Molecular Testing Guideline Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

Philip T. Cagle, MD, Marc Ladanyi, MD, Neal I. Lindeman, MD

April 24, 2013

Guideline Publication

- **Lindeman NI, Cagle PT, …, Ladanyi M. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC), Association for Molecular Pathology (AMP).[Published online ahead of print at www.archivesofpathology.org]. *Arch Pathol Lab Med. doi: 10.5858/arpa.2012-0720-OA.***

- **Lindeman NI, Cagle PT, …, Ladanyi M. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC), Association for Molecular Pathology (AMP).[Published online ahead of print at http://journals.lww.com/jto/toc/publishahead]. *J Thorac Oncol. doi: 10.1097/JTO.0b013e318290868f.***

- **Lindeman NI, Cagle PT, …, Ladanyi M. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC), Association for Molecular Pathology (AMP).[Published online ahead of print at http://www.journals.elsevierhealth.com/periodicals/jmdi]. *J Mol Diagn. doi:10.1016/j.jmoldx.2013.03.001.***

Neal I. Lindeman, MD

Disclosures

Dr. Lindeman has disclosed the following:

- **Partners Health Care has filed a patent on *EGFR* Mutation Testing. NIL is not a patent holder.**

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Definition of grades of recommendations

Grade of recommendation	Description
A	Body of evidence can be trusted to guide practice
B	Body of evidence can be trusted to guide practice in most situations
C	Body of evidence provides some support for recommendation(s) but care should be taken in its application
D	Body of evidence is weak and recommendation must be applied with caution

Clinical Practice Guideline Questions

I. When should molecular testing for NSCLC be performed?

II. How should *EGFR* testing be performed?

III. How should *ALK* testing be performed?

IV. Should other genes be routinely tested in lung adenocarcinoma?

V. How should molecular testing of lung adenocarcinomas be implemented and operationalized?

Philip T. Cagle, MD, FCAP

Disclosures

Dr. Cagle has disclosed the following:

- **Archives of Pathology & Laboratory Medicine, Editor-in-Chief (Recused from the journals' approval process of this guideline)**

Question 1: Which Patients Should Be Tested for *EGFR* Mutations and *ALK* Rearrangements?

- **1.1a: Recommendation: *EGFR* molecular testing should be used to select patients for EGFR-targeted TKI therapy**, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.
- **1.1b: Recommendation: *ALK* molecular testing should be used to select patients for ALK-targeted TKI therapy**, and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.

Different Outcomes in All Stages of Non-Small Cell Lung Cancer Patients With and Without *EGFR* Mutations, Treated With Tyrosine Kinase Inhibitor

Outcome	Percentage		*P* value
	EGFR mutation Positive	*EGFR* mutation Negative	
Response rate	68%	11%	*P*<.001
Disease control rate	86%	42%	*P*<.001

Different Outcomes in All Stages of Non-Small Cell Lung Cancer Patients With and Without *EGFR* Mutations, Treated With Tyrosine Kinase Inhibitor

Outcome	Mean ± SD		*P* value
	EGFR mutation Positive	*EGFR* mutation Negative	
Time to Progression/ Progression Free Survival (months)	12.0 ± 7.86	3.4 ± 2.59	*P*<.001
Median Survival Time (months)	23.3 ± 18.4	12.1 ± 13.9	*P*<.001

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

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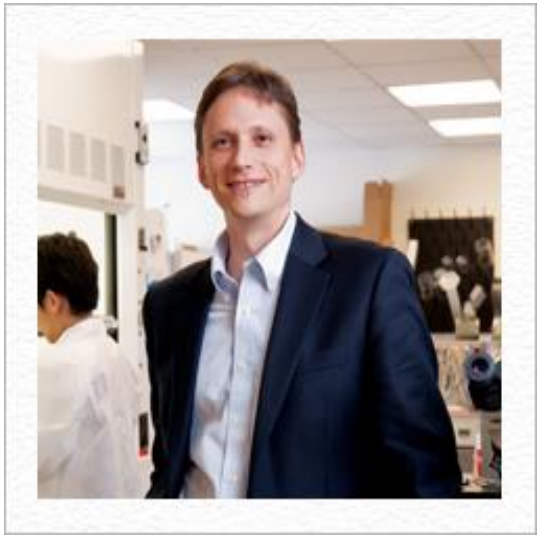
Outcomes in advanced adenocarcinoma patients with *ALK* rearrangements at a mean treatment duration of 6.4 months with crizotinib

Outcome	Percentage
Overall Response rate (%)	57%
Stable Disease	33%
Disease control rate (%) at 8 weeks	87%
Estimated 6 month probability of Progression free survival	72%



Question 1: Which Patients Should Be Tested for *EGFR* Mutations and *ALK* Rearrangements?

- **1.1a: Recommendation:** *EGFR* molecular testing should be used to select patients for EGFR-targeted TKI therapy, **and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.**
- **1.1b: Recommendation:** *ALK* molecular testing should be used to select patients for ALK-targeted TKI therapy, **and patients with lung adenocarcinoma should not be excluded from testing on the basis of clinical characteristics.**

Which Patients Should be Tested for *EGFR* Mutations: Clinical Features?

- ***EGFR*** **mutations more common in**
  - **women than men**
  - **never-smokers than former or current smokers**
  - **Asians than other ethnic groups**

Which Patients Should be Tested for *ALK* Fusion Genes: Clinical Features?

- ***ALK*** **rearrangements more common in**
  - **never/light smokers versus former or current smokers**
  - **Average age of patients is younger**

Clinical Criteria Excludes Too Many Potential Recipients Who Might Benefit

- **Not recommended to use these clinical characteristics to exclude patients for *EGFR* mutation or *ALK* rearrangement testing**
- **Despite associations, there are many exceptions**
- **Excludes significant numbers of patients who might benefit from treatment**

Which Patients Should Be Tested for *EGFR* Mutations and *ALK* Rearrangements?

- **1.2: Recommendation.—**

  In the setting of lung cancer resection specimens,

  ***EGFR* and *ALK* testing is recommended for adenocarcinomas and mixed lung cancers with an adenocarcinoma component,**

  regardless of histologic grade.

Which Patients Should Be Tested for *EGFR* Mutations and *ALK* Rearrangements?

- **1.2: Recommendation.—**

  In the setting of fully excised lung cancer specimens,

  ***EGFR* and *ALK* testing is NOT recommended in lung cancers that lack any adenocarcinoma component,**

  such as "pure" squamous cell carcinomas, "pure" small cell carcinomas, or large cell carcinomas lacking any immunohistochemistry (IHC) evidence of adenocarcinoma differentiation.

Major studies specifically reporting *EGFR* mutation analysis in surgically resected squamous cell carcinomas as compared to adenocarcinomas

Source, y	Predominant Ethnic Origin of Study Population	*EGFR* Mutations in Resected Adenocarcinomas, No. (%)	*EGFR* Mutations in Resected Squamous Cell Carcinomas, No. (%)
Marchetti, *et al.*, 2005	European	39/375 (10.4)	0/454
Sugio, *et al.*, 2006	Asian	136/322 (42.2)	0/102
Tsao, *et al.*, 2006	North American	14/96 (14.6)	0/63
Tsao, *et al.*, 2011	North American	32/231 (13.9)	8/162 (4.9)
Bae, *et al.*, 2007	Asian	20/55 (36.4)	0/60
Lee, *et al.*, 2010	Asian	36/117 (30.8)	0/56
Miyamae, *et al.*, 2011	Asian	-	3/87 (3.4)
Rekhtman, *et al.*, 2012	North American	-	0/95
TCGA, 2012	North American	-	2/178 (1.1)

Clin Cancer Res. 2012 Feb 15;18(4):1167-76.

Rekhtman N, Paik PK, Arcila ME, Tafe LJ, Oxnard GR, Moreira AL, Travis WD, Zakowski MF, Kris MG, Ladanyi M.

"Clarifying the Spectrum of Driver Oncogene Mutations in Biomarker-Verified Squamous Carcinoma of Lung: Lack of *EGFR/KRAS* and Presence of *PIK3CA/AKT1* Mutations."

Clin Cancer Res. 2012 Feb 15;18(4):1167-76.

RESULTS:

- **95 biomarker-verified SQCCs revealed no *EGFR/KRAS* mutations**
- **Detailed morphologic and immunohistochemical reevaluation of *EGFR/KRAS*-mutant "SQCC"**
- **10 (63%) cases reclassified as AD-SQCC**
- **5 (31%) cases reclassified as poorly differentiated adenocarcinoma morphologically mimicking SQCC (i.e., adenocarcinoma with "squamoid" morphology)**
- **1 (6%) case had no follow-up.**

Clin Cancer Res. 2012 Feb 15;18(4):1167-76.

CONCLUSIONS:

- **Our findings suggest that *EGFR/KRAS* mutations do not occur in pure pulmonary SQCC,**
- **and occasional detection of these mutations in samples diagnosed as "SQCC" is due to challenges with the diagnosis of AD-SQC and adenocarcinoma,**
- **which can be largely resolved by comprehensive pathologic assessment incorporating immunohistochemical biomarkers.**

Studies Specifically Reporting Outcome of *ALK* Rearrangement Studies in Squamous Cell Carcinomas

	n	*ALK* Rearrangement Positive, %
Takeuchi, et al., 2008	71	0
Takahashi, et al., 2010	75	0
Inamura, et al.,2008	48	0

Abbreviation: n, number of squamous cell carcinoma samples tested.

Which Patients Should Be Tested for *EGFR* Mutations and *ALK* Rearrangements?

- **1.3: Recommendation:**

  In the setting of more limited lung cancer specimens (biopsies, cytology) where an adenocarcinoma component cannot be completely excluded,

  ***EGFR* and *ALK* testing may be performed in cases showing squamous or small cell histology**

  but clinical criteria (eg, young age, lack of smoking history) may be useful in selecting a subset of these samples for testing.

Which Patients Should Be Tested for *EGFR* Mutations and *ALK* Rearrangements?

- **1.4: Recommendation:**

  To determine *EGFR* and *ALK* status for initial treatment selection, primary tumors or metastatic lesions are equally suitable for testing.

		Primary tumor	
		EGFR +	*EGFR*-
Metastatic lesions	*EGFR* +	108	6
	EGFR -	11	183

Which Patients Should Be Tested for *EGFR* Mutations and *ALK* Rearrangements?

- **1.5: Expert consensus opinion:**

  For patients with multiple, apparently separate, primary lung adenocarcinomas, each tumor may be tested but testing of multiple different areas within a single tumor is not necessary.

Question 2: When Should a Patient Specimen Be Tested for *EGFR* Mutation or *ALK* Rearrangement?

- **2.1a: Recommendation:**

  ***EGFR* mutation testing should be ordered at the time of diagnosis for patients presenting with advanced-stage disease (stage IV) who are suitable for therapy**

  or at time of recurrence or progression in patients who originally presented with lower-stage disease but were not previously tested.

Question 2: When Should a Patient Specimen Be Tested for *EGFR* Mutation or *ALK* Rearrangement?

- **2.1b: Suggestion:**

  ***ALK* rearrangement testing should be ordered at the time of diagnosis for patients presenting with advanced-stage disease (stage IV) who are suitable for therapy or at time of recurrence**

  or progression in patients who originally presented with lower-stage disease but were not previously tested.

Question 2: When Should a Patient Specimen Be Tested for *EGFR* Mutation or *ALK* Rearrangement?

