Molecular Basis of Drug Resistance

(EGFR and ALK inhibitors)

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CONTENTS

Resistance for Targeted Drugs

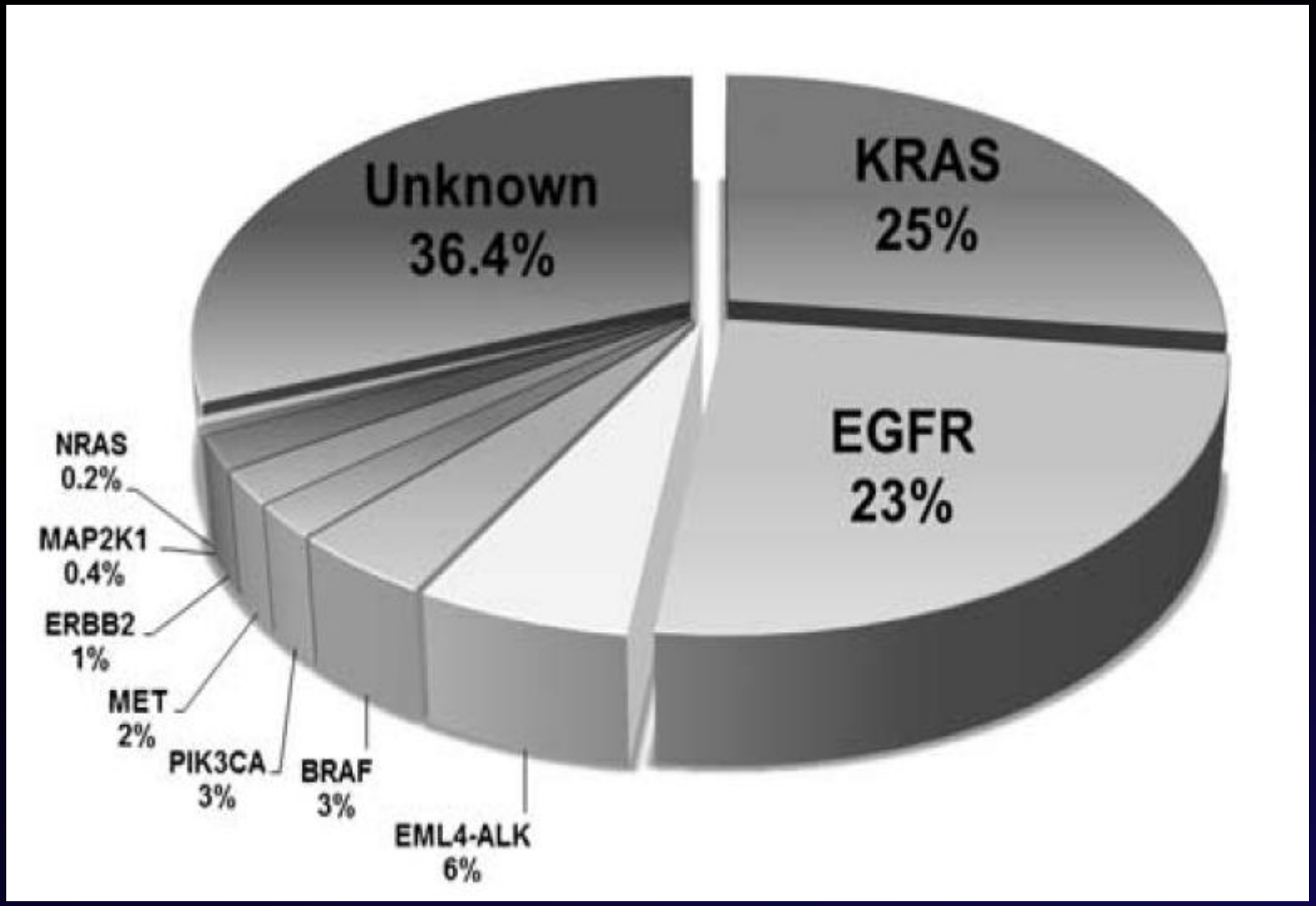
a. EGFR tyrosine kinase inhibitors

    1. primary resistance

    2. secondary resistance

b. ALK inhibitors

Frequency of major driver mutations in signaling molecules in lung adenocarcinomas



Cheng. 2012, Modern Pathology

TABLE 1
Molecular aberrations in non–small cell lung cancer

Molecular aberration	Frequency in NSCLC (%)	Comment
EGFR mutation	10–16.6	Indicates sensitivity to EGFR inhibitors
EGFR amplification	30.8–59.2	May be associated with response to EGFR inhibitors
EML4-ALK fusion	5–7	Indicates sensitivity to ALK inhibitors (eg, PF-02341066, crizotinib)
KRAS mutation	19–21	Usually in smokers; associated with poor prognosis irrespective of therapy; conflicting data with respect to resistance to EGFR inhibitors
PIK3CA mutation	2	May be involved in EGFR resistance
PIK3CA amplification	12–17	May be involved in EGFR resistance
MET mutation	12–14	Contributes to EGFR resistance
MET amplification	11.1–21	Contributes to EGFR resistance

EML4 = echinoderm microtubule-associated protein-like 4; *ALK* = anaplastic lymphoma kinase; *EGFR* = epidermal growth factor receptor; *KRAS* = GTPase *KRAS*; *MET* = hepatocyte growth factor receptor; NSCLC = non–small cell lung cancer; *PIK3CA* = phosphatidylinositol 3-kinase p110 alpha catalytic subunit isoform

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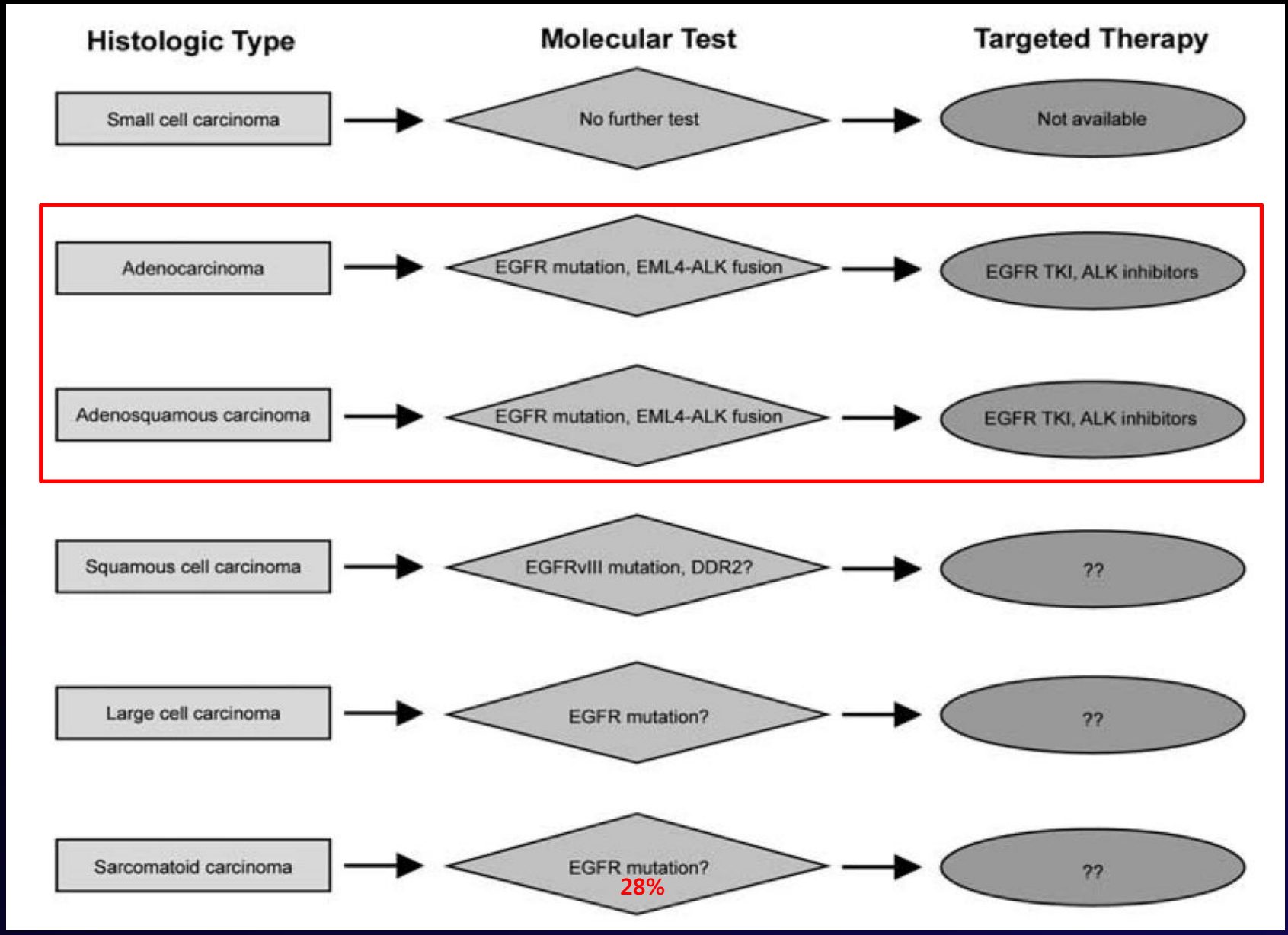
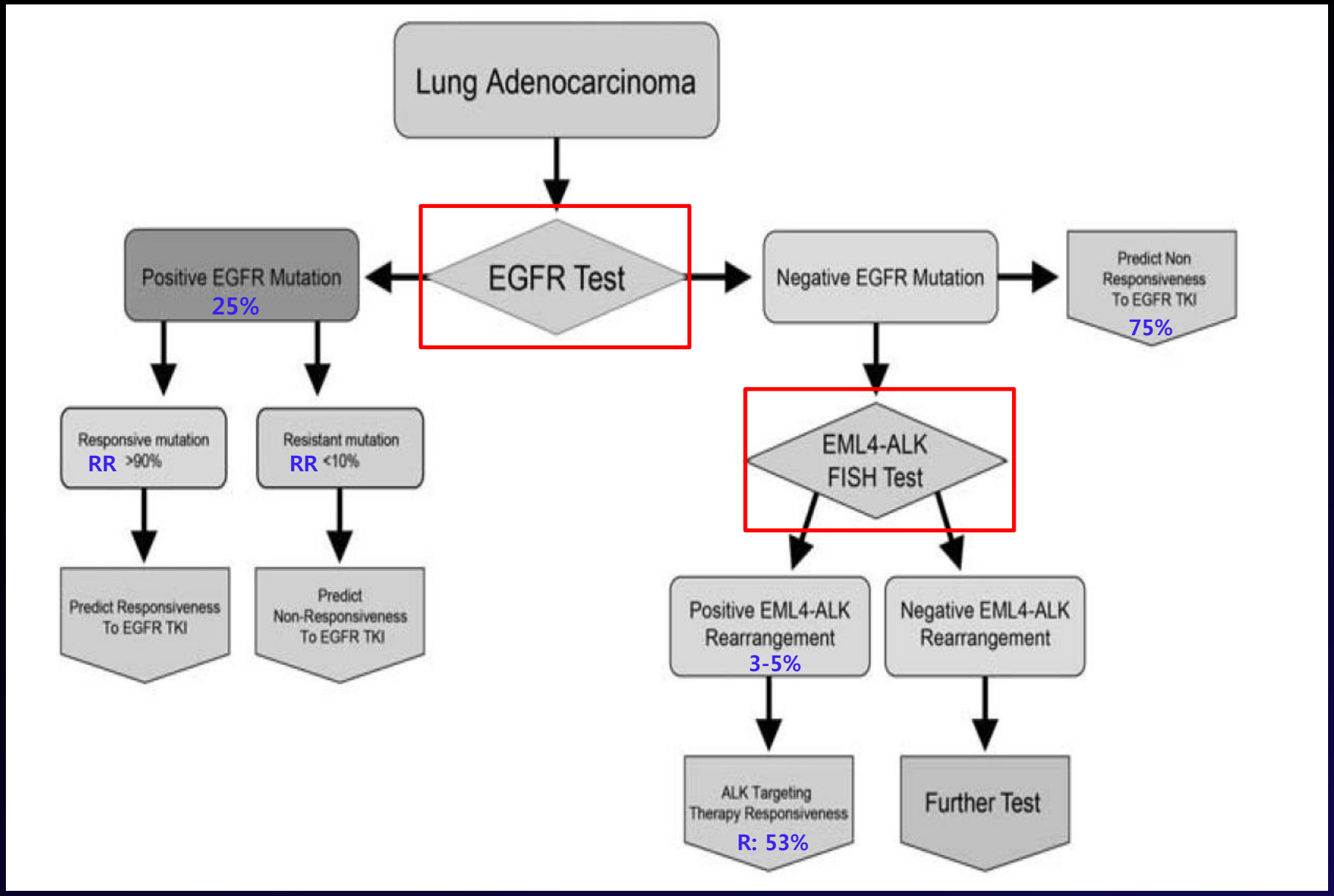
PATRICK, 2012. CLEVELAND CLINIC JOURNAL OF MEDICINE

Current molecular tests and options for targeted therapies



Cheng. 2012, Modern Pathology

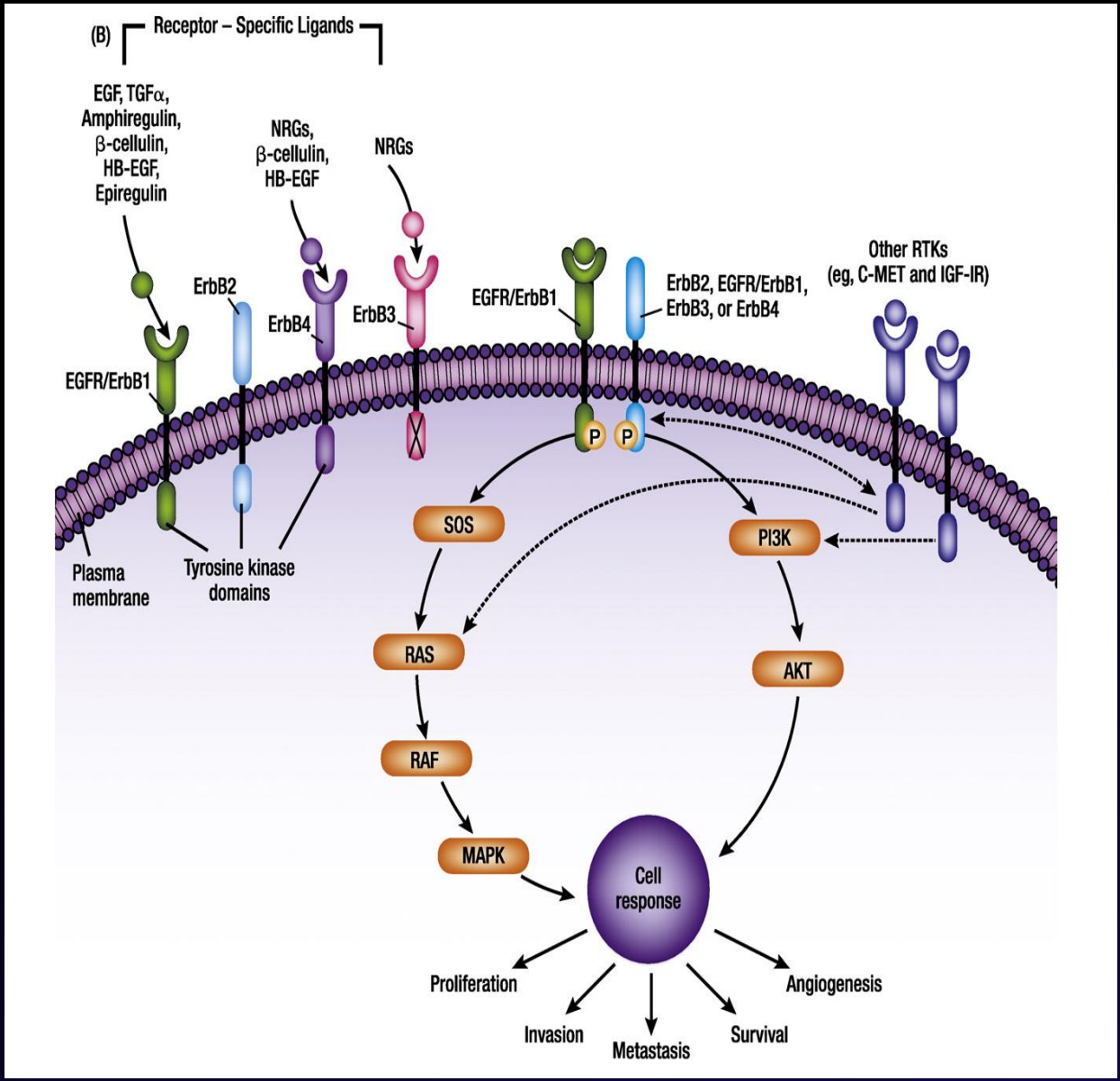
Suggested algorithm for molecular testing for patients with lung adenocarcinoma

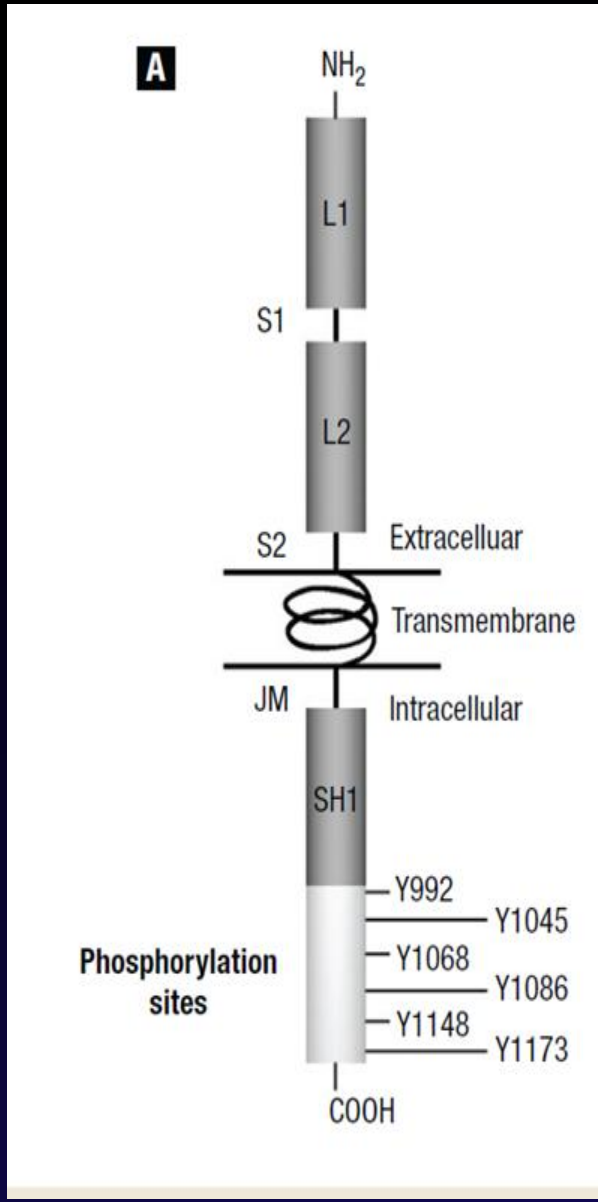


Cheng. 2012, Modern Pathology

EGFR Tyrosine Kinase Inhibitors

Signaling pathways and tyrosine kinase receptors involved in the tumorigenesis of NSCLC





Ou, 2012. Critical Reviews in Oncology/Hematology

Select Phase III Clinical Trials in Lung Cancer Involving EGFR TKIs

Trial	Year	Line	No. of Participants	Race	*EGFR* Mutant (%)	EGFR TKI	Reference Arm	TKI *v* Reference: RR (%)	TKI *v* Reference: CR (%)	TKI *v* Reference: PFS (months)	TKI *v* Reference: OS (months)
ISEL[27]	2005	Second to third	1,692	White, 75%; Asian, 21%*	12.1†	Gefitinib	Placebo	8.0 *v* 1.3	NA	3.0 *v* 2.6	5.6 *v* 5.1
BR.21[28]	2005	Second to third	731	Asian, 12%; other, 88%	23‡	Erlotinib	Placebo	8.9 *v* < 1	0.7 *v* 0	2.2 *v* 1.8	6.7 *v* 4.7
INTEREST[29]	2008	Second	1,433	White, 75%; Asian, 21%*	14.8§	Gefitinib	Docetaxel	9.1 *v* 7.6	NA	2.2 *v* 2.2	7.6 *v* 8.0
IPASS[4,30]	2009	First	1,217	East Asian, 100%	59.7‖	Gefitinib	Platinum doublet	43.0 *v* 32.2	NA	5.7 *v* 5.8	18.8 *v* 17.4
IPASS subgroup[4,30]	2009	First	261	East Asian, 100%	100	Gefitinib	Platinum doublet	71.2 *v* 47.3	NA	9.5 *v* 6.3	21.6 *v* 21.9
WJTOG3405[6,31]	2009	First	172	East Asian, 100%	100	Gefitinib	Platinum doublet	62.1 *v* 32.2	NA	9.2 *v* 6.3	35.5 *v* 38.8
NEJ002[7]	2009	First	224	East Asian, 100%	100	Gefitinib	Platinum doublet	73.7 *v* 30.7	4.4 *v* 0	10.8 *v* 5.4	30.5 *v* 23.6
OPTIMAL[8,32]	2011	First	165	East Asian, 100%	100	Erlotinib	Platinum doublet	82 *v* 36	2 *v* 0	13.1 *v* 4.6	22.7 *v* 28.9
EURTAC[9]	2012	First	174	White, 100% (Hispanic)	100	Erlotinib	Platinum doublet	64 *v* 18	3 *v* 0	9.7 *v* 5.2	19.3 *v* 19.5

Abbreviations: CR, complete response; EGFR, epidermal growth factor receptor; EURTAC, European Tarceva Versus Chemotherapy; INTEREST, IRESSA Non-Small-Cell Lung Cancer Trial Evaluating Response and Survival Against Taxotere; IPASS, Iressa Pan-Asia Study; ISEL, IRESSA Survival Evaluation in Lung Cancer; NA, not applicable; OPTIMAL, Open Label, Phase III Study Comparing First Line Tarceva vs Cisplatin Plus Gemcitabine in Chinese Advanced/Metastatic Non-Small-Cell Lung Cancer Patients With EGFR Activating Mutations; OS, overall survival; PFS, progression-free survival; RR, response rate; TKI, tyrosine kinase inhibitor.

*Excludes people of Indian origin.

†26 positive in 215 tested samples.

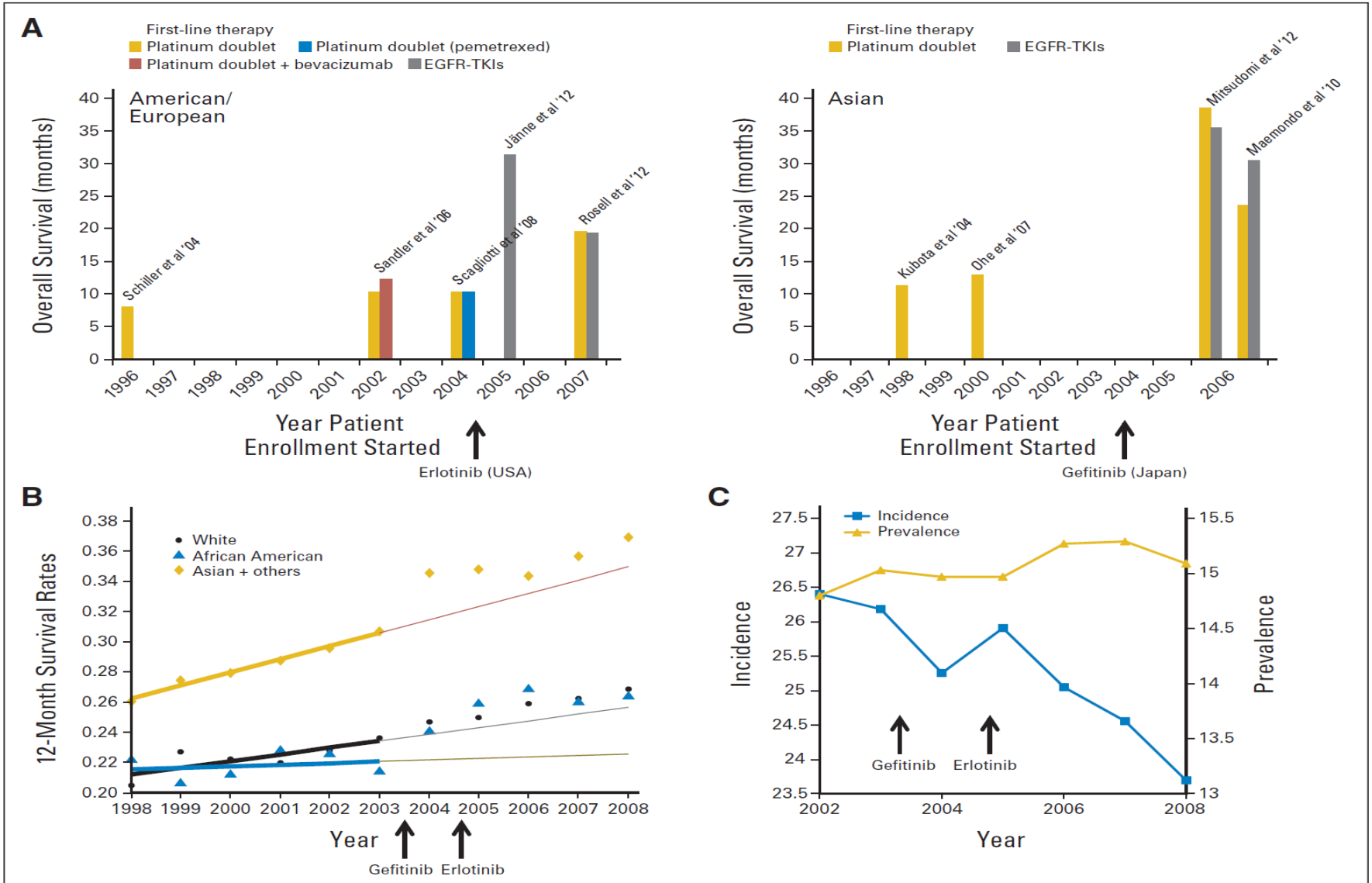
‡40 positive in 177 tested samples.

