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Molecular diagnosis of lung cancer

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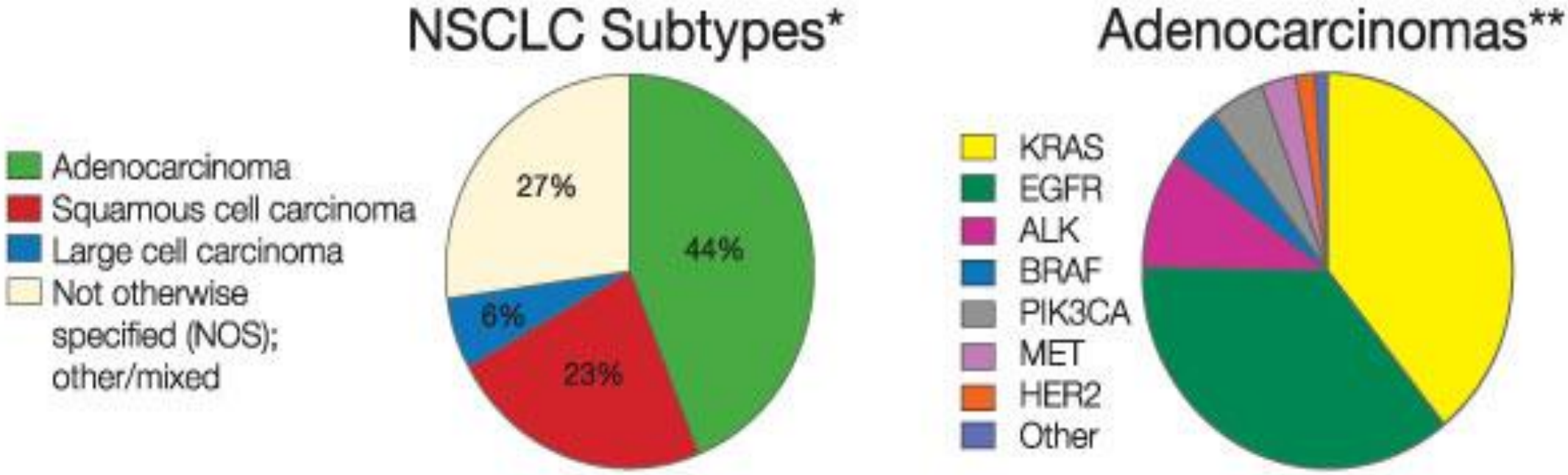
Introduction

- Recently, molecular-targeted therapies have been developed for cancer treatment, especially for lung cancer treatment.
- Non-small cell lung cancer (NSCLC） patients with epidermal growth factor receptor (EGFR) gene mutations have shown a dramatic response to EGFR tyrosine kinase inhibitors (EGFR-TKI) such as gefitinib and erlotinib.
- EML4-ALK fusion protein is present in approximately 5% of the patients with adenocarcinomas and Crizotinib is an oral tyrosine kinase inhibitor (TKI), which has recently been approved for the treatment of NSCLC aberrantly expressing ALK.
- I reviewed the current development of molecular diagnosis of cancers, especially in the aspect for target therapies against lung cancer.

Histological type in NSCLC and the patterns of mutations in adenocarcinoma



*Summary of histotype classification in NSCLC according to the California Cancer Registry

**Kris MG, Johnson BE, Kwiatkowski DJ, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: The NCI's Lung Cancer Mutation Consortium (LCMC). *J Clin Oncol* 29: 2011 (suppl; abstr CRA7506)

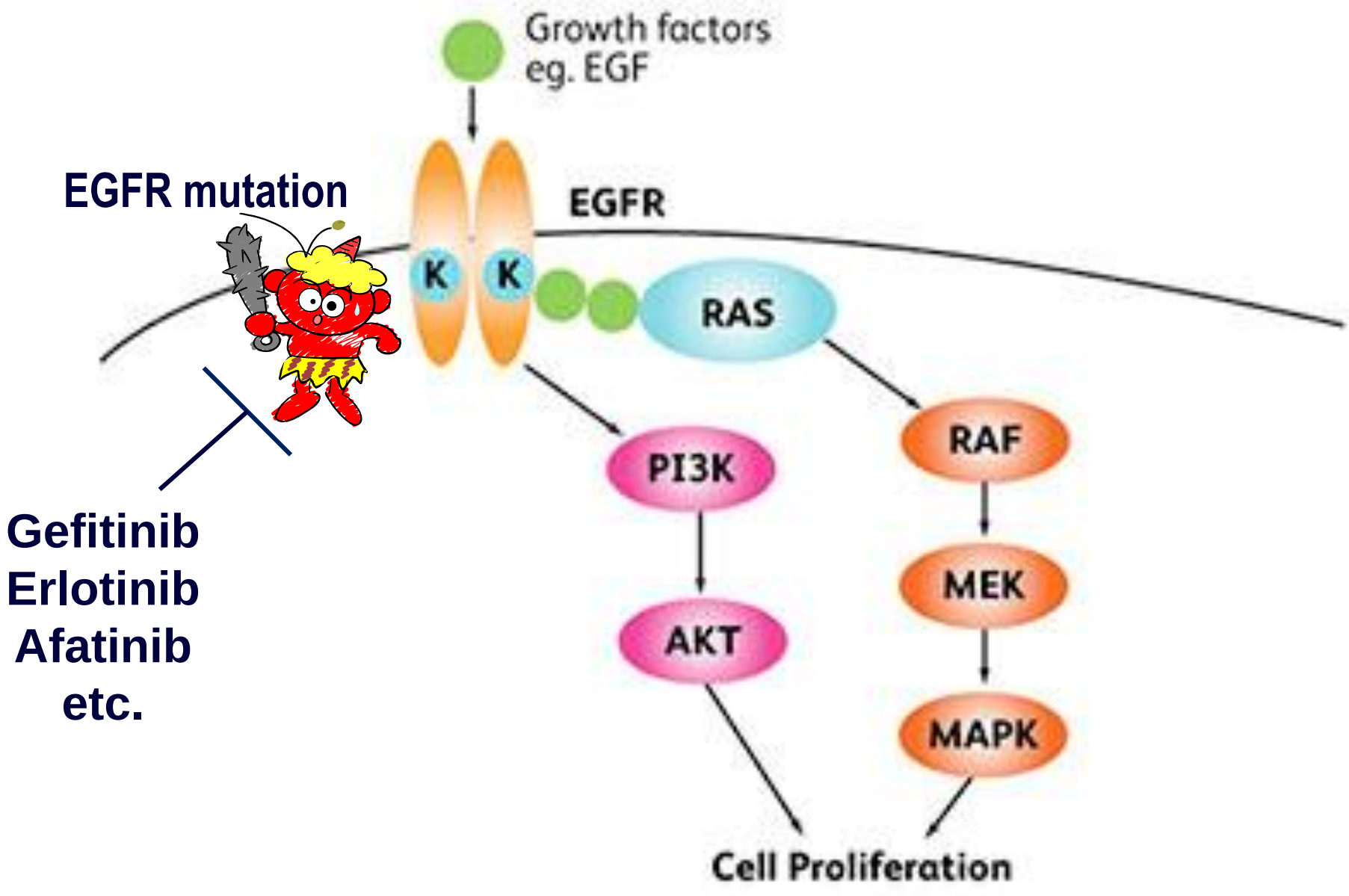
Oncogene Mutations Predict Likelihood of Resistance or Response to Targeted Therapies in Patients with NSCLC

Oncogene	Mutation Prevalence	Mutation-predicted Therapeutic Response	Predicted Overall Response Rates to Targeted Therapies
EGFR	Asians: 40% Caucasians: 10–15%	Sensitive to EGFR TKIs (most mutations)[a]	Erlotinib: ~82%–83%[4] Gefitinib: ~71%–73%[4]
KRAS	Asians: 10% Caucasians: 30%	Resistant to EGFR TKIs	0%–5%[2,5,7]
ALK	2–7%	Sensitive to ALK inhibitors	Crizotinib: 50%-61%[9,10]
		Resistant to EGFR TKIs	0%[b]

TKI, tyrosine kinase inhibitor.