- **2.2a: Expert Consensus Opinion:**

  ***EGFR* testing of tumors at diagnosis from patients presenting with stage I, II, or III disease is encouraged**

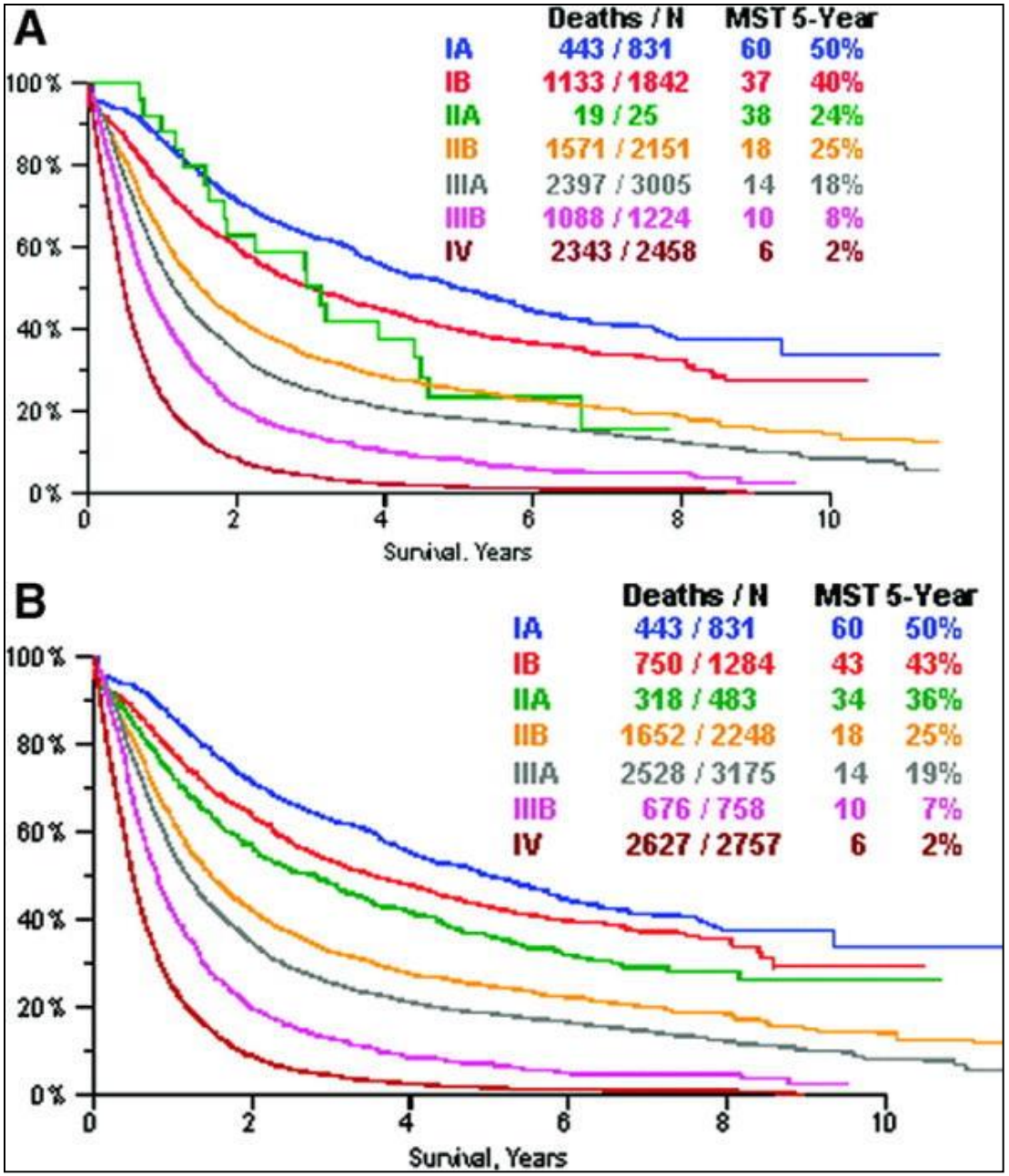
  but the decision to do so should be made locally by each laboratory, in collaboration with its oncology team.

Question 2: When Should a Patient Specimen Be Tested for *EGFR* Mutation or *ALK* Rearrangement?

- **2.2b: Expert Consensus Opinion:**

  ***ALK* testing of tumors at diagnosis from patients presenting with stage I, II, or III disease is encouraged,**

  but the decision to do so should be made locally by each laboratory, in collaboration with its oncology team.



Goldstraw et al.
Journal of Thoracic Oncology.
2(8):706-714, August 2007.

Question 2: When Should a Patient Specimen Be Tested for *EGFR* Mutation or *ALK* Rearrangement?

- **2.3: Recommendation:**

  Tissue should be prioritized for *EGFR* and *ALK* testing.

Neal I. Lindeman, MD

Question 3: How Rapidly Should Test Results Be Available?

- 3.1: Expert Consensus Opinion: *EGFR* and *ALK* results should be available within 2 weeks (**10 working days**) of receiving the specimen in the testing laboratory.
- 3.2: Expert Consensus Opinion: Laboratories with average turnaround times beyond 2 weeks need to make available a more rapid test —either in-house or through a reference laboratory — **in instances of clinical urgency.**
- 3.3: Expert Consensus Opinion: Laboratory departments should establish processes to ensure that specimens **that have a final pathologic diagnosis** are sent to outside molecular pathology laboratories within 3 working days of receiving requests and to intramural molecular pathology laboratories within 24 hours.

Turnaround Time (TAT)

- No publications relate TAT to outcome

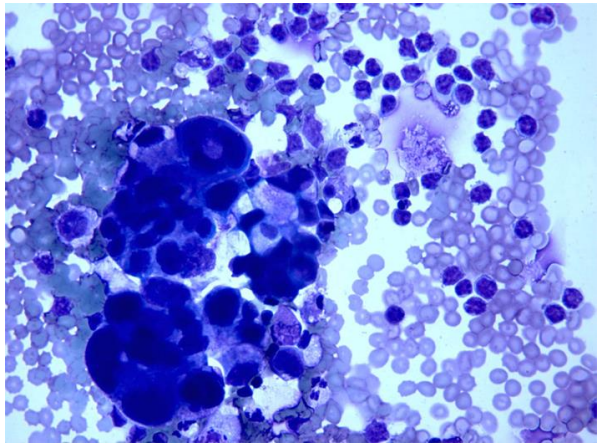
- Diagnosis must be established first
  - Need efficiency after diagnosis established
- Some patients can wait; some cannot
  - Untreated stage IV lung cancer survival: ~4 mos
    - Treatment is delayed pending test result
- Our opinion: 2 weeks or less is reasonable and feasible
  - Slowest recommended method: Sanger

Question 4: How Should Specimens Be Processed for *EGFR* Testing?

- **4.1: Expert Consensus Opinion.—**Pathologists should use formalin-fixed, paraffin-embedded (FFPE) specimens or fresh, frozen, or alcohol-fixed specimens for polymerase chain reaction (PCR)–based *EGFR* mutation tests. Other tissue treatments (eg, **acidic or heavy metal fixatives**, or **decalcifying solutions**) **should be avoided** in specimens destined for *EGFR* testing.

Question 4: How Should Specimens Be Processed for *EGFR* Mutation Testing?

- **4.2: Expert Consensus Opinion: Cytologic samples are also suitable for *EGFR* and *ALK* testing, with cell blocks being preferred over smear preparations.**

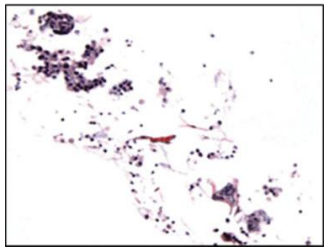


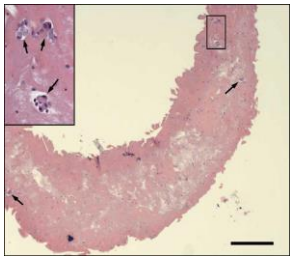
http://www.eurocytology.eu

- **Smear preparations**
  - ***EGFR* mutation: adequate if suitably cellular**
  - **ALK FISH: interpretive challenges**
    - **Overlapping nuclei**
    - **Identification of malignant cells with DAPI stain**

Question 5: What Are the Specimen Requirements for *EGFR* Testing?

- 5.1: Expert Consensus Opinion: Pathologists should determine the adequacy of specimens for *EGFR* testing by assessing **cancer cell content and DNA quantity and quality**.
- 5.2: Expert Consensus Opinion: Each laboratory should establish the **minimum proportion and number of cancer cells** needed for mutation detection during validation.
- 5.3: Expert Consensus Opinion.—A **pathologist should assess** the tumor content of **each specimen** and either perform, or guide a trained technologist to perform, microdissection for tumor cell enrichment as needed.





Question 6: How Should *EGFR* Testing Be Performed?

- 6.1: Recommendation: Laboratories may use **any** validated *EGFR* testing **method with sufficient performance characteristics**.
- 6.2: Expert consensus opinion: Laboratories should use *EGFR* test methods that are **able to detect mutations in specimens with at least 50% cancer cell content**, **although** laboratories are strongly **encouraged to use** (or have available at an external reference laboratory) **more sensitive tests** that are able to detect mutations in specimens with as little as 10% cancer cells.

Question 6: How Should *EGFR* Testing Be Performed?

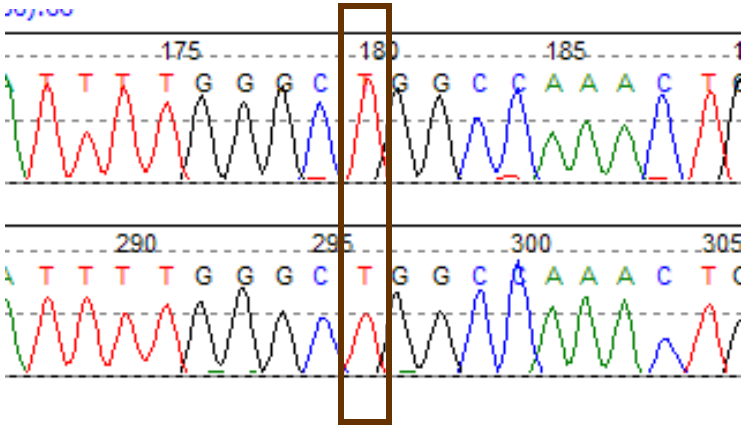
- **Sanger sequencing is OK**
  - **Initial discoveries that showed *EGFR* mutations were clinically useful used Sanger sequencing**

- **BUT…**

- **A lot of patients have samples that are too small or too heterogeneous for Sanger sequencing**
  - **Sanger labs should make a more sensitive test available for these patients**
    - **PNA/LNA enrichment, COLD-PCR, second test, sendout**