§44 positive in 297 tested samples.

‖261 positive in 437 tested samples.

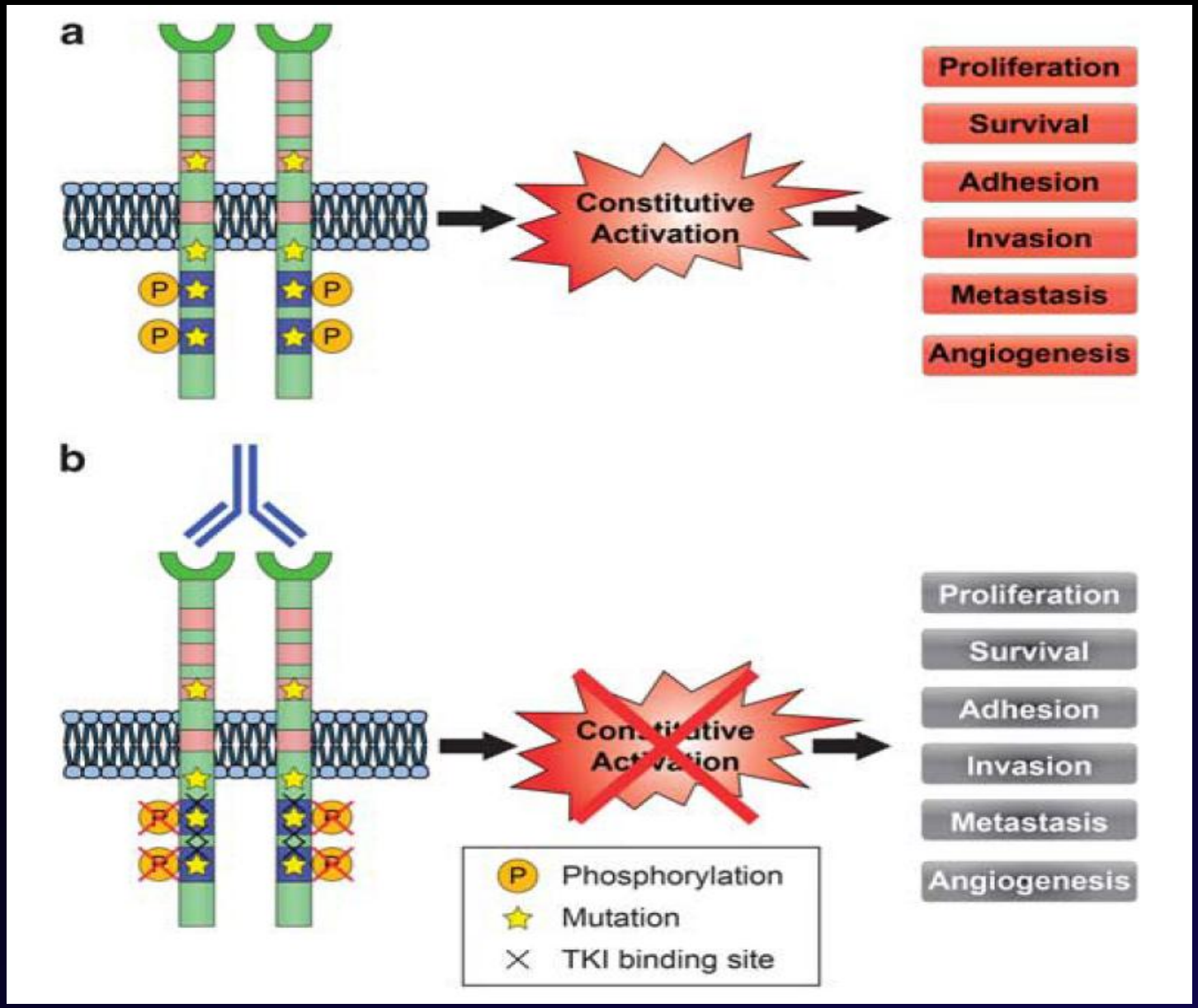
Ohashi , 2013. JCO

Impact of EGFR-TKIs on survival in patients with lung cancers harboring *EGFR* mutations



Ohashi , 2013. JCO

Mechanism of constitutive activation of EGFR results from EGFR mutation and strategies of anti-EGFR therapy



Cheng. 2012, Modern Pathology

Acquired Resistance from Reversible TKIs

- 12-month rates of progression-free survival were 24.9% with gefitinib.

  Mok, 2009. NEJM

- Median progression-free survival for 217 patients who received erlotinib were 14 months.

  Rosell, 2009 NEJM

- Despite this initial response, patients with NSCLCs containing *EGFR* mutations acquire resistance to EGFR inhibitors, and the median time to disease progression is **about 12 months**

  Sequist, 2011. Sci Transl Med.

Genotypic and Histological Evolution of Lung Cancers Acquiring Resistance to EGFR Inhibitors

Lecia V. Sequist[1,2,†,*], Belinda A. Waltman[2,*], Dora Dias-Santagata[2,3,*], Subba Digumarthy[2,4], Alexa B. Turke[1,2], Panos Fidias[1,2], Kristin Bergethon[3], Alice T. Shaw[1,2], Scott Gettinger[5], Arjola K. Cosper[1], Sara Akhavanfard[2,3], Rebecca S. Heist[1,2], Jennifer Temel[1,2], James G. Christensen[6], John C. Wain[1,2,7], Thomas J. Lynch[5], Kathy Vernovsky[1], Eugene J. Mark[2,3], Michael Lanuti[1,2,7], A. John Iafrate[2,3], Mari Mino-Kenudson[2,3], and Jeffrey A. Engelman[1,2,†]

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Sequist, 2011. Sci Transl Med

Genotypic and Histological Evolution of Lung Cancers Acquiring Resistance to EGFR Inhibitors

Patients

- All 43 consecutive *EGFR*-mutant NSCLC patients with acquired EGFR TKI resistance
- Bx: core biopsies whenever possible

  fine-needle aspiration
- feasibility of repeat biopsy and comparative molecular analysis: 37/43 or 86%.

Sequist, 2011. Sci Transl Med

Biopsies of Resistant Cancers

- biopsies at the time that drug resistance was acquired.
- 37 patients had tumor tissue available both before and after TKI treatment. (15 men and 22 women)
- All patients had activating *EGFR* mutations;

  20 (54%) had an exon 19 deletion mutation

  15 (41%) had the exon 21 point mutation L858R.
- responded clinically to either gefitinib (5) or erlotinib (32).
- The median duration of primary TKI therapy: 14.1 Ms(4 to 69 Ms)

  1- or 2-year PFR: 64 or 30%, respectively.

Sequist, 2011. Sci Transl Med

Biopsies of Resistant Cancers

- Most patients (78%) were still taking an EGFR TKI at the time of repeat biopsy, and biopsies were performed a median of 30 months (range,5 to 99 months) after original diagnosis.

- Only four patients received chemotherapy between the development of resistance and the repeat biopsy.

- Anatomic sites of repeat biopsy most commonly included lung lesions (38%), liver lesions (16%), and mediastinal or cervical lymph nodes (16%).

Sequist, 2011. Sci Transl Med

Biopsies of Resistant Cancers

- Most biopsies (68%) were percutaneous with either CT or ultrasound guidance, but some were performed via bronchoscopy, mediastinoscopy, or another surgical procedure.

- There were no major biopsy-related complications, including no cases of clinically significant bleeding, pneumothorax, or unanticipated hospital admission.

Sequist, 2011. Sci Transl Med

Thirty-seven paired lung tumor biopsies resistant to EGFR inhibitors

ID#	Age	Sex	*EGFR* mutation	Baseline histology	Summary of changes	Primary TKI (time on TKI)	TKI status at repeat biopsy
					T790M		
1	66	M	L858R	Adeno	T790M	Erlo (6 months)	Off (2 months)
2	74	F	Exon 19 del	Adeno	T790M	Erlo (12 months)	On
3	47	F	Exon 19 del	Adeno	T790M	Gef (15 months)	On
4	60	F	Exon 19 del	Adeno	T790M	Erlo (7 months)	On
5	57	M	L858R	Adeno	T790M	Gef (5+ years)	On
6	47	M	Exon 19 del	Adeno	T790M	Erlo (12 months)	Off (14 months)
7	58	F	Exon 19 del	Adeno	T790M	Erlo (3+ years)	On
8	69	M	L858R	Adeno	T790M*	Erlo (2 years)	On
9	58	F	G719C, S768I	Adeno	T790M	Erlo (2+ years)	On
10	46	F	Exon 19 del	Adeno	T790M	Erlo (3 years)	Off (3 months)
11	53	F	Exon 19 del	Adeno	T790M	Erlo (16 months)	On
12	59	F	L858R	Adeno	T790M	Erlo (8 months)	Off (5 months)
					T790M + *EGFR* amp		
13	42	M	Exon 19 del	Adeno	T790M, *EGFR* amp	Erlo (5 months)	On
14	55	M	Exon 19 del	Adeno	T790M, *EGFR* amp	Erlo (10 months)	On
15	37	F	Exon 19 del	Adeno	T790M, *EGFR* amp	Erlo (6 months)	On
					T790M + new, additional mutations		
16	88	F	Exon 19 del	Adeno	T790M, β-*catenin*	Erlo (2+ years)	On
17	85	M	Exon 19 del	Adeno	T790M, β-*catenin*	Erlo (22 months)	On
18	75	F	Exon 19 del	Adeno	T790M, *APC*†	Erlo (18 months)	On
					***MET* amplification**		
19	61	M	L858R	Adenosquam	*MET* amp, loss *EGFR* amp	Erlo (15 months)	On
20	76	M	L858R	Adeno	*MET* amp	Erlo (13 months)	Off (5 months)
					Acquired *PIK3CA* mutation		
21	65	M	Exon 19 del	Adeno	*PIK3CA* acquisition	Erlo (21 months)	On
					Histologic transformation (one with acquired *PIK3CA* mutation)		

Sequist, 2011. Sci Transl Med

Thirty-seven paired lung tumor biopsies resistant to EGFR inhibitors

ID#	Age	Sex	*EGFR* mutation	Baseline histology	Summary of changes	Primary TKI (time on TKI)	TKI status at repeat biopsy
22	67	F	L858R	Adeno	SCLC transformation	Erlo (22 months)	On
23	54	F	Exon 19 del	Adeno	SCLC transformation	Erlo (3+ years)	On
24	56	F	L858R	Adeno	SCLC transformation, *PIK3CA*	Erlo (14 months)	On
25	40	F	Exon 19 del	Adeno	SCLC transformation	Erlo (2+ years)	Off (2 months)
26	61	F	L858R	Adeno	SCLC transformation	Erlo (18 months)	On
27	66	M	L858R	Adeno	EMT	Erlo (11 months)	On
28	59	M	Exon 20 ins‡	Adeno	EMT	Gef (11 months)	On
29	64	M	L858R	Adeno	Sarcomatoid CA, loss of β-*catenin*	Erlo (11 months)	Off (2 weeks)
					No histological or genetic changes identified		
30	62	F	L858R	Adeno	None	Erlo (6 months)	On
31	52	F	Exon 19 del	Adeno	None	Gef (17 months)	On
32	58	F	Exon 19 del	Adeno	None	Erlo (14 months)	On
33	61	F	L858R	Adeno	None	Erlo (13 months)	On
34	85	F	Exon 19 del	Adeno	None	Erlo (6 months)	On
35	62	M	L858R	NSCLC	None	Gef (3+ years)	On
36	56	M	L858R	Adeno	None	Erlo (5 months)	Off (<2 weeks)
37	51	F	Exon 19 del	Adeno	None	Erlo (8 months)	On

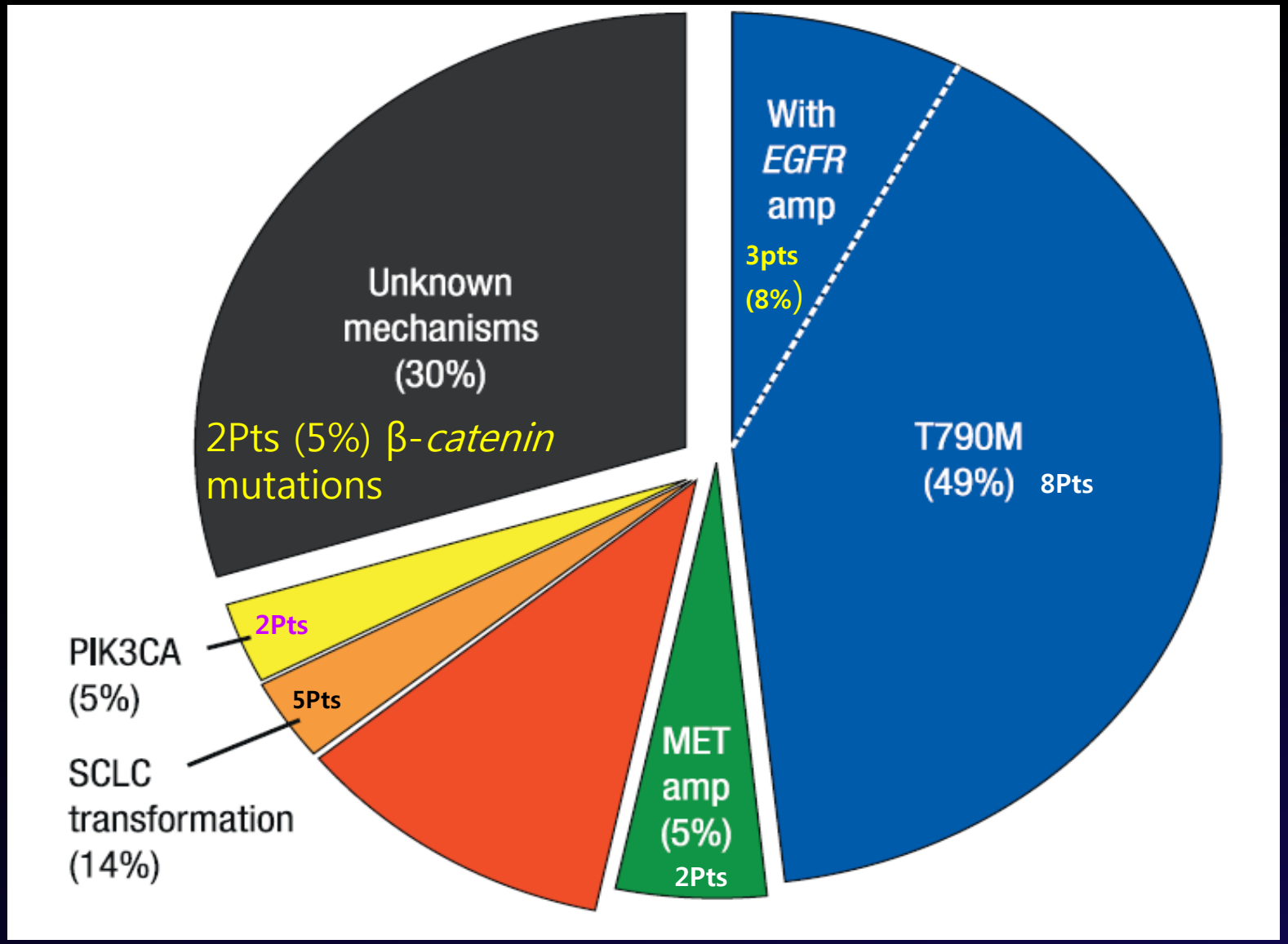
* *TP53* mutation suspected to be present, but not confirmed.

† *APC* mutation confirmed in resistant specimen, but not confirmed to be present in initial biopsy.

‡ Exon 20 insertion assay added to SNaPshot for this patient given direct sequencing result from pretreatment sample.

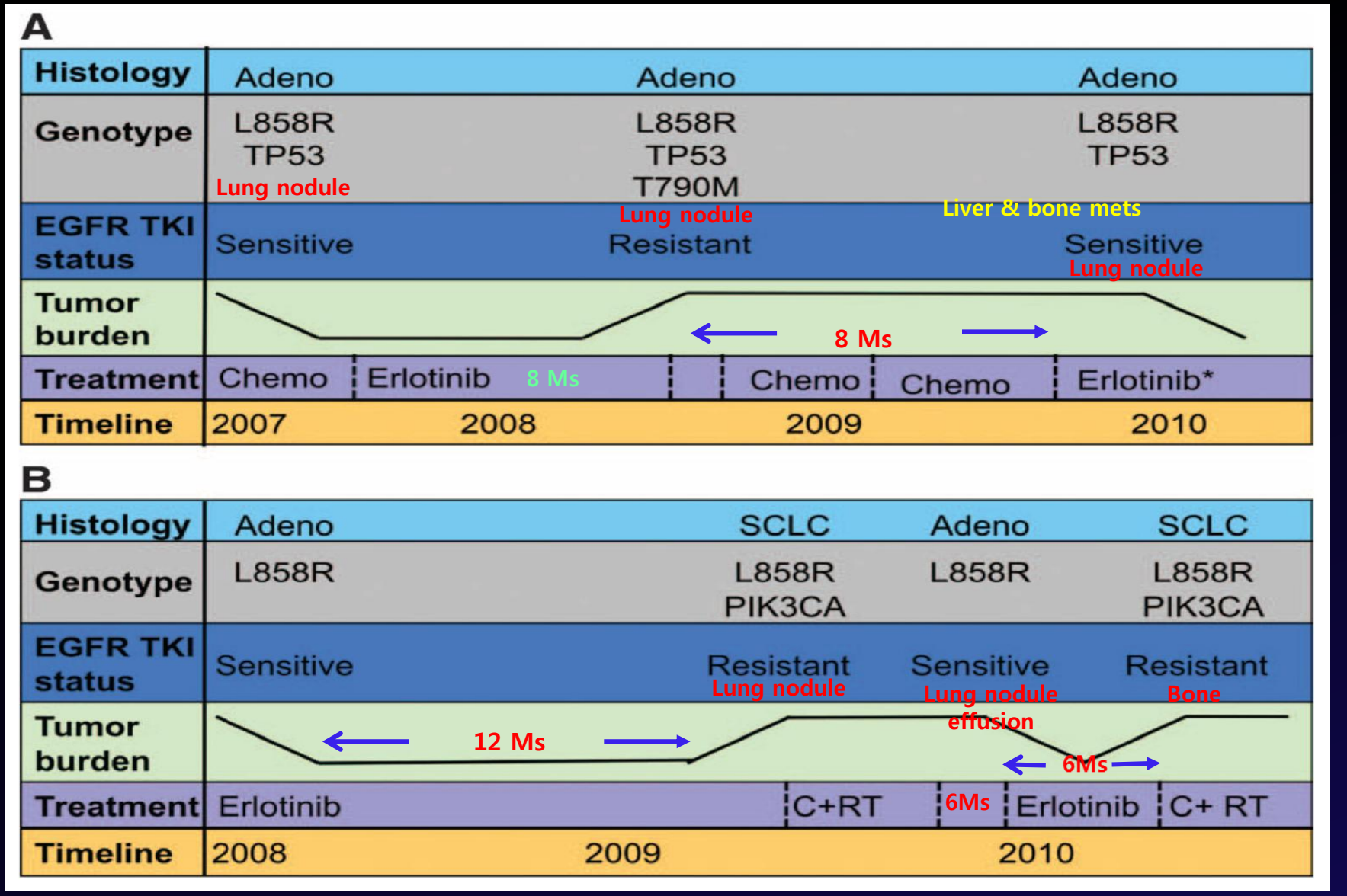
Sequist, 2011. Sci Transl Med

Prevalence of Acquired Drug Resistance Mechanisms



Sequist, 2011. Sci Transl Med

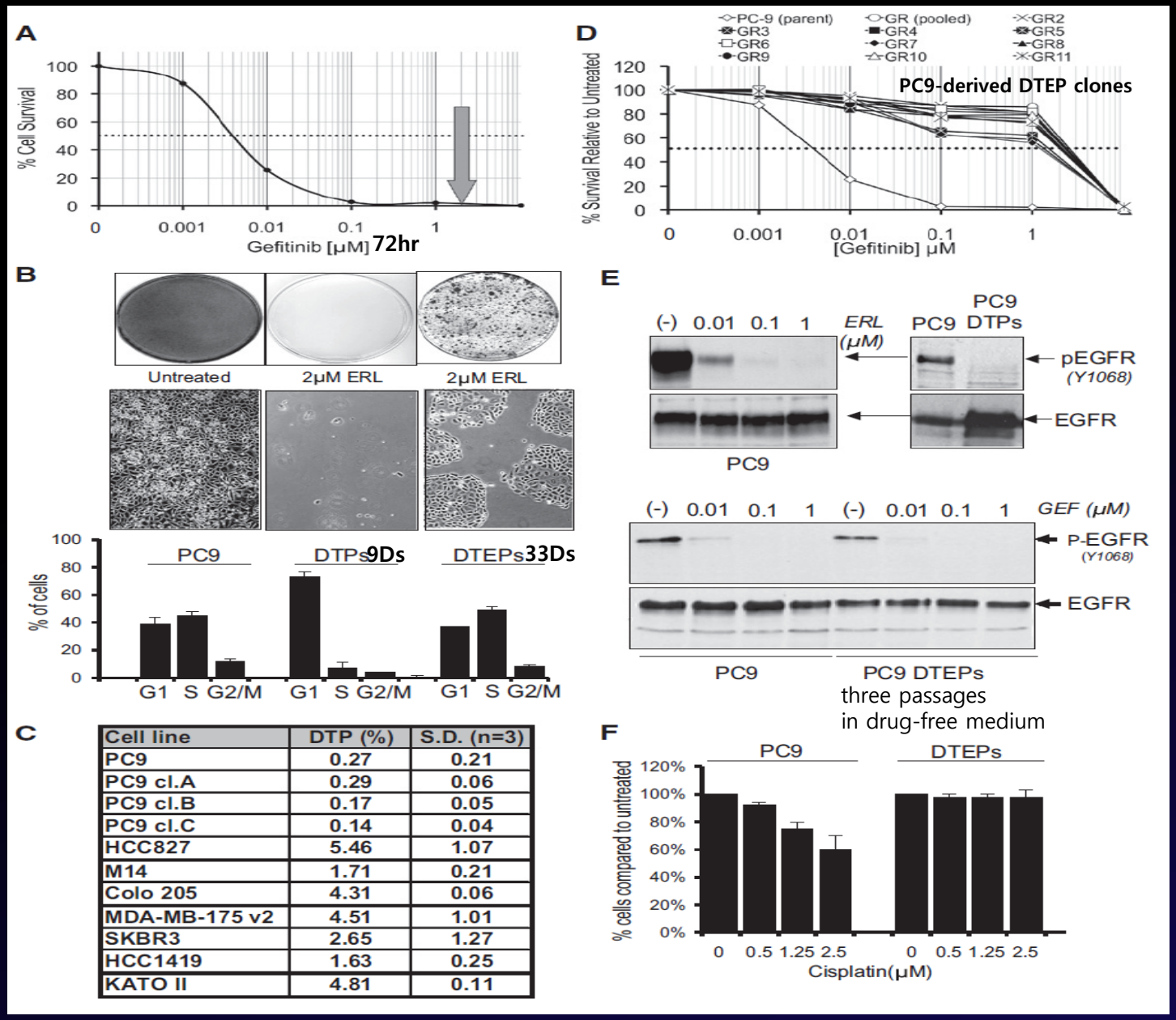
Longitudinal genotypic and phenotypic changes in response to EGFR TKI



