
Median, months	10.9	7.0
HR (95% CI)	0.45 (0.35-0.60)	
P[b]	<.0001	

Crizotinib improves quality of life and cancer-related symptoms over first-line chemotherapy

Solomon, NEJM 2014

Comparison of survival for patients with lung adenocarcinoma in second line before and after crizotinib approval

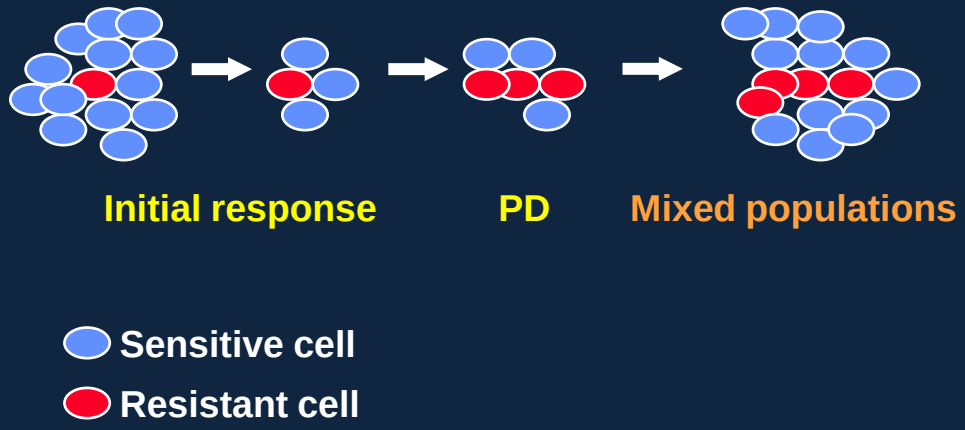


Shaw, Lancet Oncol 2011

Evidence-based strategy

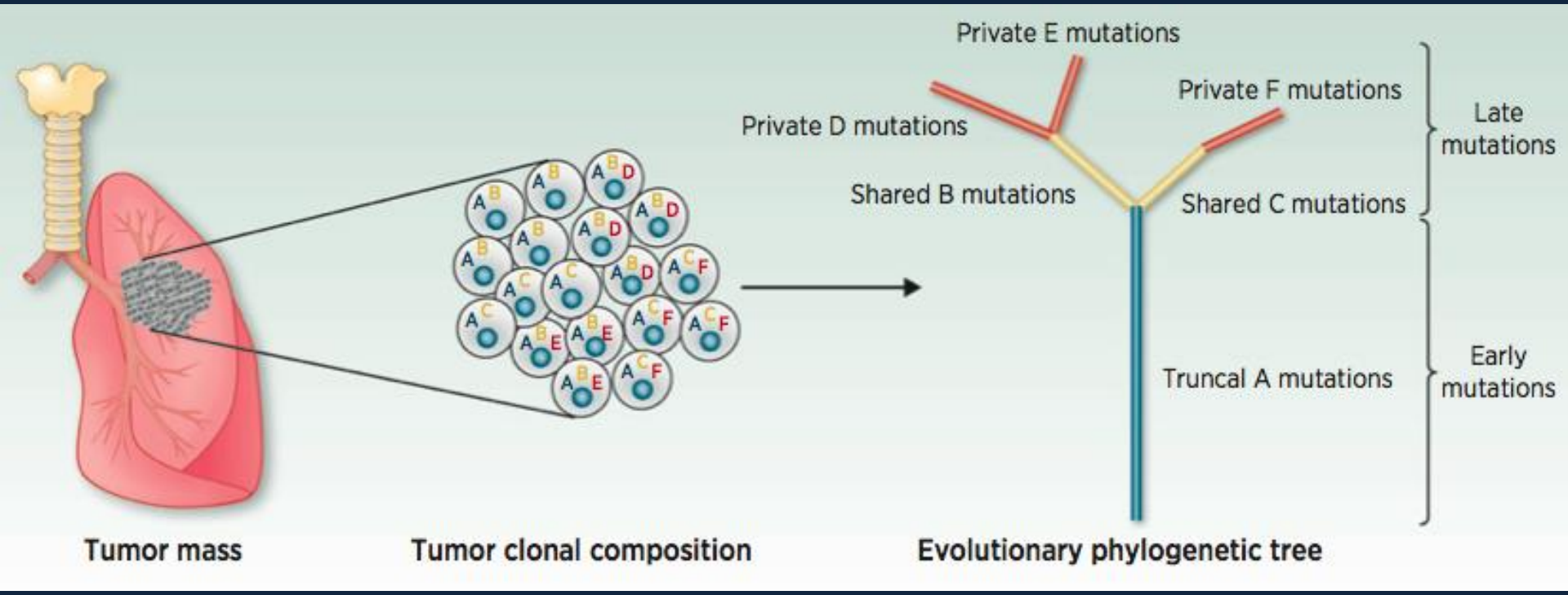
RESISTANCE

Dynamics of systemic resistance evolution
The probable scenario



- Clones heterogeneity pre-exists and continually increases, through ongoing error-prone DNA replication and selective pressure
- Balance is dictated by selective pressure and fitness of resistant cells

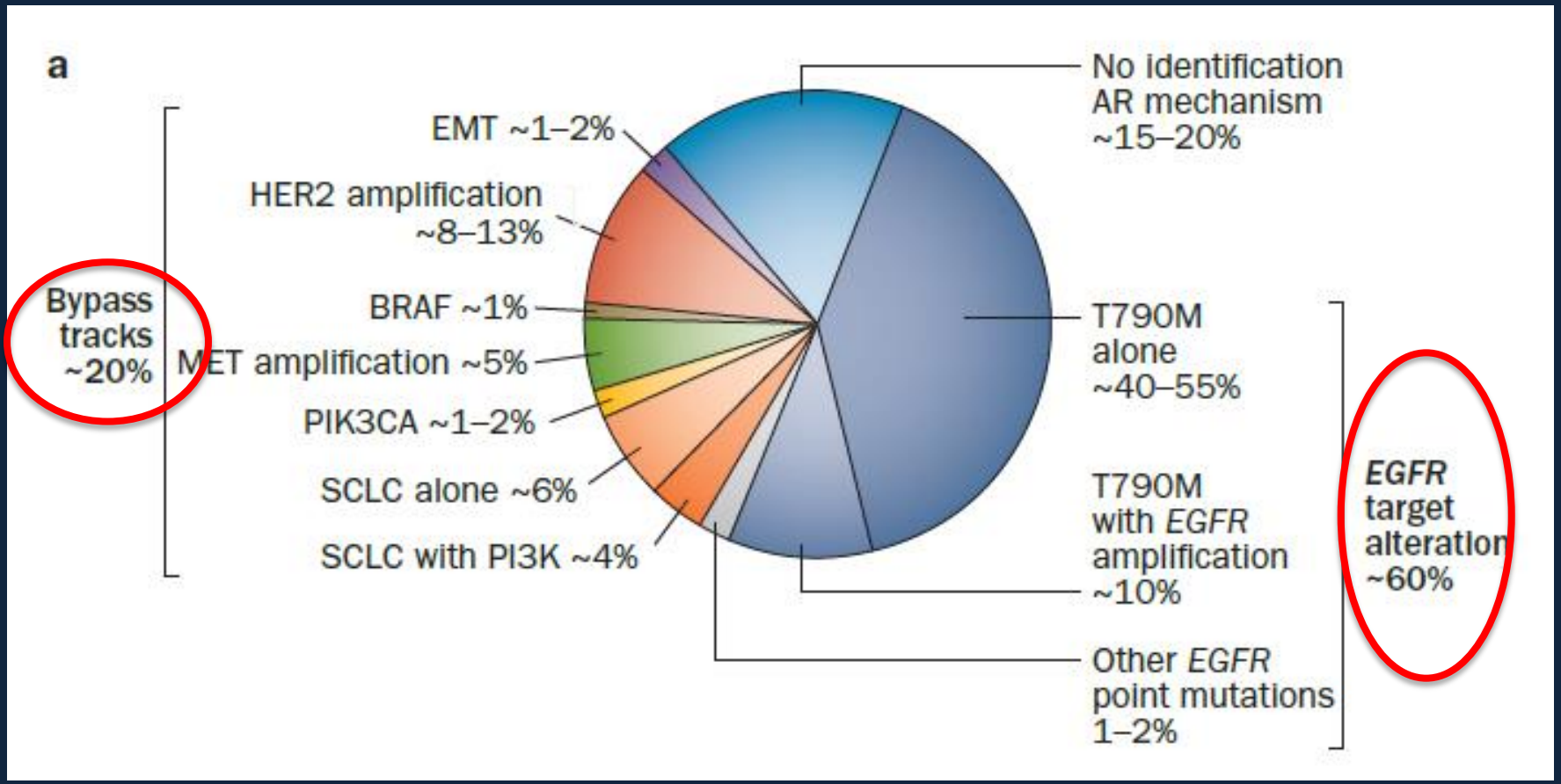
Can we prevent resistance emergence using 3rd generation TKIs ? Tumour heterogeneity