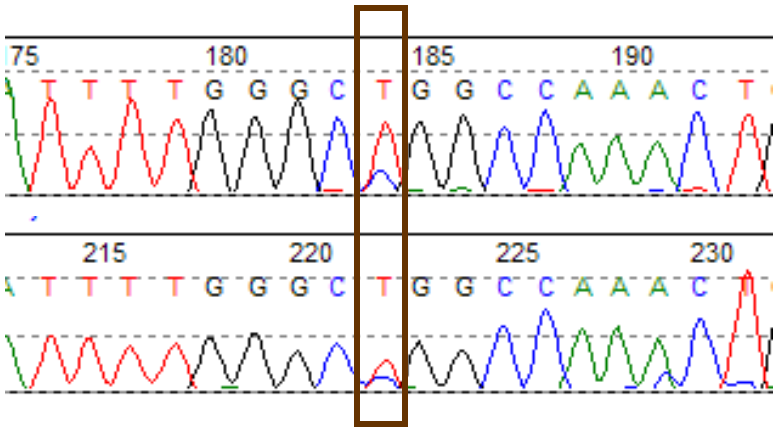
Sample with 30% Tumor content

UNMODIFIED Sanger



EGFR wild type
Rx: platinum doublet
1-yr survival: 5%

PNA-enriched Sanger



EGFR exon 21 mutation
Rx: erlotinib
1-yr survival: 30%

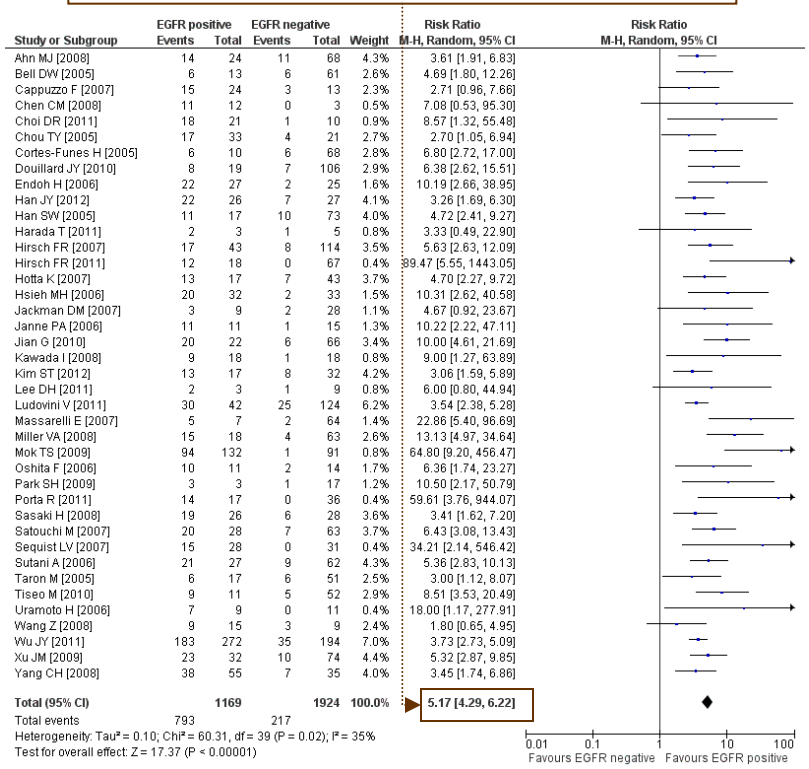
6.3 *Opinion:* Test for all *EGFR* mutations accounting individually for at least 1% of all *EGFR* mutations

EGFR exon	*EGFR* codon	Mutations[a] (amino acid)	Nucleotide substitutions	Approximate % of all *EGFR* mutations
18	E709	E709K E709A E709G E709V E709D E709Q	c.2125G>A c.2126A>C c.2126A>G c.2126A>T c.2127A>C, c.2127A>T c.2125G>C	1%
	G719	G719S G719A G719C G719D	c.2155G>A c.2156G>C c.2155G>T c.2156G>A	2-5%
19	K739, I740, P741, V742, A743, I744	Insertions 18 bp ins		1%
	E746, L747, R748, E749, A750, T751, S752, P753	Deletions 15bp del 18bp del 9 bp del 24bp del 12bp del		45%
20	A763, A767, S768, V769, D770, N771, P772, H773, V774	Insertions 3 bp ins 6 bp ins 9 bp ins 12 bp ins		5-10%
	S768	S768I	c.2303G>T	1-2%
	T790	T790M	c.2369C>T	2%[b]
21	L858	L858R L858M	c.2573T>G c.2572C>A (rare)	40%
	L861	L861Q L861R	c.2582T>A, c.2582T>G	2-5%

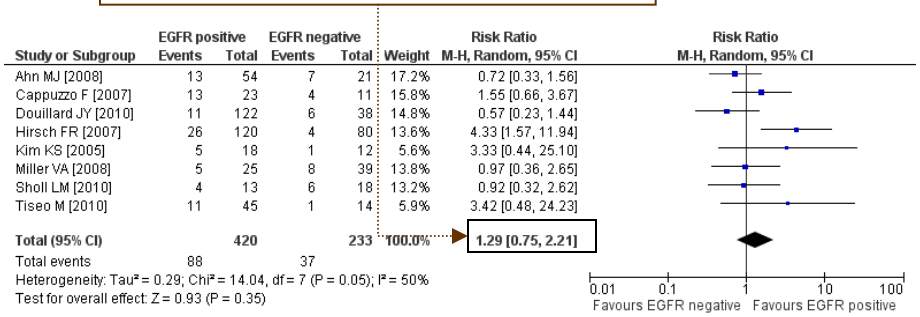
Question 6: How Should *EGFR* Testing Be Performed?

- 6.4: Recommendation: **Immunohistochemistry** for **total EGFR** is **not recommended** for selection of EGFR TKI therapy.

Mutation vs. response rate
RR=5.2



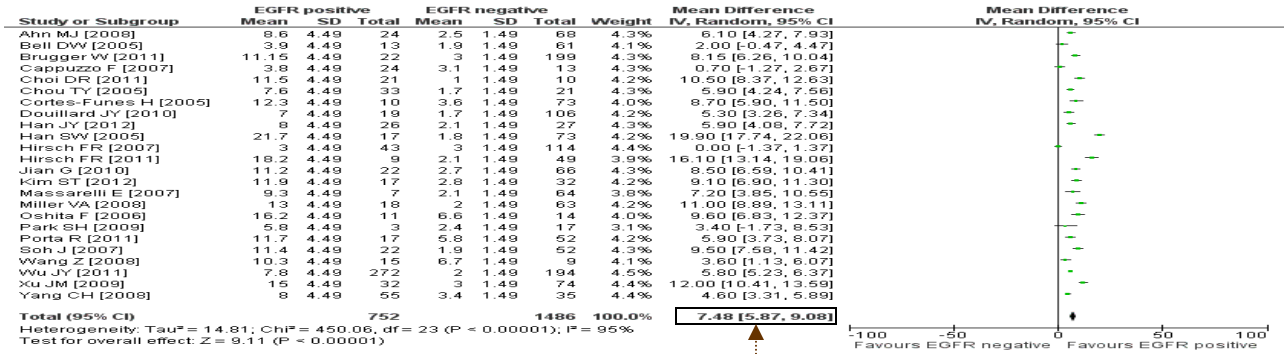
IHC vs. response rate
RR=1.3



Question 6: How Should *EGFR* Testing Be Performed?

- 6.5: Recommendation: *EGFR* copy number analysis (ie, **FISH or CISH**) is **not recommended** for selection of EGFR TKI therapy.

Mutation vs. PFS
WMD=7.5



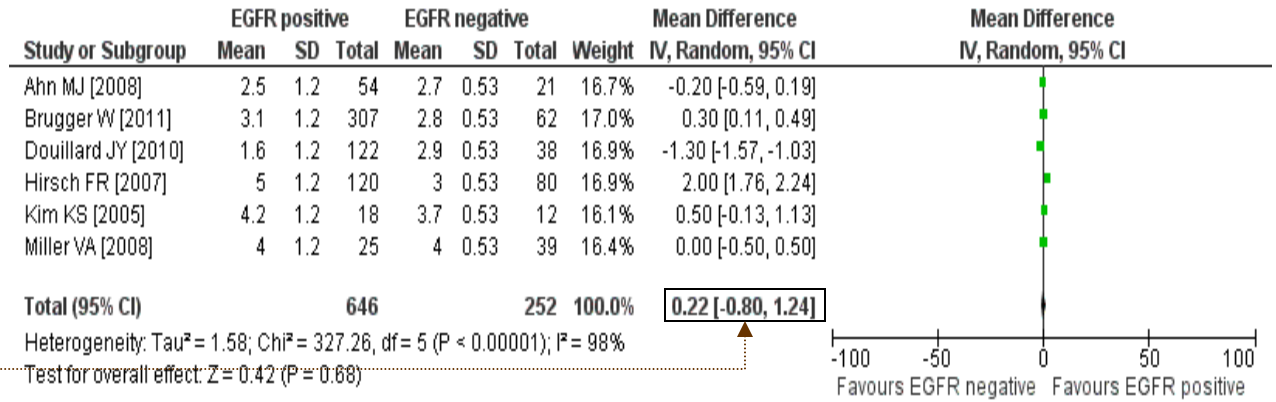
Study or Subgroup	EGFR positive Mean	SD	Total	EGFR negative Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Ahn MJ [2008]	8.6	4.49	24	2.5	1.49	68	4.3%	6.10 [4.27, 7.93]
Bell DW [2005]	3.9	4.49	13	1.9	1.49	61	4.1%	2.00 [-0.47, 4.47]
Brugger W [2011]	11.15	4.49	22	3	1.49	199	4.3%	8.15 [6.26, 10.04]
Cappuzzo F [2007]	3.8	4.49	24	3.1	1.49	13	4.3%	0.70 [-1.27, 2.67]
Choi DR [2011]	11.5	4.49	21	1	1.49	10	4.2%	10.50 [8.37, 12.63]
Chou TY [2005]	7.6	4.49	33	1.7	1.49	21	4.3%	5.90 [4.24, 7.56]
Cortes-Funes H [2005]	12.3	4.49	10	3.6	1.49	73	4.0%	8.70 [5.90, 11.50]
Douillard JY [2010]	7	4.49	19	1.7	1.49	106	4.2%	5.30 [3.26, 7.34]
Han JY [2012]	8	4.49	26	2.1	1.49	27	4.3%	5.90 [4.08, 7.72]
Han SW [2005]	21.7	4.49	17	1.8	1.49	73	4.2%	19.90 [17.74, 22.06]
Hirsch FR [2007]	3	4.49	43	3	1.49	114	4.4%	0.00 [-1.37, 1.37]
Hirsch FR [2011]	18.2	4.49	9	2.1	1.49	49	3.9%	16.10 [13.14, 19.06]
Jian G [2010]	11.2	4.49	22	2.7	1.49	66	4.3%	8.50 [6.59, 10.41]
Kim ST [2012]	11.9	4.49	17	2.8	1.49	32	4.2%	9.10 [6.90, 11.30]
Massarelli E [2007]	9.3	4.49	7	2.1	1.49	64	3.8%	7.20 [3.85, 10.55]
Miller VA [2008]	13	4.49	18	2	1.49	63	4.2%	11.00 [8.89, 13.11]
Oshita F [2006]	15.2	4.49	11	5.6	1.49	14	4.0%	9.60 [6.83, 12.37]
Park SH [2009]	5.8	4.49	3	2.4	1.49	17	3.1%	3.40 [-1.73, 8.53]
Porta R [2011]	11.7	4.49	17	5.8	1.49	52	4.2%	5.90 [3.73, 8.07]
Soh J [2007]	11.4	4.49	22	1.9	1.49	52	4.3%	9.50 [7.58, 11.42]
Wang Z [2008]	10.3	4.49	15	6.7	1.49	9	4.1%	3.60 [1.13, 6.07]
Wu JY [2011]	7.8	4.49	272	2	1.49	194	4.5%	5.80 [5.23, 6.37]
Xu JM [2009]	15	4.49	32	3	1.49	74	4.4%	12.00 [10.41, 13.59]
Yang CH [2008]	8	4.49	55	3.4	1.49	35	4.4%	4.60 [3.31, 5.89]
Total (95% CI)			752			1486	100.0%	7.48 [5.87, 9.08]

Heterogeneity: Tau² = 14.81; Chi² = 450.06, df = 23 (P < 0.00001); I² = 95%
Test for overall effect: Z = 9.11 (P < 0.00001)