* erlotinib plus an investigational agent that does not target T790M

Sequist, 2011. Sci Transl Med

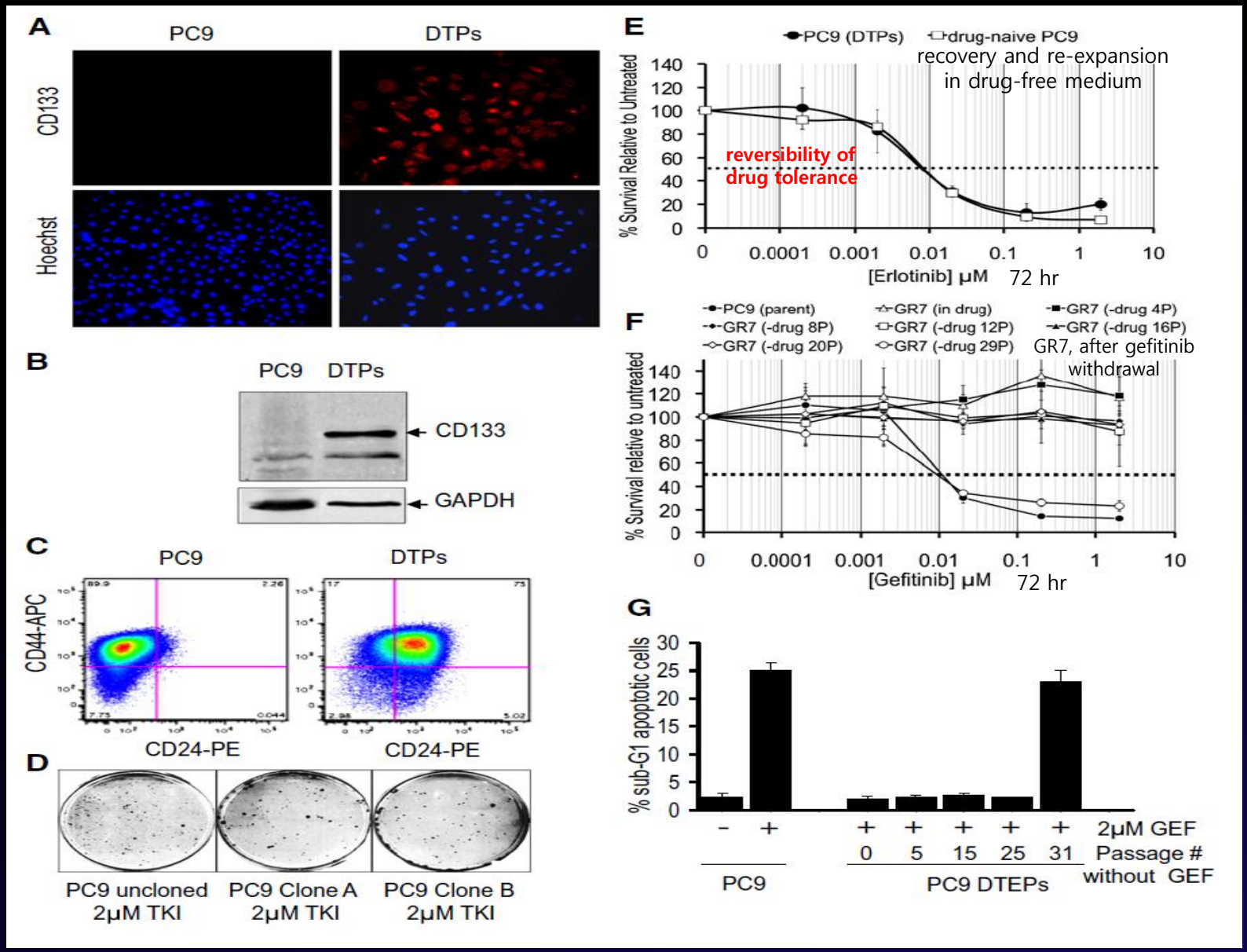
Detection of a Drug-Tolerant Subpopulation of Cancer Cells



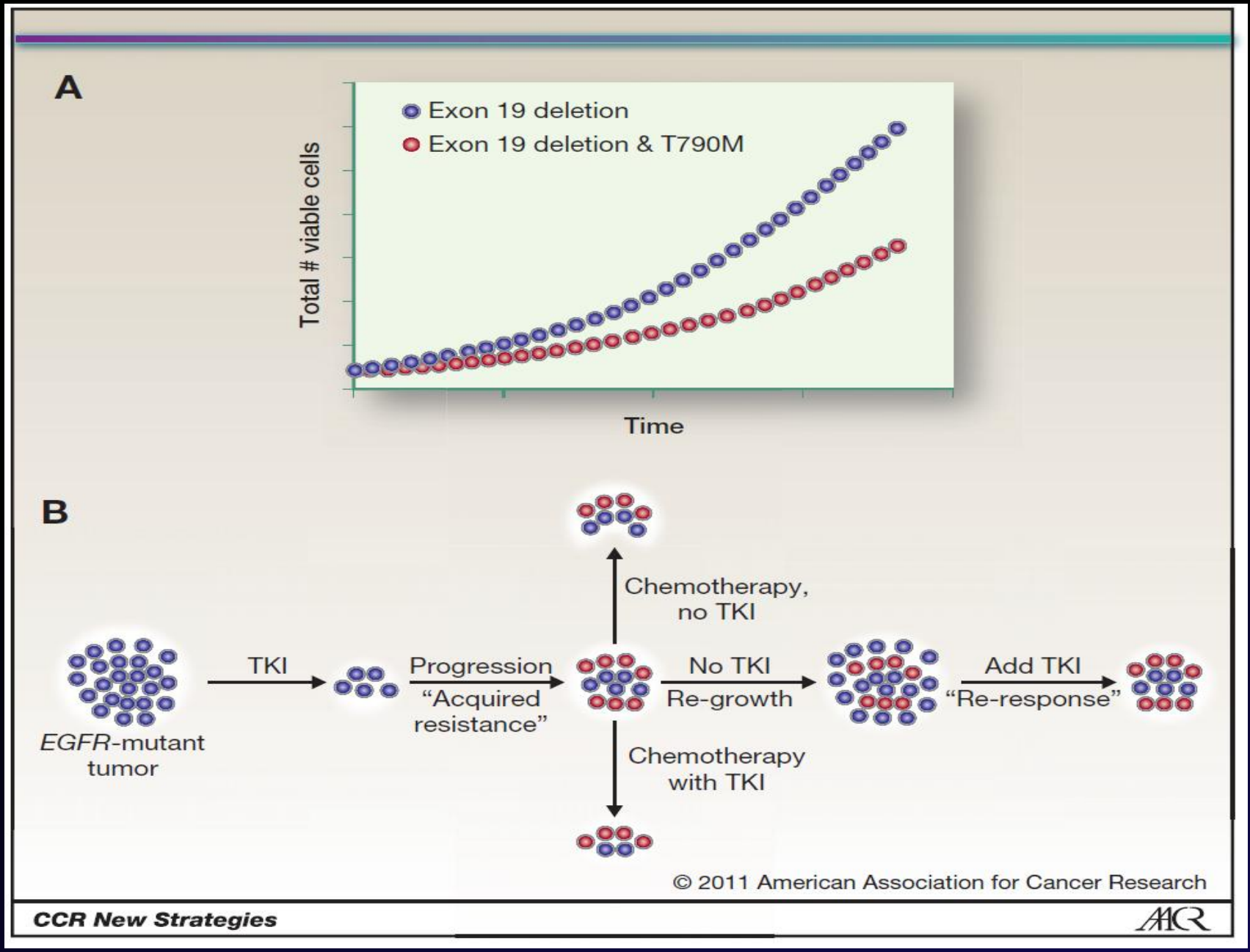
Cell line	DTP (%)	S.D. (n=3)
PC9	0.27	0.21
PC9 cl.A	0.29	0.06
PC9 cl.B	0.17	0.05
PC9 cl.C	0.14	0.04
HCC827	5.46	1.07
M14	1.71	0.21
Colo 205	4.31	0.06
MDA-MB-175 v2	4.51	1.01
SKBR3	2.65	1.27
HCC1419	1.63	0.25
KATO II	4.81	0.11

Sharma, 2010. Cell

Heterogeneity and Reversibility in the Drug-Tolerant Cell Populations



Sharma, 2010. Cell



Oxnard, CCR. 2011

Longitudinal genotypic and phenotypic changes in response to EGFR TKI

- Withdrawal of the TKI may permit their rapid expansion to a degree that overtakes the bulk of the tumor burden.
- these findings confirm that even **"genetic" mechanisms of resistance are potentially reversible.**
- a static diagnostic biopsy may be insufficient to guide **therapeutic decision** making throughout the course of a patient's disease.
- all of our patients experienced a second response to erlotinib when their resistance mechanism was no longer detectable, suggesting that **repeat biopsies** can provide molecular guidance about the likely benefit of a second treatment regimen with EGFR TKI therapy.

Sequist, 2011. Sci Transl Med

Resistance Mechanism for EGFR Tyrosine Kinase Inhibitors

a. Primary resistance to EGFR-TKIs

b. Acquired resistance to EGFR-TKIs

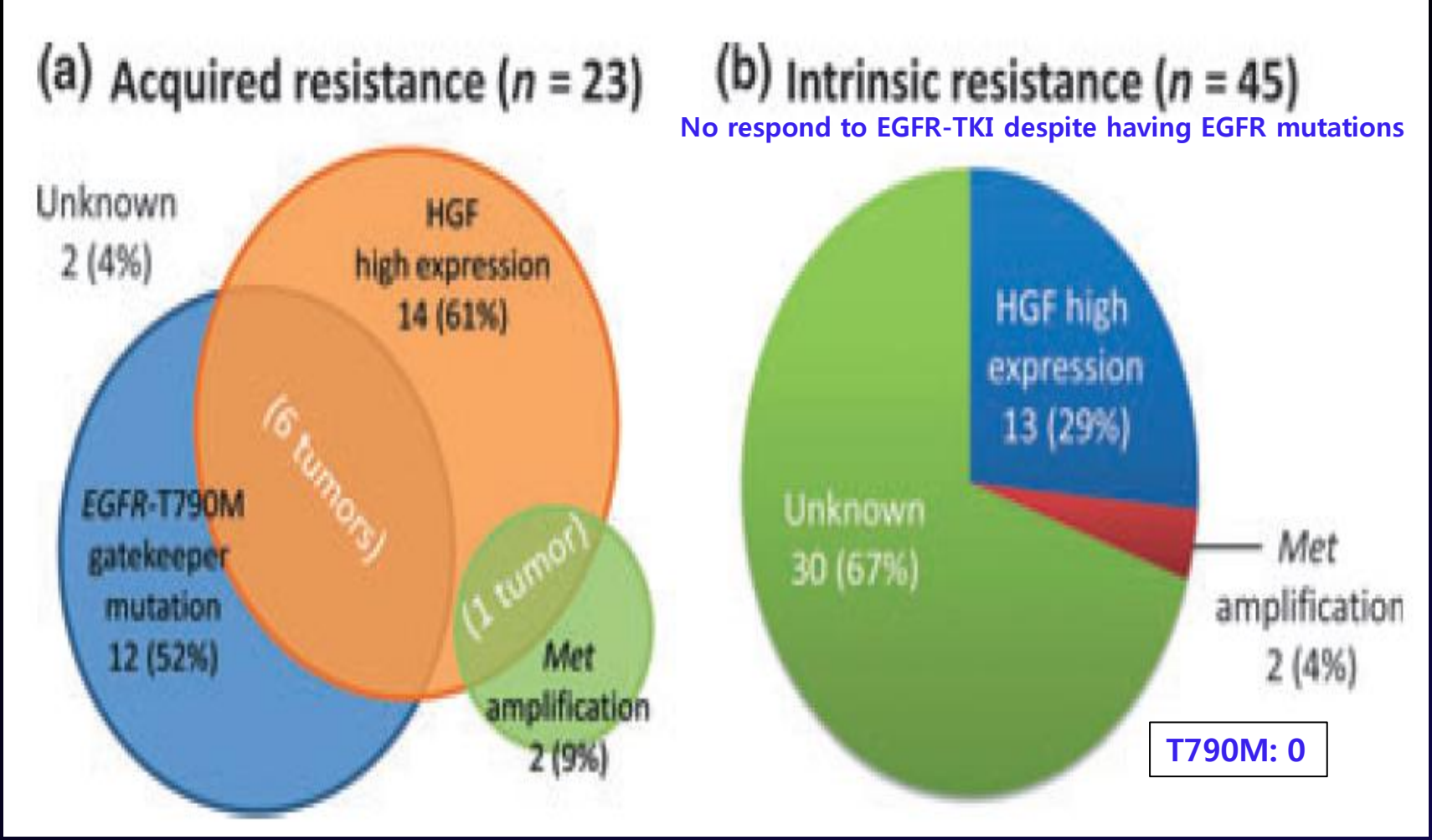
Major mechanisms of EGFR-TKI resistance in EGFR mutant lung cancer

	Acquired resistance	Intrinsic resistance	Reversible resistance	Reference
Alteration of target gene				
Gatekeeper mutation (secondary mutation:T790M)	○		?	20, 21
Activation of bypass signal				
Receptor gene amplification (Met)	○			23
Activation of ligand (HGF)	○	○	?	11
Receptor activation by epigenetic mechanism			○	50
Alteration of downstream				
PIK3CA mutation	○			26
PTEN loss	○			25
BIM suppression		○		27
Others				
SCLC transformation	○			26
EMT	○			26

○, involved. EMT, epithelial-to-mesenchymal transition; HGF, hepatocyte growth factor; PTEN, phosphatase and tensin homolog; SCLC, small cell lung cancer.

Yano, 2012, Cancer science

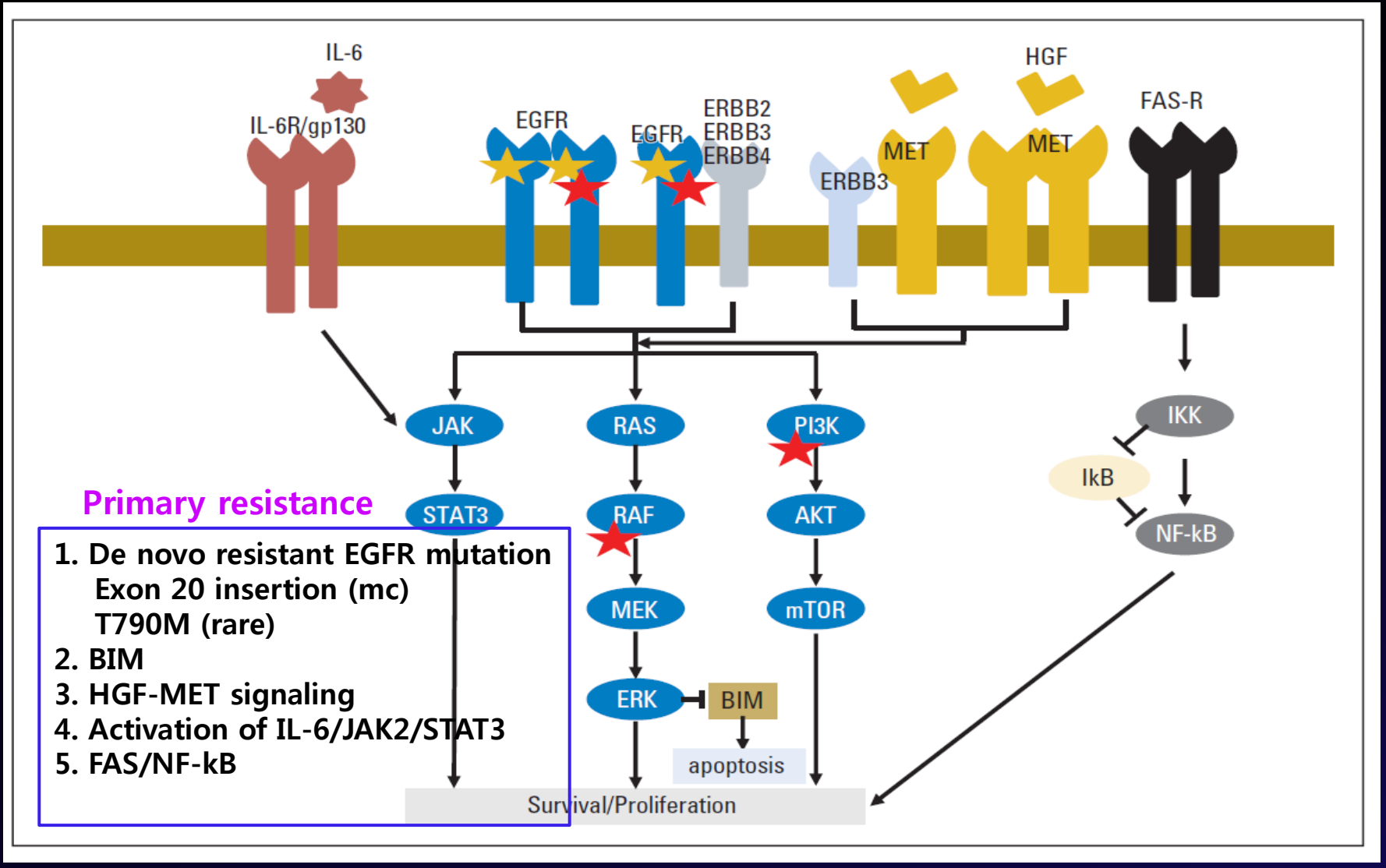
Incidence of resistance factors in EGFR mutant lung cancer resistant to EGFR-TKIs



Results of a joint study of Japanese patients with EGFR-mutant lung cancer conducted at 12 facilities.

Yano, 2012, Cancer science

Schematic representation of the EGFR signaling pathway and Molecules that may affect drug resistance



Ohashi , 2013. JCO

Mechanism of Primary Resistance

a. K-Ras mutation

b. ErbB family members:

mutation and amplification

c. Loss of PTEN

2.4 CRKL amplification

Nakata, 2012. Expert Opin. Ther. Targets

I. K-Ras Mutation

Table 2 *EGFR* and *KRAS* Mutations Associated With EGFR TKI Resistance in NSCLC

Gene	Mutation
EGFR[a]	
Exon 19	D761Y (P/A), L747S[119] (A)
Exon 20	T790M[b] (P/A)
	D770_N771 (ins NPG) (P)
	D770_N771 (ins SVQ) (P)
	D770_N771 (ins G) (P)
	N771T (P)
	V769L (P)
	S768I (P)
Exon 21	T854A[60] (A)
KRAS[c]	
Exon 2	G12C
	G12D
	G12S
	G12V
	G12A
	G13C

Abbreviations: *EGFR* = epidermal growth factor receptor; *KRAS* = Kirsten rat sarcoma viral oncogene homolog; NSCLC = non–small-cell lung cancer; TKI = tyrosine kinase inhibitor; P = primary resistance; A = acquired resistance.

[a] Data on *EGFR* resistance mutations from Sharma et al.[15]

[b] The most clinically relevant resistance mutation is T790M, which is associated with approximately 50% of acquired resistance cases.

[c] Data on *KRAS* resistance mutations from Massarelli et al[41] and Pao et al.[42]

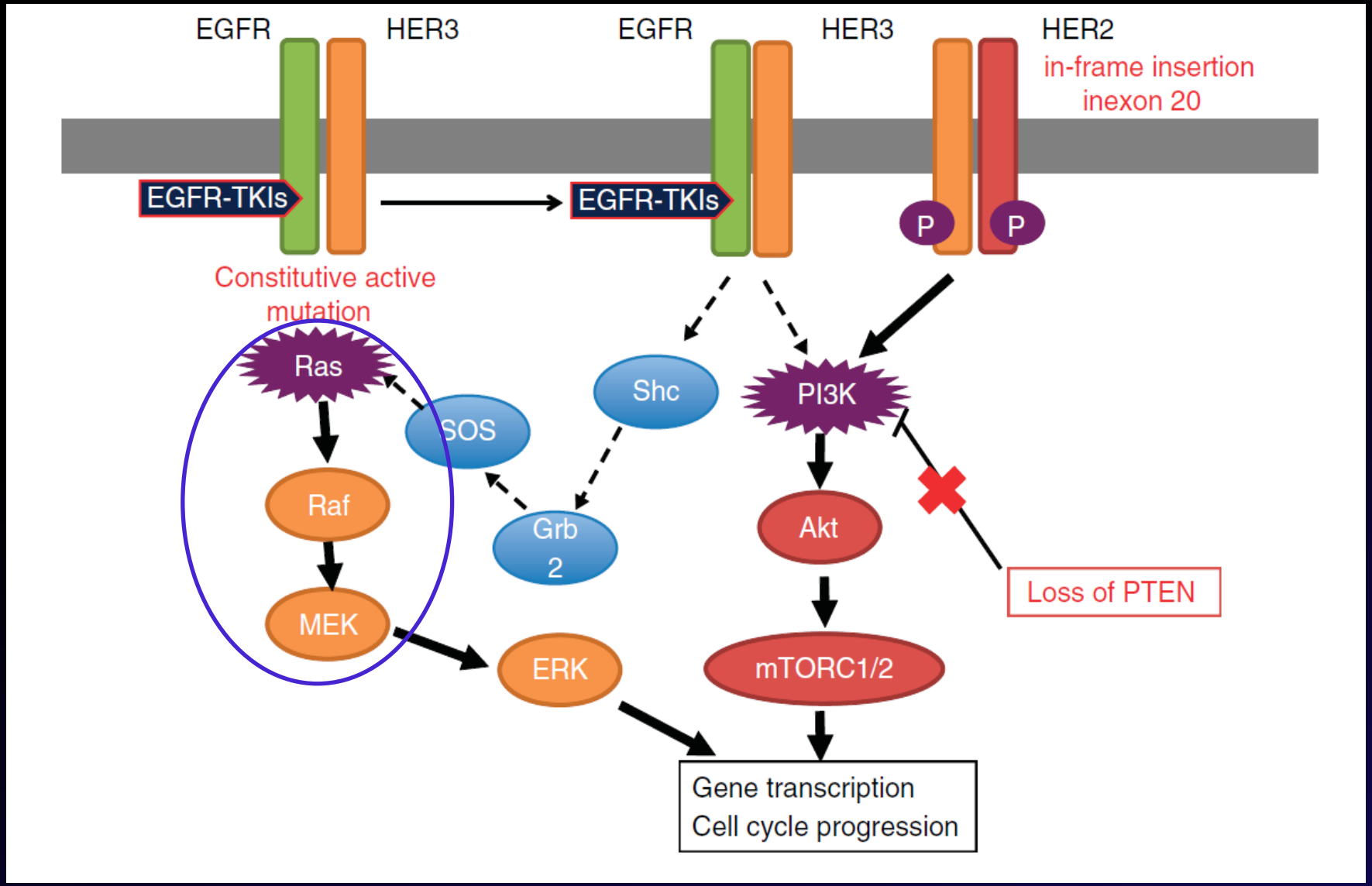
Bar, 2012. Clinical Lung Cancer

K-Ras mutation

- K-Ras mutations were associated with a lack of tumor response to EGFR-TKIs.
- none of the 9 tumors with K-Ras mutations analyzed responded to EGFR-TKI treatment.
- K- Ras mutations are mutually exclusive with mutations of the EGFR gene and that NSCLC patients with K-Ras mutations have decreased sensitivity to EGFR-TKIs.

Pao, 2005 PLoS Med

Mechanism of Primary Resistance of EGFR-TKIs



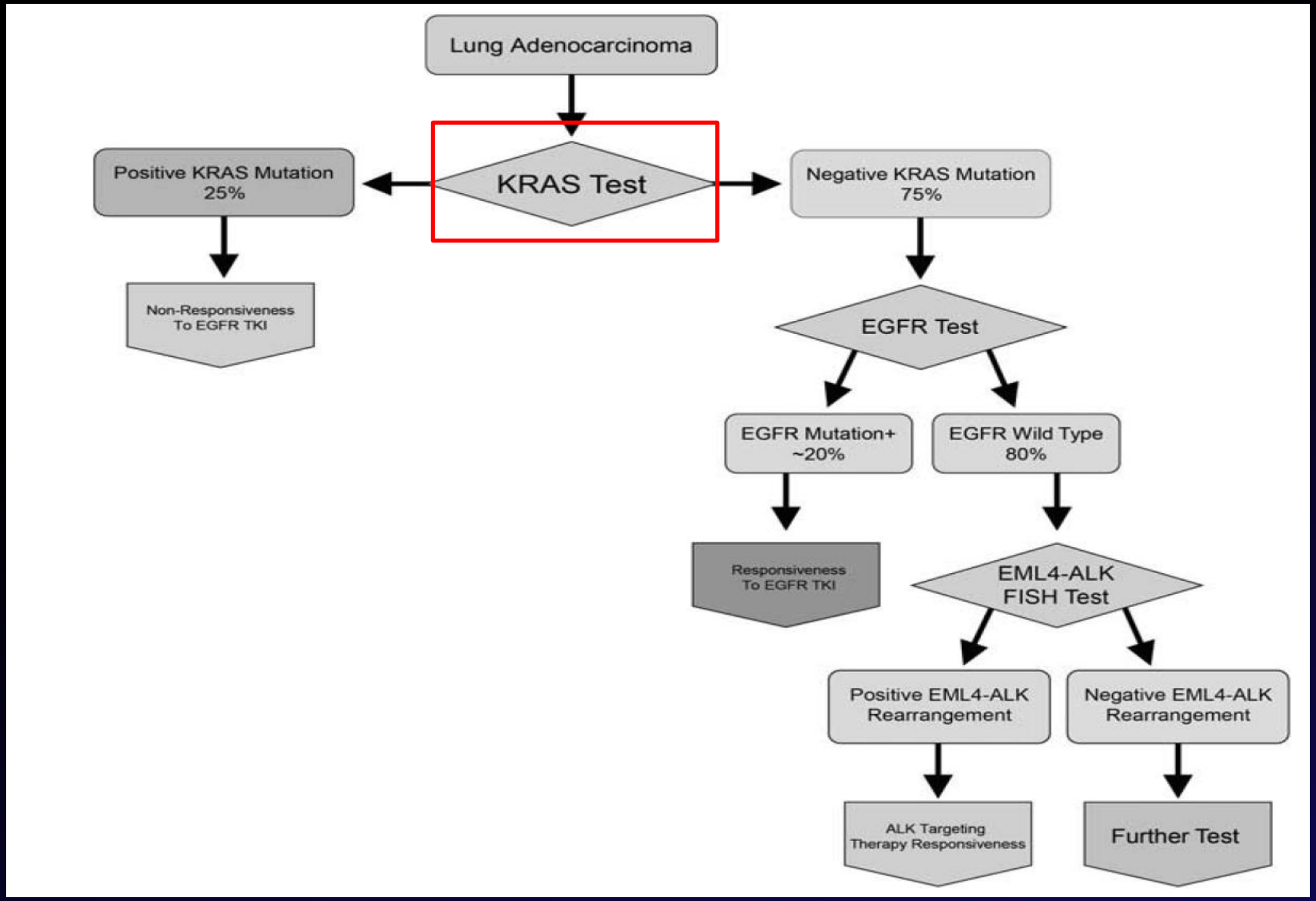
Nakata, 2012. Expert Opin. Ther. Targets

K-Ras mutation

It may be beneficial for patients who have K-ras mutations to avoid EGFR-TKI therapy by screening for K-Ras mutations in cancer tissues.