Heterogeneity is increasing over time and minor clones are continuously subjects to Darwinian selection

Jamal-Hadjani, CCR 2015

T790M mutation is present in the majority of cases at clinical progression

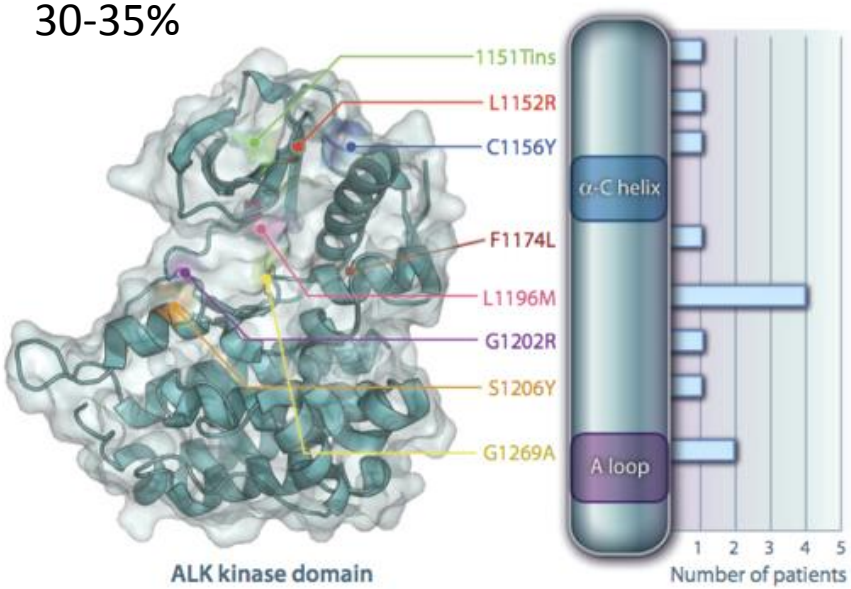


Camidge Nat Rev Clin Oncol 2014

Using a second generation ALK TKI? Resistance mechanisms

ALK dominant

30-35%



Non-ALK dominant

Alternative signaling pathways (Bypass)

- HER2 activation[7]
- EGFR activation[5]
- KIT amplification (SCF)[5]

Alternative oncogenes (Clonal)

- KRAS mutation[6]
- EGFR mutation[6]

1. Camidge DR, et al. *Lancet Oncol.* 2012;13:1011–1019; 2. Kim D, et al. ESMO 2012: Abstr 1230PD; 3. Shaw AT, et al. ESMO 2012: Abstr LBA1_PR; 4. Takeda M, et al. *J Thorac Oncol.* 2013;8(5):654-7; 5. Katayama R, et al. *Sci Transl Med.* 2012;4(120):120ra17; 6. Doebele RC, et al. *Clin Cancer Res.* 2012;18:1472–1482; 7. Tanizaki J, et al. *Clin Cancer Res.* 2012;18(22):6219-26; 8. Lovly & Pao. *Sci Transl Med.* 2012;4(120):120ps

Using a second generation ALK TKI?

Inhibitor	Targets	Development stage	Recent reports
Ceritinib	ALK/ROS	FDA approved Phase III/CUP	Shaw, NEJM 2014 Kim, ASCO 2014

All characterized by :

- A stronger ALK affinity
- Binding to several secondarily mutated ALK proteins
- Improved brain penetration

X-396	ALK/ROS	Phase I/II	Horn, ASCO 2014
RXDX-101	ALK/ROS/TRK	Phase I/II	De Braud, ASCO 2014
PF-06463922	ALK/ROS/TRK	Phase II	Johnson, J Med Chem 2014
CEP-37440	ALK/FAK	Phase I/II	-

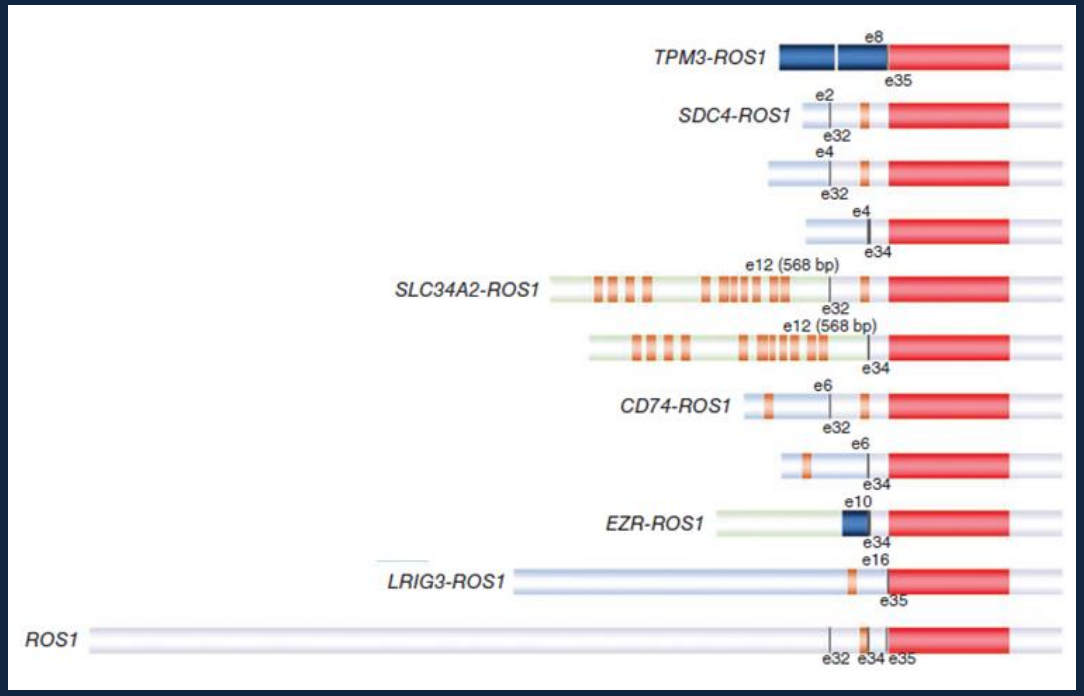
Adapted from Award MM, Shaw A. *Clin Adv Hematol Oncol.* 2014;12(7):429-439

Evidence-based strategy

BEYOND EGFR AND ALK

Targeting ROS1

ROS1: Receptor tyrosine kinase of the insulin receptor family, little known about its specific function
ROS1 fusion with the transmembrane solute carrier protein SLC34A2 results in a constitutive kinase activity in a NSCLC cell line