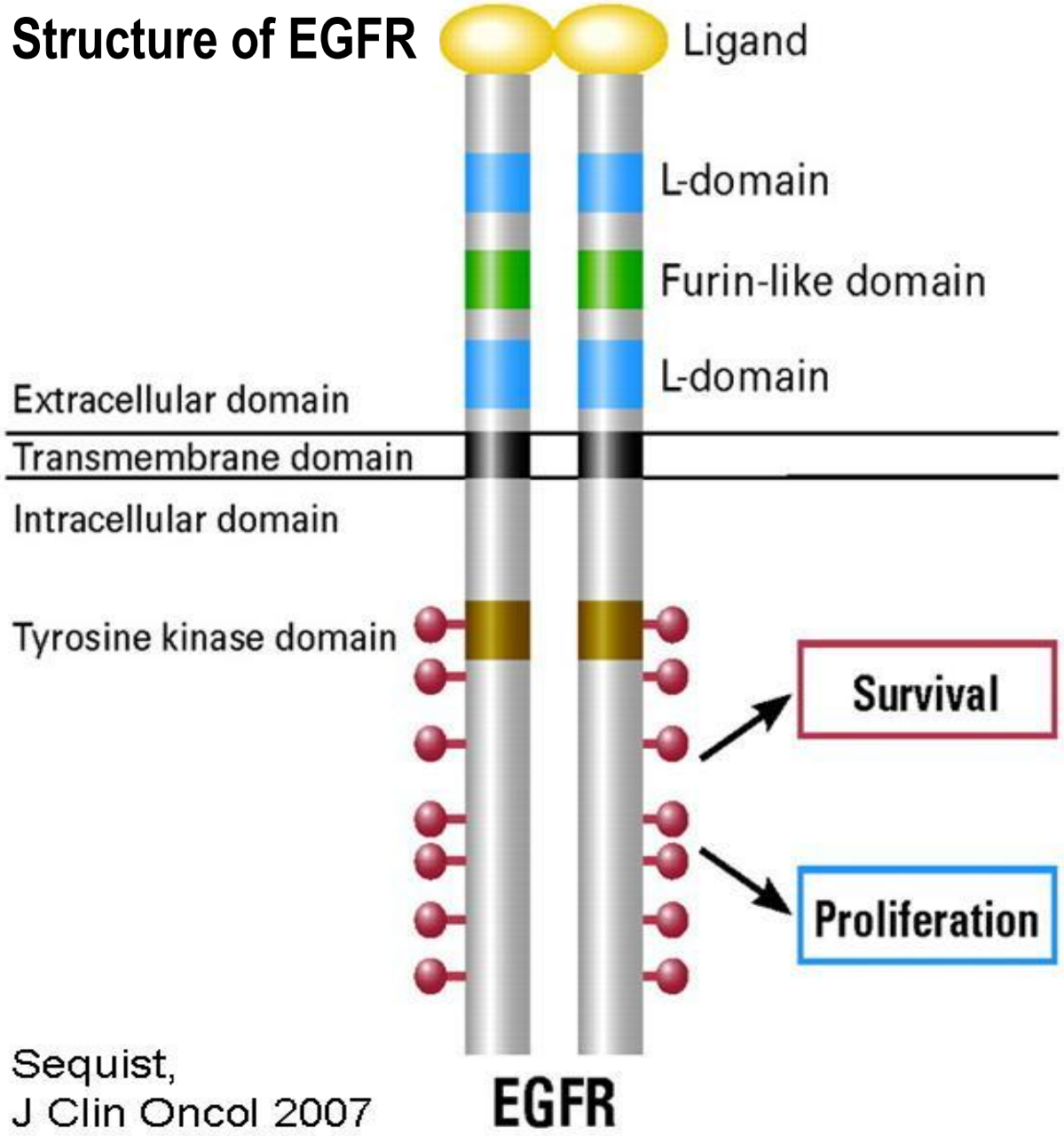
[a] Common mutations (exon 19 deletions and L858R) are associated with response to EGFR TKIs; other mutations such as T790M and exon 20 insertion are associated with decreased response or secondary resistance to TKIs.[4]

[b] Based on one study that included 10 patients positive for *EML4-ALK*.[8]



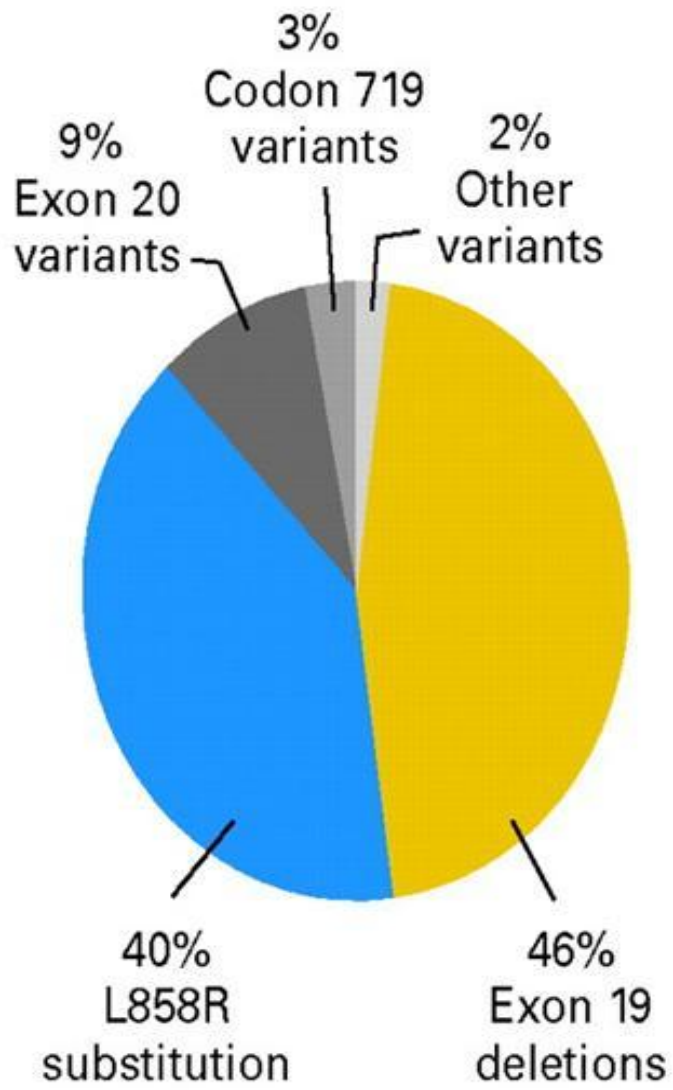
EGFR activating mutations in the tyrosine kinase domain lead to tumor growth. In such cases, molecular targeting therapy for EGFR is effective.

Structure of EGFR

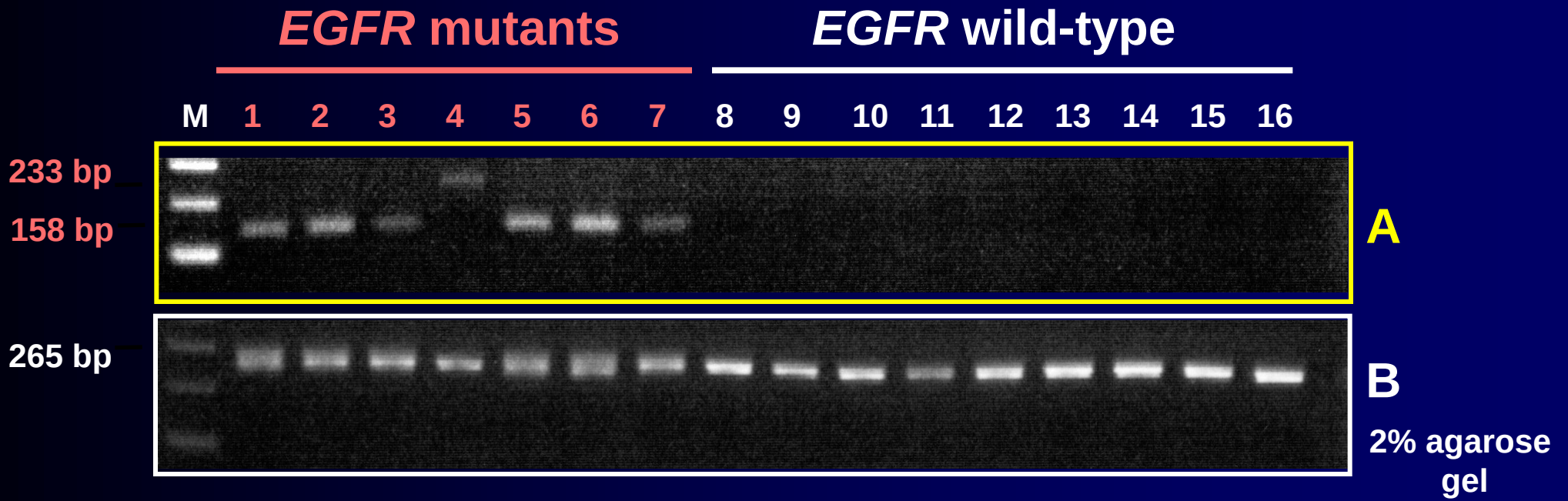


Sequist,
J Clin Oncol 2007

Mutations of EGFR



Detection of *EGFR* deletions (exon 19) and L858R (exon 21) by a single PCR (*Arifin et al., Research Signpost, 2009*)