Nakata, 2012. Expert Opin. Ther. Targets

Alternative algorithm for molecular testing for patients with lung adenocarcinomas



Cheng. 2012, Modern Pathology

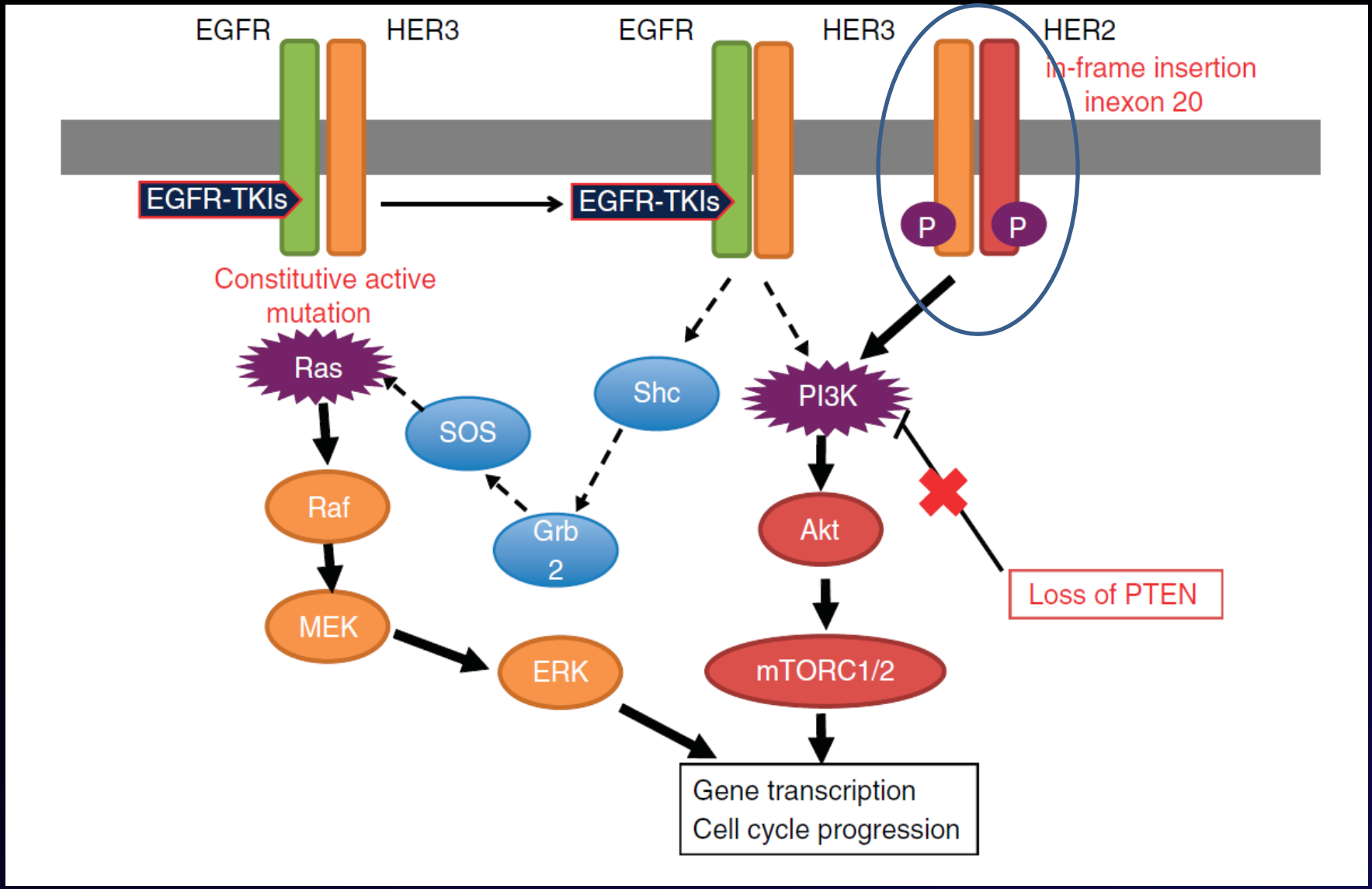
II. Erb B Family members: Mutation and Amplification

Erb B2 (HER 2): Mutation and Amplification

- Somatic mutations of the HER2 gene were identified in a very small fraction of lung adenocarcinomas.
- the mutations of HER2 are mutually exclusive with those of EGFR.
- Most types of HER 2 mutations are in-frame insertion mutations in exon 20, leading to constitutively activate the HER2 kinase.
- HER2 amplification is associated with the sensitivity to EGFR-TKIs in NSCLC patients with EGFR mutations, indicating that HER2 amplification could be associated with gefitinib sensitivity.

Nakata, 2012. Expert Opin. Ther. Targets

The mechanism of Primary Resistance of EGFR-TKIs



Nakata, 2012. Expert Opin. Ther. Targets

ErbB family members: mutation and amplification

- Expression levels of **phospho-HER2 and total HER3 protein** are associated with resistance to gefitinib in head and neck squamous cell carcinoma (HNSCC) cell line.
- Mutations in HER4, another ErbB family member, have been detected in NSCLC patients, but neither their biological characteristics nor their response to EGFR-TKIs has been studied.

Nakata, 2012. Expert Opin. Ther. Targets

III. Loss of PTEN

Loss of PTEN

- Somatic mutations of the PTEN gene are often found in various types of human cancers, including NSCLCs.
- Loss of PTEN function results in Akt hyperactivation caused by an increased concentration of PIP3.
- Mutations of the PTEN gene in NSCLCs are associated with resistance to EGFR-TKIs.

Nakata, 2012. Expert Opin. Ther. Targets

Loss of PTEN

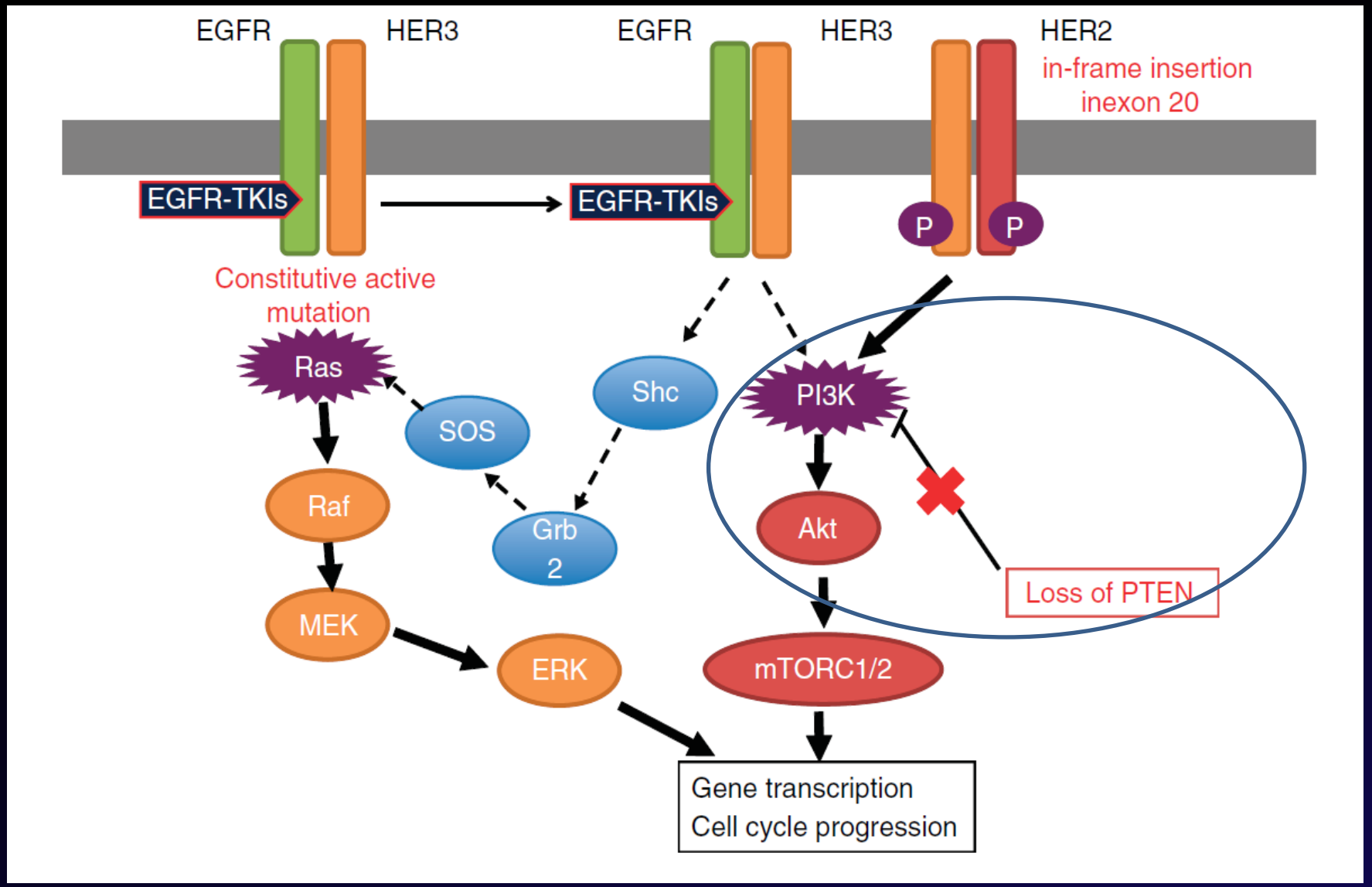
- Akt is activated independent of EGFR signaling in resistant cells, and reintroduction of a PTEN gene restores gefitinib-induced Akt inhibition, indicating that loss of PTEN is associated with acquired resistance.

  Bianco, 2003. Oncogene

- Downregulation of PTEN expression and expression of constitutive active form of Akt is modestly upregulated in erlotinib-resistant A-431 cells.

  Yamasaki, 2007, Cancer Res.

Mechanism of Primary Resistance of EGFR-TKIs



Nakata, 2012. Expert Opin. Ther. Targets

Mechanism of Secondary Resistance

a. Secondary T790M mutation of the EGFR gene

b. Amplification of the MET oncogene

c. overexpression of hepatocyte growth factor (HGF)

d. Insulin-like growth factor 1 receptor signaling

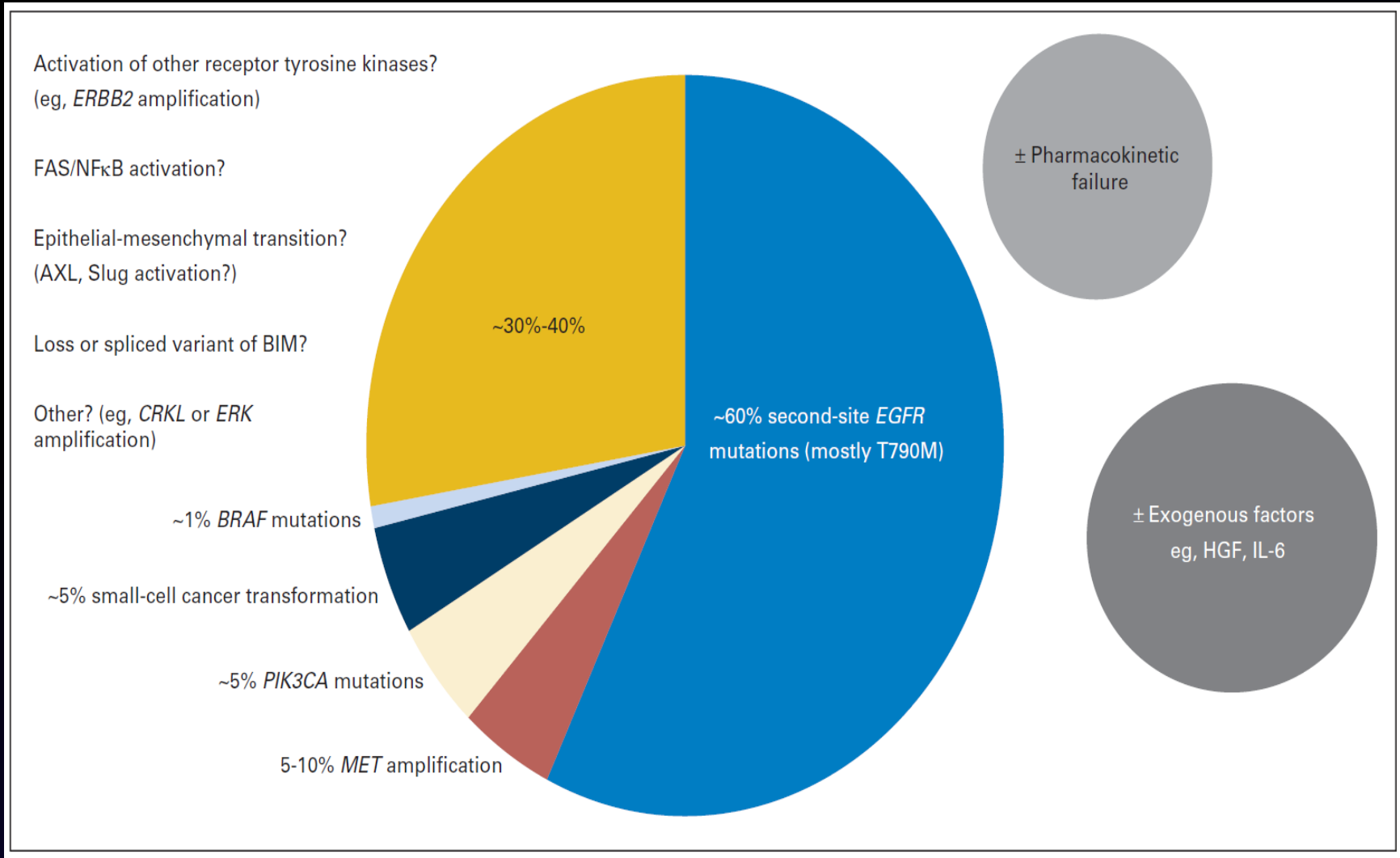
e. PI3KCA

e. Small cell histology

f. EMT

g, Miscellanous (microRNA, CRKL amplification, FAS–NFκB activation, autophagy)

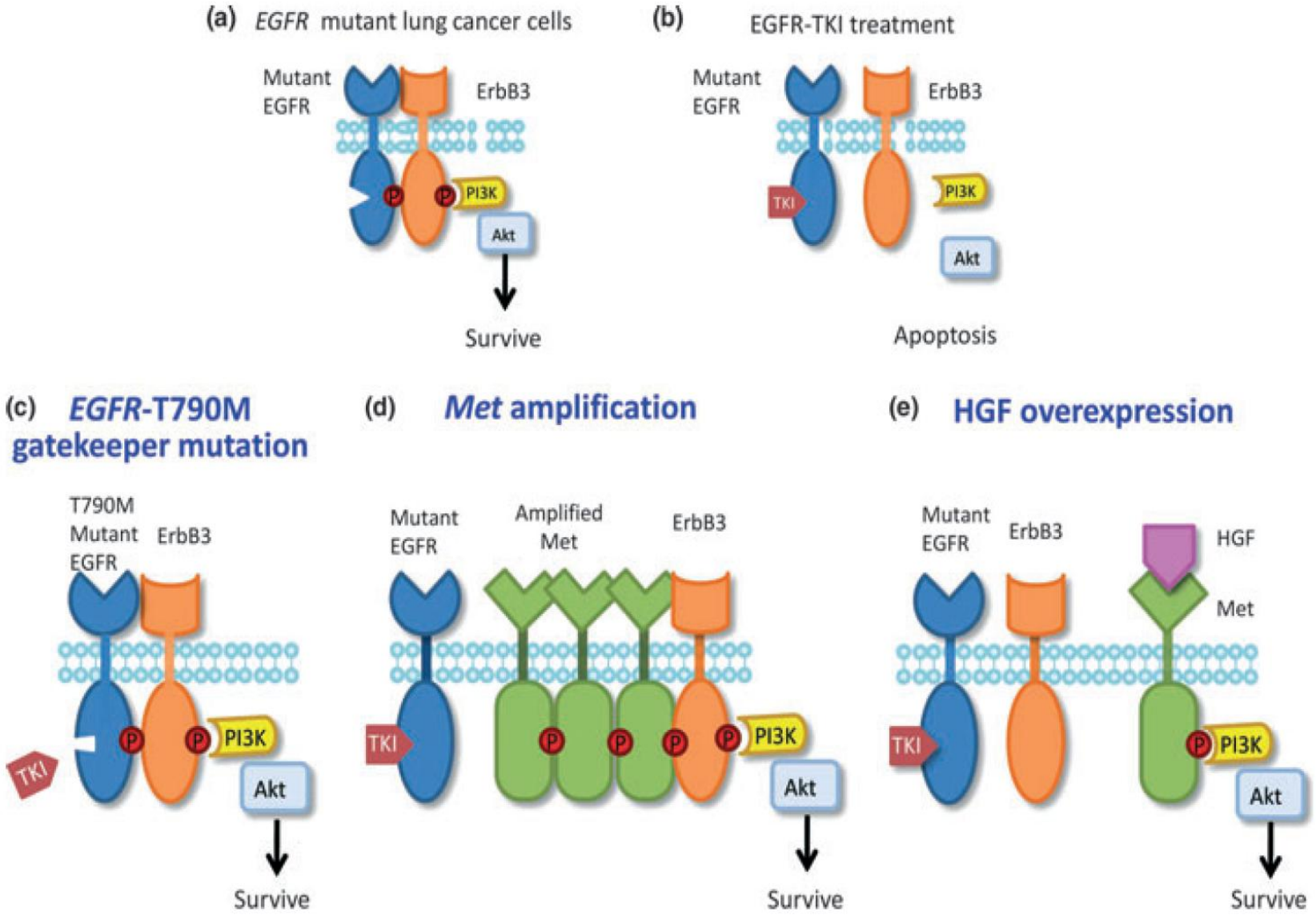
Mechanisms of acquired resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors



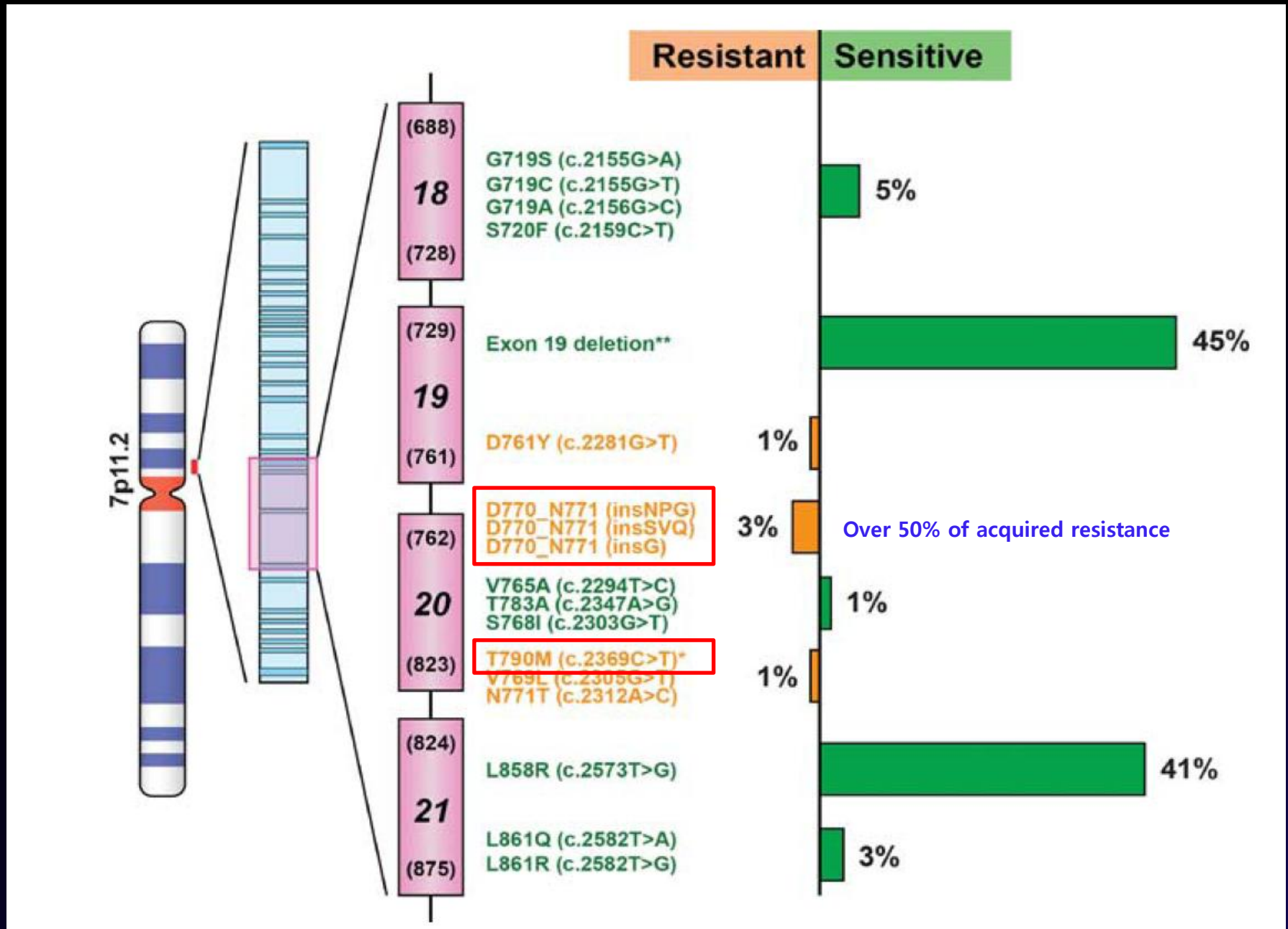
Ohashi , 2013. JCO

I. Secondary T790M mutation

Resistance signals to EGFR-TKIs in EGFR mutant lung cancer cells



Yano, 2012, Cancer science



Cheng. 2012, Modern Pathology

Secondary T790M mutation of the EGFR gene

- The presence of a secondary mutation of the EGFR gene in the threonine (T) 790 to methionine (M) (T790M) was reported in 2005.
- The threonine residue 790 is known as the "gatekeeper residue" and is located in the ATP-binding site, adjacent to the catalytic cleft of the kinase domain.
- T790Mmutation causes higher affinity to ATP and relatively lower affinity to EGFR-TKIs in EGFR.
- its mutation leads to the stabilization of the active conformation of the EGFR tyrosine kinase
- T790M mutation of the EGFR gene causes increased kinase activity

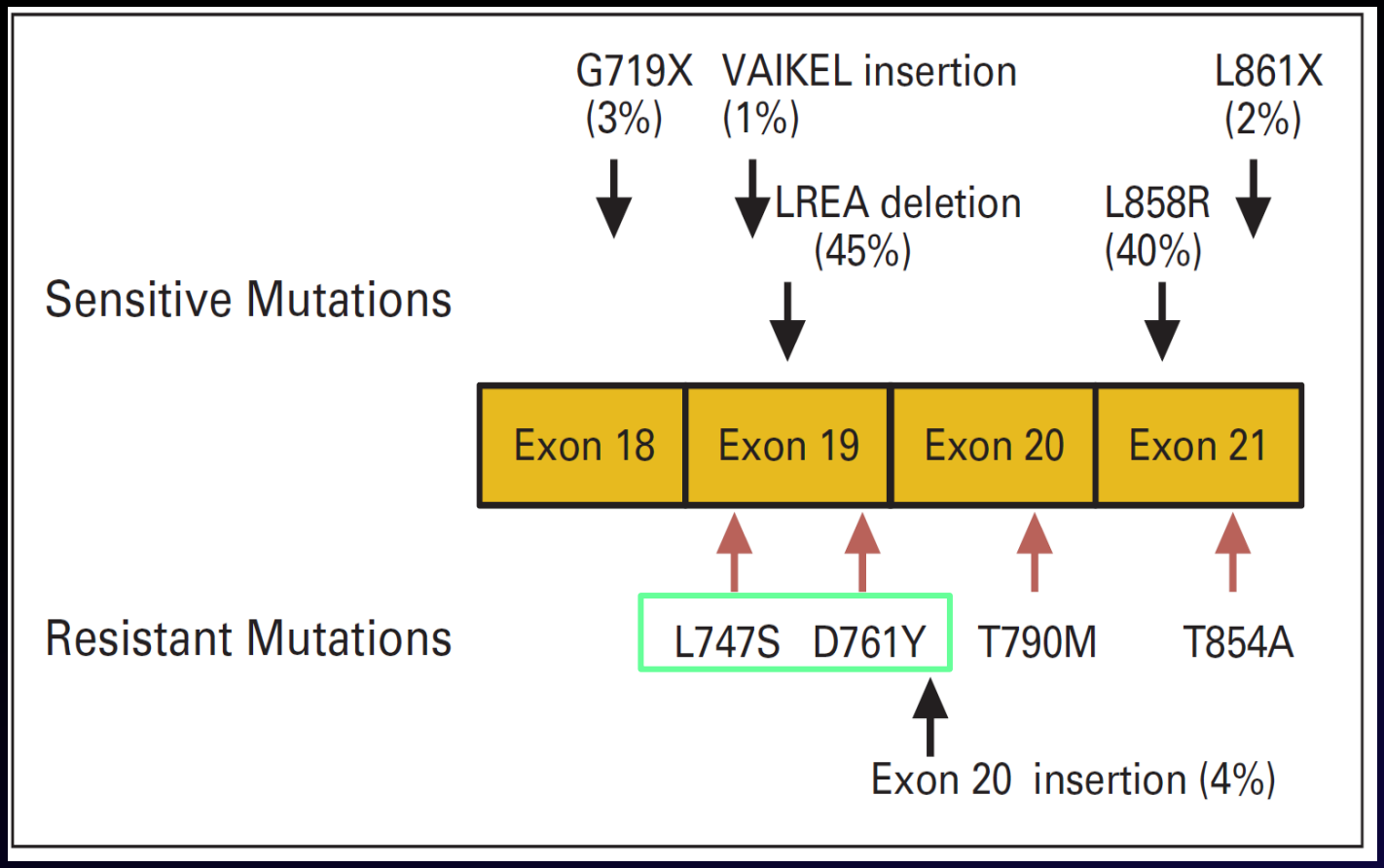
Nakata, 2012. Expert Opin. Ther. Targets

Secondary T790M mutation of the EGFR gene

- Tumor cells harboring the EGFR T790M mutation constitute a small minority of the cells before treatment with EGFR-TKIs.
- After treatment, the T790M mutant tumor cells were found to account for approximately 50% of the cells because of selective growth of T790M mutant cells in response to EGFR-TKIs.
- The T790M EGFR mutant exhibited higher level of tyrosine phosphorylation than wild type EGFR, and the T790M/L858R double mutant exhibited a substantial increase in phosphorylation levels compared with the L858R mutant alone.