Mean Difference IV, Random, 95% CI

-100 -50 0 50 100
Favours EGFR negative Favours EGFR positive

ISH vs. PFS
WMD=0.22



Study or Subgroup	EGFR positive Mean	SD	Total	EGFR negative Mean	SD	Total	Weight	Mean Difference IV, Random, 95% CI
Ahn MJ [2008]	2.5	1.2	54	2.7	0.53	21	16.7%	-0.20 [-0.59, 0.19]
Brugger W [2011]	3.1	1.2	307	2.8	0.53	62	17.0%	0.30 [0.11, 0.49]
Douillard JY [2010]	1.6	1.2	122	2.9	0.53	38	16.9%	-1.30 [-1.57, -1.03]
Hirsch FR [2007]	5	1.2	120	3	0.53	80	16.9%	2.00 [1.76, 2.24]
Kim KS [2005]	4.2	1.2	18	3.7	0.53	12	16.1%	0.50 [-0.13, 1.13]
Miller VA [2008]	4	1.2	25	4	0.53	39	16.4%	0.00 [-0.50, 0.50]
Total (95% CI)			646			252	100.0%	0.22 [-0.80, 1.24]

Heterogeneity: Tau² = 1.58; Chi² = 327.26, df = 5 (P < 0.00001); I² = 98%
Test for overall effect: Z = 0.42 (P = 0.68)

Mean Difference IV, Random, 95% CI

-100 -50 0 50 100
Favours EGFR negative Favours EGFR positive

Marc Ladanyi, MD

Disclosures

Dr. Ladanyi has disclosed the following:

- **Consultancy: Arqule / Daiichi Sankyo (April 2010), NanoString (September 2012)**
- **Lecture Fees Paid by Entity: Genzyme (March 2010), Infinity (July 2010), Sequenom (November 2009), Remedica Medical Education (June 2012)**
- **Family and Business Partners: Wife: Continuing Medical Education (CME) activities for Abbott**
- **Institutional Financial Interest: Memorial Sloan-Kettering Cancer Center (MSKCC) licensed patent for EGFR T790M testing to MolecularMD. ML is not a patent holder.**

Question 7: What Is the Role of *KRAS* Analysis in Selecting Patients for Targeted Therapy With EGFR TKIs?

- **7.1: Recommendation: *KRAS* mutation testing is not recommended as a sole determinant of EGFR TKI therapy.**
  - *KRAS* mutations are mutually exclusive with EGFR mutations (and ALK fusions)
  - *KRAS* mutations are the most common oncogene mutations in lung adenocarcinoma (approx. 30-35%)
  - *KRAS* mutations are "easy" to study: >95% are in codons G12 and G13 so can be detected by sequencing just exon 2 of *KRAS*
  - *KRAS* mutations predict lack of response to EGFR TKIs

KRAS Mutations: A Negative Predictor for Response to EGFR TKIs

Table 1. Retrospective Analyses of EGFR Tyrosine Kinase Inhibitors in Lung Adenocarcinoma

Author	Drugs	Patients tested for *KRAS* mutations (mutant/WT)	Response rate *KRAS* mutant	Response rate *KRAS* WT
Jackman[12]	Erlotinib	41 (6/35)	0%	14%
Zhu[13]	Erlotinib	206 (30/176)	5%	10%
Miller[9]	Erlotinib	80 (18/62)	0%	30%
Massarelli[14]	Erlotinib/Gefitinib	70 (16/54)	0%	7%
Hirsch[10]	Gefitinib	138 (36/102)	1%	7%
Hirsch[15]	Gefitinib	152 (12/140)	0%	8%
Han[16]	Gefitinib	69 (9/60)	0%	27%

WT: wild type (non-mutated).

Riely GJ, Ladanyi M. *KRAS* Mutations: an old oncogene becomes a new predictive biomarker. J Mol Diagn 10:493-495, 2008.

Impact of *KRAS* mutations on outcomes in patients for treated with EGFR Tyrosine Kinase Inhibitors

Outcome	Percentage		n (N)	RR [95% CI]	*P* value
	KRAS Mutations	No *KRAS* Mutations			
Response rate (%)	3%	24%	12(1041)	0.33 [0.18, 0.60]	***P*<.001**

Outcome	Mean ± SD		n (N)	*P* value
	KRAS Mutations	No *KRAS* Mutations		
Time to Progression/ Progression Free Survival (months)	3.4 ± 2.7	5 ± 3.7	7(918)	***P*=.002**
Median Overall Survival time (months)	9.2 ± 5.6	13.2 ± 7.1	7(737)	***P*=.006**

Abbreviations: CI, Confidence interval; n, Number of studies; N, Number of patients; RR, Relative Risk, Mantel-Haenszel, Random Effects model, [95% CI]

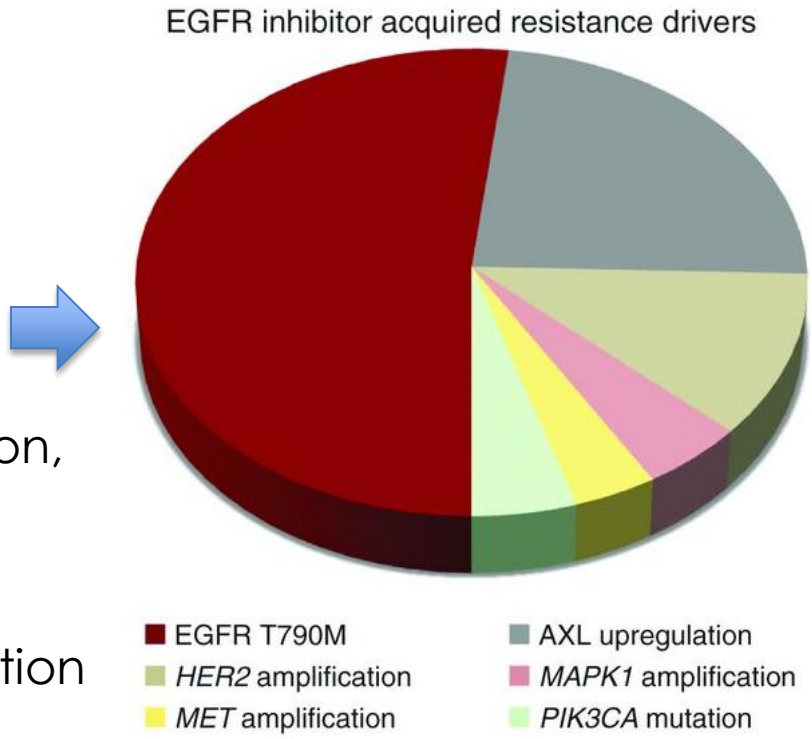
- … but a lack of *KRAS* mutation is only associated with a 24% response rate to EGFR TKI because most (approx. 70%) of *KRAS-non-mutated* cases also lack *EGFR* mutations.
- A rapid and inexpensive *KRAS* assay may be performed to exclude *KRAS-mutated* tumors from *EGFR* mutation testing as part of an algorithm designed to maximize testing efficiency.

Question 8: What Additional Testing Considerations Are Important in the Setting of Secondary or Acquired EGFR TKI Resistance?

- **8.1: Recommendation: If a laboratory performs testing on specimens from patients with acquired resistance to EGFR kinase inhibitors, such tests should be able to detect the secondary *EGFR* T790M mutation in as few as 5% of cells.**
  - As a secondary, acquired mutation, the T790M is not present in every tumor cell.
  - Biopsies of previously treated, recurrent tumors often have low tumor cell content, further increasing the need for more sensitive mutation detection.
  - In vitro studies suggest that cell population–level EGFR TKI resistance becomes detectable in the presence of as little as 5% T790M-bearing cells

Detection of *EGFR* T790M in tumors from patients with relapse after initial response to EGFR TKI treatment

Study or Subgroup	T790M	Total	Percent
Chen HJ, *et al*., 2009	14	29	48%
Kosaka T, *et al*., 2006	7	14	50%
Onitsuka T, *et al*., 2010	7	10	70%
Oxnard, *et al*., 2011	58	93	62%
Total	**86**	**146**	**59%**

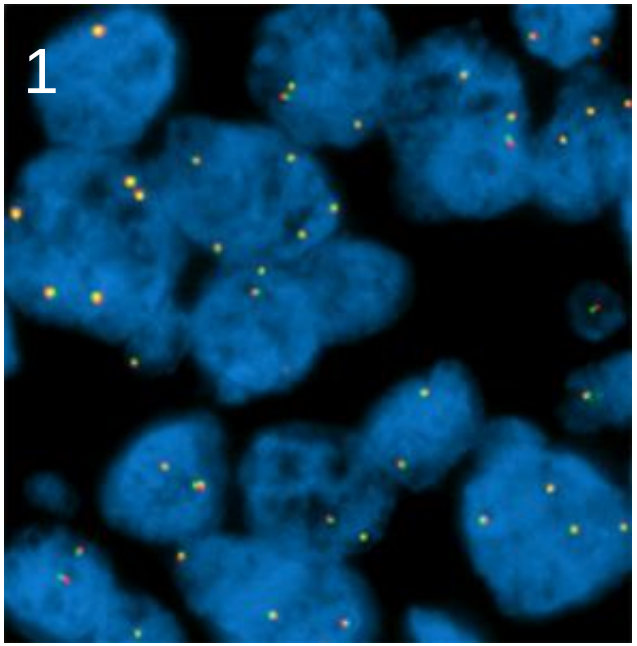


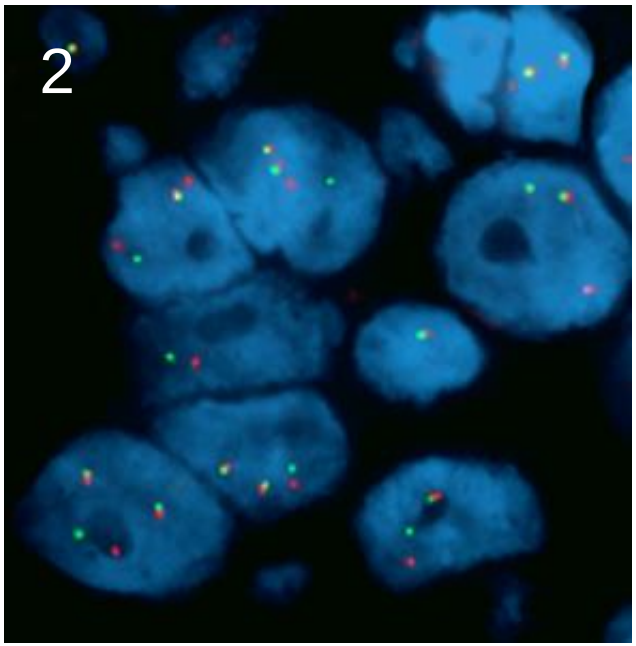
- The EGFR tyrosine kinase domain mutation, T790M, is caused by a single base substitution, C to T, at nucleotide 2369.
- This mutation is found as a second mutation on the *EGFR* allele harboring the initial ‘‘sensitizing’’ *EGFR* mutation

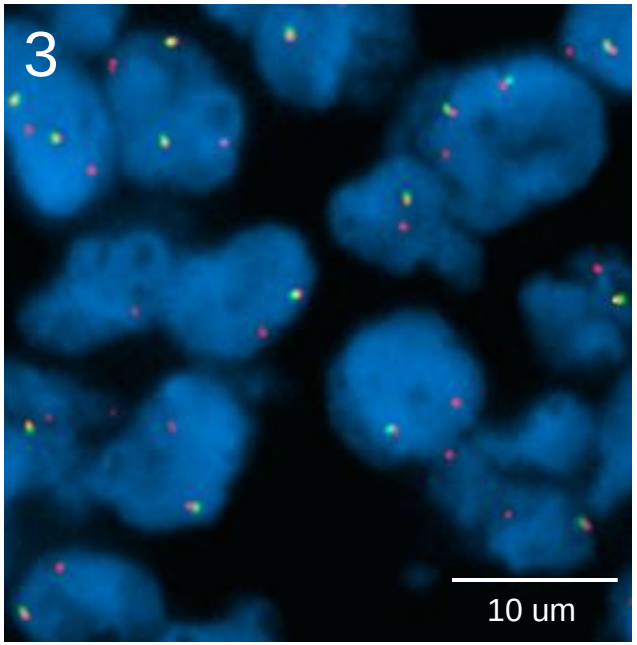
Blakely C M , Bivona T G Cancer Discovery 2012;2:872-875

Question 9: What methods should be used for *ALK* testing?

