Should we test for EGFR and ALK in completely resected NSCLC
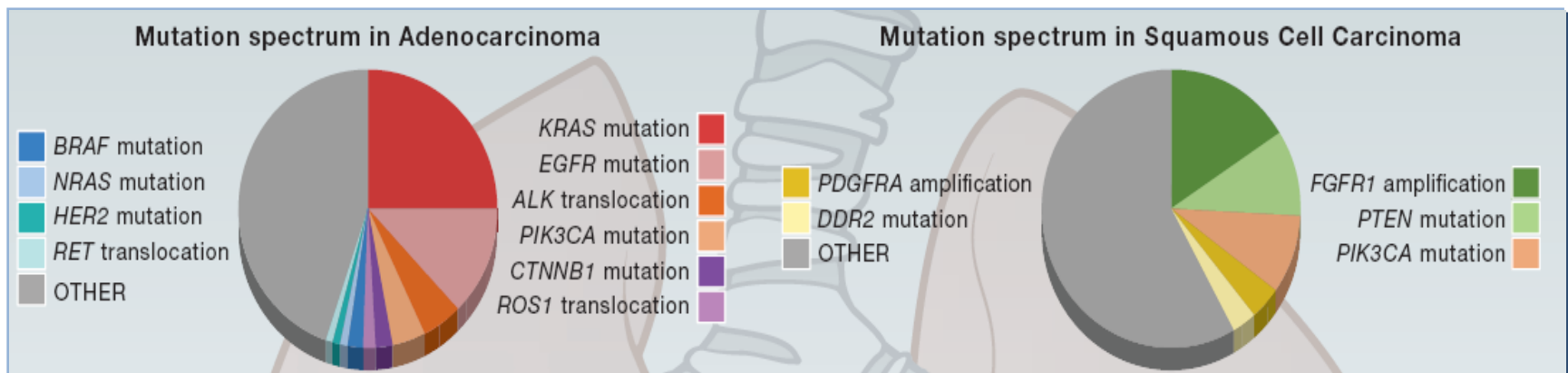
Yi-long Wu