Rikova, Cell 2007; Takeuchi, Nat Med 2011; Bergethon JCO 2012

ROS1 FISH+ : Clinical Features and Outcomes

25% to 50% of pulmonary adenocarcinoma are *EGFR-/KRAS-/ALK-*

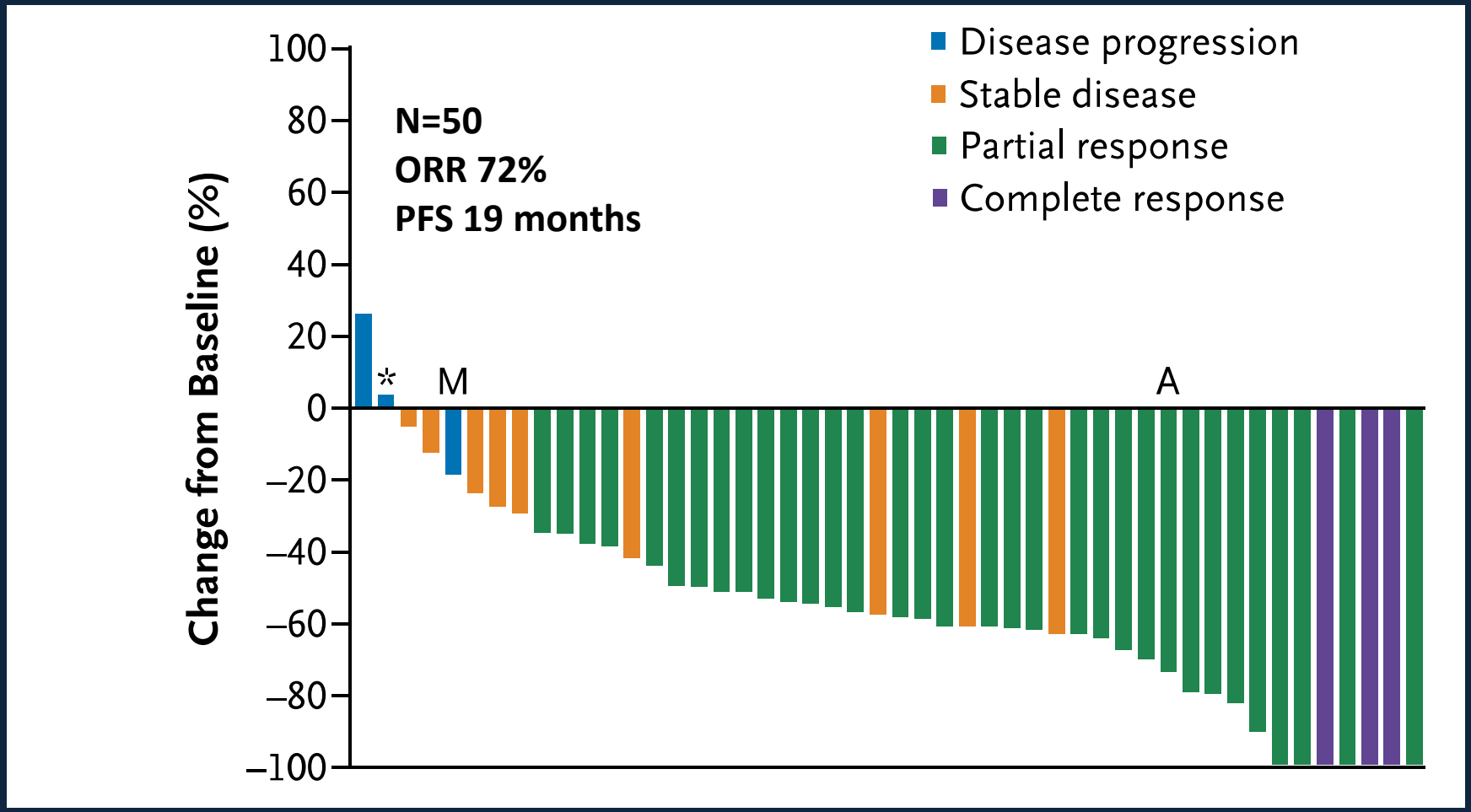
→ *Incidence of ROS1 7.4% -12.1%*

[1] Mescam-Mancinia Lung Cancer 2014
[2] Go J Thor Oncol 2013

Smoking (%)	**11** **6 (light)**	**50**	**NA**	**38**	**NA**	**38**
Women (%)	**61**	**55**	**NA**	**63**	**66**	**62**
Predom. Histology	**acinar**	**acinar**	**NA**	**microp., solid**	**lepidic, acinar**	**NA**

[1] Bergethon J Clin Oncol 2012 [2] Chen J Thor Oncol 2014 [3] Rimkunas Clin Cancer Res 2012 [4]Mescam-Mancinia Lung Cancer 2014 [4] Go J Thor Oncol 2013 [5] Warth Histopathology 2014 [6]Takeuchi Nat Med 2012

Crizotinib in ROS1+ NSCLC



Shaw, NEJM 2015

Targeting HER2

Lung cancer

Intragenic ERBB2 kinase mutations in tumours

Table 1 ERBB2 mutations in primary tumours

Sample	*Tumour/histology*	*Nucleotide**	*Amino acid**
PD1353a	NSCLC adenocarcinoma	2322 ins/dup(GCATACGTGATG)	ins774(AYVM)
PD0258a	NSCLC adenocarcinoma	2322 ins/dup(GCATACGTGATG)	ins774(AYVM)
PD0317a	NSCLC adenocarcinoma	2322 ins/dup(GCATACGTGATG)	ins774(AYVM)
PD0319a	NSCLC adenocarcinoma	2335 ins(CTGTGGGCT)	ins779(VGS)
PD0270a	NSCLC adenocarcinoma	TT2263-4CC	L755P

- 120 primary NSCLC, 4.2% with mutations in HER2 kinase domain, 9.8% (5/51) in adenocarcinomas

Stephens, NATURE | VOL431 | 30 SEPTEMBER 2004 | www.nature.com/nature

Epidemiology HER2 mutation

- 403 stage I-III adenocarcinomas in caucasian : 2.2%
- 78% in frame duplications/ insertions in exon 20
- Frequency higher in females and in never smokers

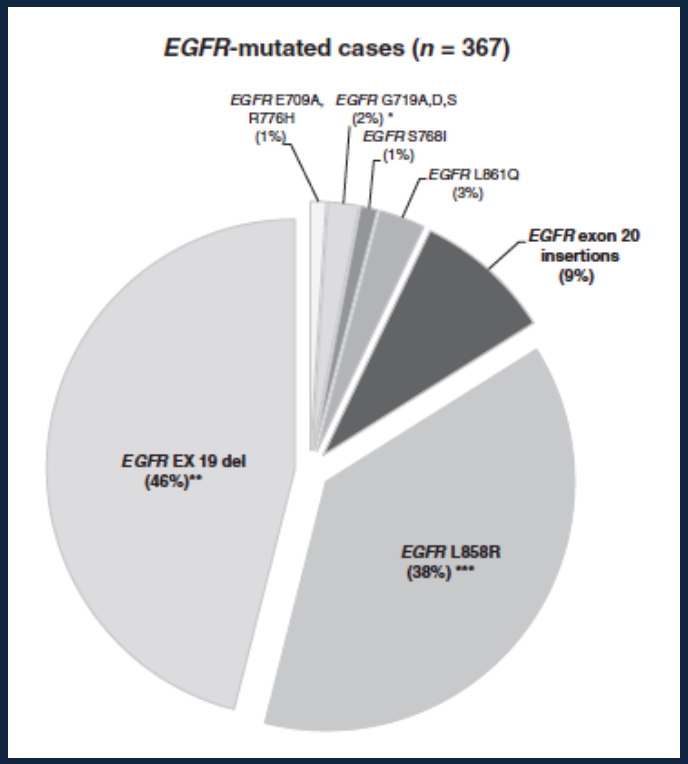
- 394 adenocarcinoma, HER2 mutations preferentially in oriental ethnicity: 3.9% vs 0.7%
- All insertions in exon 20, more frequent in never smokers and adenocarcinoma

- 6% of EGFR/KRAS/ALK-negative specimens
- More frequent among never-smokers
- **HER2 mutation was not associated with concurrent HER2 amplification**

- HER2 mutations in 13 of 504 japanese patients (2.6%) undergoing surgery for NSCLC. No difference in the overall survival