- **9.1: Recommendation: Laboratories should use an *ALK* FISH assay using dual-labeled break-apart probes for selecting patients for ALK TKI therapy; ALK immunohistochemistry, if carefully validated, may be considered as a screening methodology to select specimens for *ALK* FISH testing.**
  - FISH was the methodology used in the initial studies that demonstrated major clinical responses of patients with ALK-rearranged tumors to treatment with crizotinib, a targeted ALK TKI







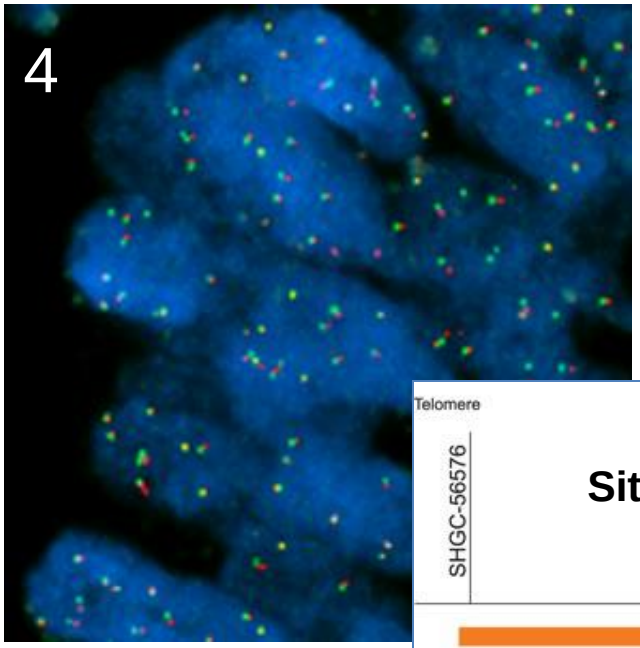
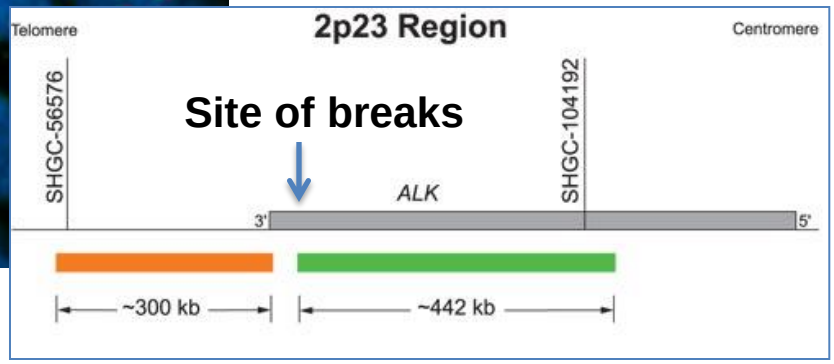


Figure 1. Negative for *ALK* rearrangement

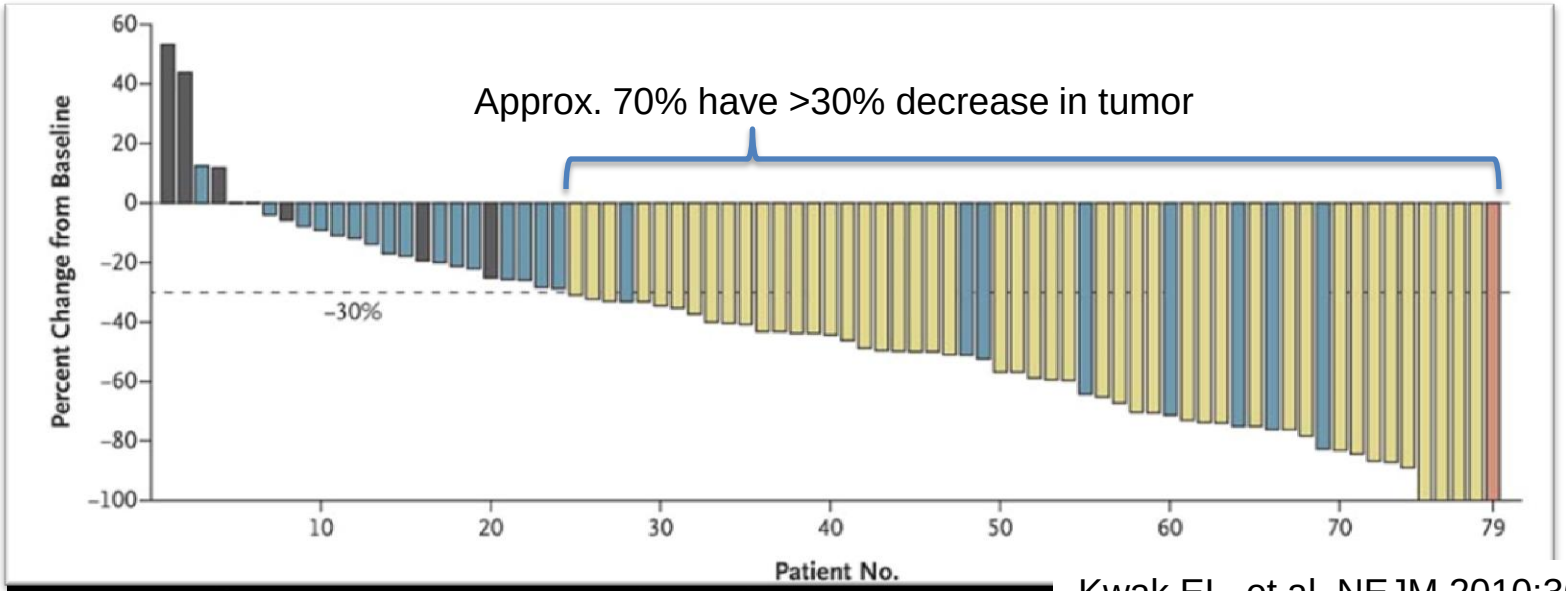
Figure 2. Positive for *ALK* rearrangement
(split 3’ ALK-5’ ALK)

Figure 3. Positive for *ALK* rearrangement
(single 3’ ALK)

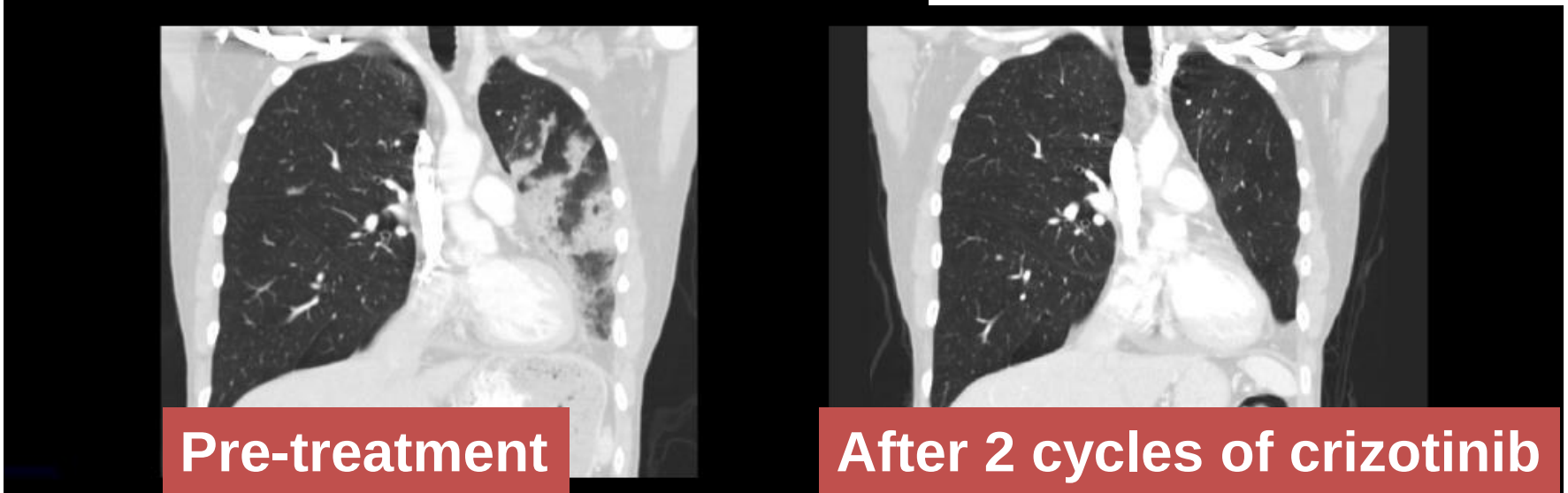
Figure 4. Negative for *ALK* rearrangement
with ALK high copy number



Crizotinib in *EML4-ALK* fusion positive lung adenocarcinoma



Kwak EL, et al. NEJM 2010;363:1693-1703.

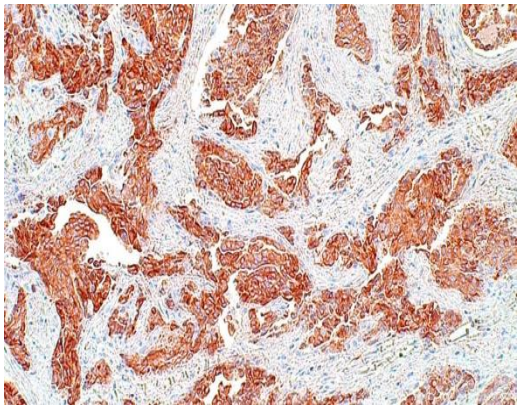


Comparing *ALK* FISH with Immunohistochemistry (IHC)

		Concordance		Discordance	
IHC Antibody	**n(N)**	**FISH+/IHC+**	**FISH-/IHC-**	**FISH+/IHC-**	**FISH-/IHC+**
IHC - CD246	4(391)	25	344	20	2
IHC - D5F3/D9E4	3(148)	46	101	1	0
IHC - 5A4	1(640)	28	602	0	10

Abbreviations: n, Number of studies; N, Number of patients

- a properly validated ALK IHC method may be used as a screening modality, and tumors that fail to demonstrate ALK immunoreactivity with a sensitive IHC method may not need to be tested by ALK FISH