Nakata, 2012. Expert Opin. Ther. Targets

Other acquired mutation of the EGFR gene

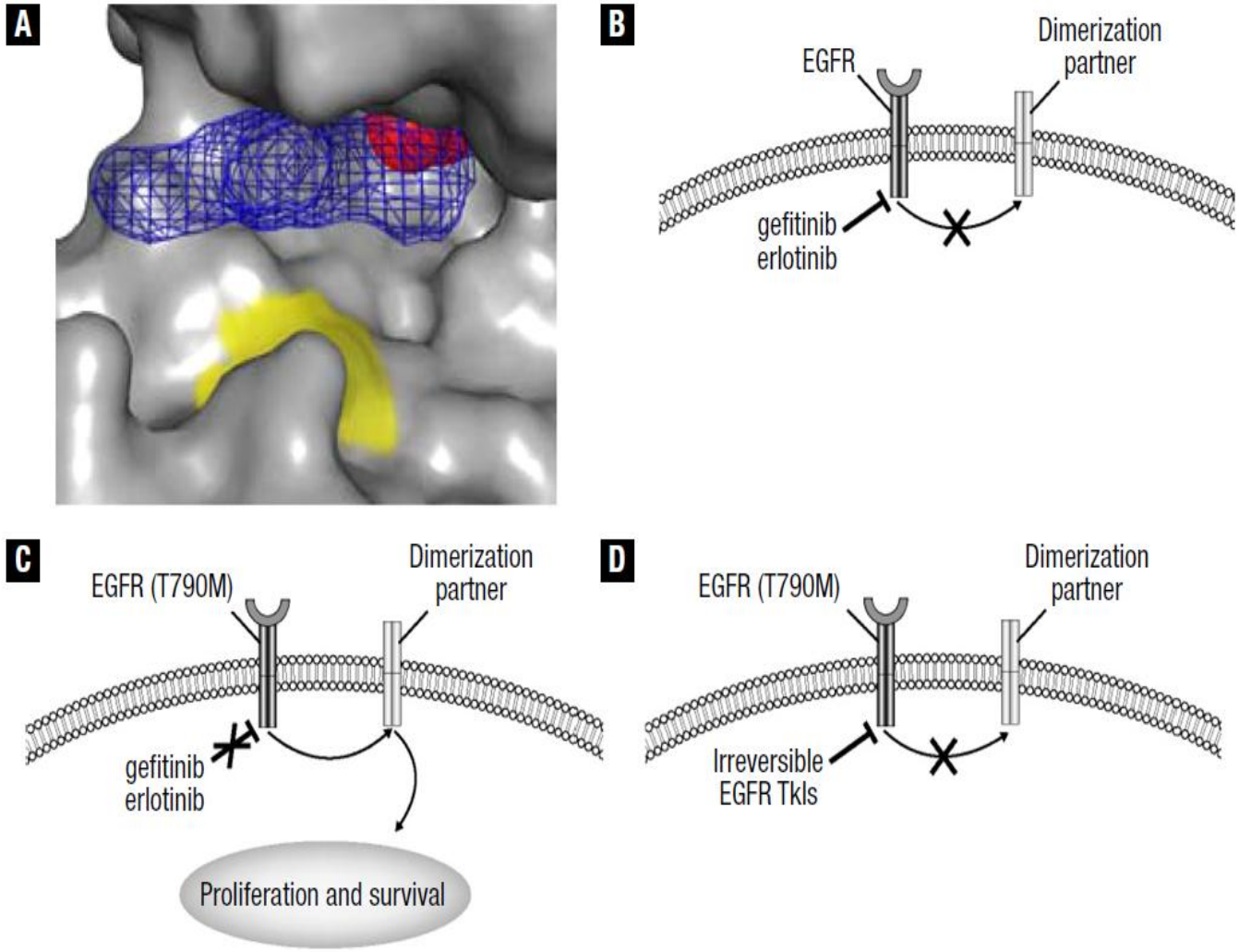


Costa, 2007. PLoS Med

Balak, 2006 CCR

- EGFR mutant carrying D761Y or L747S was 100-fold less resistant to EGFR-TKIs than the mutant carrying T790M in vitro.

Irreversible TKI inhibitors

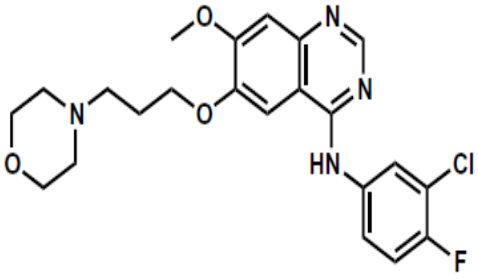


Molecular Surface Model of the Catalytic Cleft of T790M Mutated EGFR in Active Conformation. Red Spheres Denote the Acquired Methionine Residue. The Blue Mesh Represents the Location of Erlotinib Bound in the Catalytic Cleft.

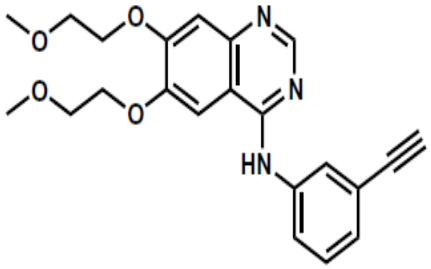
Bar, 2012. Clinical Lung Cancer

2nd and 3rd generation EGFR inhibitors

1st Generation EGFR inhibitors

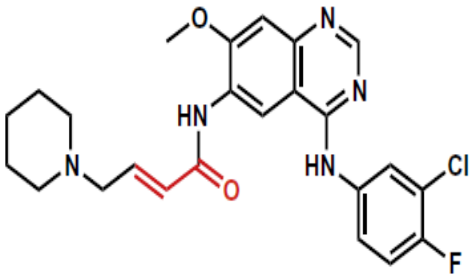


Gefitinib (Iressa™), AstraZeneca
Major Activity:
EGFR

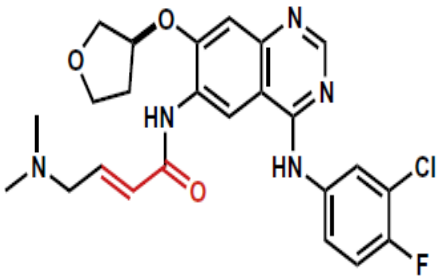


Erlotinib (Tarceva™), Genentech
Major Activity:
EGFR

2nd Generation EGFR inhibitors

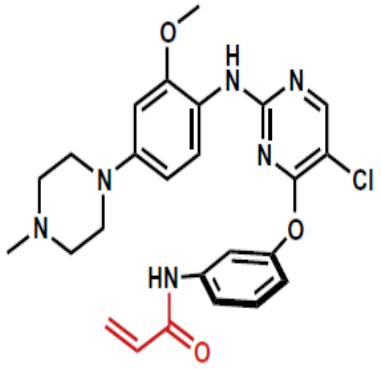


PF00299804
Dacomitinib, Pfizer, Phase III
NCT01000025/NCT01360554
Major Activity:
EGFR/HER2



BIBW 2992
Afatinib, Boehringer-Ingelheim, Phase III
NCT00949650/NCT01085136/NCT01121393
Major Activity:
EGFR/HER2/HER4

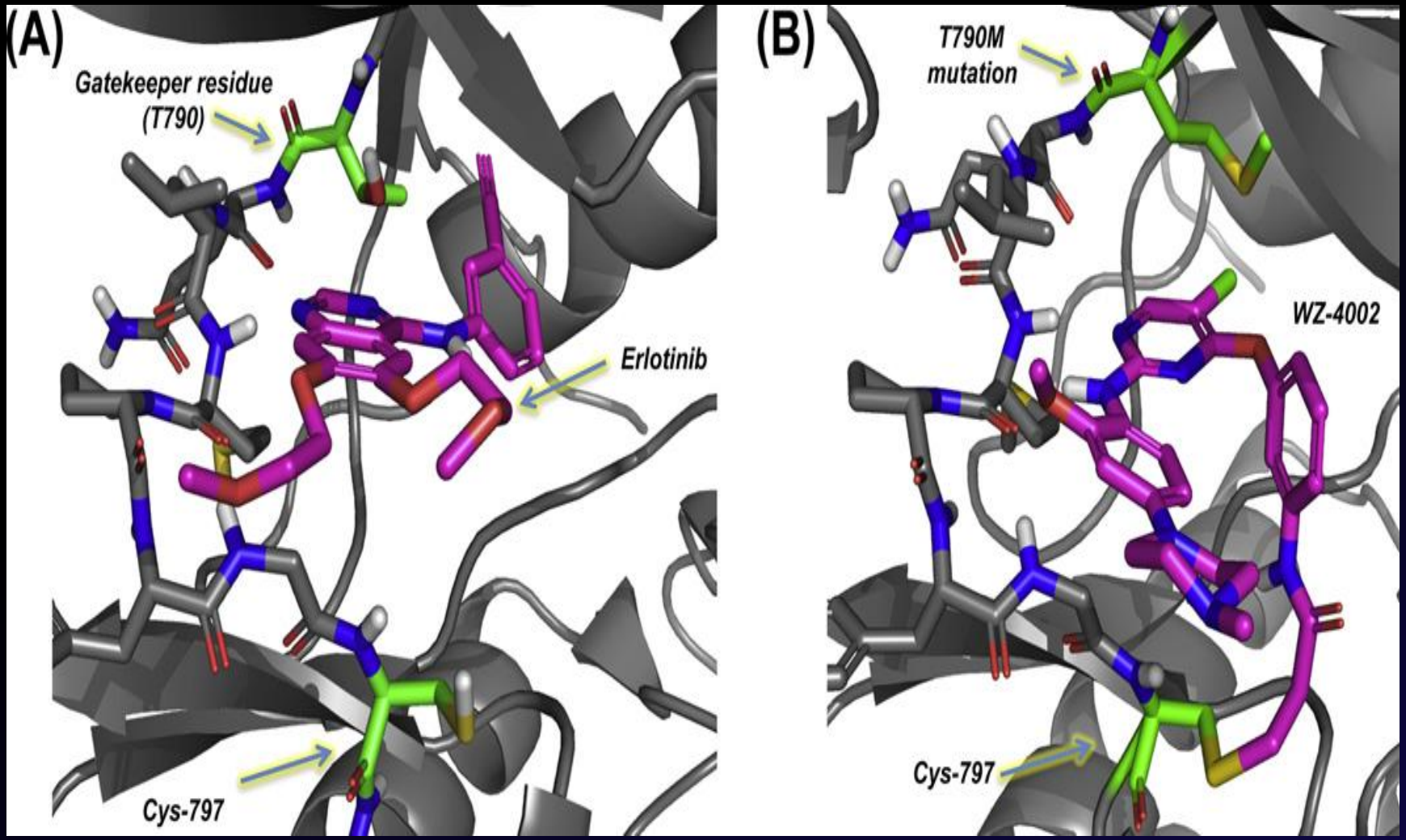
3rd Generation EGFR inhibitors



WZ-4002, Gatekeeper Pharmaceuticals, Preclinical
Major Activity:
$EGFR^{T790M}$

CO-1686, Clovis Oncology, Phase I/II
- Structure not yet disclosed -
NCT01526928
Major Activity:
$EGFR^{T790M}$

Giroux, 2013. Bioorg Med Chem Lett.



(A) Erlotinib (magenta) bound to the EGFR tyrosine kinase, the gatekeeper residue (T790) is highlighted in green; cysteine-797 which forms a covalent bond with 2nd and 3rd generations irreversible EGFR inhibitors is highlighted in green; (B) Structure of WZ-4002 covalently bound to EGFRT790M via cysteine-797.

Giroux, 2013. Bioorg Med Chem Lett.

Irreversible EGFR TKI Inhibitors Trial

Study Drug	Phase	Name	Schema	Pts	Results
Afatinib	IIb/III	Lux-lung 1	Afatinib vs placebo	585	PFS of 3.3 months in Afatinib arm as compared to 1.1 months in placebo OS of 10.7 month in Afatinib arm 11.9 months with placebo
	II	Lux-lung 2	Single arm, afinitib 50 mg or 40 mg until disease progression.	129	PFS of 14 months, OS of 24 months, ORR 60%. DCR of 86%
	III	Lux-lung 3	Afatinib vs cisplatin/pemetrexed as first line chemotherapy	330	Awaiting results. (NCT00949650)
	III	Lux-lung 5	Afatinib plus paclitaxel vs choice of chemotherapy following progression on afatinib monotherapy	300	Recruiting patients (NCT01085136)

Ayoola, 2012 Cancer Investigation

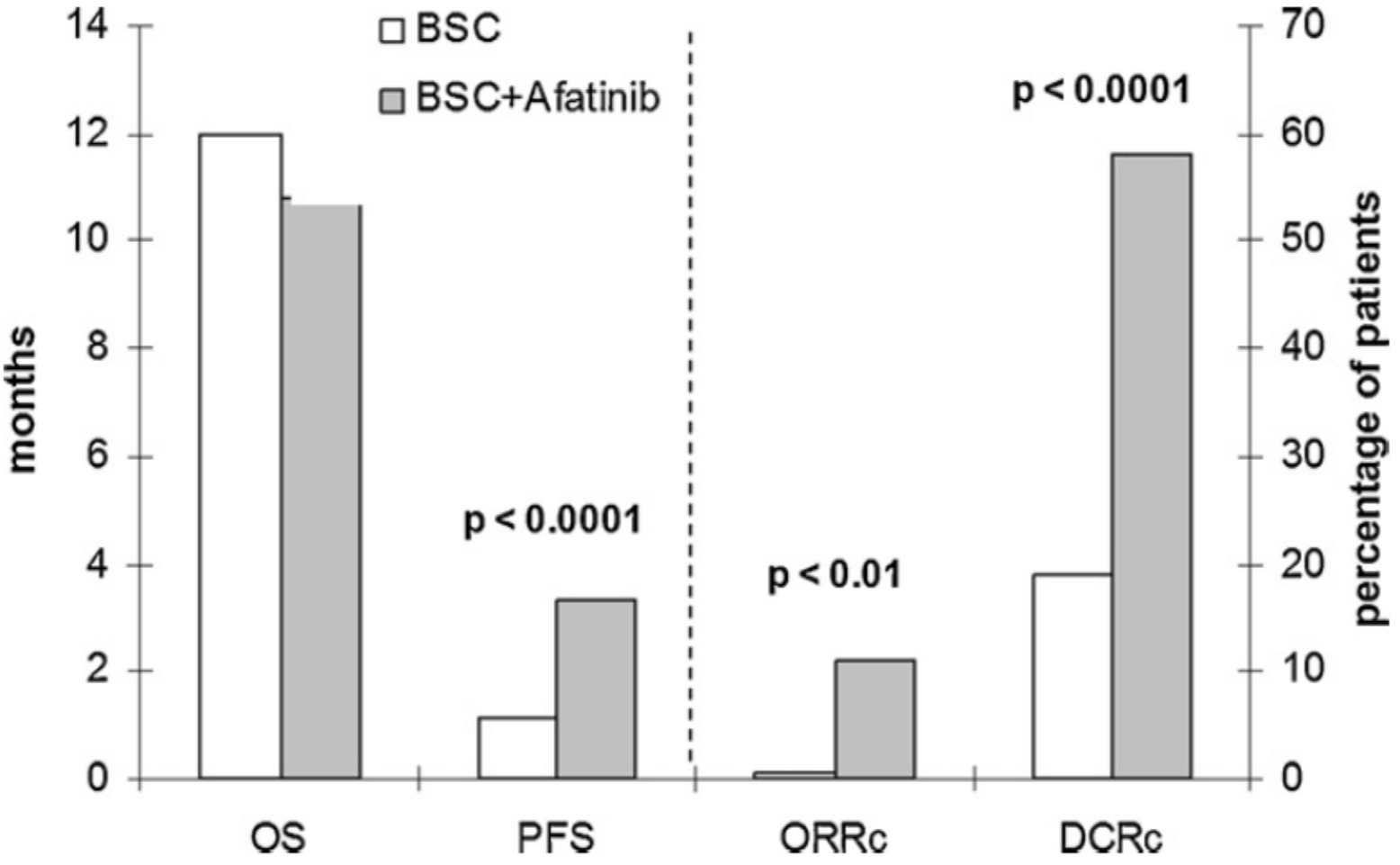


Fig. 2. Data from LUX-Lung 1 trial [18]: OS and PFS (left axis), and response rates (right axis) of afatinib + BSC compared to BSC. BSC: best supportive care; OS: overall survival; PFS: progression free survival; DCRc: confirmed disease control rate (CR + PR + SD); ORRc: confirmed overall response rate (CR + PR).

Bar, 2012. Clinical Lung Cancer

Irreversible EGFR TKI Inhibitors Trial

Study Drug	Phase	Name	Schema	Pts	Results
	lll	Lux-lung 6	Afatinib vs cisplatin/gemcitabine as first line chemotherapy	330	Recruiting patients (NCT01121393)
PF-00299804	ll		Adenocarcinoma vs other histologies	66	Preliminary result-DCR of 67% in adenocarcinoma group as compared with 40% in nonadenocarcinoma group
	ll		Study drug vs erlotinib in second or third line setting	188	Improvement in PFS, ORR with study drug; 17% vs 4.3% with erlotinib; clinical benefit rate with study drug 27.7% vs 13.8%; with erlotinib
	ll		Treatment naïve Non or light smokers	29	1 pt with CR, 6 pts with PR, 16 pts with SD > 16 weeks

Ayoola, 2012 Cancer Investigation

Irreversible inhibitors

Despite the promising activity of newer, irreversible EGFR inhibitors in patients with *EGFR* mutations, their efficacy has been minimal in patients with acquired resistance to gefitinib and erlotinib

Nakata, 2012. Expert Opin. Ther. Targets

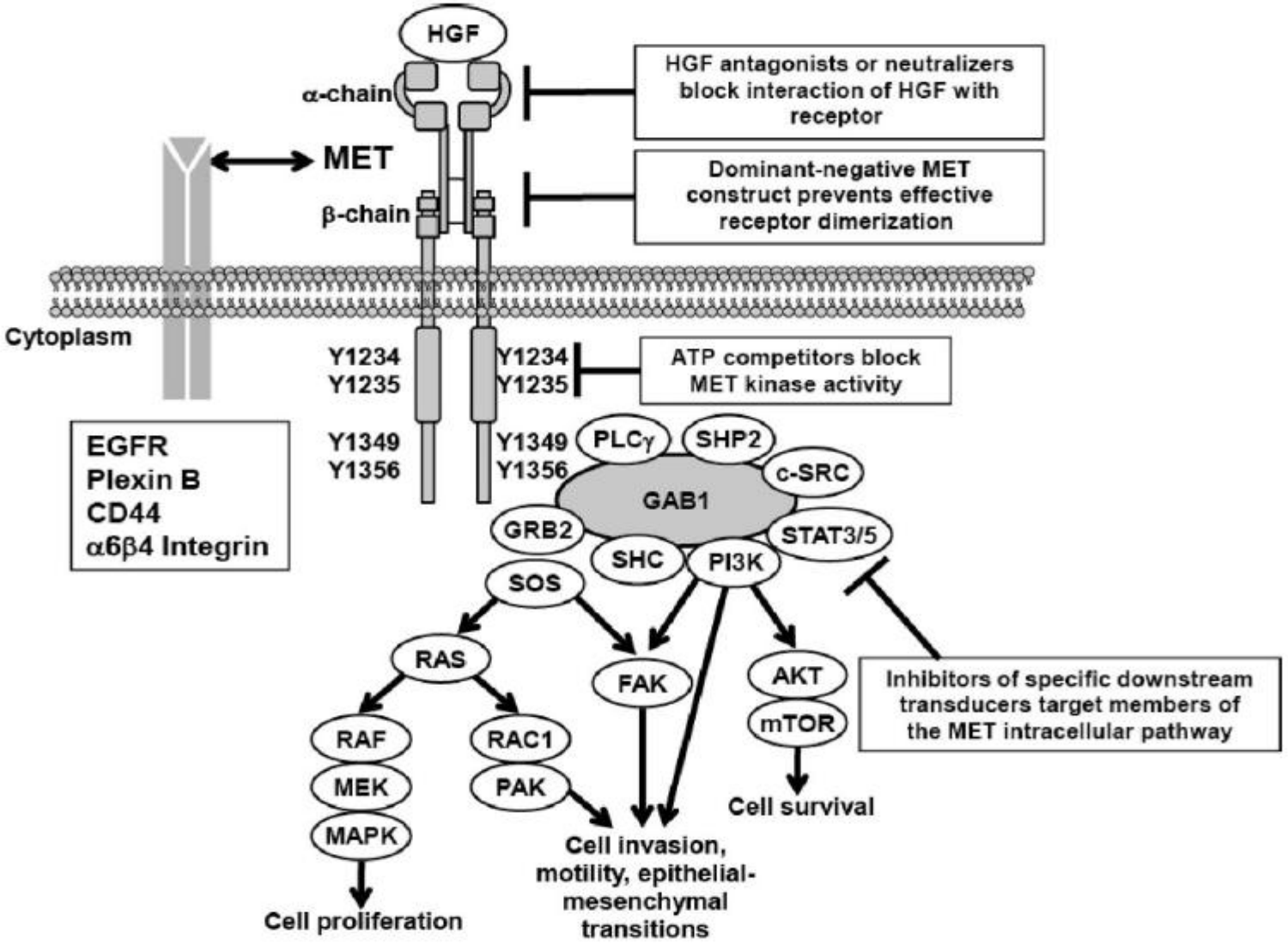
Irreversible inhibitors

- Amplified T790M mutations may promote resistance to irreversible EGFR inhibitors suggests that these patients may not respond to the current irreversible EGFR inhibitors and should be directed to other potential therapeutic strategies such as combined PI3K and MEK inhibition, newer, more potent T790M–specific EGFR inhibitors, or combinations of anti-EGFR therapies.