Buttitta, Int J Cancer 2006, Shigematsu, CCR 2005, Arcila CCR 2012; Tomizawa, Lung Ca

Similarity with EGFR exon 20 insertions



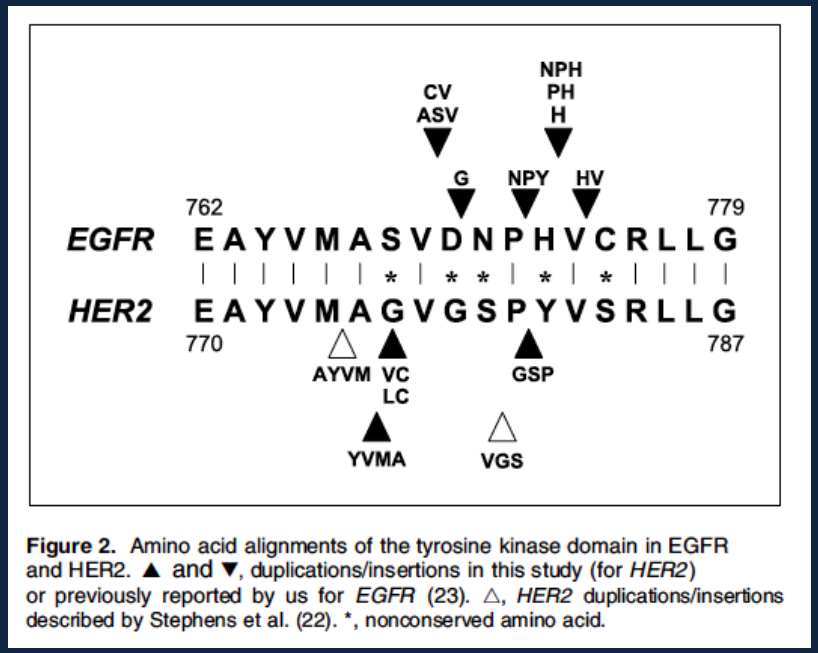


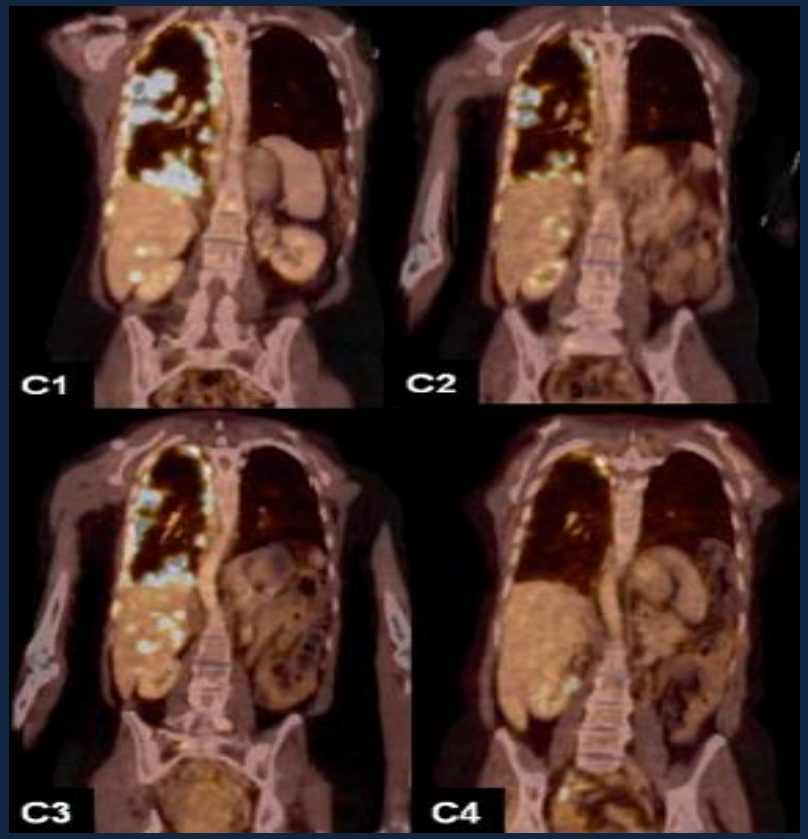
Figure 2. Amino acid alignments of the tyrosine kinase domain in EGFR and HER2. ▲ and ▼, duplications/insertions in this study (for *HER2*) or previously reported by us for *EGFR* (23). △, *HER2* duplications/insertions described by Stephens et al. (22). *, nonconserved amino acid.

- Causes a shift in the helical axis that narrows the ATP binding cleft, resulting in both increased TK activity and TK inhibitor sensitivity

Shigematsu, CCR 2005; Arcila, Mol Cancer Ther 2013

HER2 mutation-dedicated clinical trial Afatinib

Clinical activity of afatinib (BIBW 2992) in adenocarcinoma patients with mutations in the kinase domain of HER2



Five patients with metastatic HER2 mutated adenocarcinomas were identified.

PR in 3/3, even after failure of other EGFR- and/or HER2-targeted treatments

ASCO 2013: 5 additional evaluable all with SD.

« DCR: 80-100% »

De Greve, Lung Cancer 2012 and ASCO 2013

Our French/European experience

- *HER2* mutation was identified in 65 patients out of 3800 patients (1.7%)

22 anti-HER2 treatments were administered after conventional chemotherapy in 16 patients:

- Overall response rate ORR 50%
- Disease control rate DCR 82%
  - DCR of 93% for trastuzumab-based (n = 15)
  - 100 % for afatinib (n = 3)
  - 0% to other HER2-targeted drugs (n = 3)

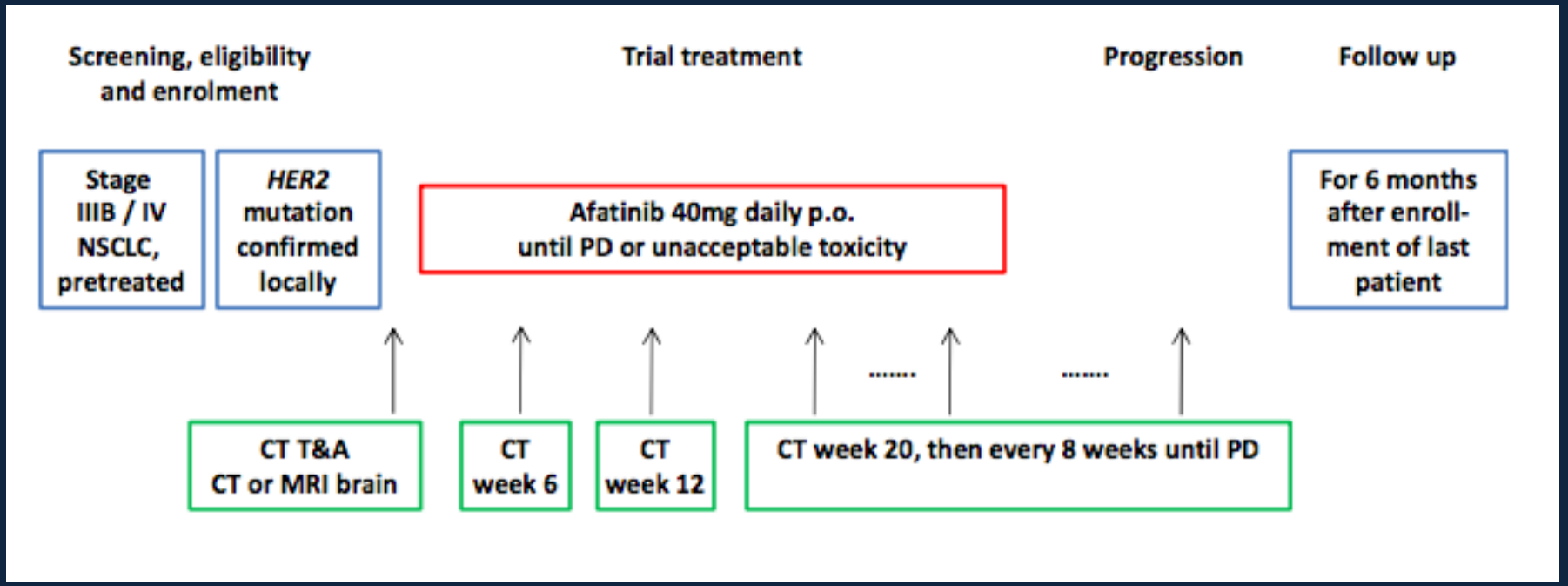
Progression free survival for patients with HER2-therapies was 5.1 mos