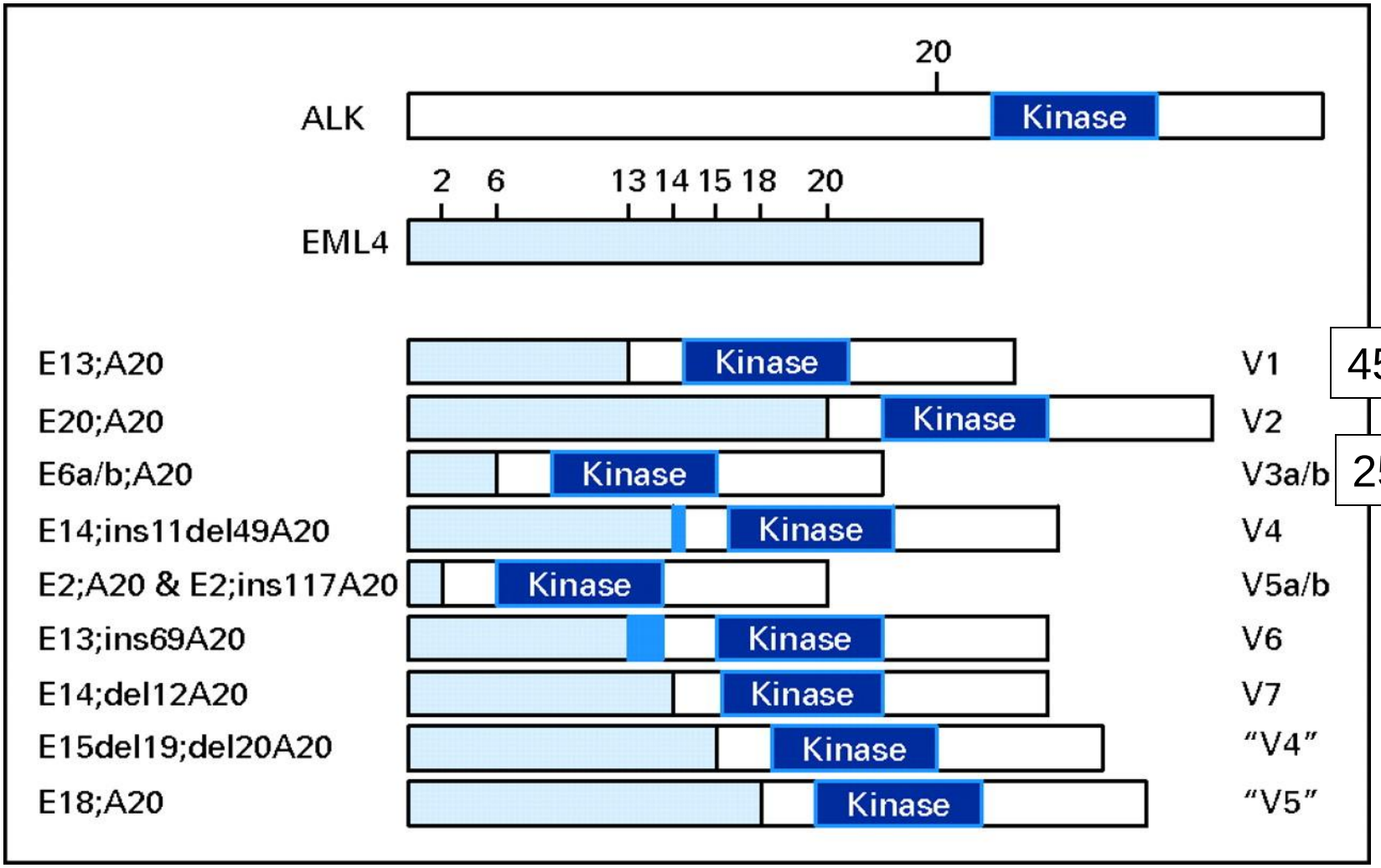


ALK D5F3 Rabbit mAb

Question 9: What methods should be used for *ALK* testing?

- **9.2: Recommendation: RT-PCR is not recommended as an alternative to FISH for selecting patients for ALK inhibitor therapy.**

ALK fusions: multiplicity of *EML4-ALK* variants + rare other *ALK* fusion partners complicate comprehensive detection by RT-PCR



Horn L , Pao W JCO 2009;27:4232-4235

Question 9: What methods should be used for *ALK* testing?

- **9.3: Expert consensus opinion: A pathologist should be involved in the selection of sections for *ALK* FISH testing, by assessing tumor architecture, cytology, and specimen quality**
  - For ALK FISH, a pathologist should choose slides or indicate regions of slides for scoring in which tumor cells are most numerous and can be distinguished from admixed normal cells under fluorescence, typically through a combination of cytologic and architectural features that can be appreciated without stains or visualization of cytoplasm.

Question 9: What methods should be used for *ALK* testing?

- **9.4: Expert consensus opinion: A pathologist should participate in the interpretation of *ALK* FISH slides, either by performing the analysis directly or by reviewing the interpretations of cytogeneticists or technologists with specialized training in solid tumor FISH analysis.**
  - The FISH technologist should work closely with a pathologist who can identify tumor-rich areas.
  - The FISH technologist should also have had training on the morphologic appearance of lung cancer, and should have easy access to assistance from a pathologist with training in FISH.

Question 9: What methods should be used for *ALK* testing?

- **9.5: Expert consensus opinion: Testing for secondary mutations in *ALK* associated with acquired resistance to ALK inhibitors is not currently required for clinical management.**
  - A diverse set of secondary mutations in ALK have been reported to confer acquired resistance to crizotinib (L1152R, C1156Y, F1174L, L1196M, L1198P, D1203N, G1269A).
  - The spectrum of acquired resistance mechanisms and their implications for further management require further studies.

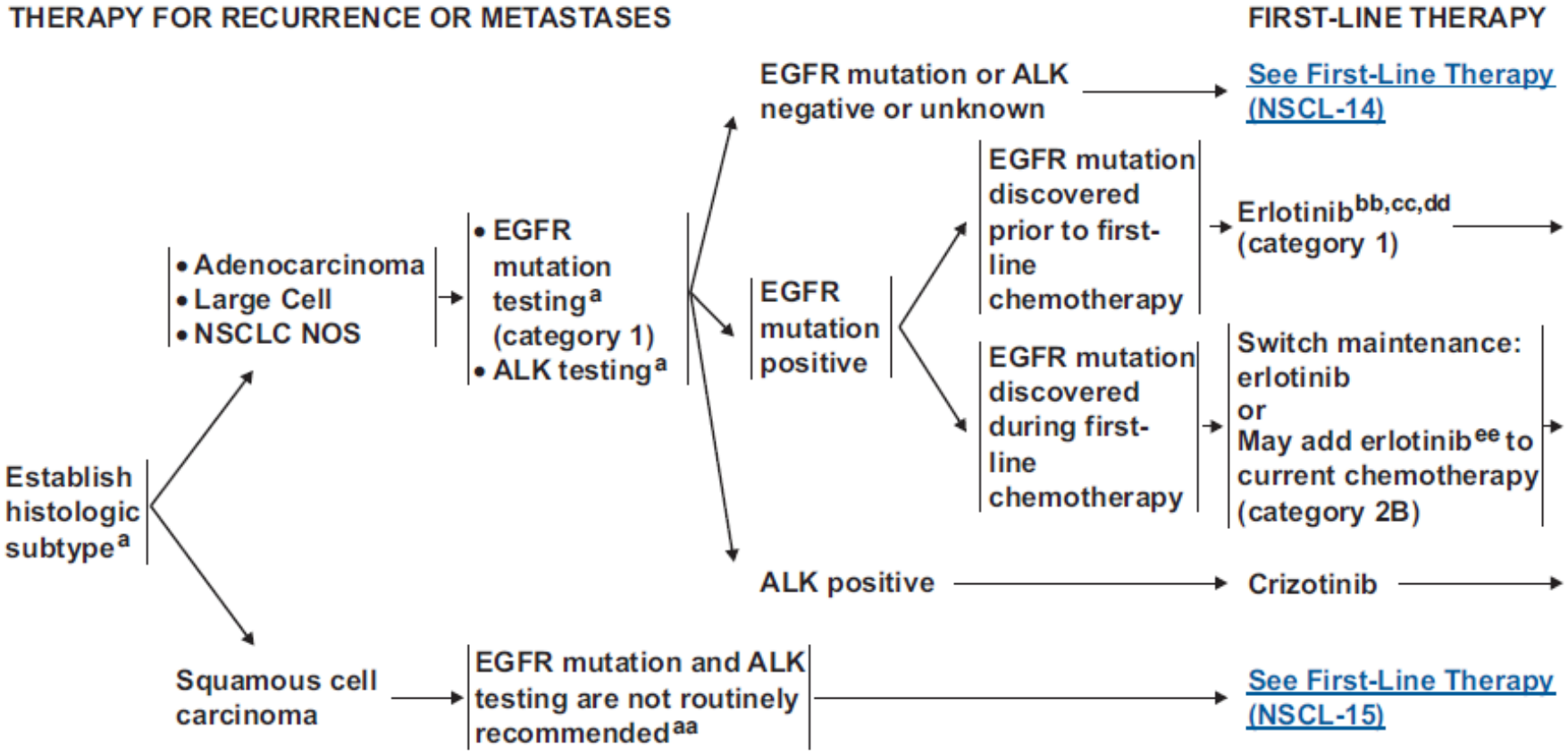
Question 10: Are Other Molecular Markers Suitable for Testing in Lung Cancer?

- **10.1a: Recommendation: Testing for *EGFR* should be prioritized over other molecular markers in lung adenocarcinoma.**
- **10.1b: Suggestion.—After *EGFR* testing, testing for *ALK* should be prioritized over other proposed molecular markers in lung adenocarcinoma, for which published evidence is insufficient to support testing guideline development at the present time.**
  - In advanced stage patients diagnosed by small biopsies, precious tumor tissue must be reserved for these analyses, before any other molecular analysis is considered.

Priority of Testing for *EGFR* and *ALK* in major clinical guidelines

National Comprehensive Cancer Network®

NCCN Guidelines Version 3.2012
Non-Small Cell Lung Cancer

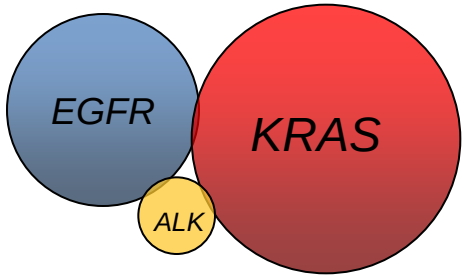


Neal I. Lindeman, MD

Question 11: Must All Adenocarcinomas Be Tested for Both *EGFR* and *ALK*?

- 11.1: Expert consensus opinion: Laboratories **may implement testing algorithms** to enhance the efficiency of molecular testing of lung adenocarcinomas, provided the overall **turnaround time** requirements are met.

- *EGFR*, *ALK*, and *KRAS* are largely mutually exclusive
  - If a mutation is found in one, further testing is unnecessary
    - This may not apply to novel mutations



Question 12: How Should *EGFR* and *ALK* Results Be Reported?

- 12.1: Expert consensus opinion: *EGFR* mutation testing reports and *ALK* FISH reports should include a results and **interpretation** section readily **understandable** by oncologists and by nonspecialist pathologists.

- Formal nomenclature should be used, but also translated

> nuc ish(ALKx2)(5'ALK sep 3'ALKx1)[56/100]
>
> FISH for *ALK* showed a split (positive) signal in 56% of 100 cancer cells analyzed
>
> This result demonstrates an *ALK* rearrangement and suggests that this lung cancer is likely to respond to treatment with a targeted inhibitor of the ALK kinase, such as crizotinib.

Question 13 &14: How Should *EGFR* and *ALK* Testing Be Validated? How Should Quality Assurance Be Maintained?