A: mixture of EGFR mutation (deletions in exon 19 and L858R) specific primers

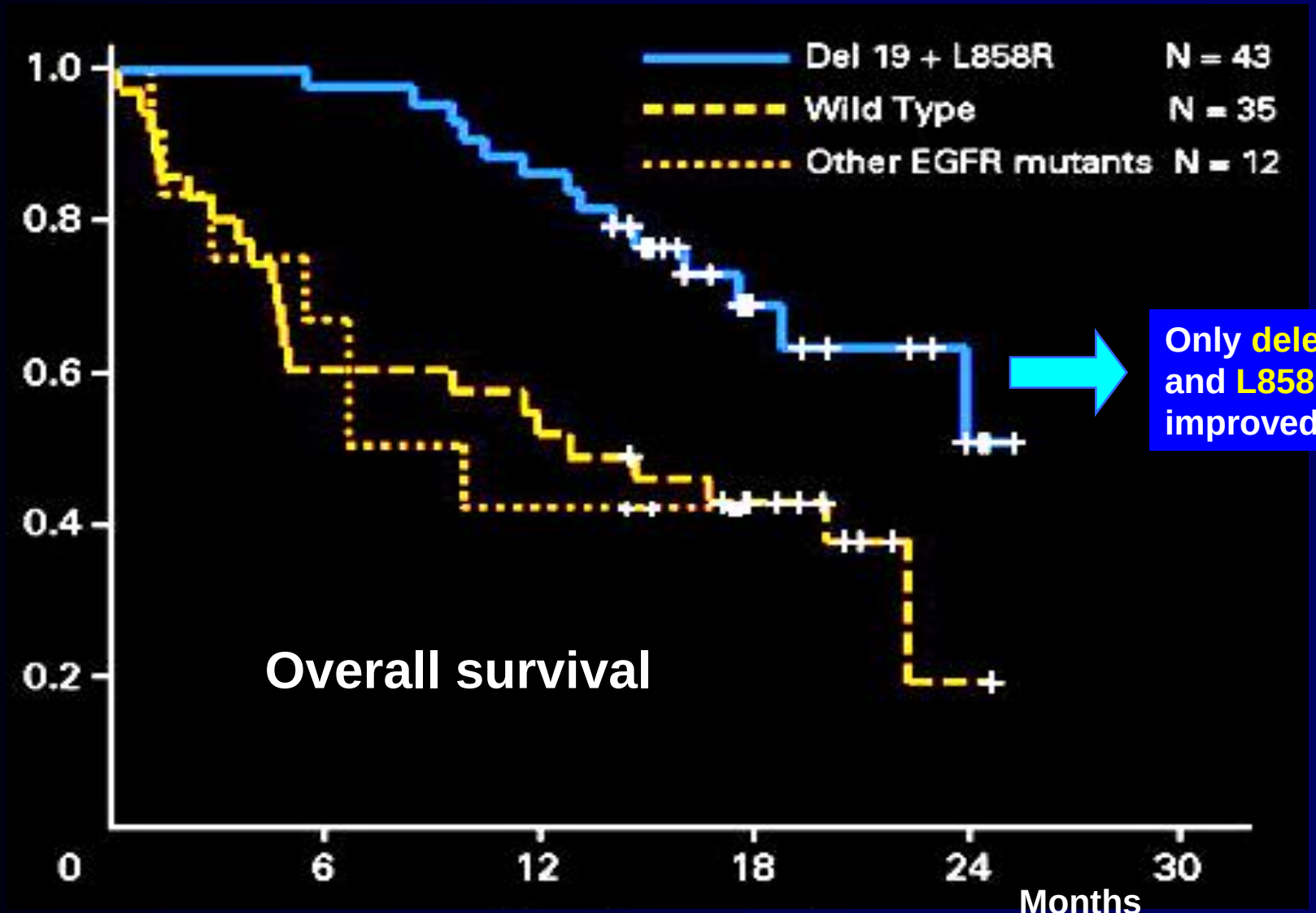
delE746-A750 (2235-2249: 1,2,6) , delE746-A750 (2236-2250: 5)

delL747-T751 (2239-2253: 3,7) , L858R (4)

B: common primer (control, not necessary for detection)

We could identify the cases with EGFR activating mutations.

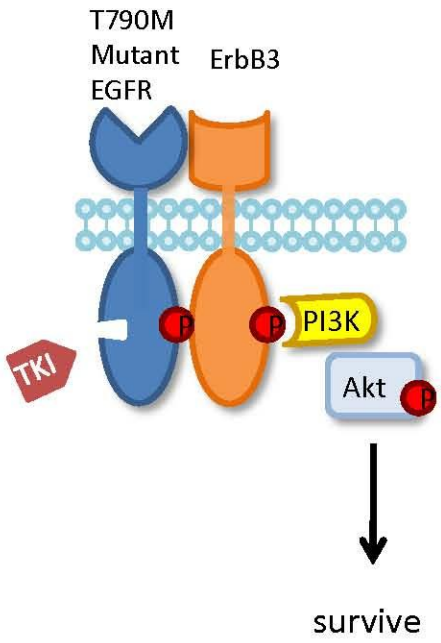
EGFR mutation in NSCLC patients treated with first-line gefitinib monotherapy (*Yang et al., J Clin Oncol 26: 2745-53, 2008*)



Only deletion in exon 19 and L858R in exon 21 improved the prognosis

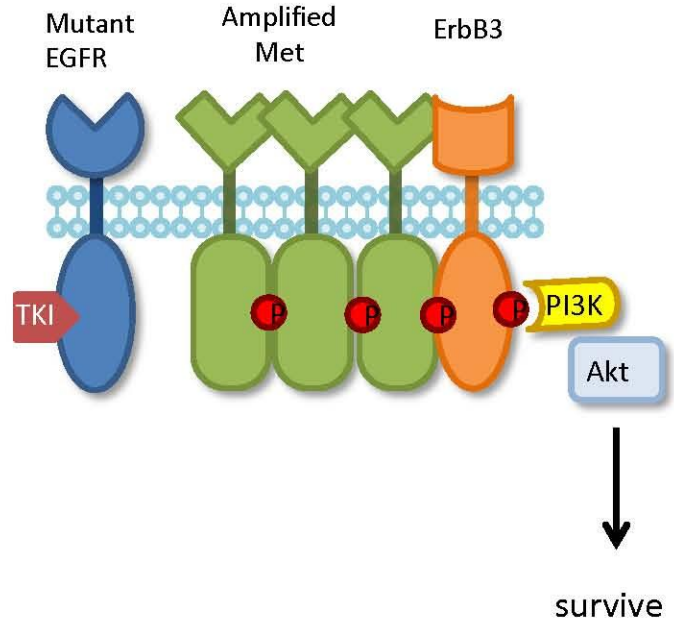
EGFR-TKI resistant mechanisms in EGFR mutated lung cancer

EGFR-T790M
Secondary mutation



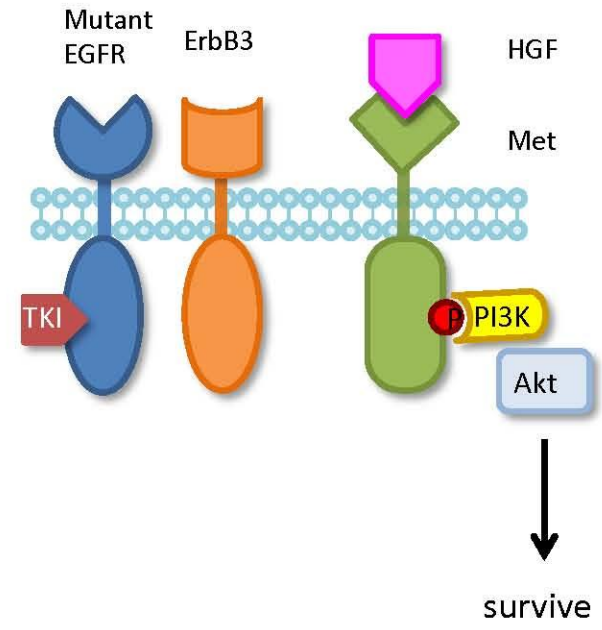
Kobayashi S et al,
NEJM 352:786, 2005

Met
Gene amplification

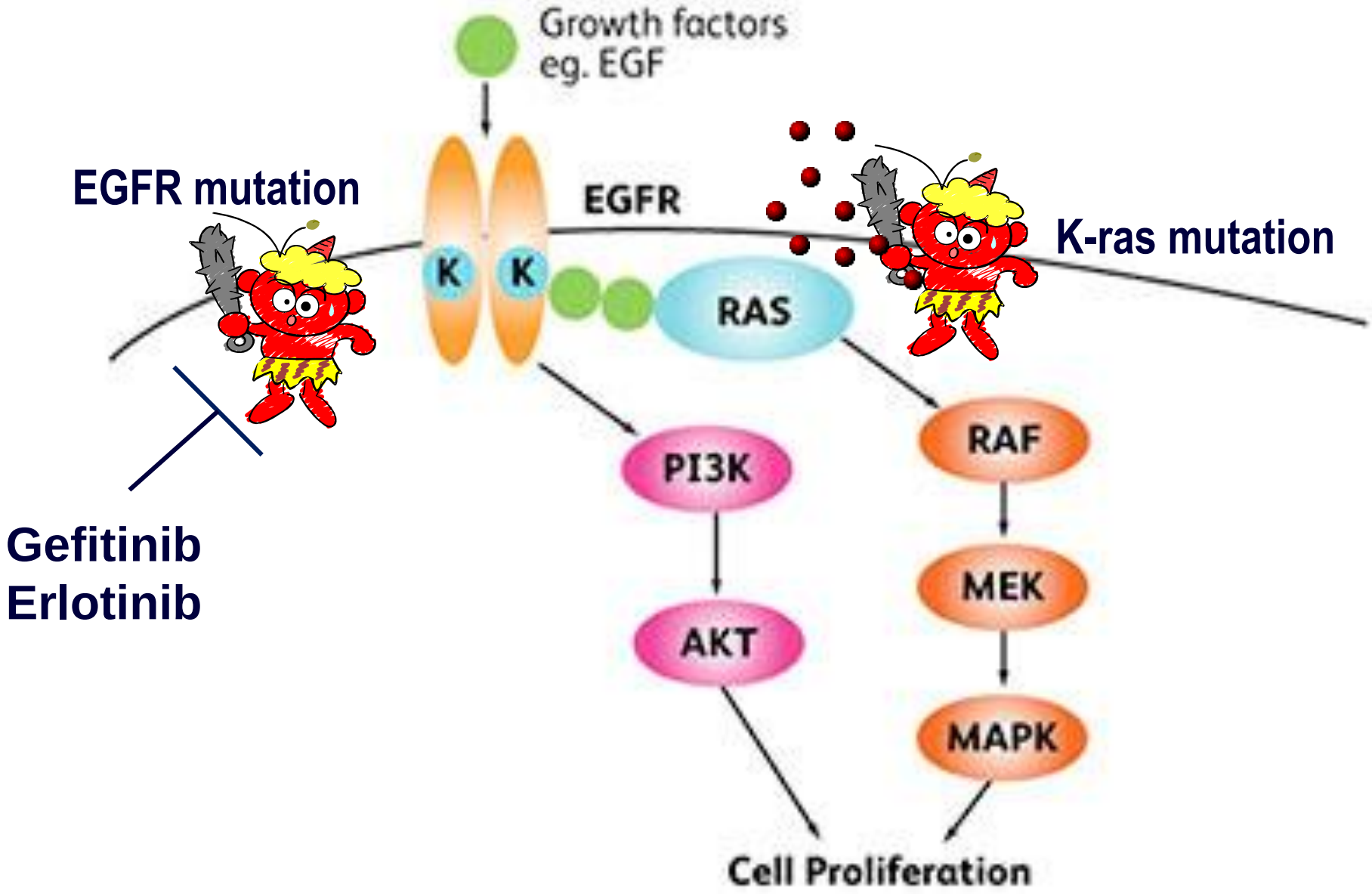


Engelman JA et al,
Science 316:1039, 2007

HGF
Over expression



Yano S et al,
Cancer Res 68:9479, 2008



KRAS exists in the pathways downstream from EGFR. When *KRAS* mutations occur, EGFR inactivation cannot block this pathway. Thus, patients with *KRAS* mutations tend to be resistant to gefitinib and erlotinib.