	First-Line Treatment	
Patient	Treatment	Best Disease Response
11	VIN-HER	PR
15	CAR-PAC-TRAS	SD
19	TXT-MASA	PD
24	VIN-TRAS	PR
26	CAR-PAC-TRAS	PR
27	VIN-TRAS	PR
28	VIN-TRAS	SD
30	LAP	PD
31	NVB-HER	PR
32	LAP	PD
37	VIN-TRAS	PD
41	DOC-TRAS	PR
43	VIN-TRAS	PR
44	VIN-TRAS	PR
45	VIN-TRAS	SD
47	TRAS	PR

Mazieres and Peters, JCO 2013

Afatinib in pretreated patients with advanced NSCLC harbouring HER2 exon 20 mutations

NICHE = afatinib in NSCLC with HER2 mutation



Sponsor: European Thoracic Oncology Platform (ETOP)

Co-chairs: Solange Peters, Lausanne, Switzerland

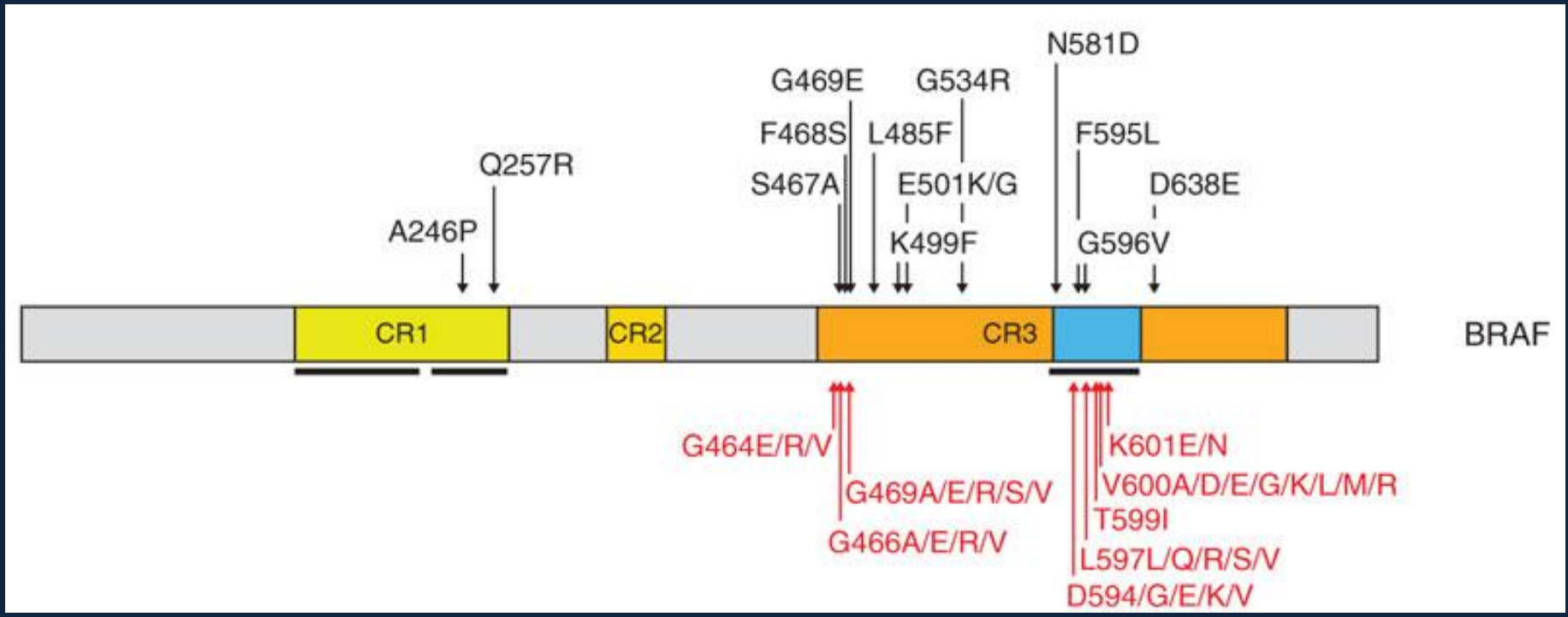
Rafal Dziadziuszko, Gdansk, Poland

BRAF Mutated Lung Cancer: Clinical Features and Outcomes

First author	Paik	Marchetti	Ilie	Cardarella	Luk	Litvak	Brustugun	Villaruz
Patients (n)	697	1046	450	883	273	63	979	951
BRAF (n)	18 (2.6%)	37 (3.5%)	40 (8.9%)	36 (4.1%)	7 (2.6%)	63 (NA)	17 (1.7%)	21 (2.2%)
V600E (%)	50	57	52	50	57	57	NA (100)	81
Smokers (%)								
- V600E	100	52	57	72	100	57	71	76
- Non-V600E	100	100	89	89	100	43	NA	100
Female (%)								
- V600E	78	76	52	56	75	53	59	53
- Non-V600E	44	7	26	50	33	56	NA	25
Survival outcome								
- BRAF mutant vs wt	same	same	NA	same	NA	same	NA	same
- V600E vs non-V600E	NA	worse	worse	same	NA	better	NA	NA