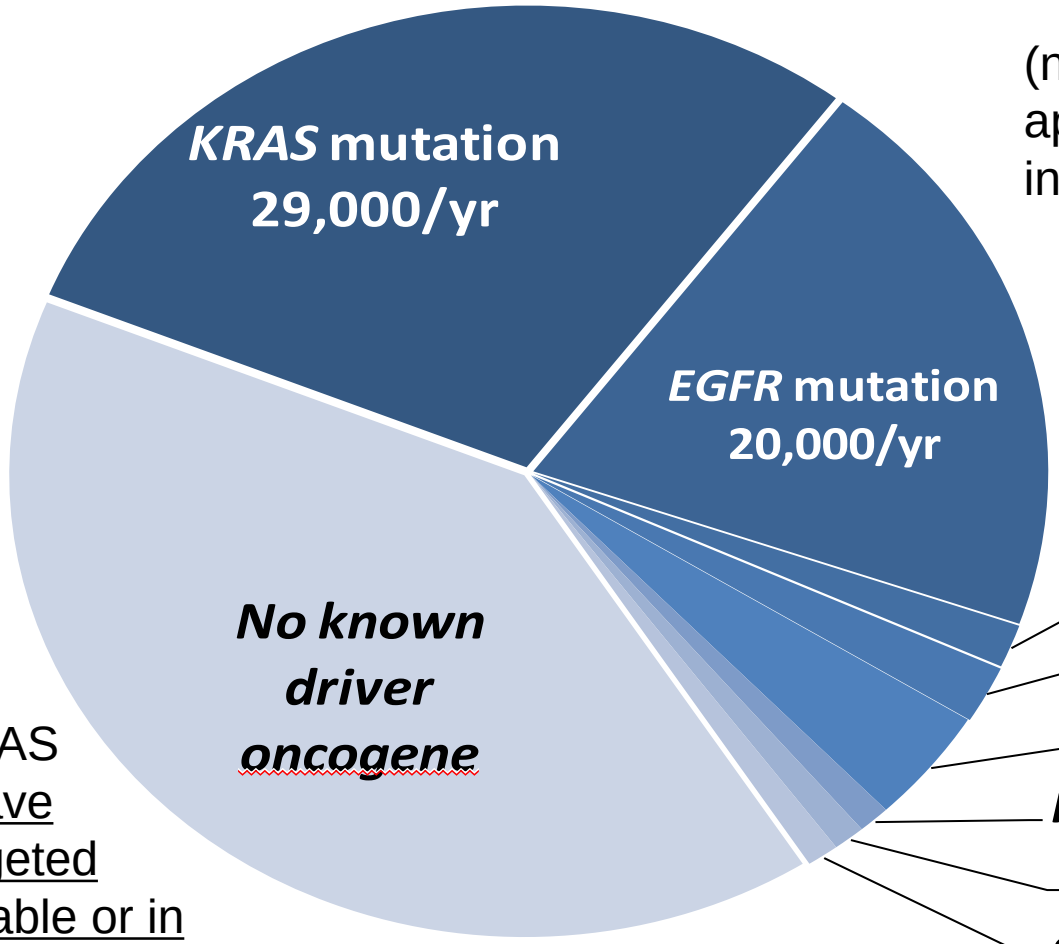
- 13.1: Expert consensus opinion: *EGFR* and *ALK* testing validation should follow the **same** guidelines **as** for **other** molecular diagnostics and FISH **tests**.

- 14.1: Expert consensus opinion: Laboratories should follow **similar** quality control and quality assurance policies and procedures for *EGFR* and *ALK* testing in lung cancers **as** for **other** clinical laboratory **assays**. In particular, laboratories performing *EGFR* and *ALK* testing for TKI therapy should enroll in proficiency testing, if available.

Marc Ladanyi, MD

Lung Adenocarcinoma molecular testing guidelines : what's next

Mutually exclusive oncogene mutations in lung adenocarcinoma



(numbers based on approximate US annual incidence of 100,000)

BRAF – *1500/yr*

ERBB2 – *2000/yr*

ALK – *4000/yr*

ROS1 – *1000/yr*

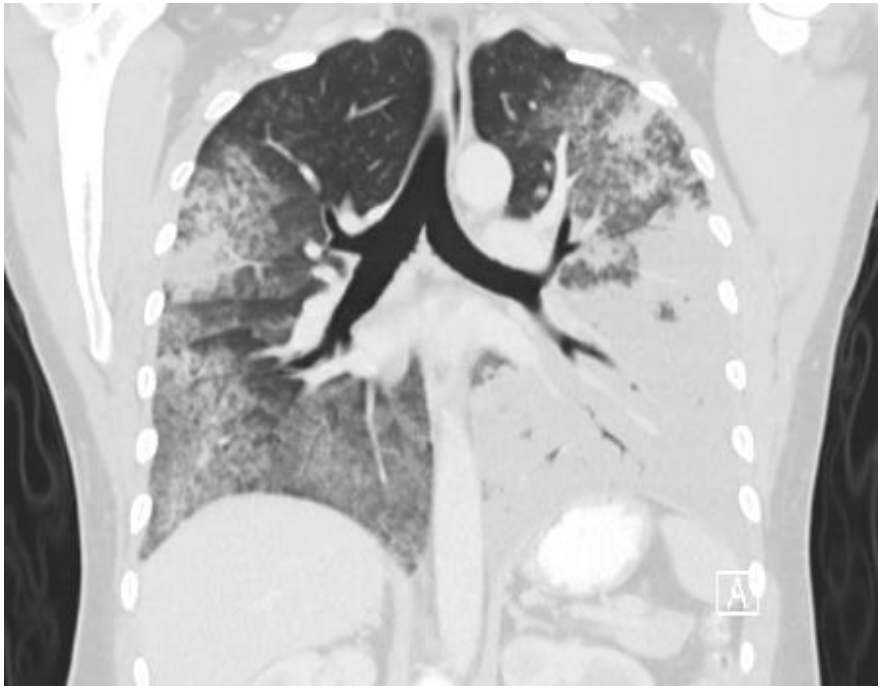
RET – *1000/yr*

Others:***MET, MAP2K1, NRAS***

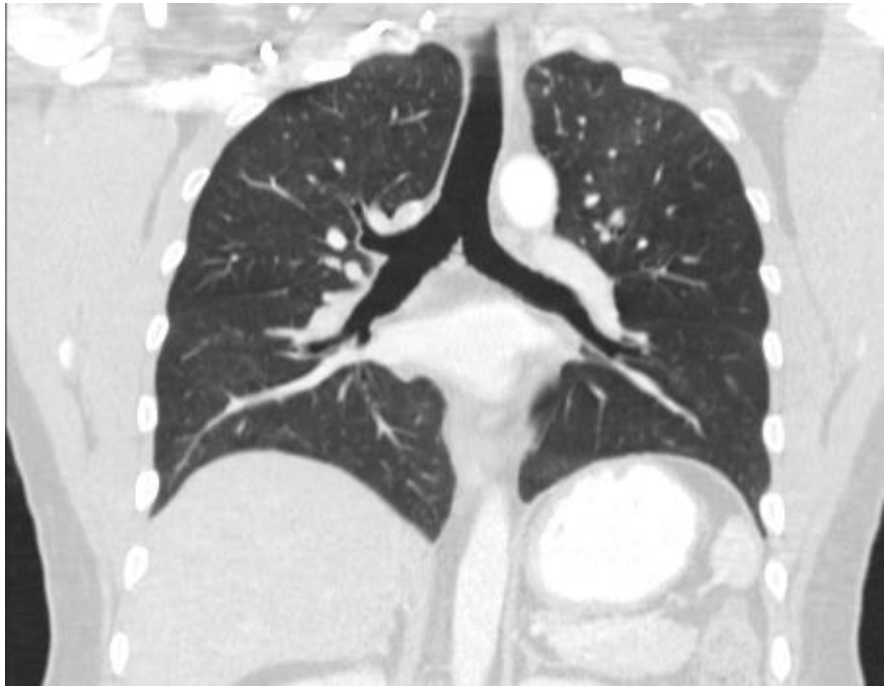
Except for RAS genes, all have effective targeted agents available or in clinical development.

Marked response to Crizotinib in a patient with *ROS1-fusion-positive* Lung Adenocarcinoma

Note: Crizotinib is a TKI for ALK/MET/ROS1.



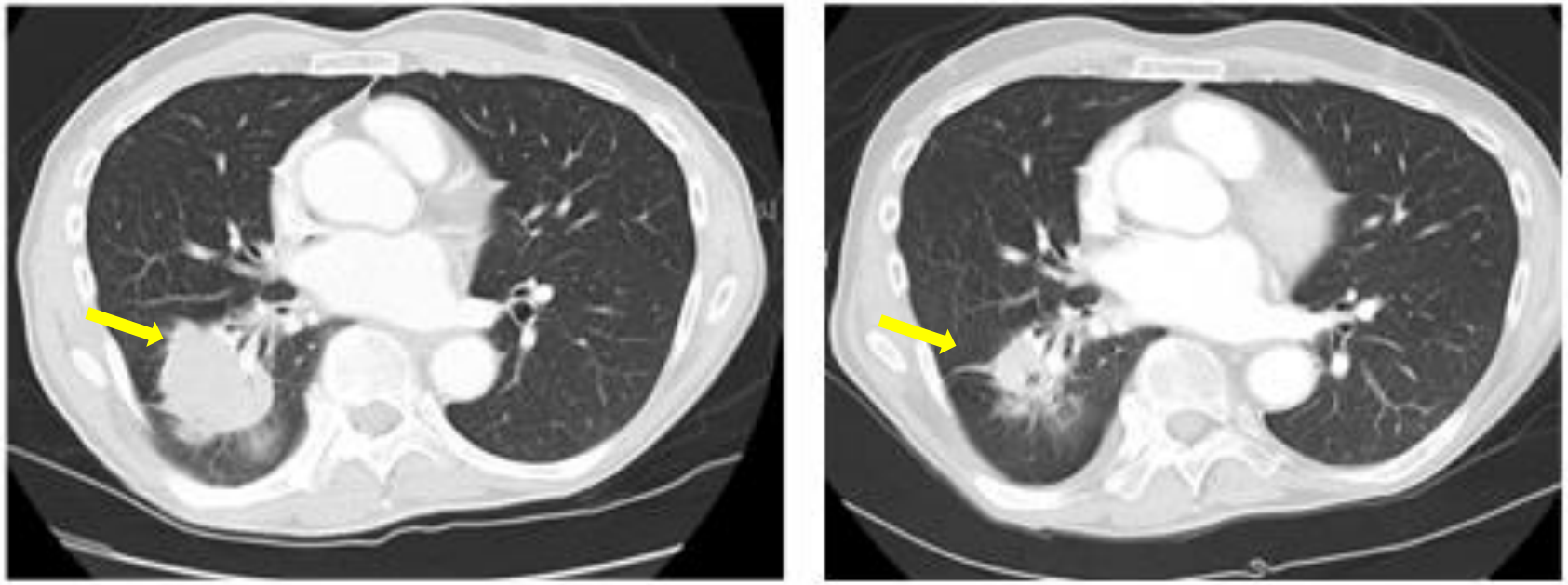
Baseline



After 3 months of crizotinib

Bergethon, Shaw, Ou et al., JCO 30(8): 863-70, 2012

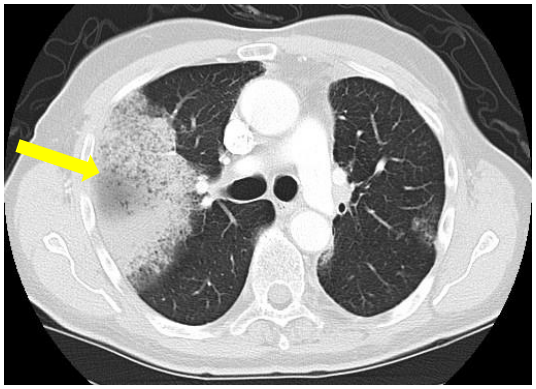
Marked response to the ERBB2 TKI Dacomitinib in a patient with an *ERBB2-mutated* lung adenocarcinoma