Ercan, 2010. Oncogene

II. Amplification of the MET Oncogene

MET signal transduction pathways



ROBINSON, 2013 THE ONCOLOGIST

MET Gene

- Dysregulation of MET expression by overexpression, constitutive kinase activation, gene amplification, paracrine or autocrine activation via HGF, *MET* mutation, and epigenetic changes.
- Amplification and/or overexpression of MET and/or HGF: correlate with poor clinical prognosis in patients with NSCLC

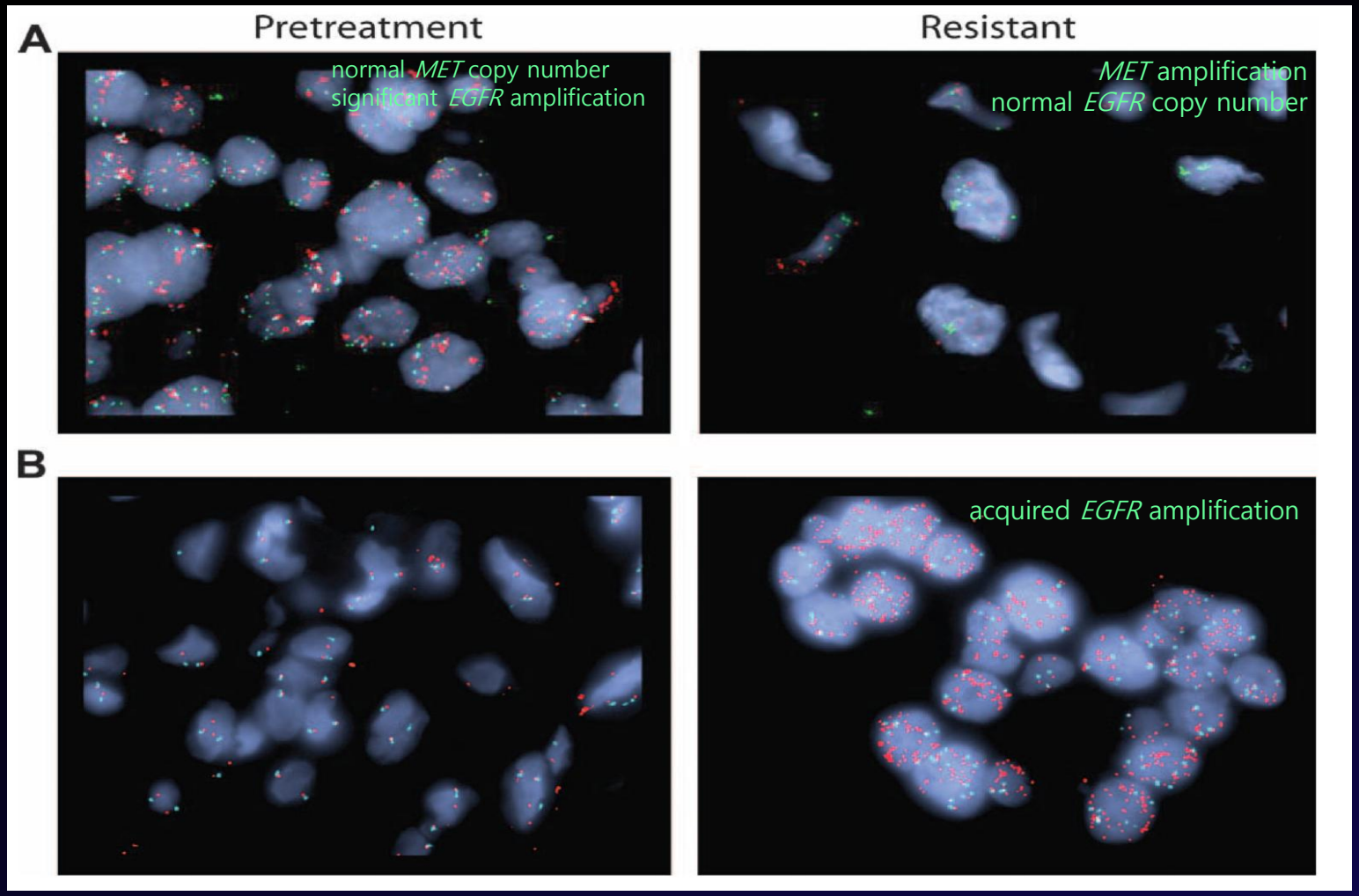
ROBINSON, 2013 THE ONCOLOGIST

MET Gene

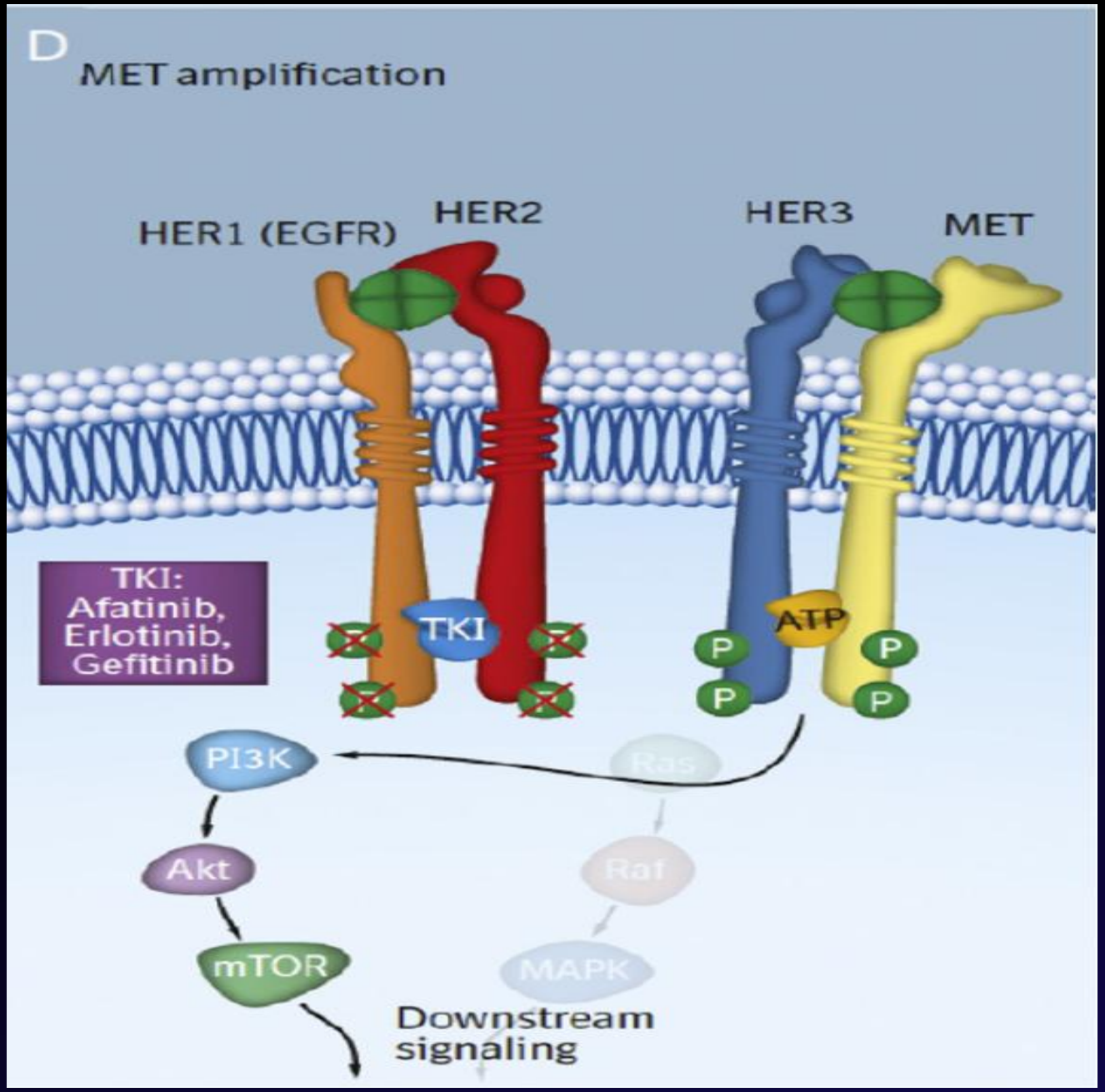
- Role of MET:
  - Cell motility and morphogenesis, metastatic lesions typically exhibit higher expression levels of MET than primary tumors
  - MET plays an important role in tumor metastasis.
- MET was also strongly expressed in 67% of adenocarcinomas, and expression of activated phospho-MET was observed preferentially along the invasive fronts of NSCLC tumor tissue.

ROBINSON, 2013 THE ONCOLOGIST

Acquired genetic amplifications in drug-resistant lung tumors

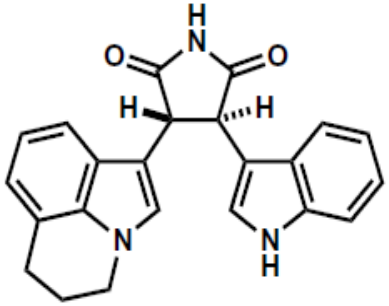


Sequist, 2011. Sci Transl Med

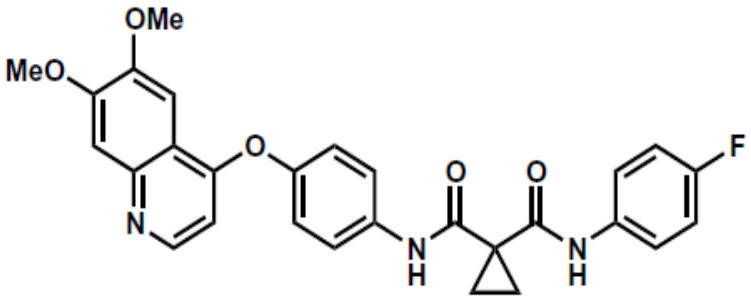


Bar, 2012. Clinical Lung Cancer

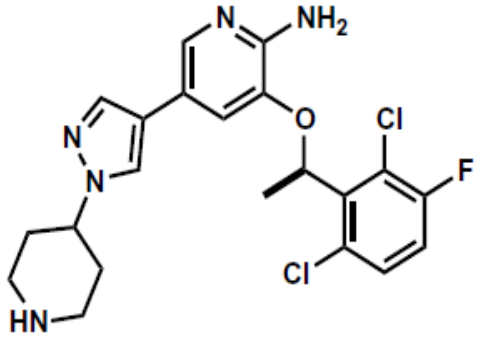
Representative chemical structures of c-MET and HSP90 inhibitors given in combination with EGFR inhibitors in NSCLC patients.



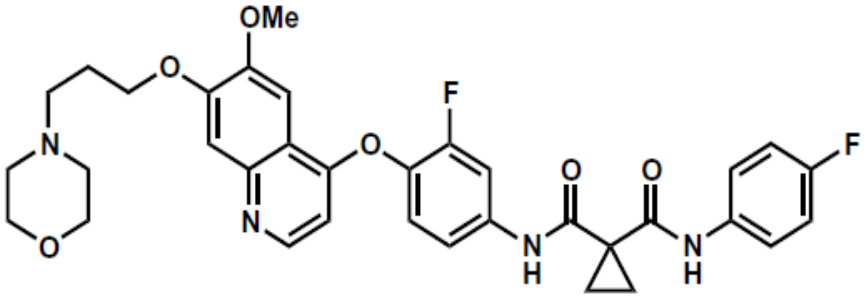
Tivantinib, ArQule
Phase III, +/- erlotinib
NCT01244191 and 01377376



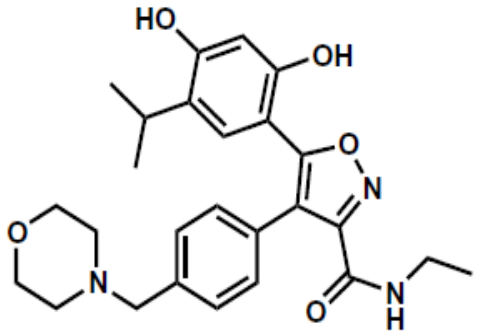
Cabozantinib, Exelixis
Phase II, +/- erlotinib
NCT00596648



Crizotinib (Xalkori™), Pfizer
Phase I, +/- dacomitinib
NCT01121575



Foretinib, Glaxo-SmithKline
Phase II, +/- erlotinib
NCT01068587



AUY922, Novartis
HSP90 inhibitor
Phase I/II, +/- erlotinib
NCT01259089

Giroux, 2013. Bioorg Med Chem Lett.

III. Overexpression of hepatocyte growth factor (HGF)

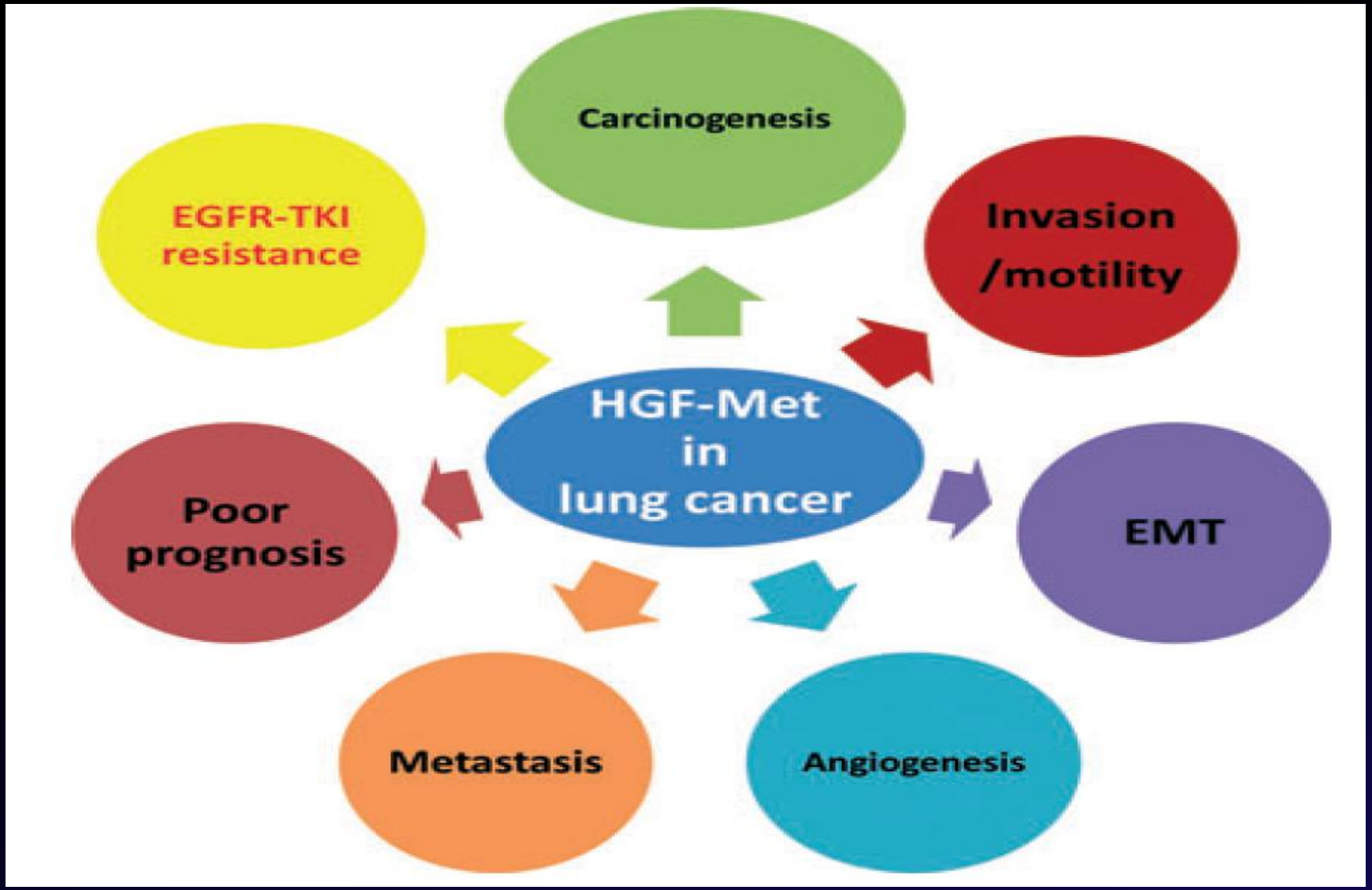
Overexpression of HGF

- Overexpression of HGF stimulates the PI3K/Akt pathway through MET phosphorylation independent of ErbB3 phosphorylation.

- Gefitinib-sensitive lung cancer cells became resistant to gefitinib when co-cultured with HGF-producing fibroblasts. Furthermore, the resistance induced by fibroblast-derived HGF was abolished by anti-HGF antibodies or HGF antagonists, such as NK4.

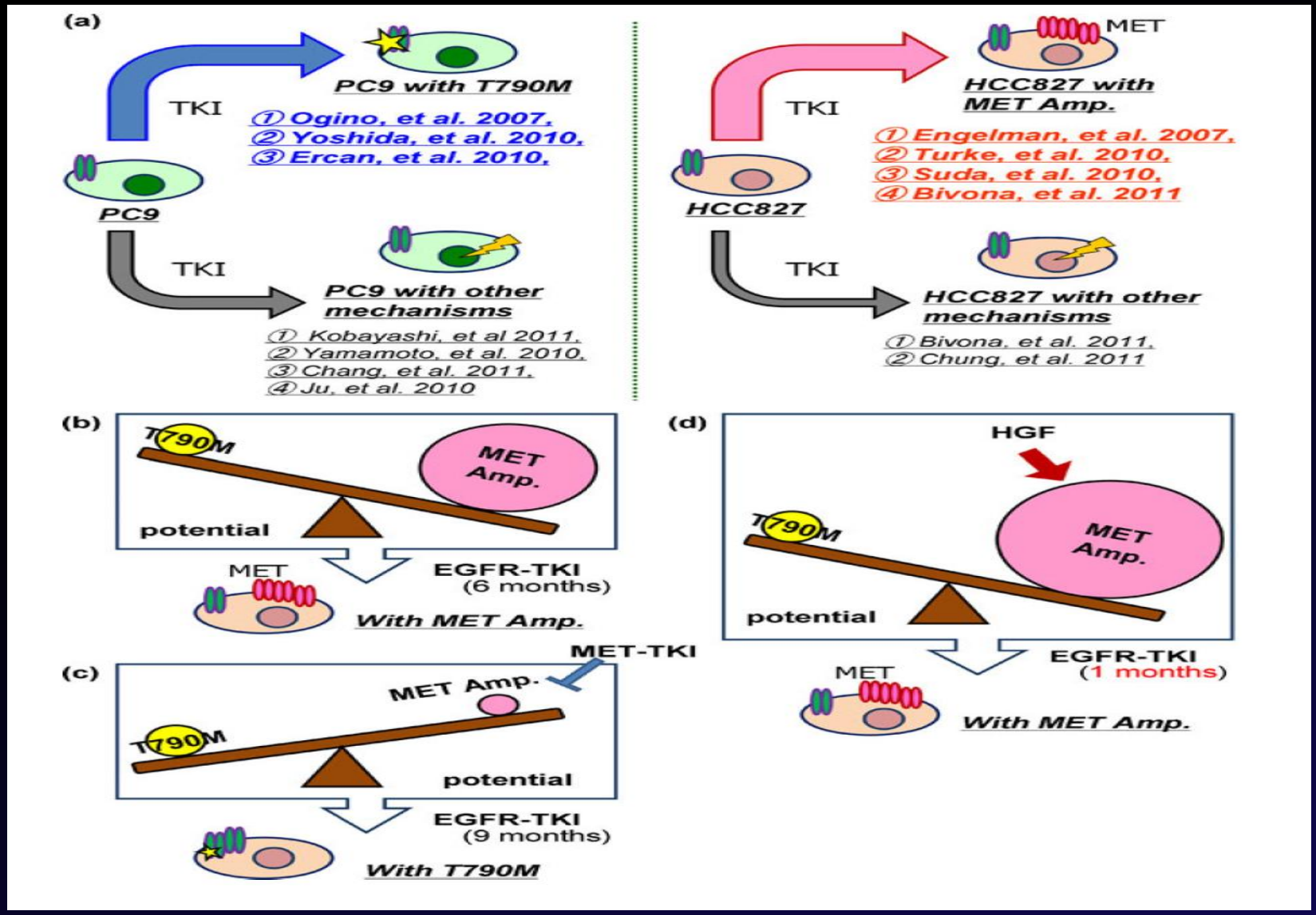
Wang, 2009. CCR

Role of hepatocyte growth factor (HGF)-Met in lung cancer.



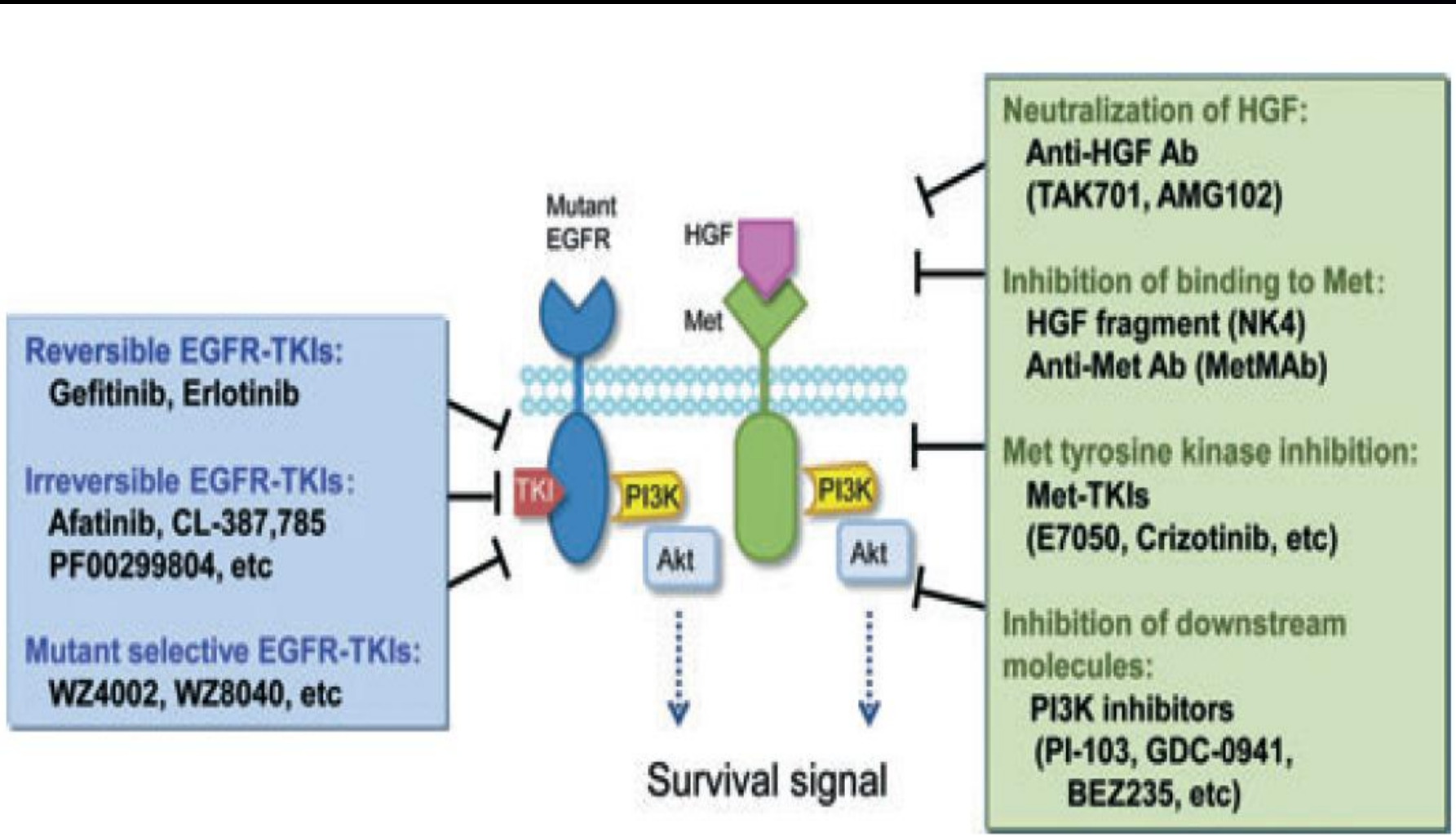
Yano, 2012, Cancer science

Destiny and ductility in acquiring resistance to TKIs in EGFR-mutated lung cancers



Suda, 2012. Cancer Metastasis Rev

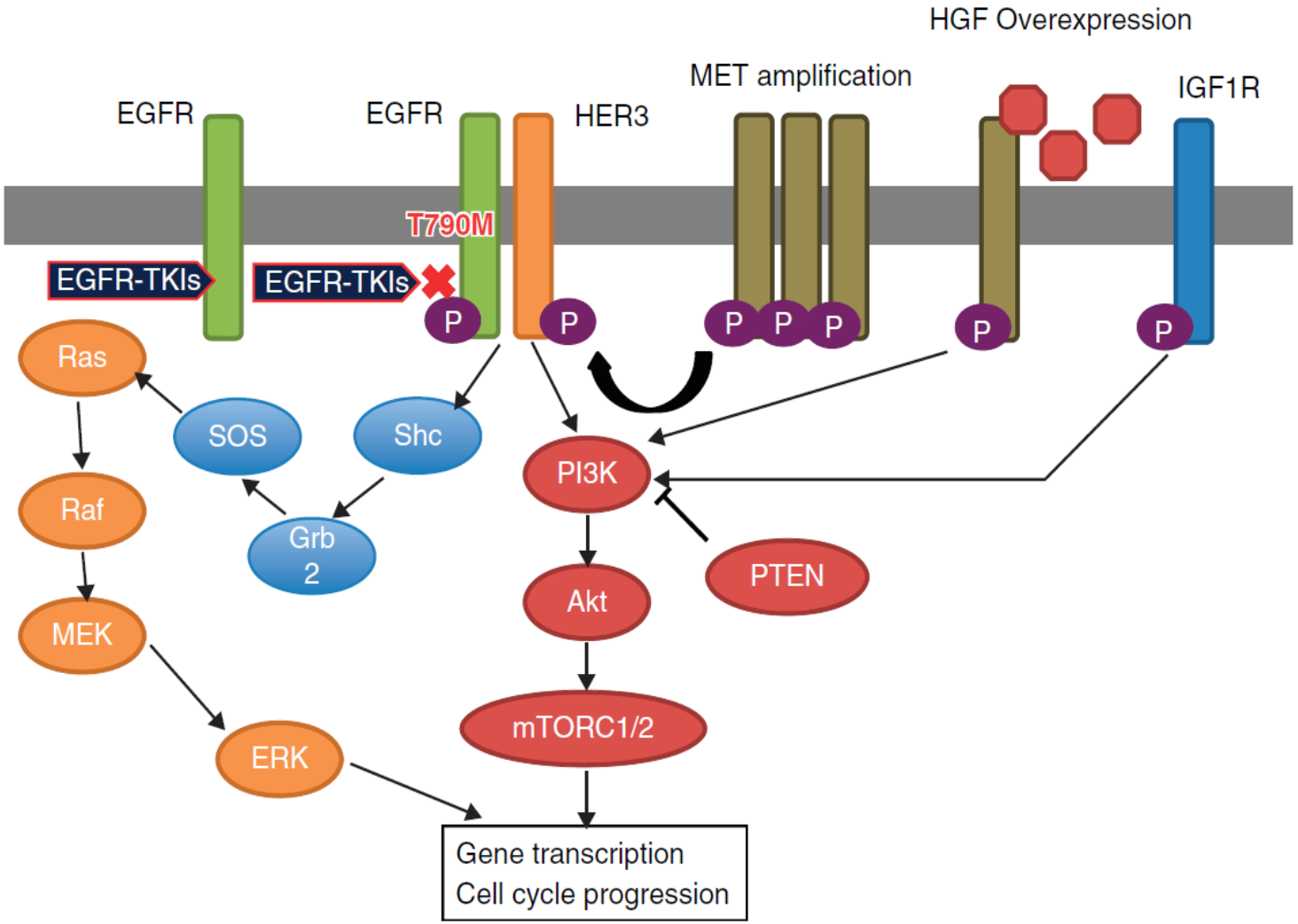
Strategies to treat HGF-triggered resistance



Yano, 2012, Cancer science

IV. Insulin-like growth factor 1 receptor (IGF1R) signaling

IGFR activation induces activation of PI3K/Akt pathway independent of EGFR activation



Nakata, 2012. Expert Opin. Ther. Targets

V. Transformation to SCLC

- *EGFR* mutations in small-cell lung cancers in patients who have never smoked.