Detection of K-ras mutation: Codons 12, 13, & 61
MASA(Mutant Allele Specific Amplification) method

mutant allele-specific PCR primers for codon 12

Wild type

5'- ACT TGT GGT AGT TGG AGC TGG-3’

Set 1 for first-letter mutation

5'- ACT TGT GGT AGT TGG AGC TC -3’

5'- ACT TGT GGT AGT TGG AGC TT -3’

5'- ACT TGT GGT AGT TGG AGC TA -3’

Set 2 for second-letter mutation

5'- CTT GTG GTA GTT GGA GCT GC -3’

5'- CTT GTG GTA GTT GGA GCT GT -3’

5'- CTT GTG GTA GTT GGA GCT GA -3’

Reverse

5’-CTC ATG AAA ATG GTC AGA GAA ACC-3’

PCR
100 ng DNA Sample

95℃ 12min x 1 cycle

94℃ 1min
64℃ 2min
x 35 cycles

72℃ 7min x 1 cycle

K-ras mutation analysis – codon 12-

PC: Positive Control
NC: Negative Control

69 73 89 85 NC PC

69 73 89 85 PC NC

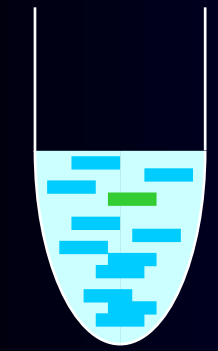
Set 1

Set 2

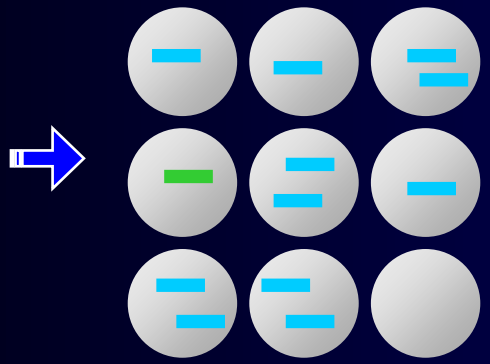
First letter Mutation

Second letter Mutation

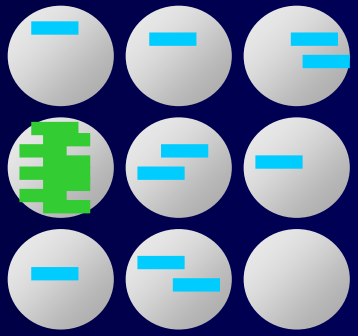
Digital PCR: Principle



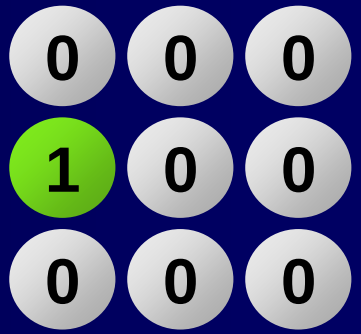
Target template
Non-target template



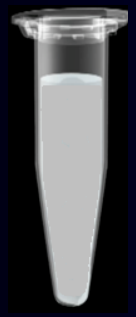
Target DNA and non-target DNA are randomly distributed into droplet