Guangdong Lung Cancer Institute

Guangdong General Hospital

Guangzhou China

Treatment strategy based on TNM staging and molecular profile

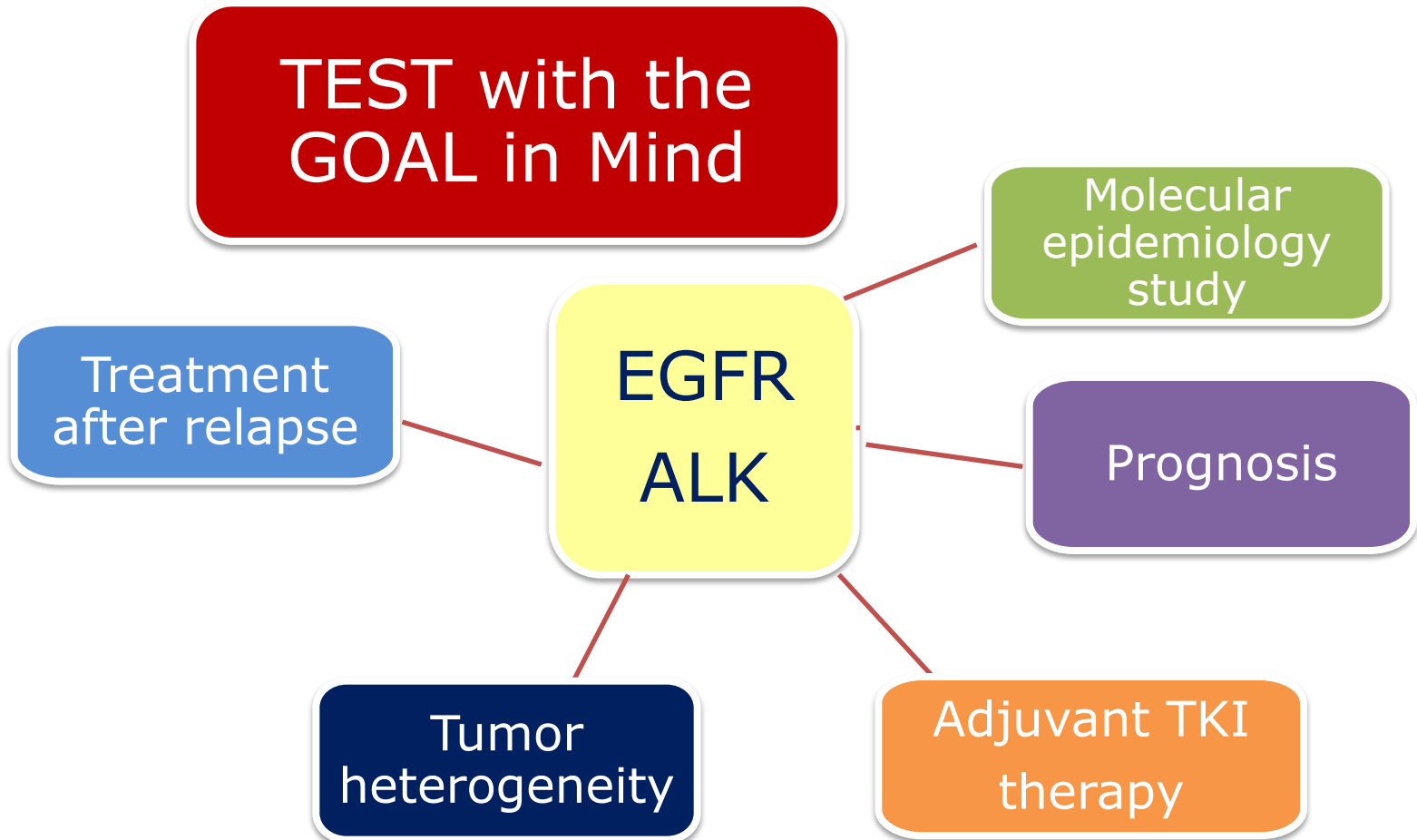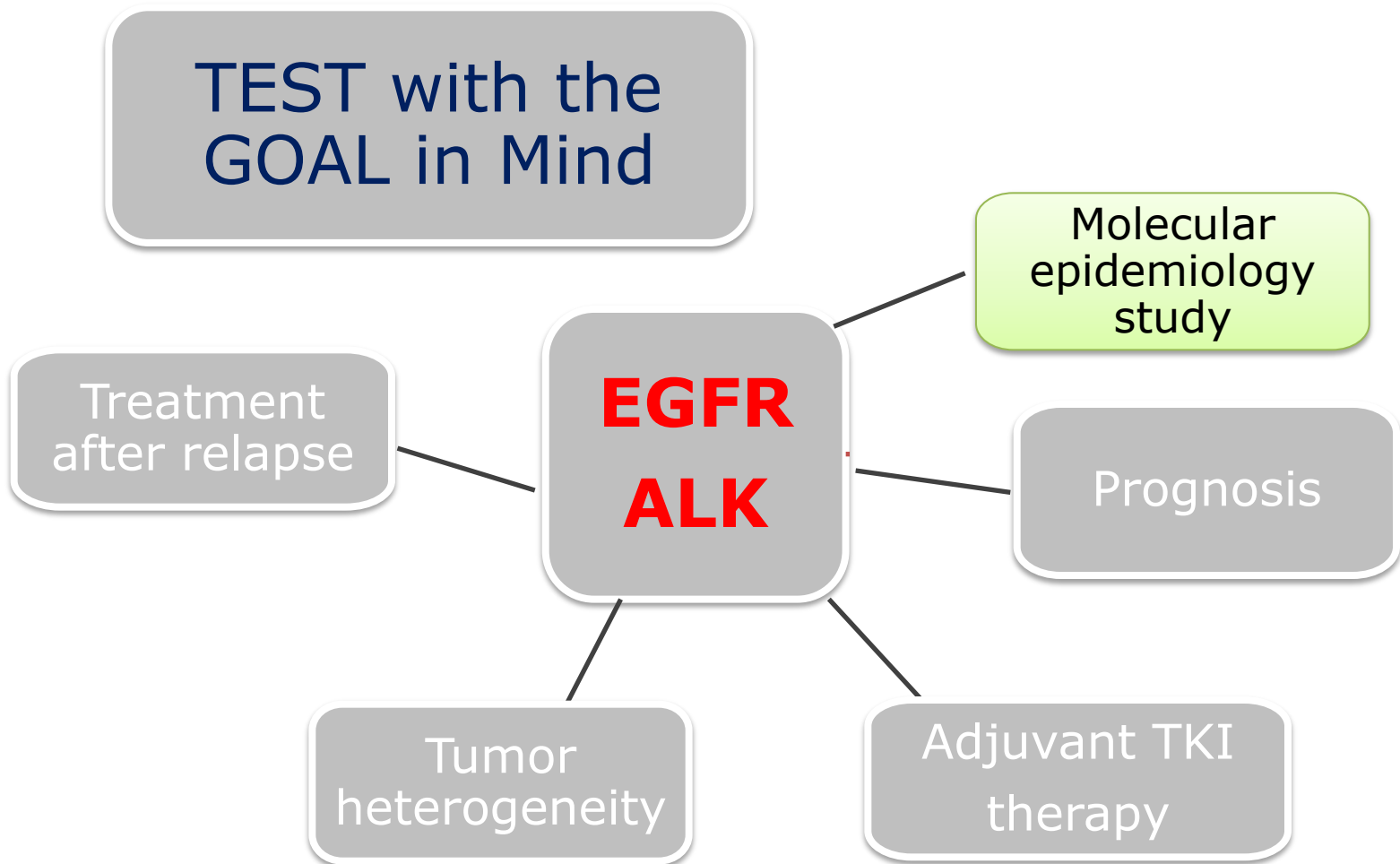| Stage	51%	General treatment recommendations	5-year overall survival [b]	
			clinical stage	pan. stage
IA	14%	Surgical resection	50%	73%
IB	10%	Surgical resection, can consider adjuvant chemotherapy in selected cases (e.g. tumor size > 4cm)	43%	58%
IIA	6%	Surgical resection followed by adjuvant chemotherapy	36%	46%
IIB	5%	Surgical resection followed by adjuvant chemotherapy	25%	36%
IIIA	16%	Multimodality treatment: chemotherapy, radiation, +/- surgery	19%	24%
IIIB	8%	Multimodality treatment: chemotherapy and radiation	7%	9%
IV	41%	Chemotherapy, consider targeted therapies according to driver mutations	2%	N/A