Nguyen and Peters, JTO 2015

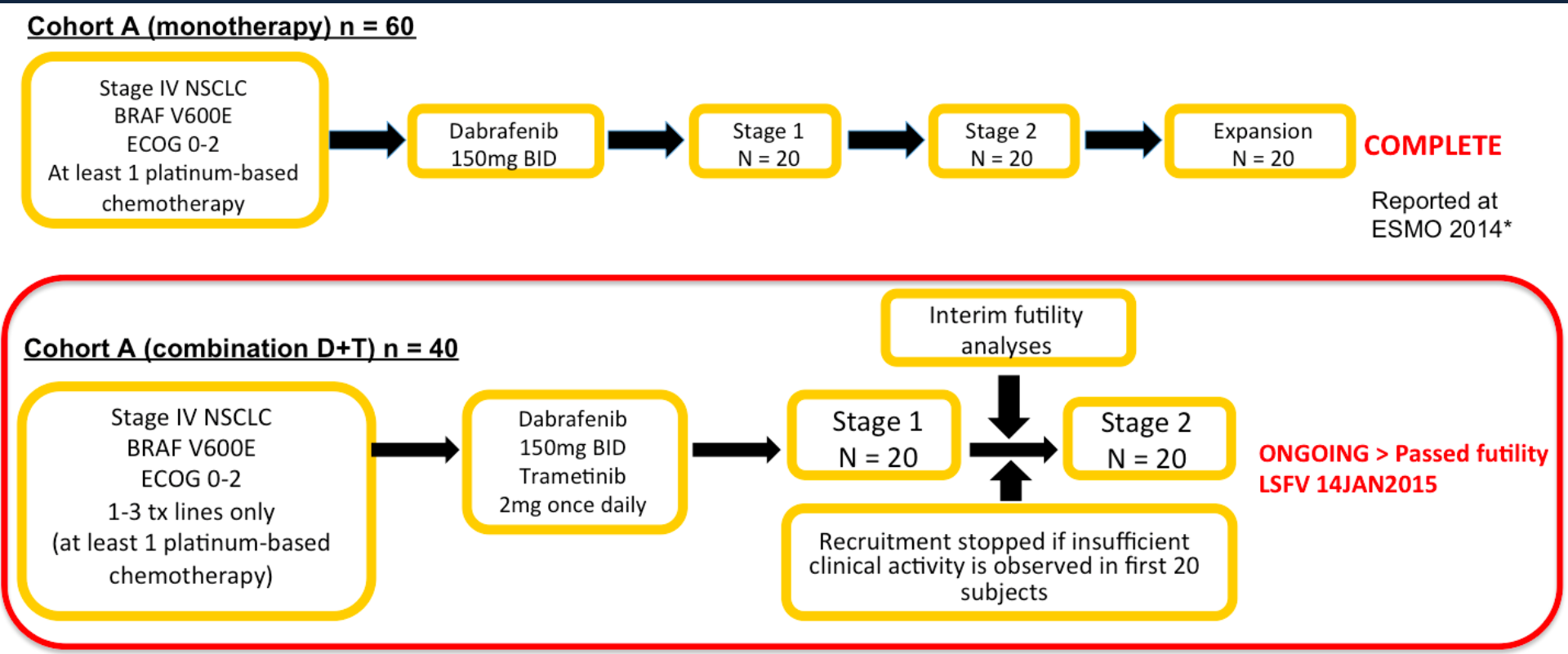
Non BRAF 6000E Mutated Lung Cancer



- Other BRAF mutations are seen in lung cancer and are thought to be involved in oncogenesis (some kinase inactivating)
- Studies typically focused on preselected V600E (exon 15) and some exon 11 codons.
- However, some studies did not separate V600E versus other mutations for associations.