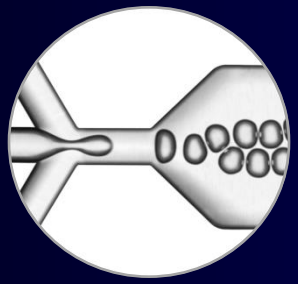


Perform PCR

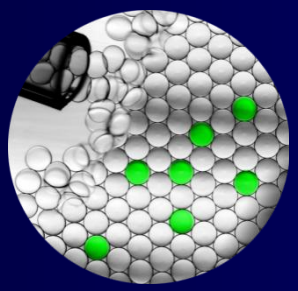


Positive droplets in each sample are read

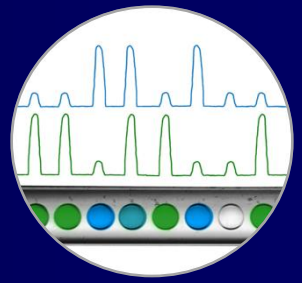




Droplet Generator

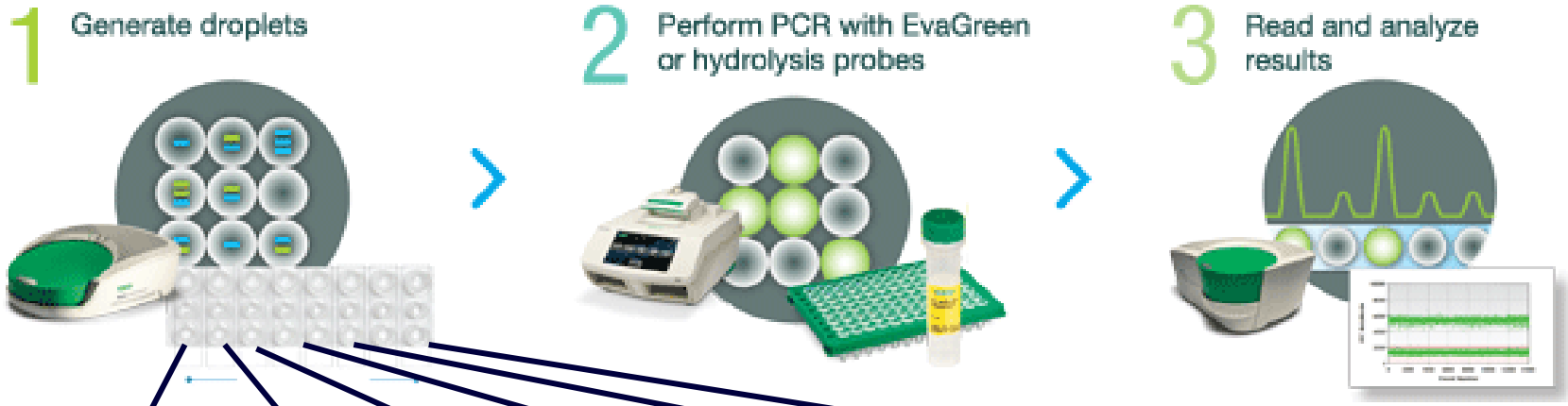


Thermal Cycler

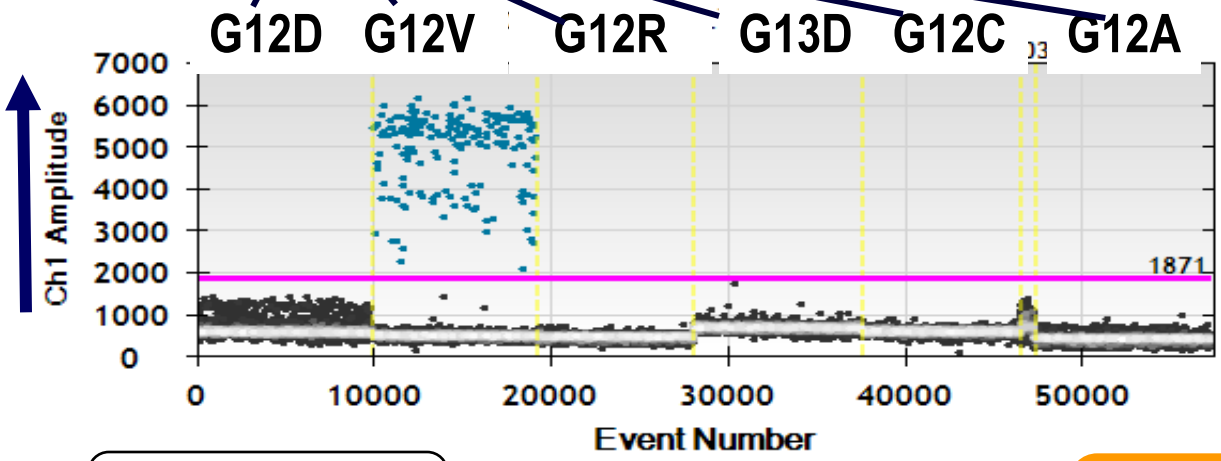


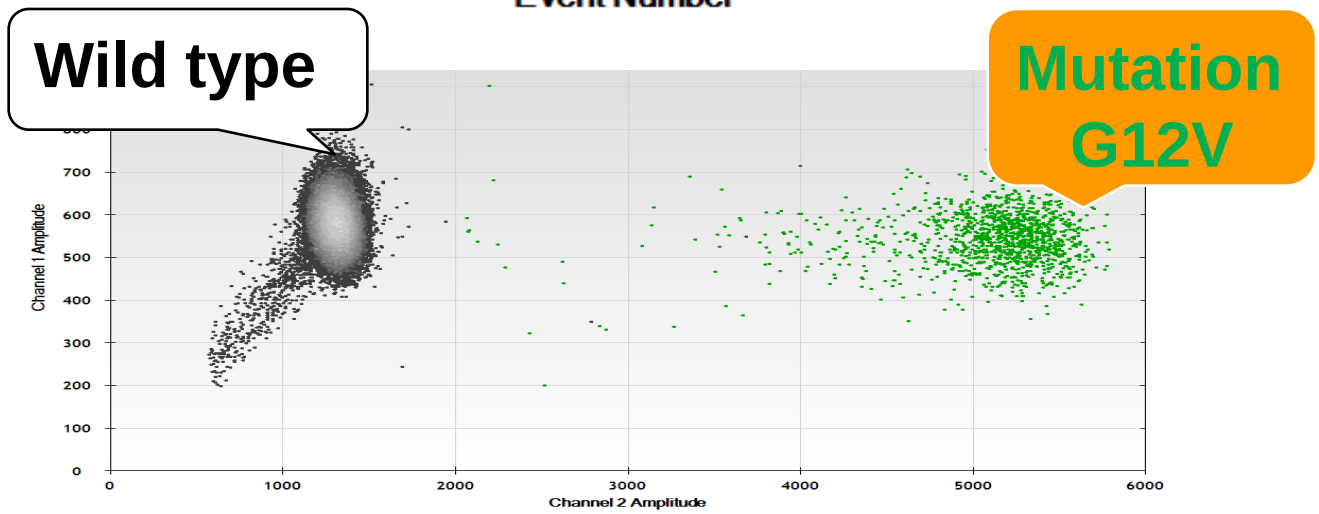
Droplet Reader

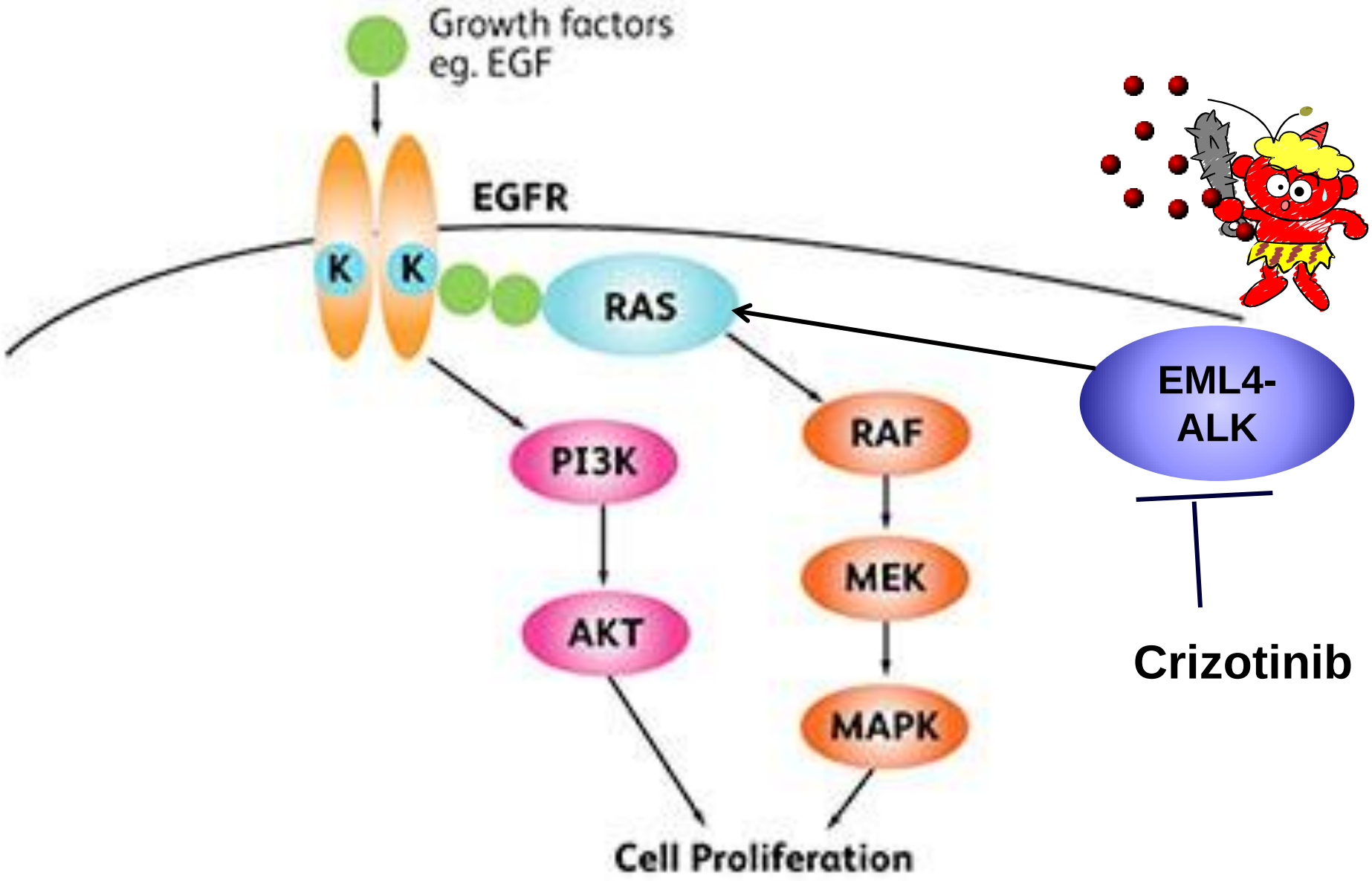
Digital PCR for KRAS mutation



Positive droplet numbers

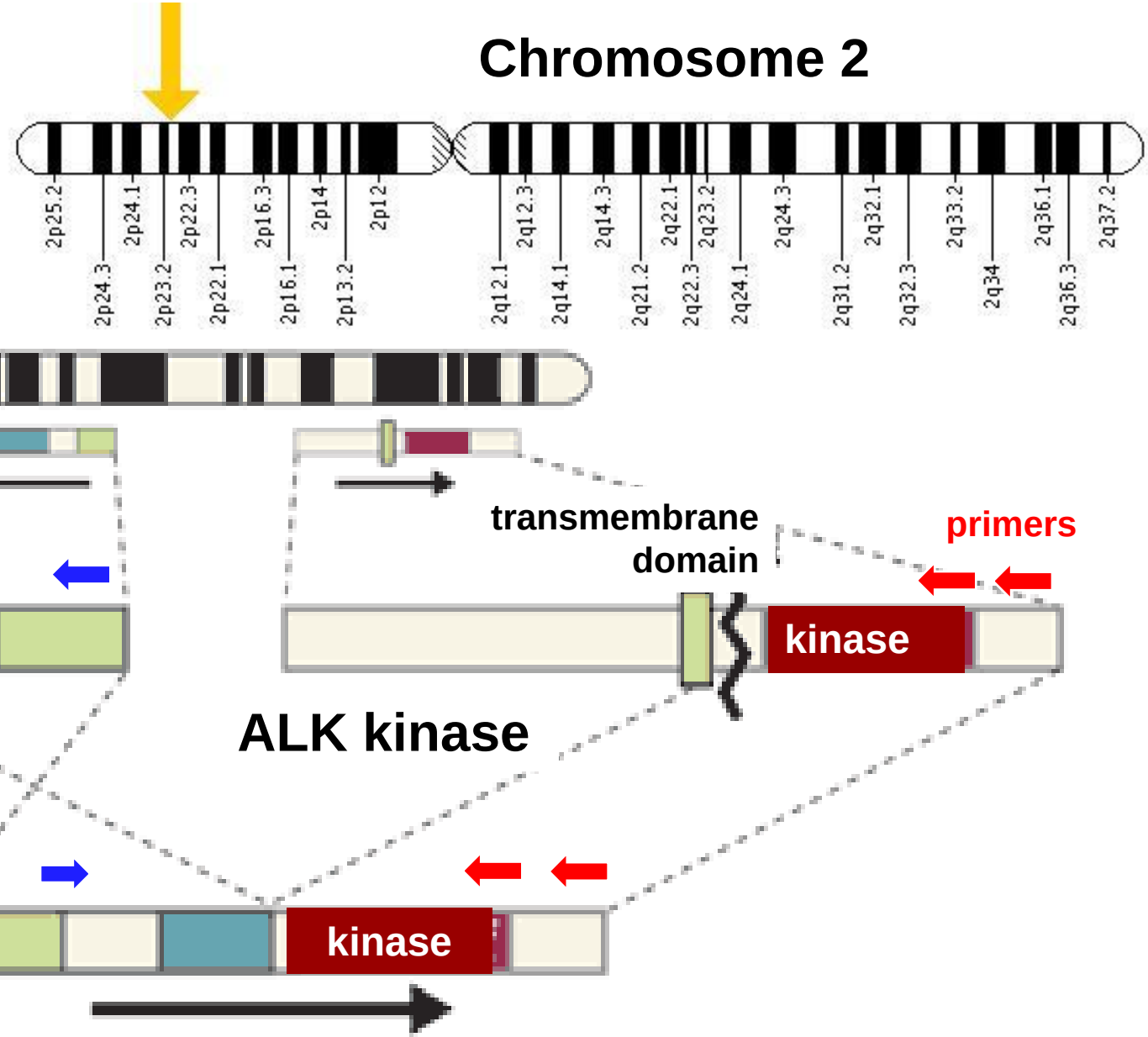






EML4-ALK fusion gene activates this pathway by Ras activation. In such cases, ALK inhibitors such as Crizotinib are effective.