  Zakowski, 2006. N. Engl. J. Med.

- EGFR mutation in gefitinib responsive small-cell lung cancer.

  never-smoking female diagnosed with CD56-positive advanced SCLC harboring an exon 19 deletion in *EGFR*, who had a good partial response to first-line gefitinib.

  Okamoto, 2006. Ann. Oncol.

- Sequential occurrence of non-small cell and small cell lung cancer with the same *EGFR* mutation.

  ***EGFR*-mutant metastatic adenocarcinoma that transformed into SCLC after developing resistance**

  Morinaga, 2007. Lung Cancer

- Epidermal growth factor receptor mutation status and clinico-pathological features of combined small cell carcinoma with adenocarcinoma of the lung.

Fuku, 2007. Cancer Sci.

6 patients with combined NSCLC-SCLC histology

one was a never-smoking female with an L858R *EGFR* mutation in both the SCLC and adenocarcinoma components.

one patient had a pretreatment adenocarcinoma that transformed into a combined SCLC-adenocarcinoma after developing clinical resistance to an EGFR TKI.

The other four patients had *EGFR*-mutant SCLC or mixed histology tumors at baseline

- Epidermal growth factor receptor mutations in small cell lung cancer.

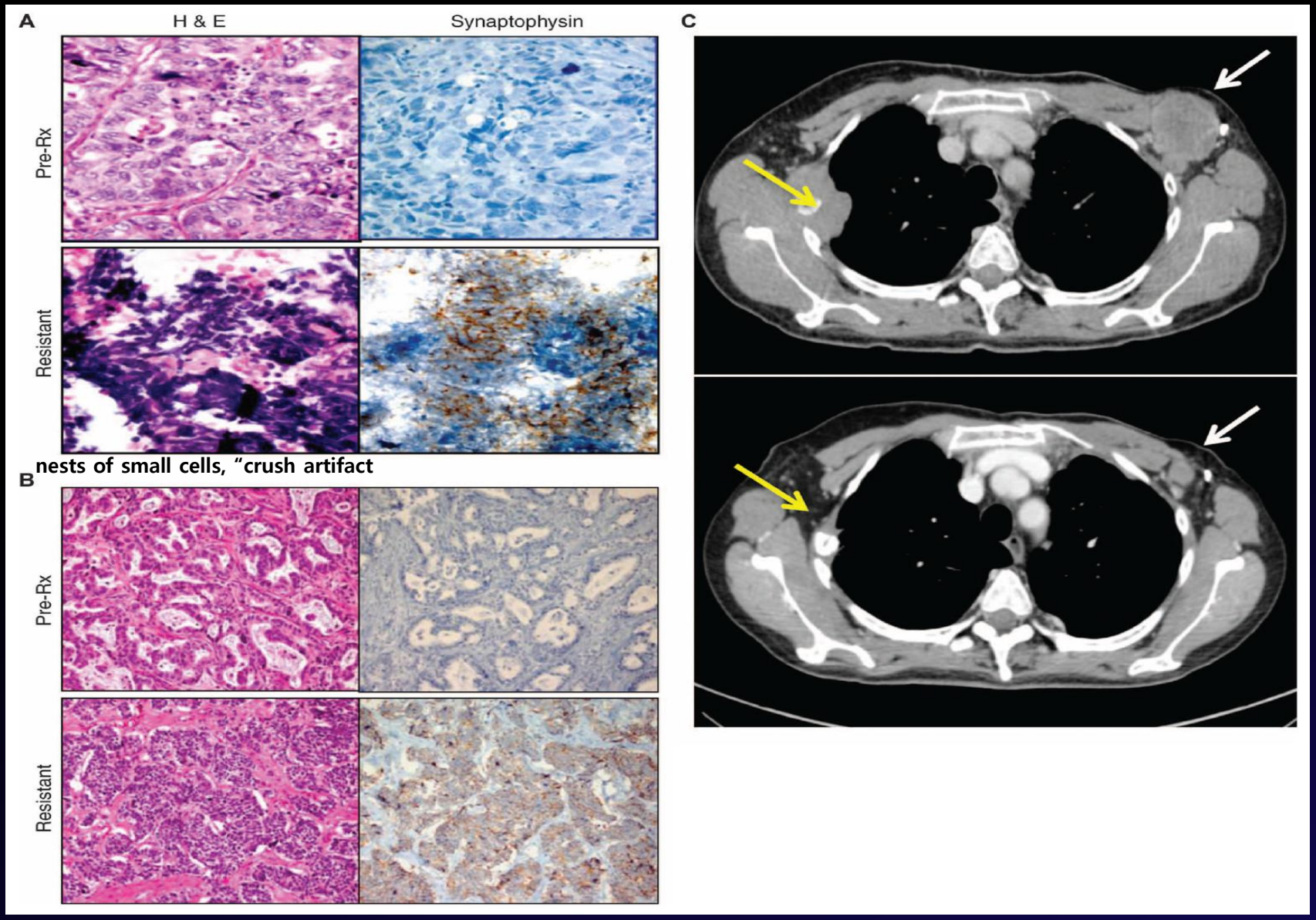
Tatematsu, 2008. Clin. Cancer Res.

Patients with lung tumors showing an NSCLC to SCLC transformation.

Age (years)	Gender	ID no.	Biopsy	Histology	Synaptophysin	Chromogranin	CD56	Genotype
67	Female	22	Pre-	Adenocarcinoma	–	–	–	L858R
			Post-	SCLC	+	+	+	L858R
54	Female	23	Pre-	Adenocarcinoma	–	–	Weak+	Exon 19 deletion
			Post-	SCLC	Strong+			Exon 19 deletion
56	Female	24	Pre-	Adenocarcinoma	–	–		L858R
			Post-	SCLC	+	+		L858R, PIK3CA
40	Female	25	Pre-	Adenocarcinoma	–	–	–	Exon 19 deletion
			Post-	SCLC	+	–		Exon 19 deletion
61	Female	26	Pre-	Adenocarcinoma	–	–	–	L858R
			Post-	SCLC	+	+		L858R

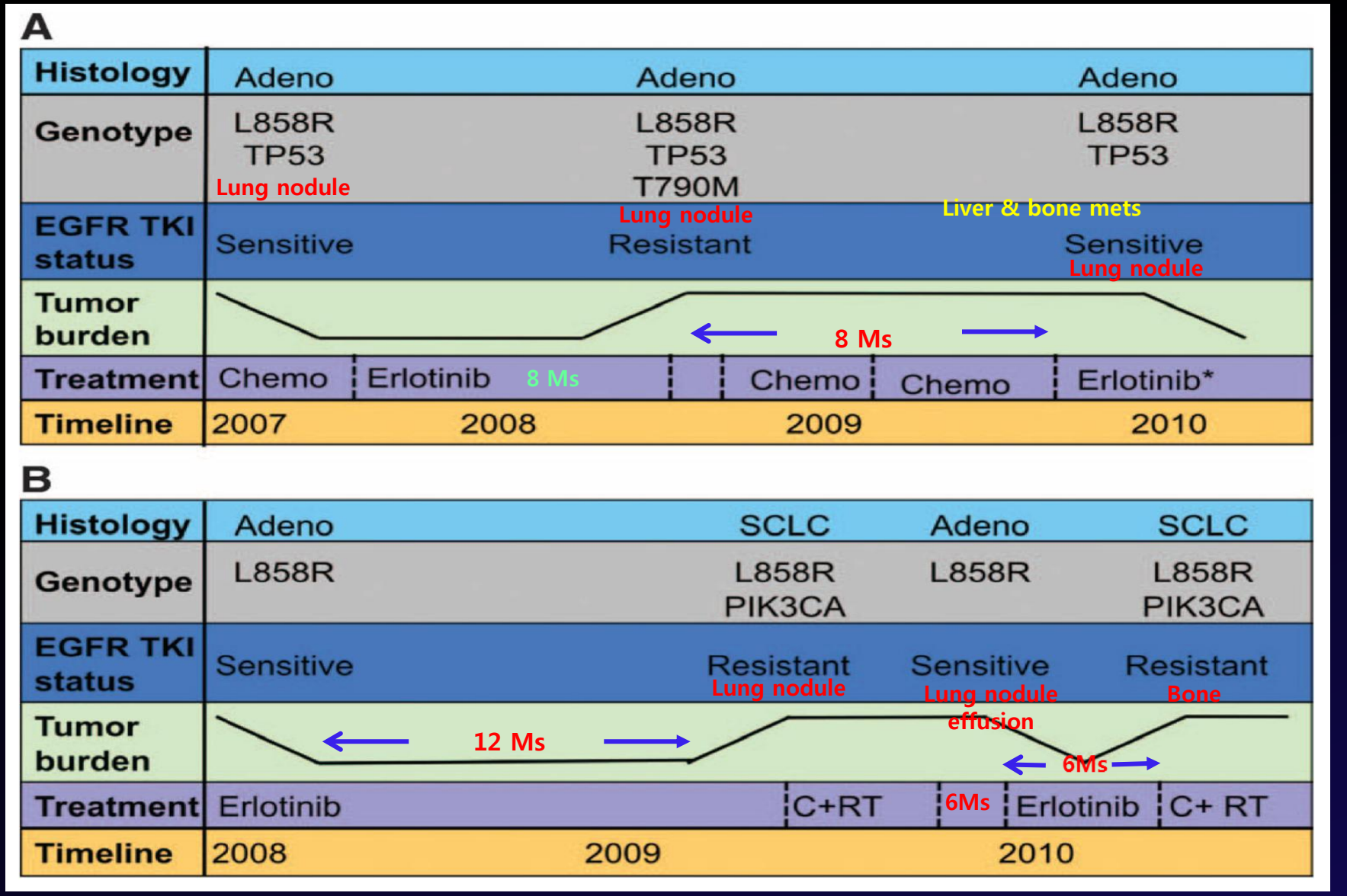
Sequist, 2011. Sci Transl Med

Drug resistance and transformation of NSCLC to SCLC



Sequist, 2011. Sci Transl Med

Longitudinal genotypic and phenotypic changes in response to EGFR TKI



* erlotinib plus an investigational agent that does not target T790M

Sequist, 2011. Sci Transl Med

Transformation to SCLC

- The biological underpinnings of the SCLC transformation are unknown and are of great interest.

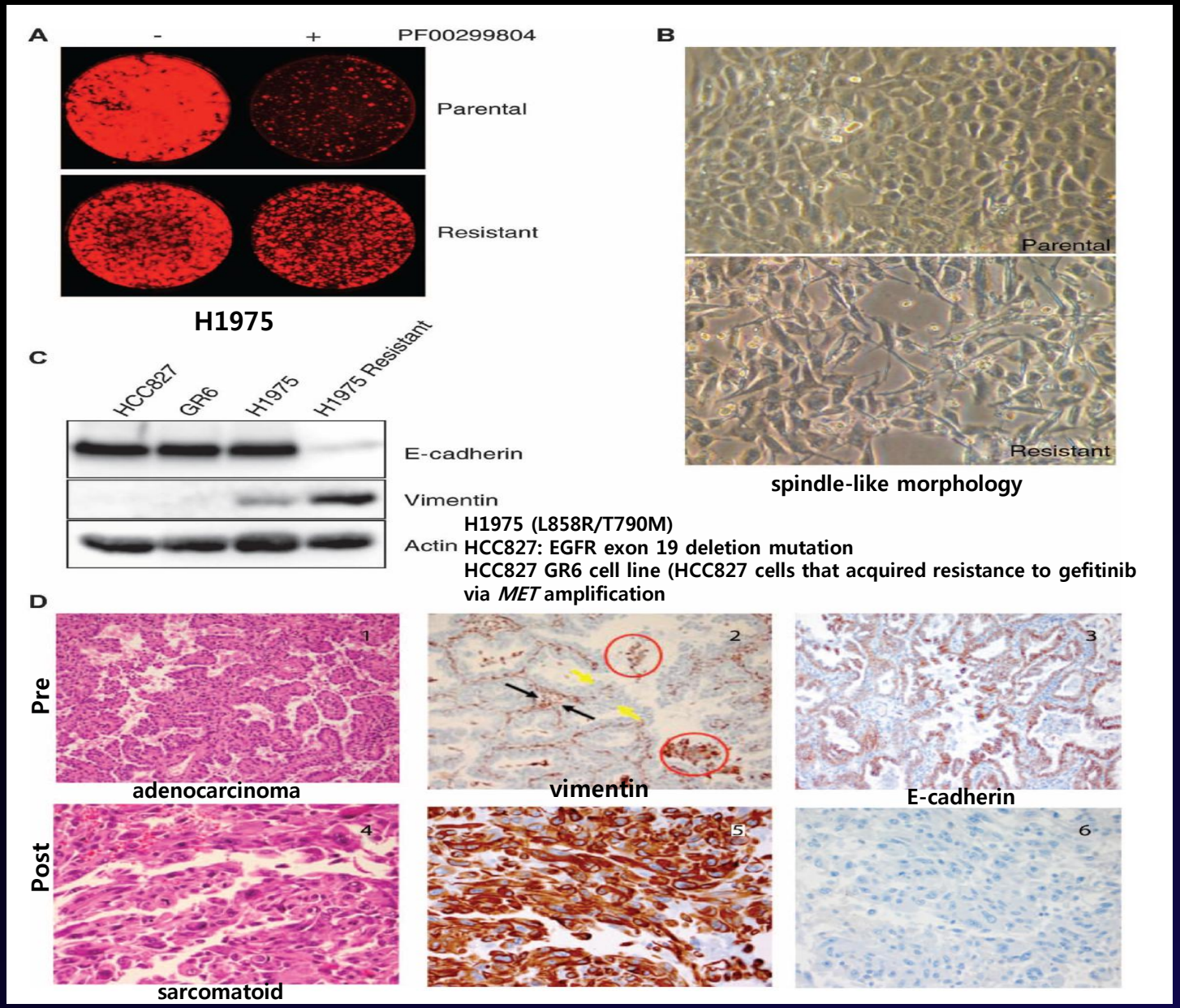
- The finding that the same *EGFR*-mutant cancer can manifest both as an adenocarcinoma and as a SCLC hints at the existence of a pluripotent population of *EGFR* mutant cancer cells or cancer stem cells that are the source of resistance.

- Perhaps, these patients developed drug resistance through a genetic or epigenetic event that concurrently led to a shift in phenotypic appearance.

Sequist, 2011. Sci Transl Med

VI. EMT

EMT and Acquired resistance



Sequist, 2011. Sci Transl Med

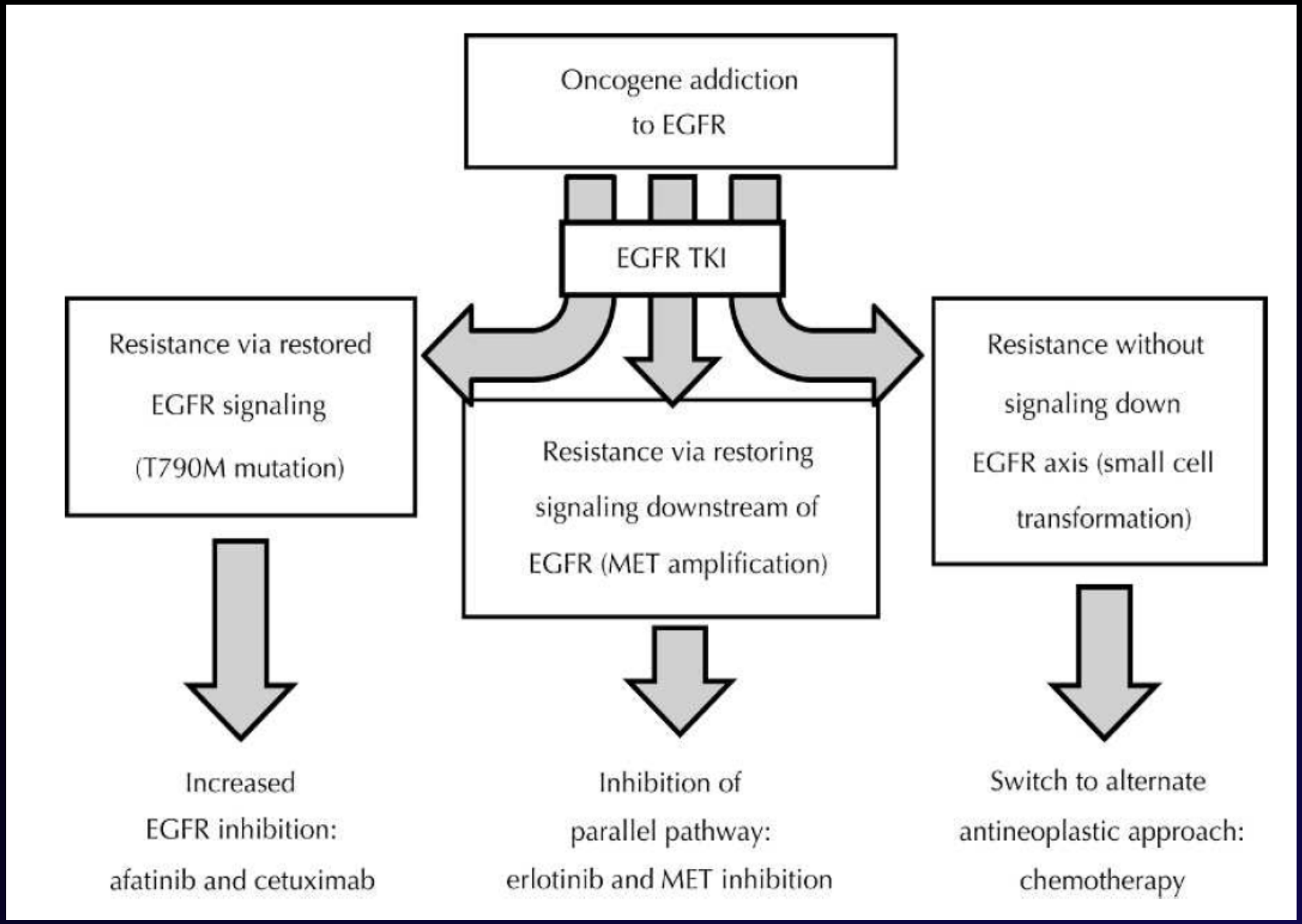
EMT

- The molecular mechanisms connecting the resistance of the cancer cells to the mesenchymal phenotype remain unknown.

- Recent findings that *KRAS*-mutant lung cancers with mesenchymal features are resistant to both KRAS knockdown and combined PI3K and MEK inhibition suggest that mesenchymal cells may have an intrinsic lack of sensitivity to the intracellular signaling pathway down-regulation that is normally the hallmark of sensitivity to EGFR TKIs.

Strategies to overcome the resistance to EGFR-TKIs

Three distinct strategies toward overcoming acquired resistance



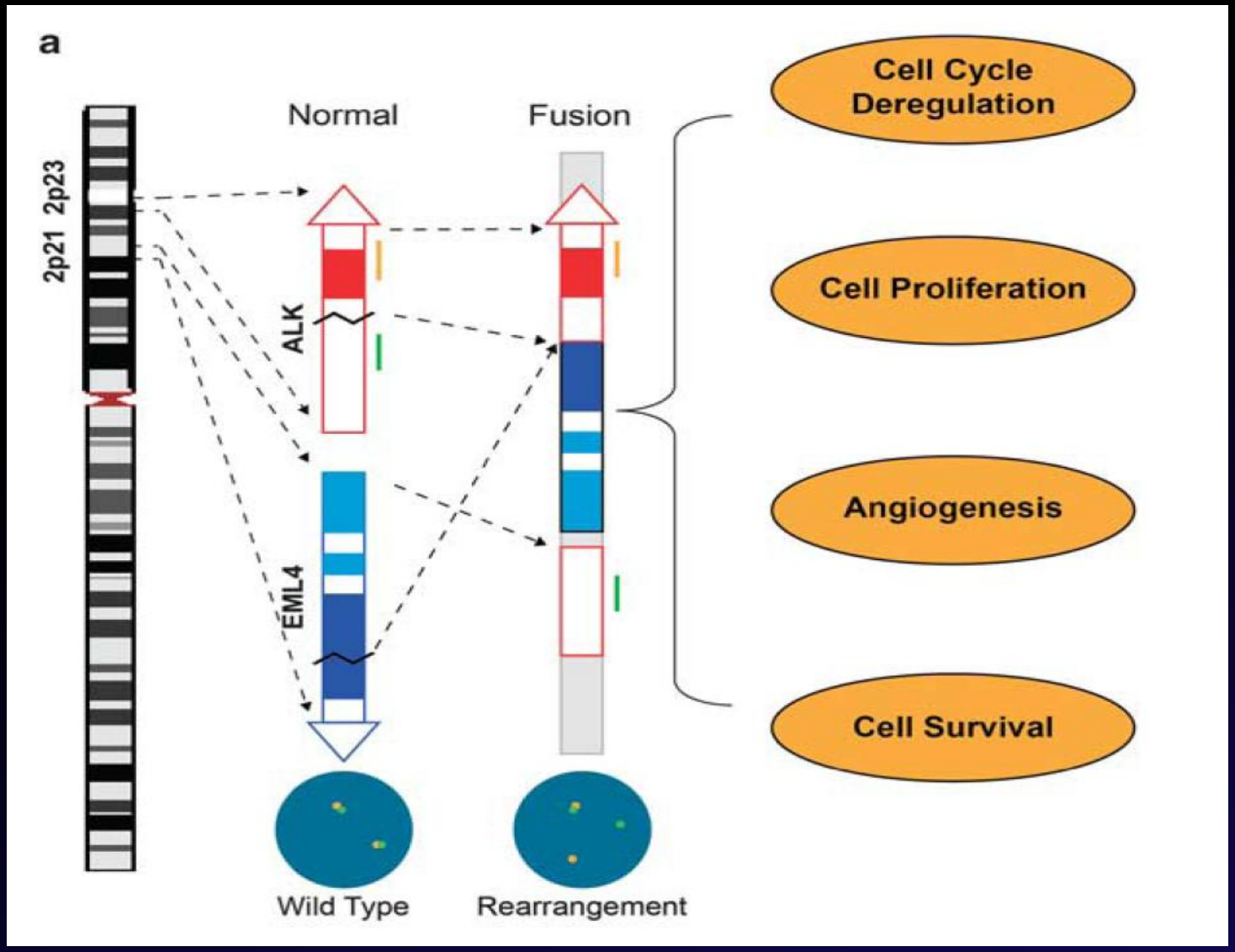
Oxnard, 2012. Arch Pathol Lab

Summary

- Development of resistance to EGFR-TKIs is a inevitable problem.
- Mechanisms of resistance to EGFR-TKIs, such as EGFR mutations, the upregulation of EGFR downstream molecules, and the activation of alternative tyrosine kinase pathways.
- Full understanding of the sensitivity and resistance to EGFR-TKIs remains elusive and further detailed investigation is required.
- The goal should now be to become as creative as possible with new strategies and therapies to make *EGFR*-mutant lung cancer a chronic rather than fatal disease.