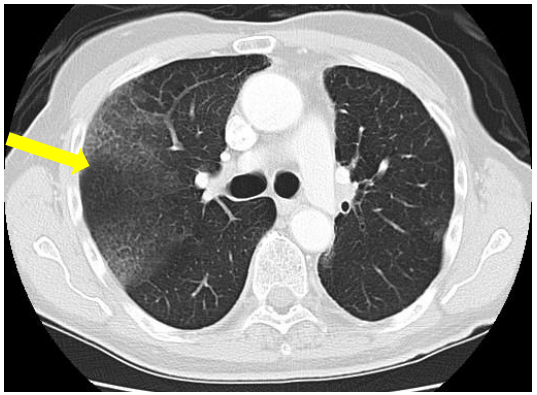
Memorial Sloan-Kettering Cancer Center

MSKCC protocol #10-080, P.I.: Mark Kris, MD

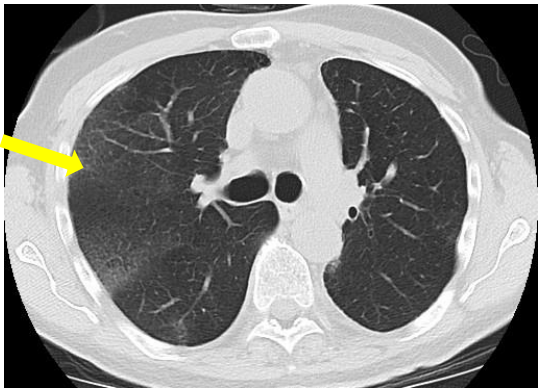
Marked response to the BRAF kinase inhibitor Dabrafenib in a patient with *BRAF V600E* Lung Cancer



Baseline



6 weeks on Dabrafenib

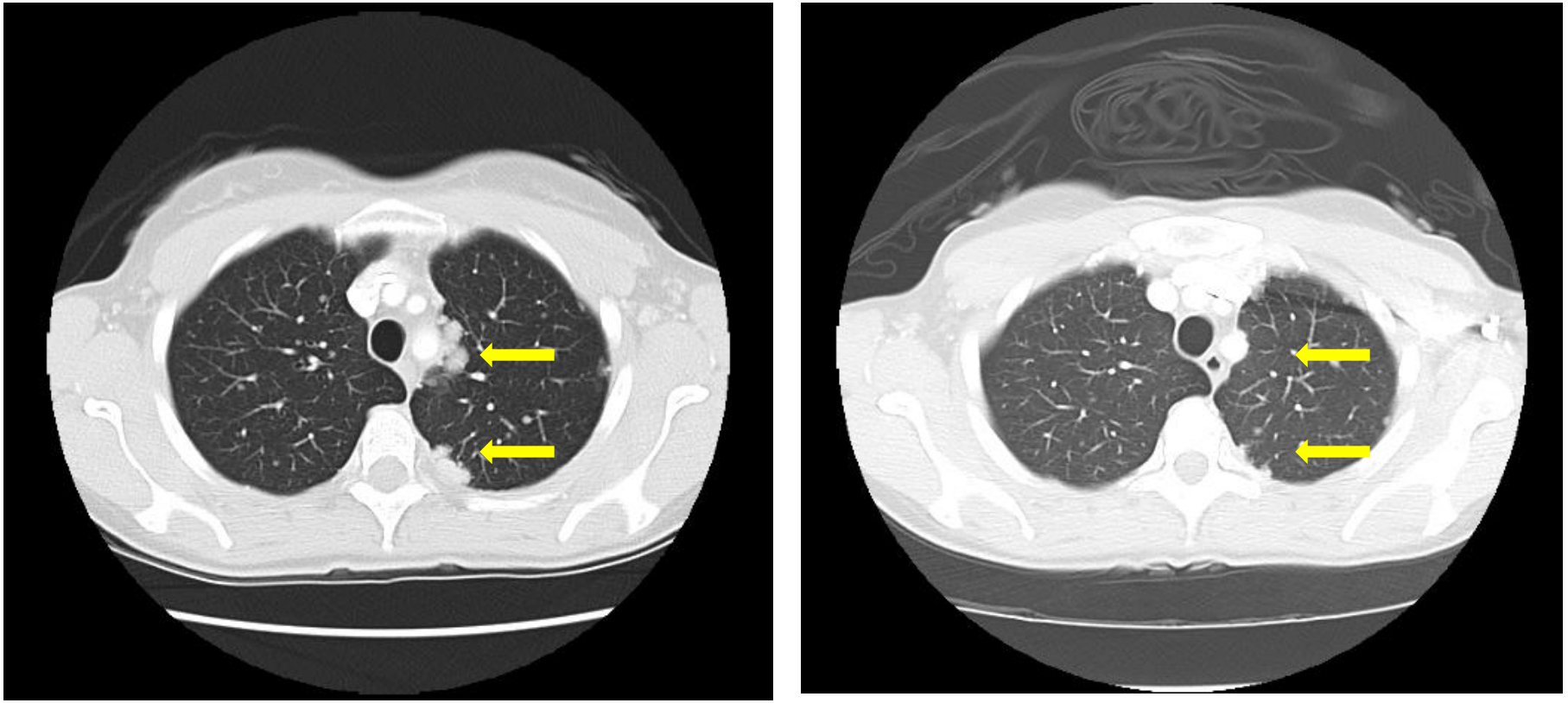


4 months on Dabrafenib

Memorial Sloan-Kettering Cancer Center

Greg Riely, MD PhD, MSKCC

Marked response to the RET TKI Cabozantinib in a patient with *RET* fusion positive Lung Adenocarcinoma



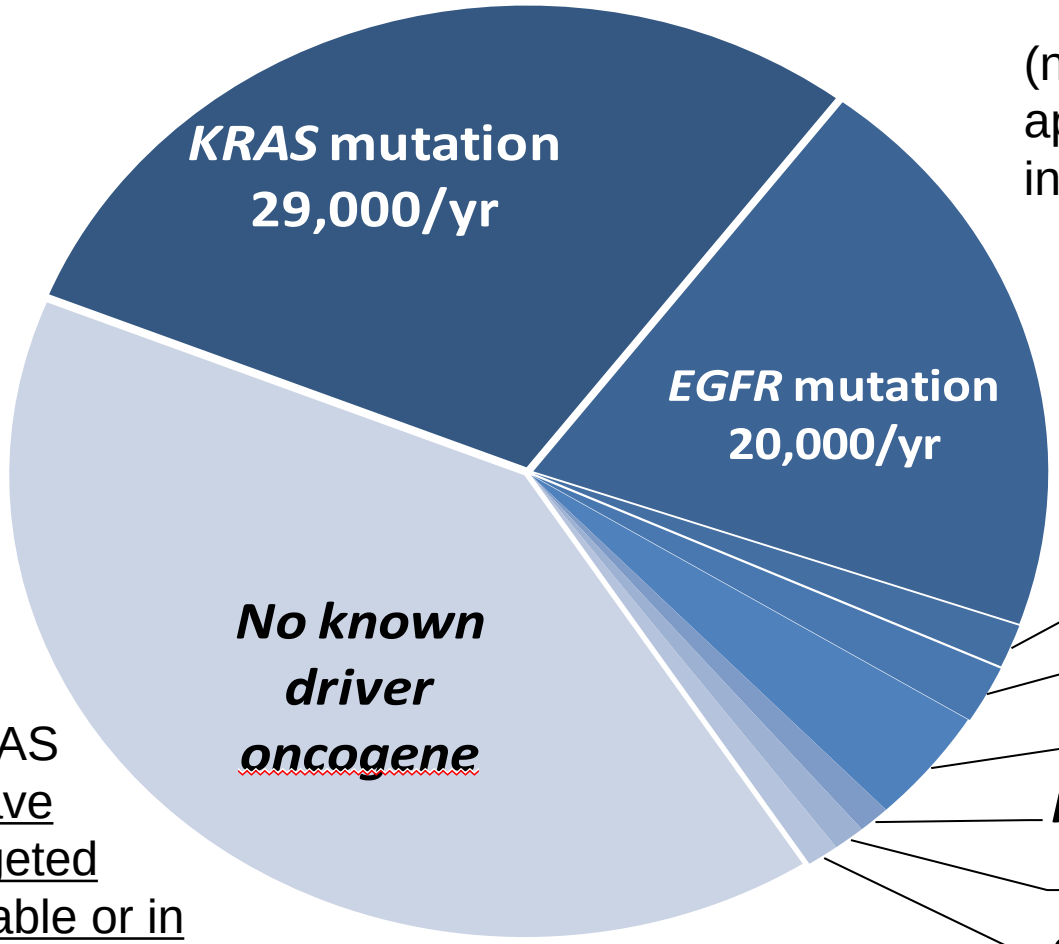
Partial response (47% shrinkage) after 28 days of cabozantinib.

Memorial Sloan-Kettering Cancer Center

Drilon A, et al. Response to cabozantinib in patients with RET fusion-positive lung adenocarcinomas. Cancer Discov. March 26, 2013

Lung Adenocarcinoma molecular testing guidelines : what's next

Mutually exclusive oncogene mutations in lung adenocarcinoma



(numbers based on approximate US annual incidence of 100,000)

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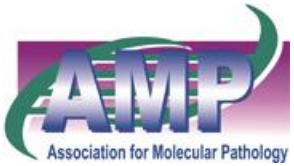
Except for RAS genes, all have effective targeted agents available or in clinical development.

Questions?


COLLEGE OF AMERICAN PATHOLOGISTS
cap


IASLC
INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER
Conquering Thoracic Cancers Worldwide


AMP
Association for Molecular Pathology

CAP Center Process-Guideline Development

Different Outcomes in All Stages of Non-Small Cell Lung Cancer Patients With and Without *EGFR* Mutations, Treated With Tyrosine Kinase Inhibitor

Outcome	Percentage		n (N)	RR [95% CI]	*P* value
	EGFR mutation Positive	*EGFR* mutation Negative			
Response rate (%)	68%	11%	51(3644)	5.16[4.41, 6.04]	***P*<.001**
Disease control rate (%)	86%	42%	28(2204)	1.99[1.73, 2.29]	***P*<.001**

Outcome	Mean ± SD		n (N)	WMD [95% CI]	*P* value
	EGFR mutation Positive	*EGFR* mutation Negative			
Time to Progression/ Progression Free Survival (months)	12.0 ± 7.86	3.4 ± 2.59	27(2347)	8.66 [6.31, 11.00]	***P*<.001**
Median Survival Time (months)	23.3 ± 18.4	12.1 ± 13.9	27(2489)	10.66 [8.36, 12.96]	***P*<.001**

Abbreviations: CI, Confidence interval; n, Number of studies; N, Number of patients; RR, Relative risk; SD, standard deviation; WMD, Weighted mean difference;

Randomized Clinical Trial Data on EGFR Tyrosine Kinase Inhibitor (TKI) Therapy Versus Chemotherapy as First-Line Therapy for Patients With *EGFR*-Mutated Lung Cancers

Study	No. of Patients With *EGFR*-Mutated Lung Cancers	Response Rate (EGFR TKI Versus Chemotherapy)	Progression-free Survival in Months (EGFR TKI Versus Chemotherapy)
EURTAC	173(86 erlotinib and 87 chemo)	58% vs. 15%	9.7 vs. 5.2 (HR 0.37)
OPTIMAL	154 (82 erlotinib and 72 chemo)	83% vs 36%	13.1 vs. 4.6 (HR 0.16)
NEJ 002	228(114 gefitinib and 114 chemo)	74% vs. 31%	10.8 vs. 5.4 (HR 0.30)
WJTOG 3405	177 (58 gefitinib and 59 chemo)	62% vs 32%	9.2 vs 6.3 (HR 0.49)
IPASS	261 (132 gefitinib and 129 chemo)	71% vs 47%	9.5 vs. 6.3 (HR 0.48)
LUX LUNG3	345 (230 afatinib and 115 chemo)	56% vs. 23%	11.1 vs. 6.9 (HR 0.58)

Abbreviations: Chemo, chemotherapy; HR, hazard ratio