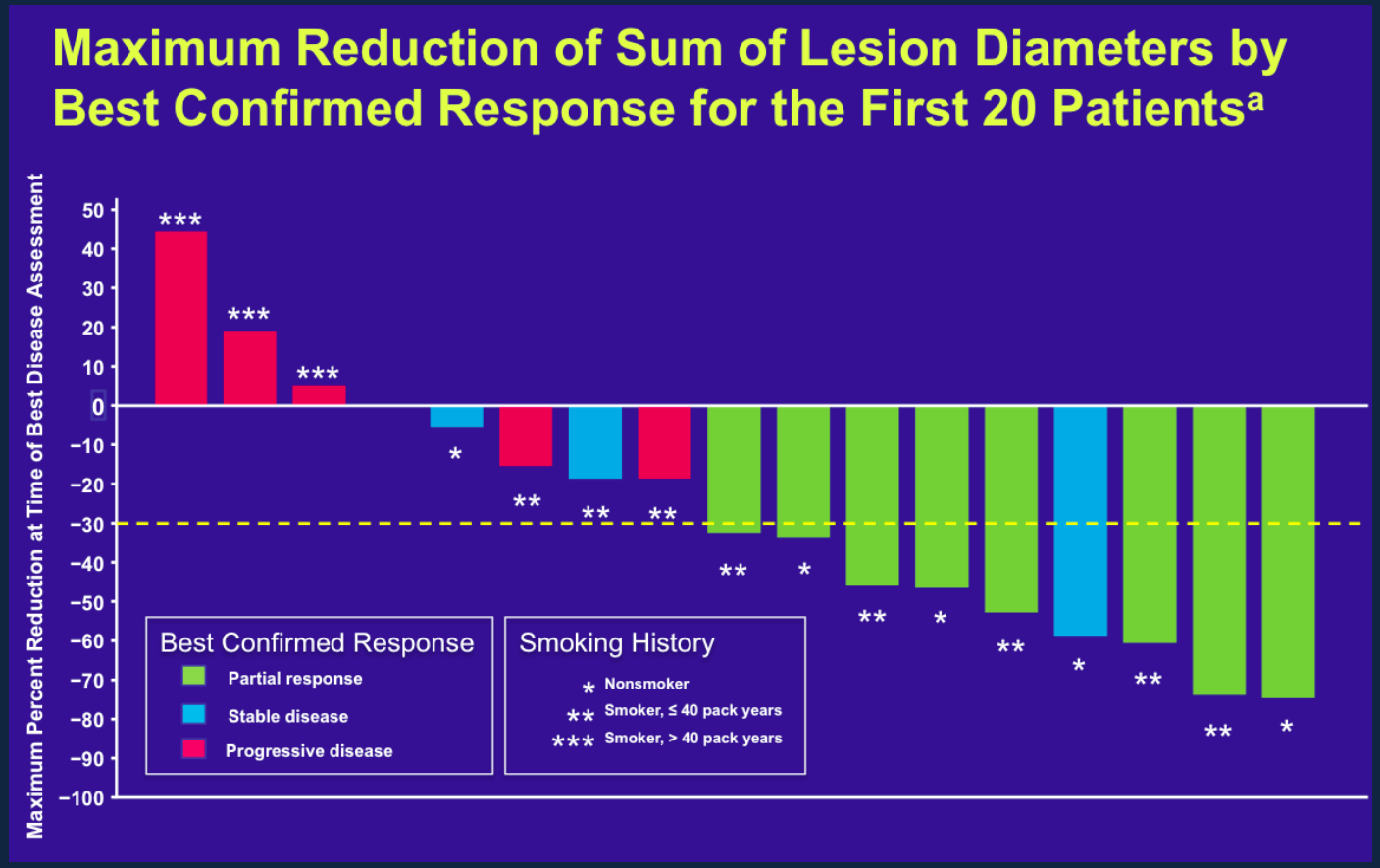
Pandit Nat Gen, 2015; Ali ECCO 2015

BRAF inhibitor phase 2 trial in NSCLC



Planchard, ASCO 2013/2015

Dabrafenib phase 2 trial in BRAF V600E NSCLC

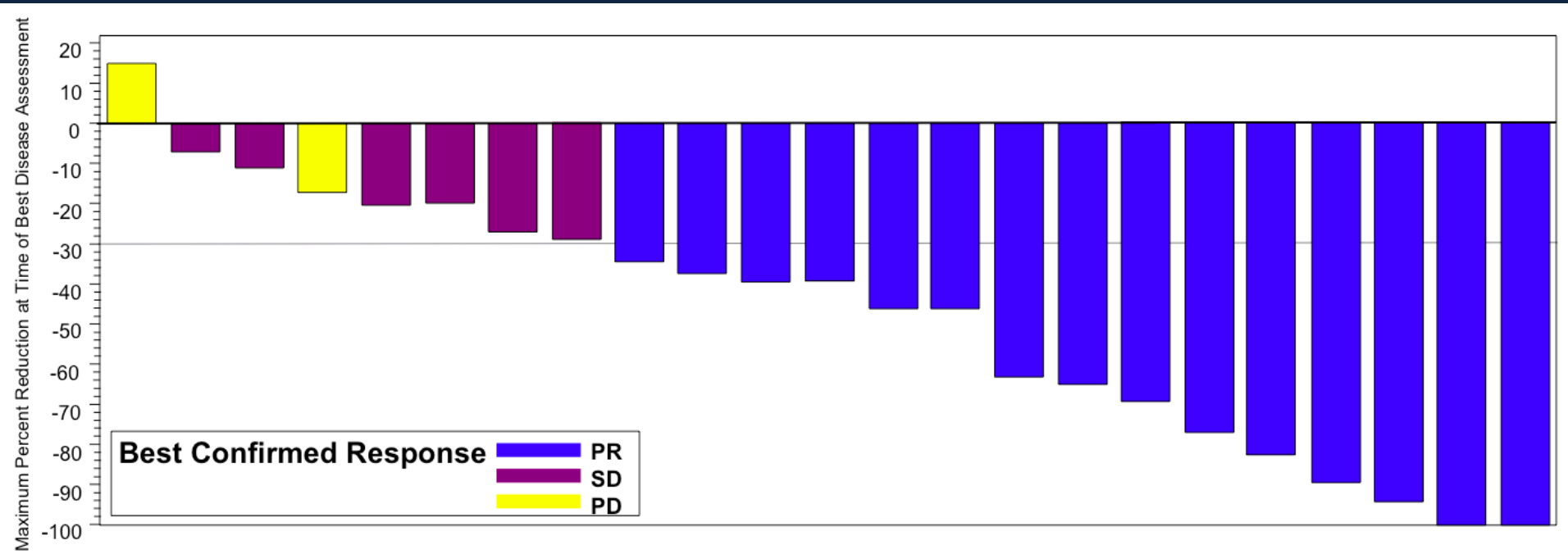


ORR = 32%, and DCR = 56%

Median PFS = 5.5 months

Planchard, ASCO 2014

Dabrafenib & trametinib phase 2 trial in BRAF V600E NSCLC



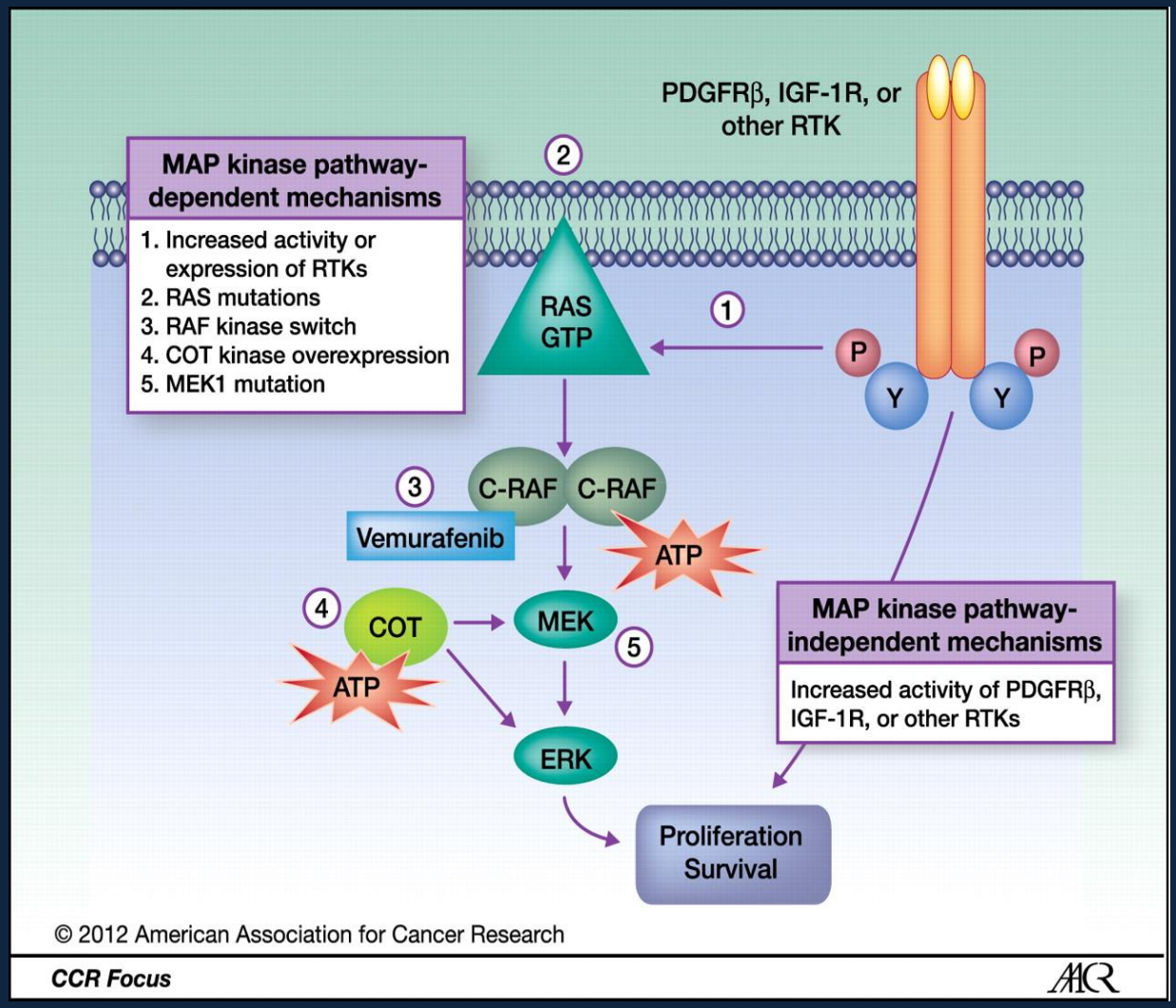
ORR = 63%, and DCR = 88%

Median PFS = not reached

D + T in previously untreated V600E NSCLC is actively recruiting

Johnson, ASCO 2015

MAPK pathway is relatively resistant to an isolated BRAF inhibition



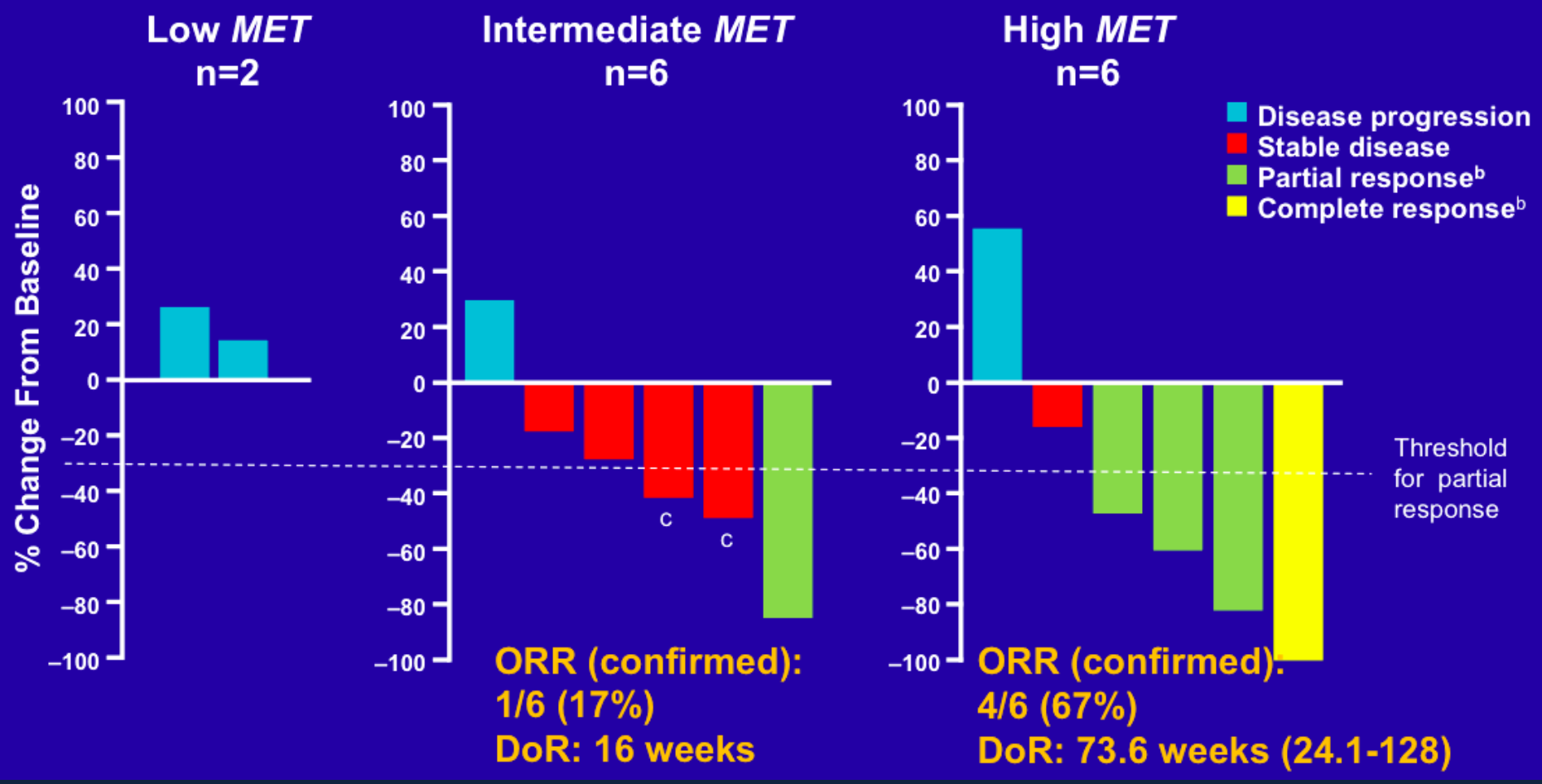
MET amplification

MET/CEP7 ratio	Number of specimens	MET amplification classification	% of total
<1.8	741	Negative	92.6%
≥1.8–≤2.2	29	Low	3.6%
>2.2–<5.0	24	Intermediate	3.0%
≥5.0	6	High	0.8%
Total	800		100.0%

- 7.4 % in this serie.
- 33% overlap with other driver alterations. More males and non-smokers.
- Definition « amplification » to be used?
  - Gene copy number: ≥5? (more frequent because includes polisomy)
  - Ratio: ≥1.8?