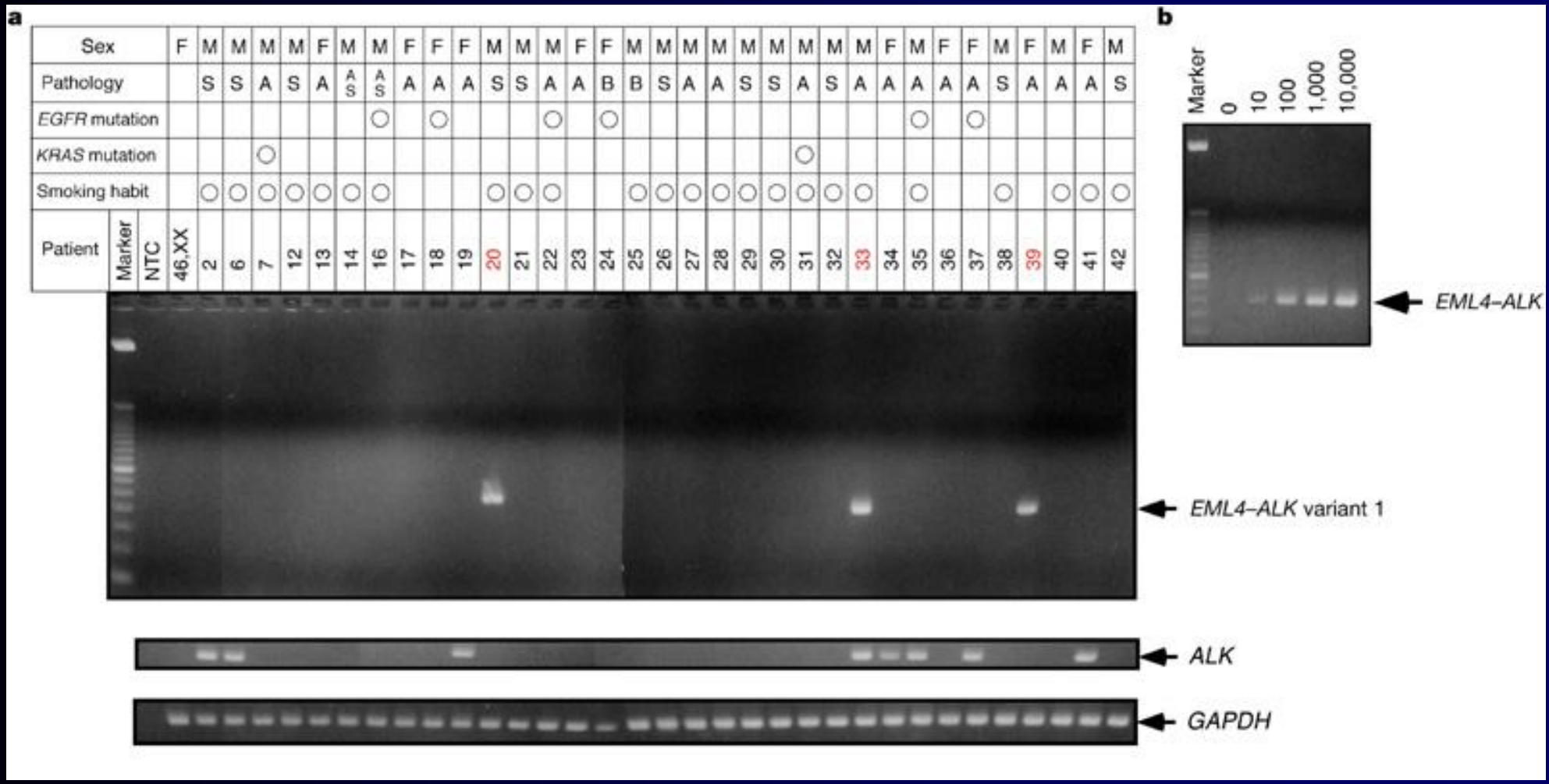
ALK aberrations: Lung cancer



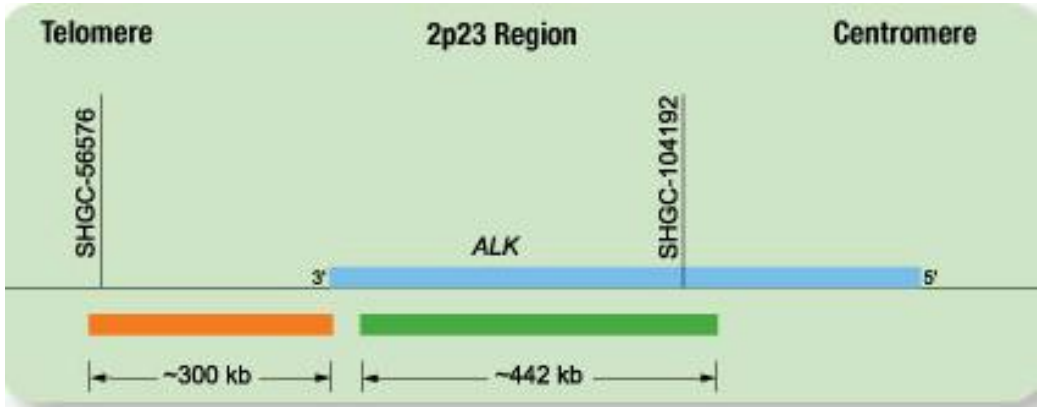
Activated EML4-ALK fusion kinase

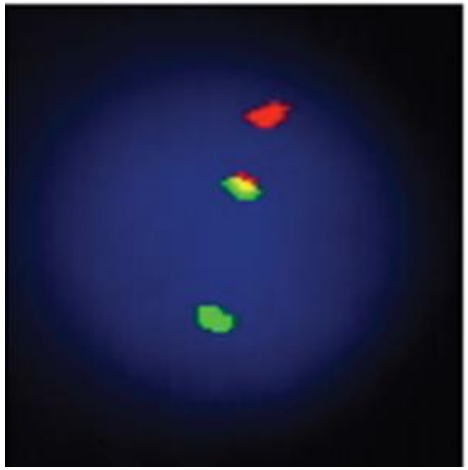
In lung cancer, ALK gene is translocated with inversed EML4 gene and activates fusion kinase. We designed the primes as shown in this slide and performed RT-PCR.