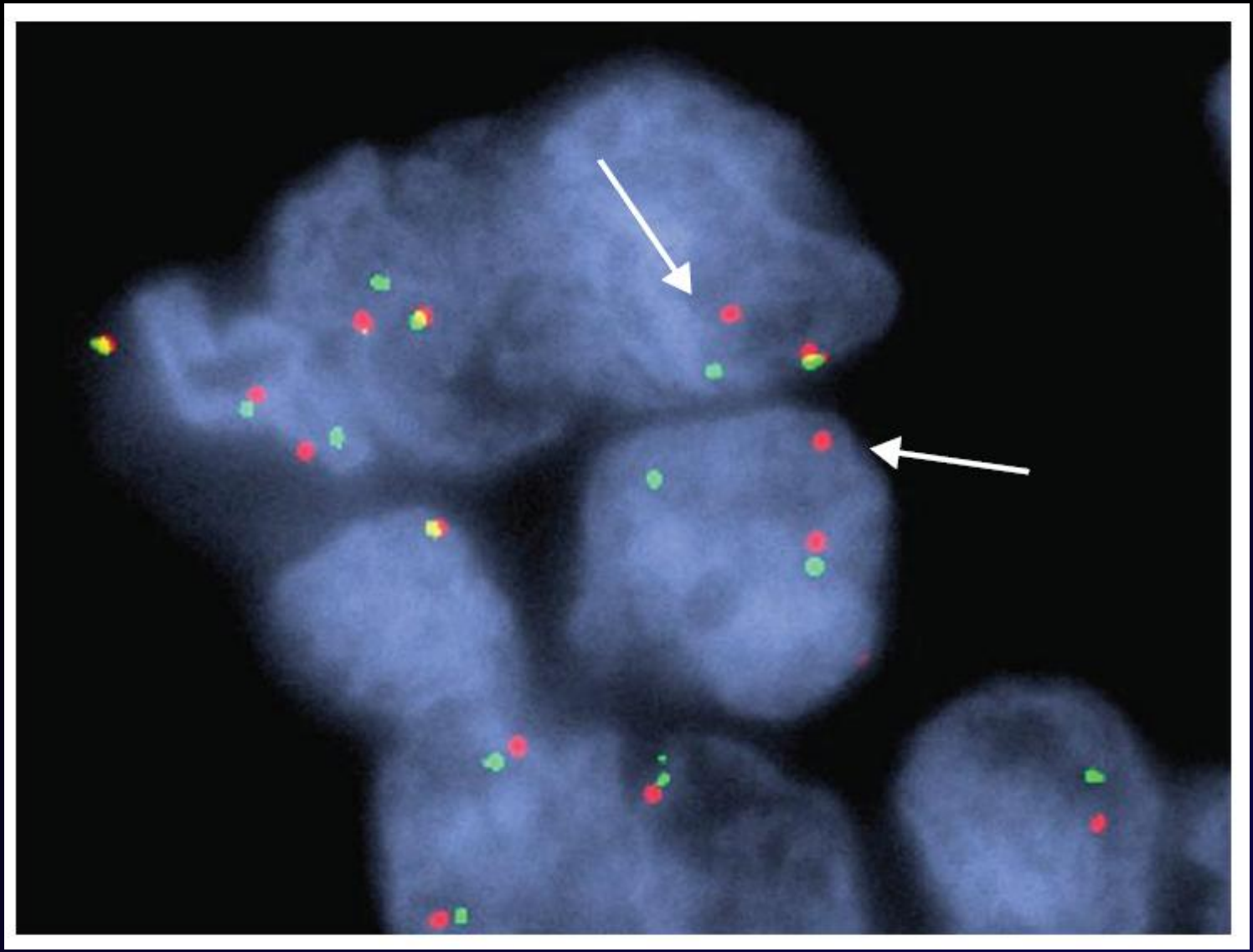
ALK Inhibitors

Schematic of EML4–ALK rearrangement



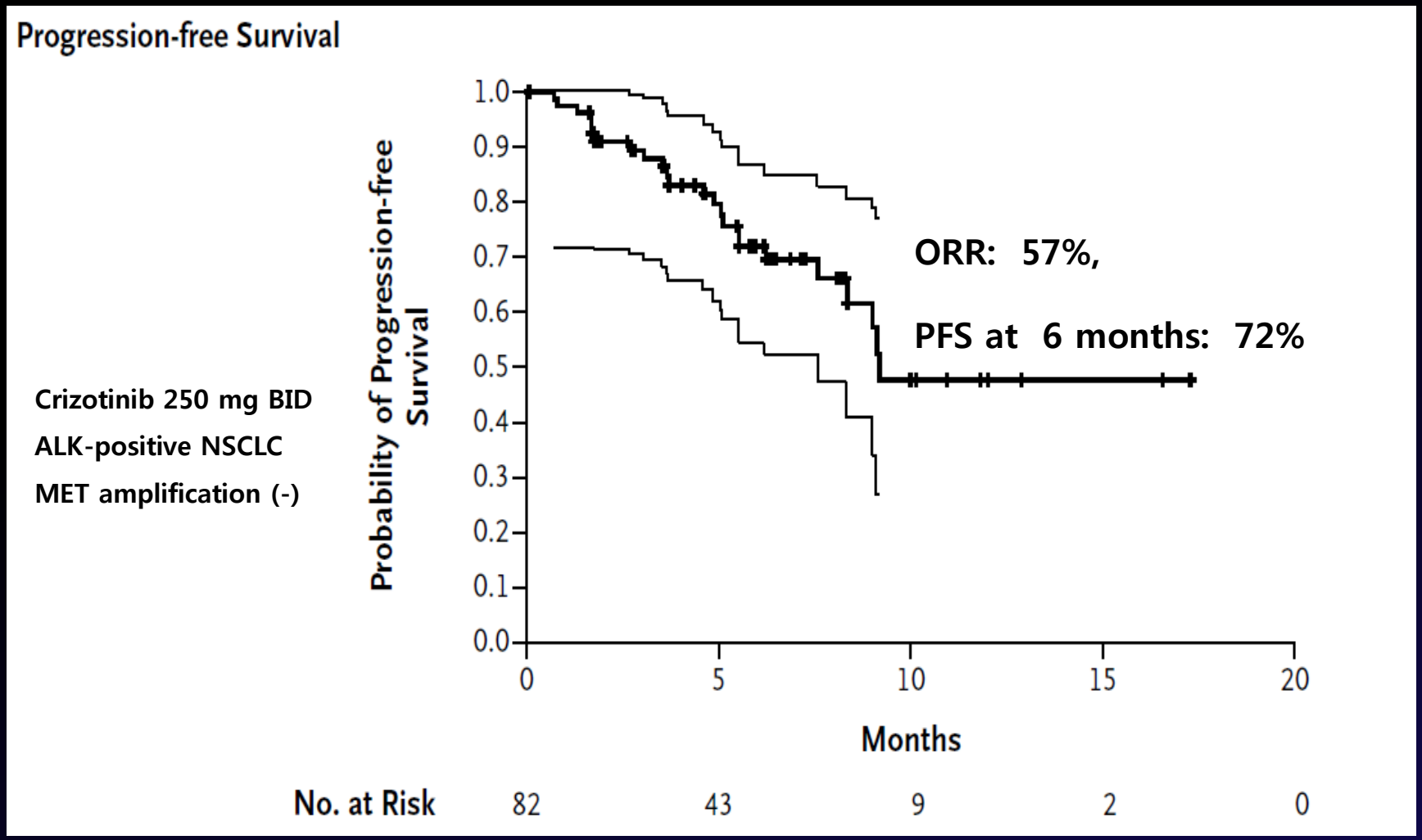
Cheng. 2012, Modern Pathology

Fluorescence in situ hybridization assay for diagnosing anaplastic lymphoma kinase (*ALK*) rearrangement



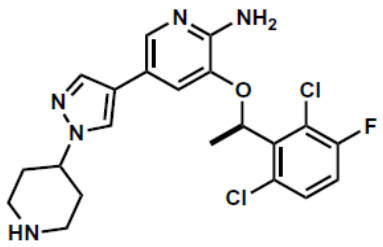
Shaw, 2009. JCO

Anaplastic Lymphoma Kinase Inhibition in Non–Small-Cell Lung Cancer

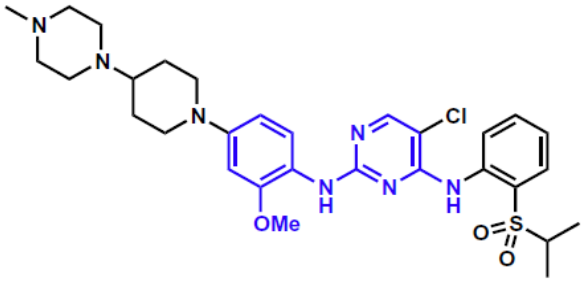


Kwak EL, 2010. N Engl J Med

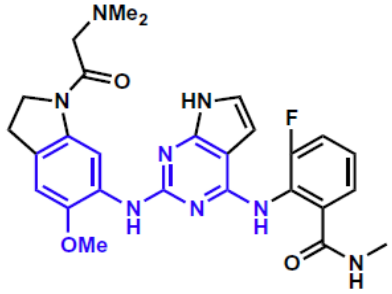
Representative chemical structures of ALK inhibitors in preclinical and clinical studies



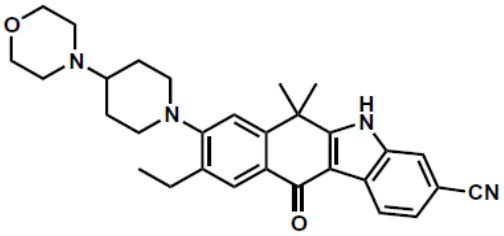
Crizotinib (Xalkori™), Pfizer
FDA-approved, Phase III
NCT00932893/NCT01154140
Active Against:
ALK^{WT}/c-Met



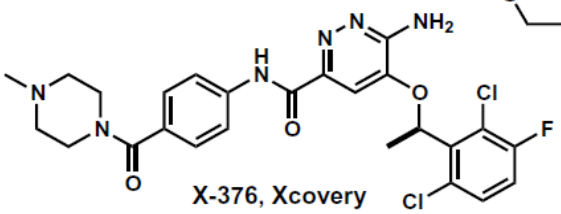
TAE-684, Novartis[51]
Not developed
Active Against:
ALK^{WT}
L1196M, F1174L mutants



GSK-1838705A, Glaxo-SmithKline[54]
Preclinical data
Active Against:
ALK^{WT}/IGFR/IR
L1196M

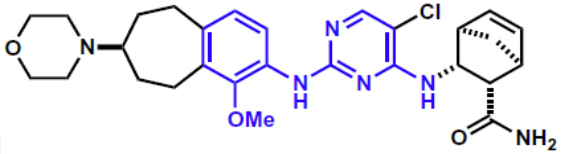


CH-5424802, Chugai Pharmaceuticals[49]
Phase I/II
NCT01588028
Active Against:
ALK^{WT}
L1196M, C1156Y, F1157L mutants



X-376, Xcovery

X-396, Xcovery[50]
Structure Undisclosed
Phase I
NCT01625234
Active Against:
ALK^{WT}
L1196M, C1156Y mutants



CEP-28122, Cephalon (Teva)[56]

CEP-37440 (*Structure Undisclosed*)
Preclinical
Active Against:
ALK^{WT}/FAK

AP-26113, Ariad Pharmaceuticals[51]
Phase I/II
NCT01449461
Active Against:
ALK^{WT}/EGFR
L1196M

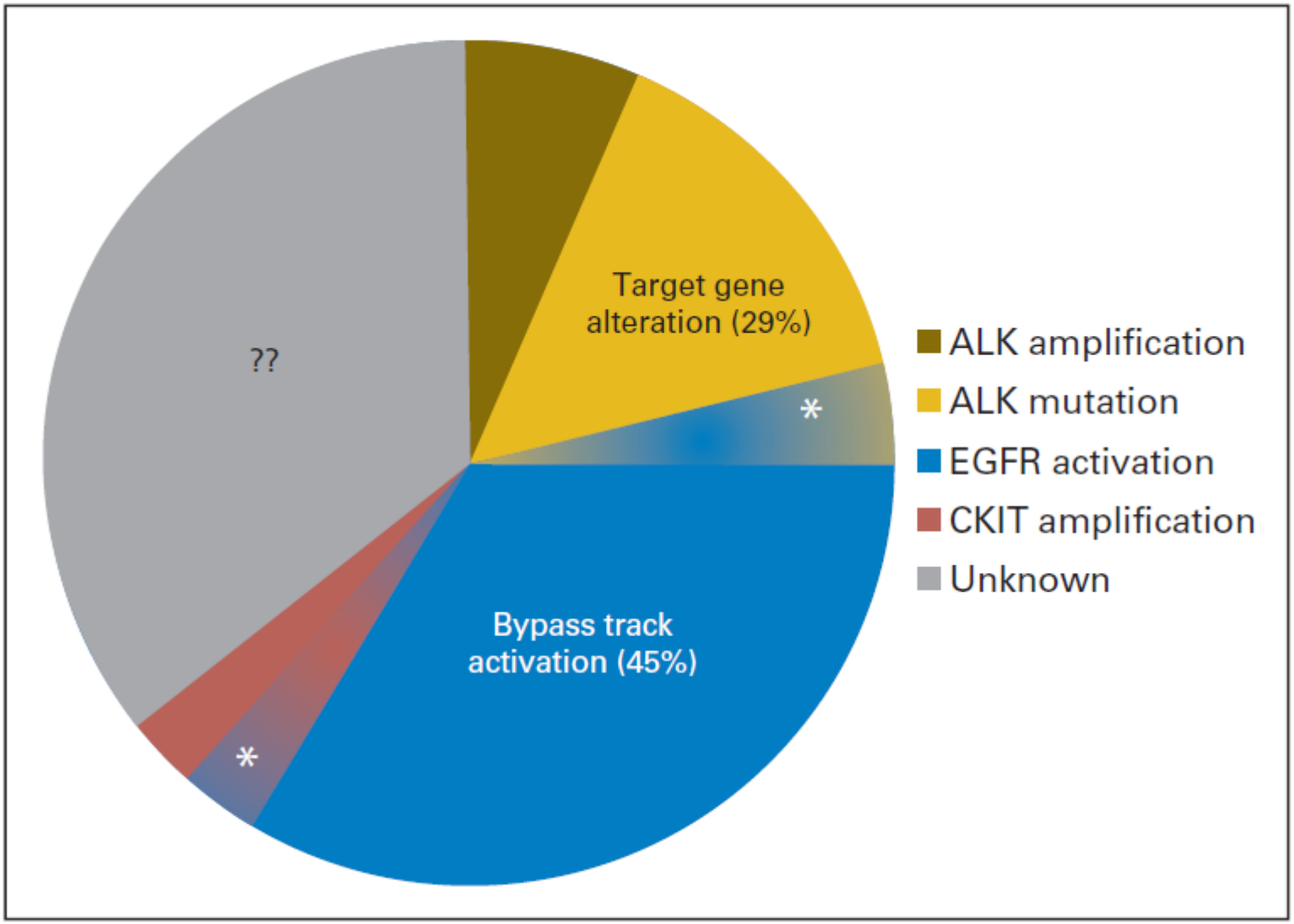
ASP-3026, Astellas Pharmaceuticals[52]
Phase I
NCT014011504/NCT01284192
Active Against:
ALK^{WT}

LDK-378, Novartis[53]
Phase I
NCT01283516
Active Against:
ALK^{WT} and
other ALK mutants

NMS-E628, Nerviano Medical Sciences[55]
Approaching IND filing
Active Against:
ALK^{WT} and other ALK mutant

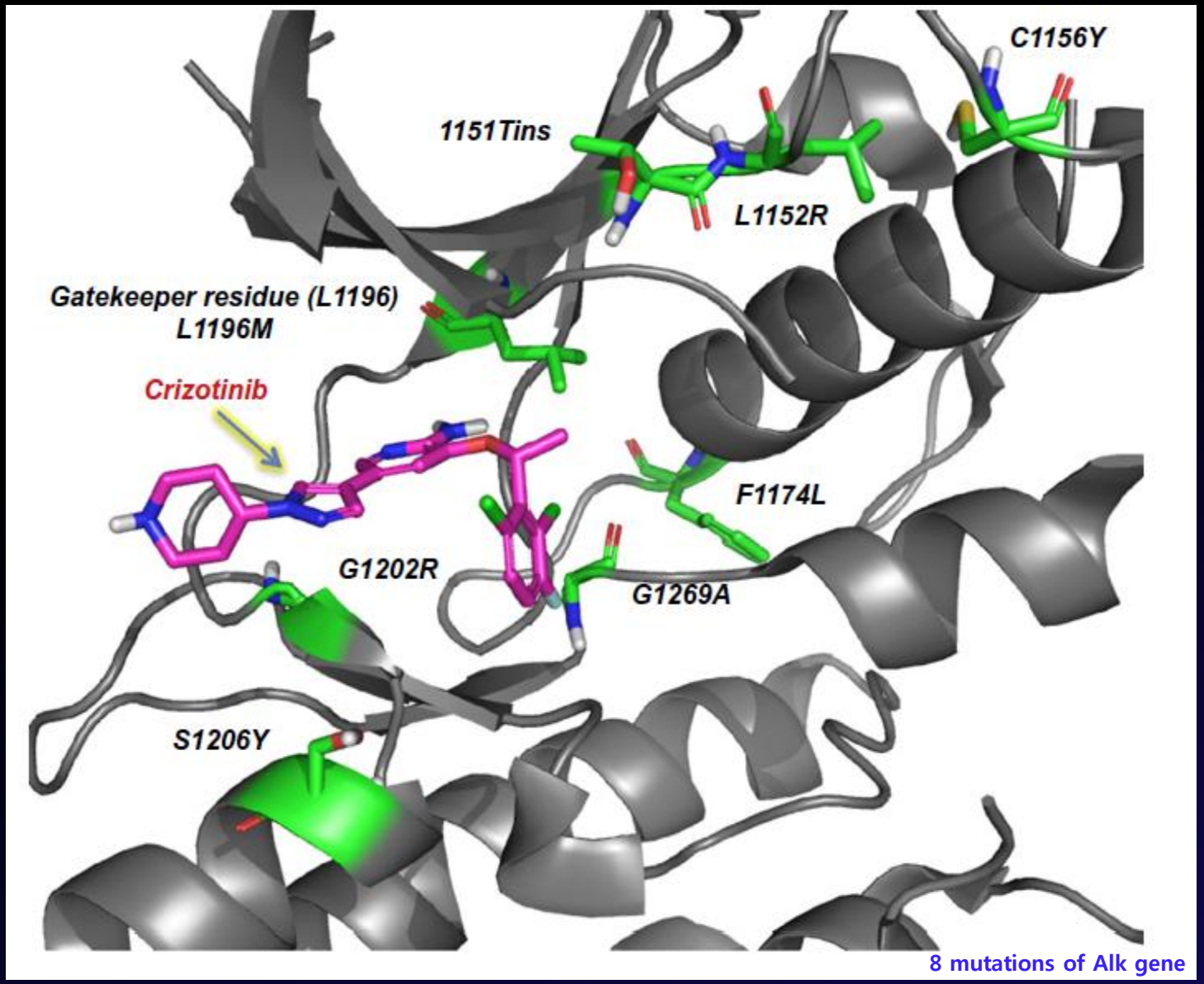
Giroux, 2013. Bioorg Med Chem Lett.

crizotinib resistance mechanisms in anaplastic lymphoma kinase (*ALK*) –positive non–small-cell lung cancer



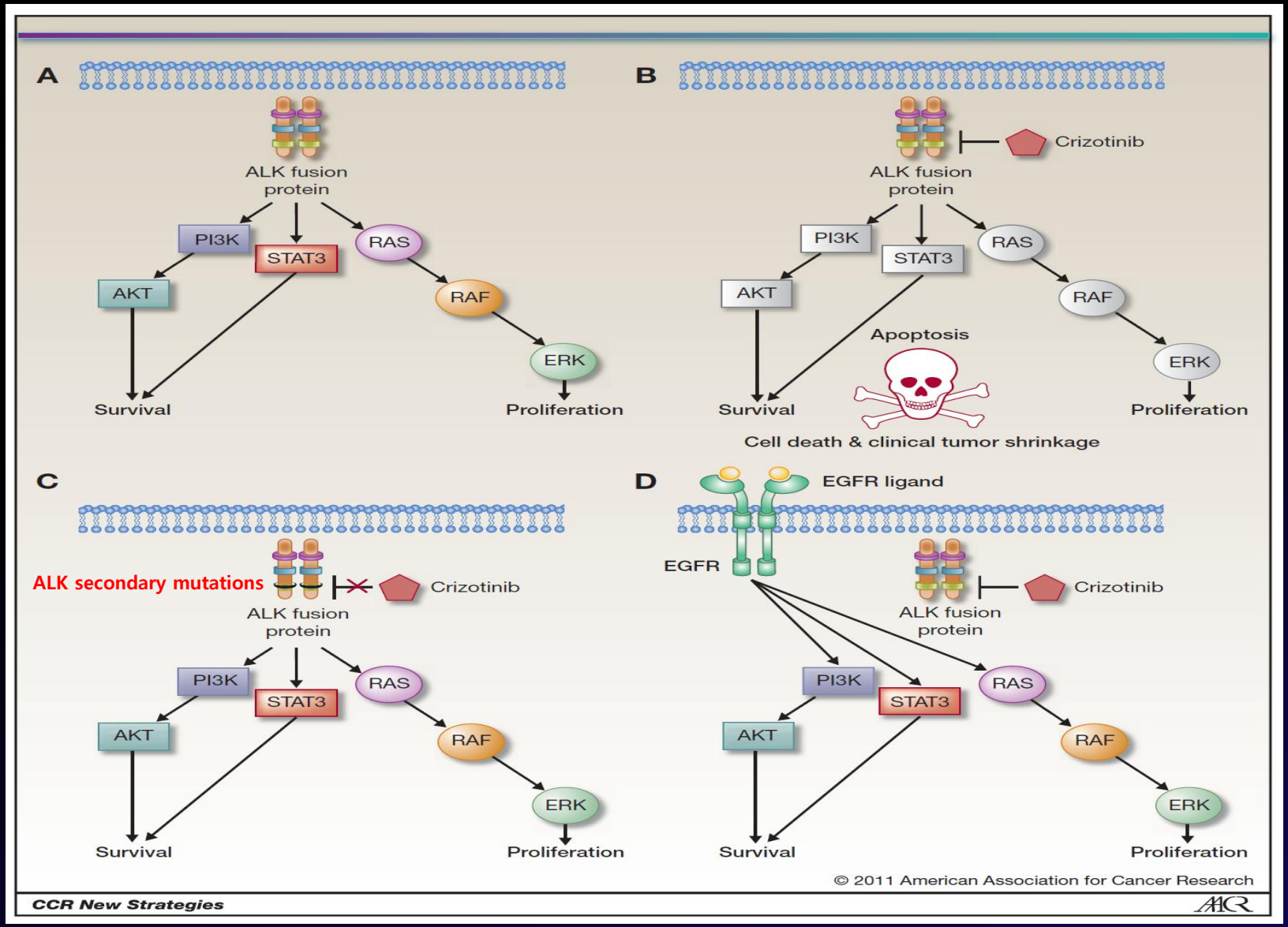
Shaw, 2013. JCO

Crizotinib (magenta) bound to the ALK kinase domain with locations of secondary mutations known to confer acquired resistance highlighted in green.



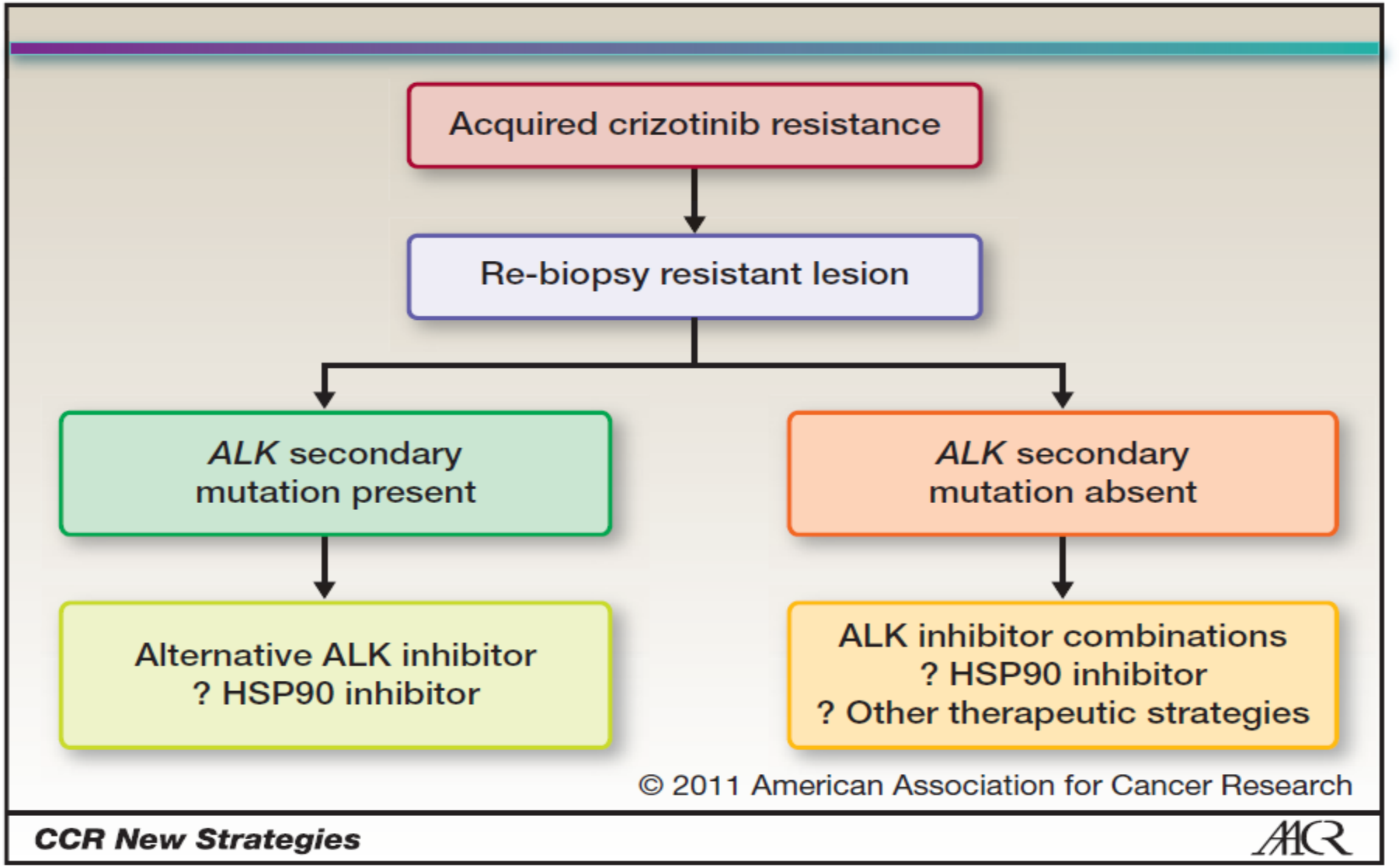
Giroux, 2013. Bioorg Med Chem Lett.

ALK signaling in drug-sensitive and drug-resistant ALK-rearranged cancers



Sasaki, 2011. Clin Cancer Res.

Potential therapeutic strategies for crizotinib-resistant ALK-rearranged NSCLC



Sasaki, 2011. Clin Cancer Res.

Summary

- patients with *ALK*-positive lung cancer invariably relapse with crizotinib as a result of the development of resistance.
- Approximately one third of resistance may be attributed to alterations in ALK itself, including a diverse array of resistance mutations as well as *ALK* fusion gene amplification.
- An additional one half of patients may experience activation of bypass tracks such as EGFR or c-KIT.
- highlighting the need for novel combinatorial strategies to overcome crizotinib resistance

Conclusion

- Cancers with various resistance mechanisms may have distinct prognoses.
- Invasive biopsies should be performed for knowledge of cancer biology.
- Technologies to assess cancers via non-invasive measures such as circulating tumor cell analyses, plasma DNA analyses, or molecular radiology may eventually obviate the need for invasive procedures.

Conclusion

- The knowledge gained from repeat biopsy directly affected treatment decisions and outcomes.

- the era of targeted therapies will mandate continual assessment of each cancer's evolution over the course of treatment to determine how it became resistant to therapy and to identify the optimal strategies to prevent or overcome it.