Camidge et al, ASCO 2014; LCMC WCLC 2015

MET amplification can be targeted



Camidge et al, ASCO 2014

MET exon 14 mutations/skipping

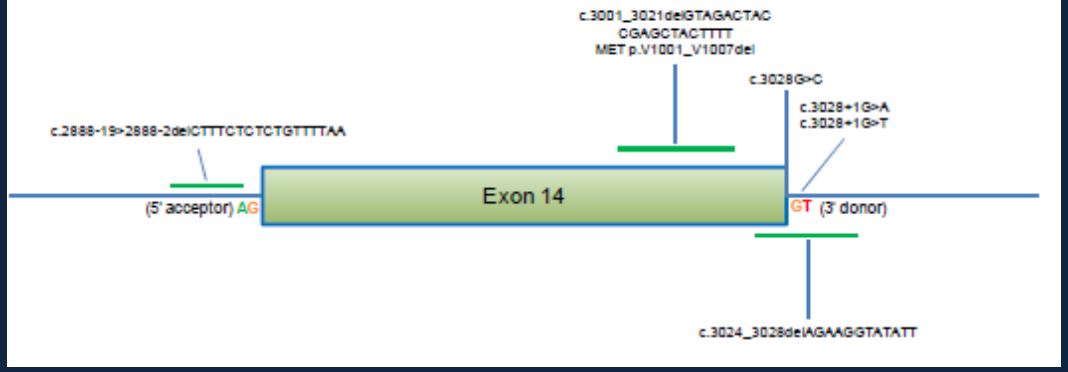
- **Foundation medicine database in cancer:**

126 distinct alterations (splice donor/acceptor site)

3% adeno (131/40402) – 2.3% other lung (62/2659)

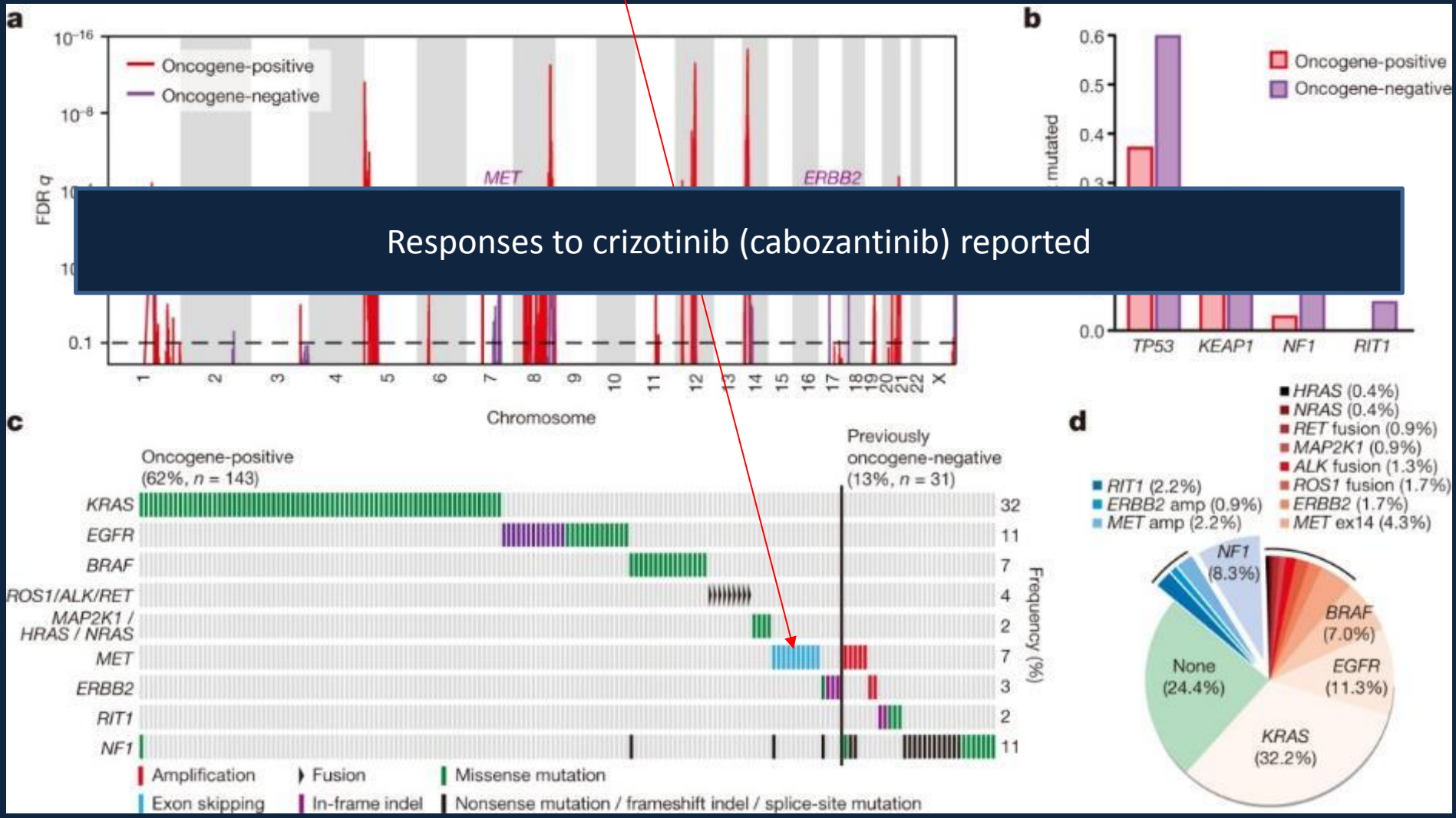
Not other concomittant driver, nor concomitant with amplification

Figure 2: *MET* exon 14 mutations. The distribution of *MET* exon 14 mutations that were discovered is shown in the diagram below. Mutations in the RNA splice and donor acceptor sites lead to RNA skipping and deletion of the juxtamembrane domain with loss of Cbl E3-ligase binding. Mutations deleting the Y1003 residue result in a similar event. These mutations lead to decreased ubiquitination and consequent overexpression of MET.



Paik et al, Cancer Discovery 2015; Frampton WCLC 2015, Kerr ECCO/ESMO 2015

TCGA 230 Adenos
~ 4% MET exon 14 skipping.



Responses to crizotinib (cabozantinib) reported

Other MET mutations not detected

Collisson, Nature 2015

Many NSCLC subtypes : How to prioritize clinical research ?

Umbrella

Test impact of different drugs on different mutations in a single type of cancer

- BATTLE
- I-SPY2
- Lung-MAP Squamous Lung Master

Basket

Test the effect of a drug(s) on a single mutation(s) in a variety of cancer types

- Imatinib Basket
- BRAF+
- NCI MATCH

Oncogene-addicted NSCLC

Everything has become way more complex

- For almost every single indentified driver, early trials with targeted agents are ongoing (phase I/II)
- The “NSCLC”-dedicated trials will probably become a rare concept in the years to come
- Prospective molecularly-driven trials will require large international networks of centers, political and economical support as well as a strong multidisciplinary collaboration

Thanks for your kind attention