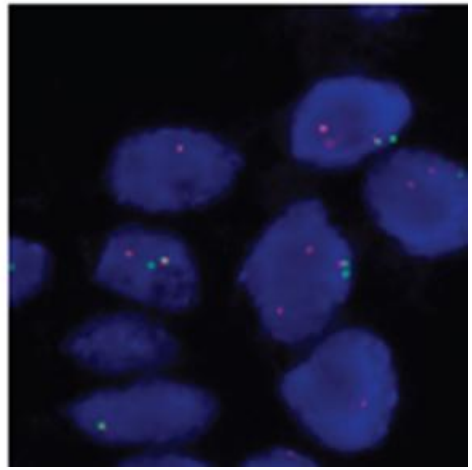
Detection of EML4-ALK fusion



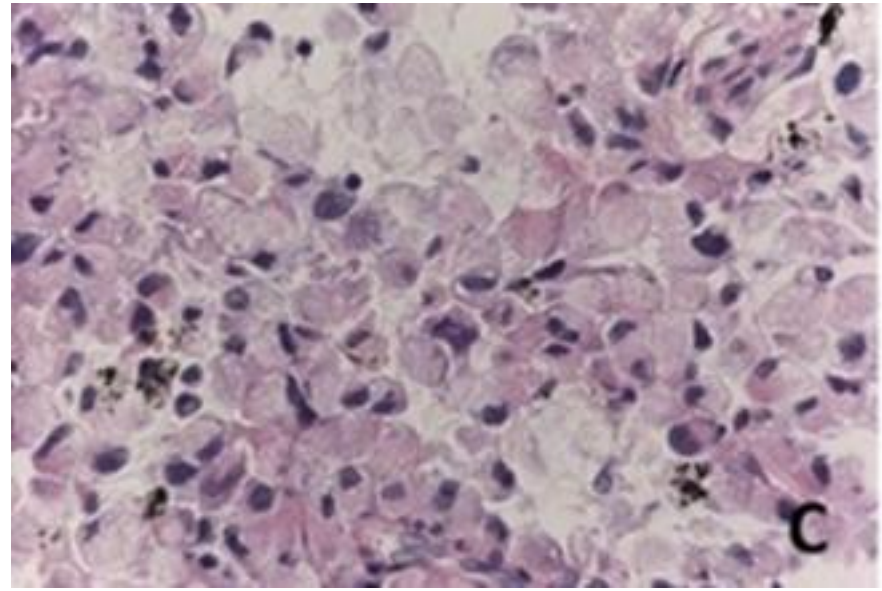
FISH for ALK fusion gene







IHC for ALK fusion gene

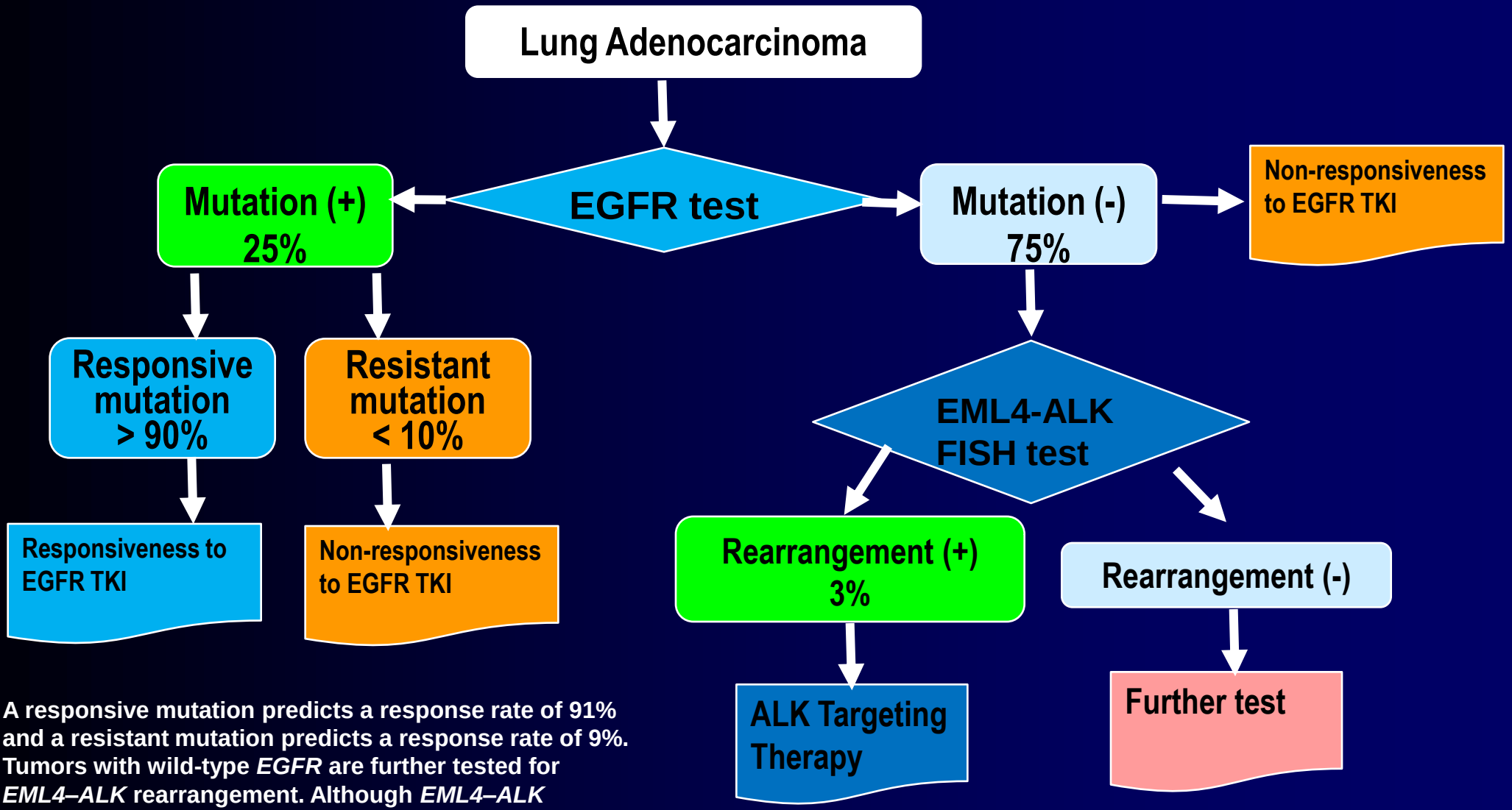


Detection methods for ALK fusion genes

	Pros	Cons
RT-PCR	Rapid, very sensitive and more accurate Very accurate	Need for high-quality RNA Not applicable for unknown partners Difficult to apply to archival tissues Difficult to confirm the presence of tumor cell
FISH	Applicable for any partners Screening methods for clinical trial Applicable to archival tissues	Expense Relative long turnaround time Less sensitive
IHC	Applicable for any partners Relative turnaround time Established in many labs Applicable to archival tissues Cheep	Indirect demonstration of the fusion gene Occasional false negative results Highly depends on antibody and detection method

Algorithm for molecular testing for patients with lung adenocarcinoma

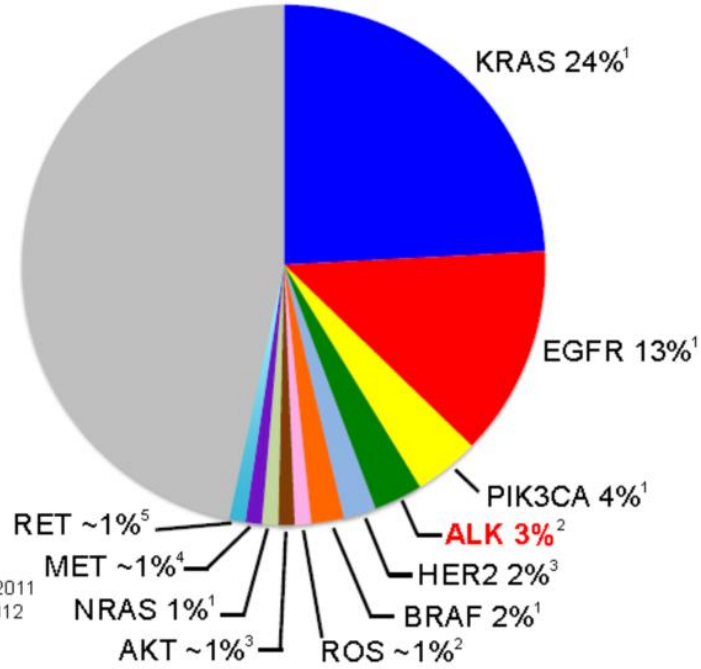
in selecting patients who could benefit from *EGFR* and *EML4–ALKA* targeted therapy



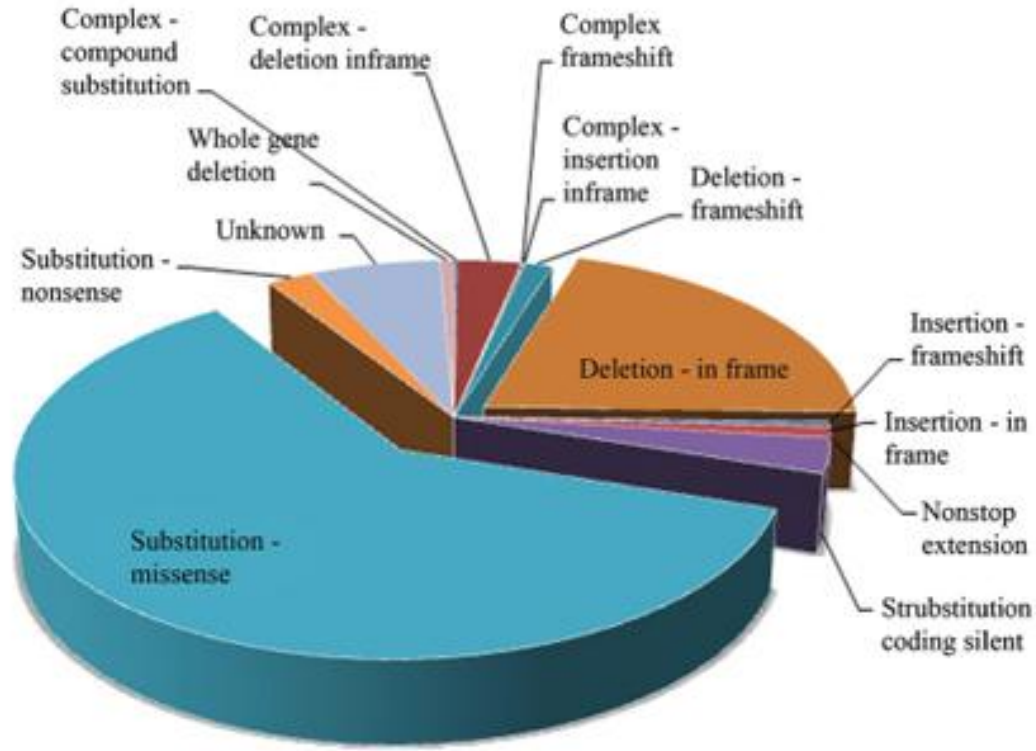
A responsive mutation predicts a response rate of 91% and a resistant mutation predicts a response rate of 9%. Tumors with wild-type *EGFR* are further tested for *EML4–ALK* rearrangement. Although *EML4–ALK* rearrangement is found in only 3% of patients with lung adenocarcinoma, its presence predicts a 53% probability of response to targeted therapy.

a 53% probability of response to targeted therapy

Oncogenic Drivers in NSCLC



Sequist et al., Ann Oncol 22:2616, 2011
Takeuchi et al., Nat Med, Feb 12 2012
Shaw et al., NEJM 365:158, 2011
Kris et al., WCLC 2011
Takeuchi et al., Nat Med, Feb 12 2012



Mutation type in Lung cancer genes

Figure 1 - Mutation types in lung cancer genome. Mutation types included three major types: substitution, deletion and insertion. Each of the major mutation types was categorized into frameshift mutation or in-frame mutation. The latter, although not causing a shift in the triplet reading frame, can, however, lead to the encoding of abnormal protein products.

Table 1. Lung cancer genetic aberrations and associated targeted therapy.

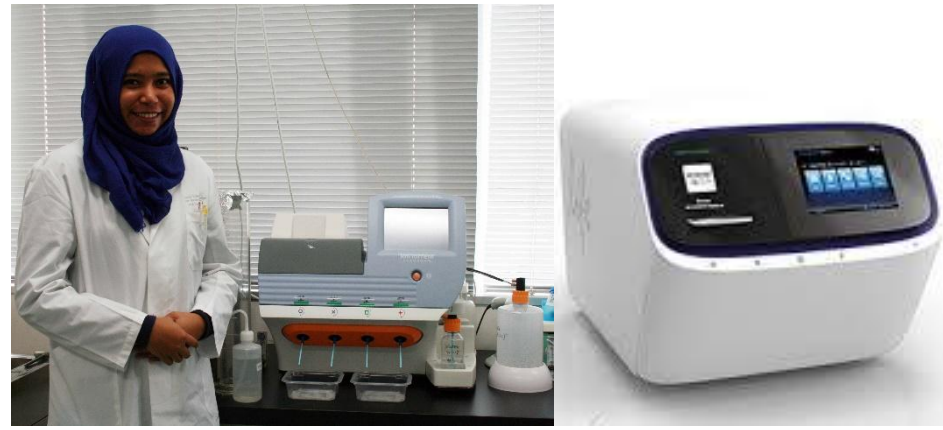
Biomarker gene	Aberration	Targeted therapeutic	Frequency of aberration [%]
EGFR	Mutation or amplification	Gefitinib, erlotinib, cetuximab	[10-25] (35% in Asian patients)
HER2 (ERBB2)	Mutation or amplification	Trastuzumab	[5-10]
BRAF	Mutation	Sorafenib	[2-3]
p53	Mutation or deletion	Advexin a p53 adenoviral vector	[30-50]
VEGF	Overexpression	Bevacizumab, afibercept	
PI3K	Modified and activated	BEZ235, LY294002	[1-3]
mTOR	Activated	Rapamycin, RAD001, CCL-779	[70-75]
RAS	Mutation leading to activation	Tipifarnib, lonafarnib	[10-15] (20-30% in Adenocarcinoma)
MEK	Activated	Trametinib, salumetinib	[1-2]
c-KIT	Overexpressed	Imatinib	[1-2]
EML/ALK	Fusion	Crizotinib	[5-13]

J. Thoracic Dis. 2014Molecular markers to predict clinical outcome and radiation induced toxicity in lung cancer

Joshua D. Palmer[1], Nicholas G. Zaorsky[1,2], Matthew Witek[1], Bo Lu[1]

Cancer Panel: Next Generation Sequencer

ABL1	EZH2	JAK3	PTEN
AKT1	FBXW7	IDH2	PTPN11
ALK	FGFR1	KDR	RB1
APC	FGFR2	KIT	RET
ATM	FGFR3	KRAS	SMAD4
BRAF	FLT3	MET	SMARCB1
CDH1	GNA11	MLH1	SMO
CDKN2A	GNAS	MPL	SRC
CSF1R	GNAQ	NOTCH1	STK11
CTNNB1	HNF1A	NPM1	TP53
EGFR	HRAS	NRAS	VHL
ERBB2	IDH1	PDGFRA	
ERBB4	JAK2	PIK3cCA	

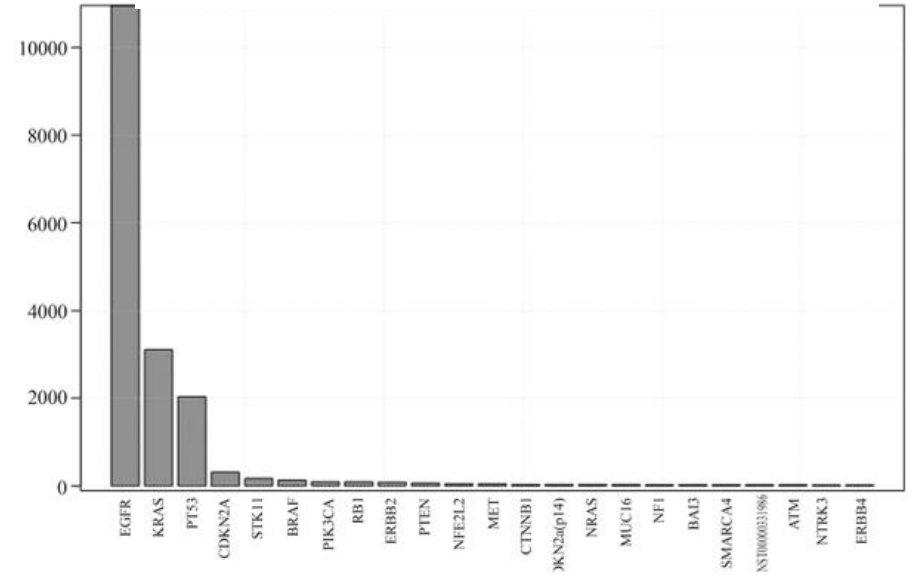


EGFR mutation

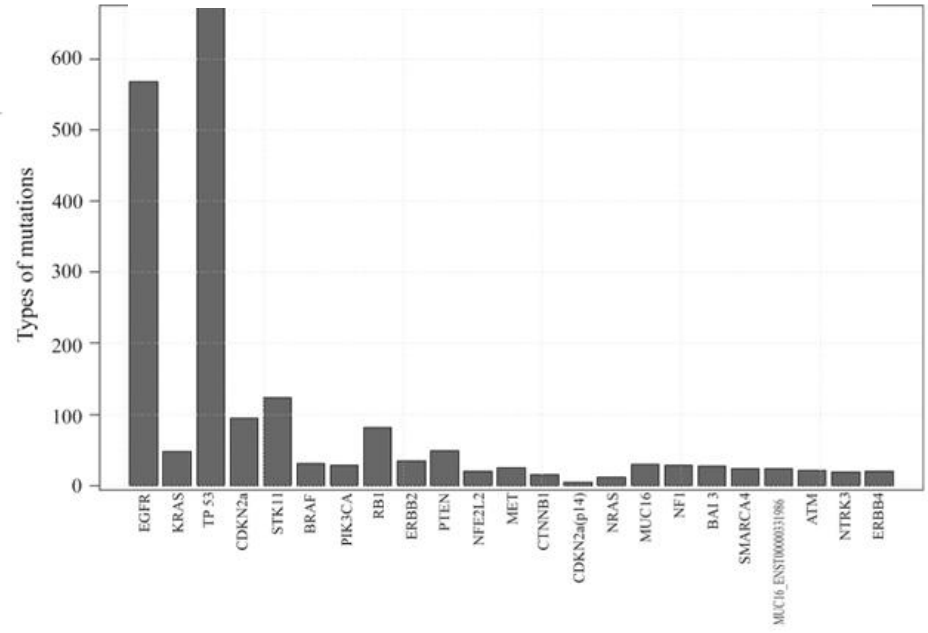
Table 2 - Genes with high mutation frequency in lung cancer (n = 145).

Gene symbol	Gene symbol	Gene symbol	Gene symbol	Gene symbol
EVI1	EPHA3	CBL	MAP2K4	PDIA4
HYRC	UBR5	RUNX1T1_ENST00000265814	ITPR1	PAK6
EGFR	PIK3CG	RUNX1T1	FLT4	P2RY8
KRAS	PDGFRA	RET	FLT3	NRG3
TP53	KDR	PPP1R3A	BRCA2	MECOM
CDKN2A	ALK	NTRK1	ABL2	LMTK3
STK11	TTN	MEN1	USP29	LATS1
BRAF	PKHD1	KSR2	RNF213	KIAA1804
PIK3CA	LPHN3	HRAS	PTPN11	JAK3
RB1	GRM8	FGFR4	PTCH2	JAK2
ERBB2	PTPRD	ROS1	PTCH1	ITPR3
PTEN	FBXW7	ROR2	PAK3	IRS1
NFE2L2	KEAP1	PIK3C3	NPY5R	IRAK2
MET	ITK	PIK3C2G	NCOA2	INHBA
CTNNB1	SMAD4	NOTCH4	MTOR	HERC1
CDKN2a(p14)	PAK7	NOTCH3	MKRN3	HEPH
NRAS	NTRK2	NLRP3	MERTK	FBXO10
MUC16	KIT	MYO3B	LTK	ERCC6
NF1	INSRR	LMTK2	HECW1	EPHB3
BAI3	GLI3	GRM1	FLT4_ENST00000261937	EPHA4
SMARCA4	FGFR2	EPHB6	DOCK3_ENST00000266037	DNER
MUC16_ENST00000331986	CSMD3	EPHA7	CREBBP	DGKB
ATM	TLR4	ENSG00000121031	CDC42BPA	CCKBR
NTRK3	PRKDC	TLN1	AKT1	BAI2
ERBB4	NOTCH2	TERT	ZMYM2_ENST00000456228	APLNR
EPHA5	FLT1	TBX22	VEGFC	ANKK1
APC	FBXW7_NM_018315_2	TAF1L	TYK2	AKAP9_ENST00000356239
NOTCH1	EPHB1	MSH6	ROBO2	AKAP9
LRP1B	CDH11	MLL	RBL1	ADAMTSL3

Frequencies of top 23 gens



Mutation types in top 23 genes



Summary

- In non-small cell lung cancer (NSCLC) patients, molecular diagnosis including epidermal growth factor receptor (EGFR) gene mutations and EML4-ALK fusion is necessary to choose targeting therapy.
- Next generation sequencing (NGS) is a powerful procedure to identify molecular targets in NSCLC .

Collaborators

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Thank you for your attention

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