Mutation spectrum in Adenocarcinoma

- BRAF mutation
- NRAS mutation
- HER2 mutation
- RET translocation
- OTHER

- KRAS mutation
- EGFR mutation
- ALK translocation
- PIK3CA mutation
- CTNNB1 mutation
- ROS1 translocation

Mutation spectrum in Squamous Cell Carcinoma

- PDGFRA amplification
- DDR2 mutation
- OTHER

- FGFR1 amplification
- PTEN mutation
- PIK3CA mutation

Rebecca S. Heist and Jeffrey A. Engelman 2012 Cancer cell

EGFR mutation vs. ALK rearrangement

Characteristic	EGFR	EML4/ALK
Histology	Adeno TTF1+	AdenoTTF1+
Subtype	Non-musin	musin
Smoke	Non-smoker	Non-smoker
Race	East	All
Age-median	66y	52y
Gender	Female	Male>female
Prognosis	Good	Poor
Treatment	EGFR-TKI	ALK-inhibitor

Two class of disease

R0 resected NSCLC

TEST with the GOAL in Mind

Molecular epidemiology study

Treatment after relapse

EGFR ALK

Prognosis

Tumor heterogeneity

Adjuvant TKI therapy

R0 resected NSCLC

A non-interventional study on EGFR mutation status and clinical outcomes of Chinese patients with completely resected lung adenocarcinoma (ICAN study)

Yi-Long Wu1, Jun Wang2, Xiang-Yang Chu3, Zhi-Dong Liu4, Yi Shen5, Haitao Ma6, Xiang-Ning Fu7, Jian Hu8, Nai-K Zhou3, Yongyu Liu9, Xinming Zhou10, Jian-Jun Wang11, Kang Yang12, Jian Li13, Lin Xu14, Si-yu Wang15, Qun Wan16, Xu Liu17, Shun Xu18, Shanqing Li19, Zhongyuan Chen20, Honghe Luo21, Ying Chen22, Changli Wang23

1, Guangdong Lung Cancer Institute, Guangdong General Hospital, Guangzhou/CHINA, 2, Peking University People's Hospital, Beijing/CHINA, 3, 301Hospital, Beijing/CHINA, 4, Beijing Chest Hospital, Capital Medical University, Beijing/CHINA, 5, The Affilated Hospital of Medical College Qingdao University, Qingdao/CHINA, 6, The First Affiliated Hospital of Soochow University, Suzhou/CHINA, 7, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan/CHINA, 8, The First Affiliated Hospital of Medical School of Zhejiang University, Hangzhou/CHINA, 9, Liaoning Cancer Hospital & Institute, Shenyang/CHINA, 10, Zhejiang Cancer Hospital, Hangzhou/CHINA, 11, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan/CHINA, 12, The First Affiliated Hospital of Third Military Medical University, Chongqing/CHINA, 13, Peing University First Hospital, Beijing/CHINA, 14, Jiangsu Cancer Hospital, Nanjing/CHINA, 15Thoracic surgery, Sun Yat-sen university cancer center, Guangzhou/CHINA, 16, Zhongshan Hospital Fudan University, Shanghai/CHINA, 17, West China Hospital, Sichuan University, Chengdu/CHINA, 18, The First Hospital of China Medical University, Shenyang/CHINA, 19, Peking Union Medical College Hospital, Beijing/CHINA, 20, Ruijin Hospital, Jiaotong University, Shanghai/CHINA, 21, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou/CHINA, 22, Jilin Cancer Hospital, Changchun/CHINA, 23, Tianjin Medical University Cancer Institute and Hospital, Tianjin/CHINA
§ Correspandence: syylwu@live.cn

Methods

Study flow diagram

✓Aged ≥18 years
✓With histological diagnosed lung adenocarcinoma,
✓Received surgical complete resection.
✓The tumor EGFR mutation testing was performed as routine clinical practice

↓

Inform consent and eligibility

↓

Collect EGFR mutation status and disease information

↓

Follow clinical outcome till 3 years after the operation
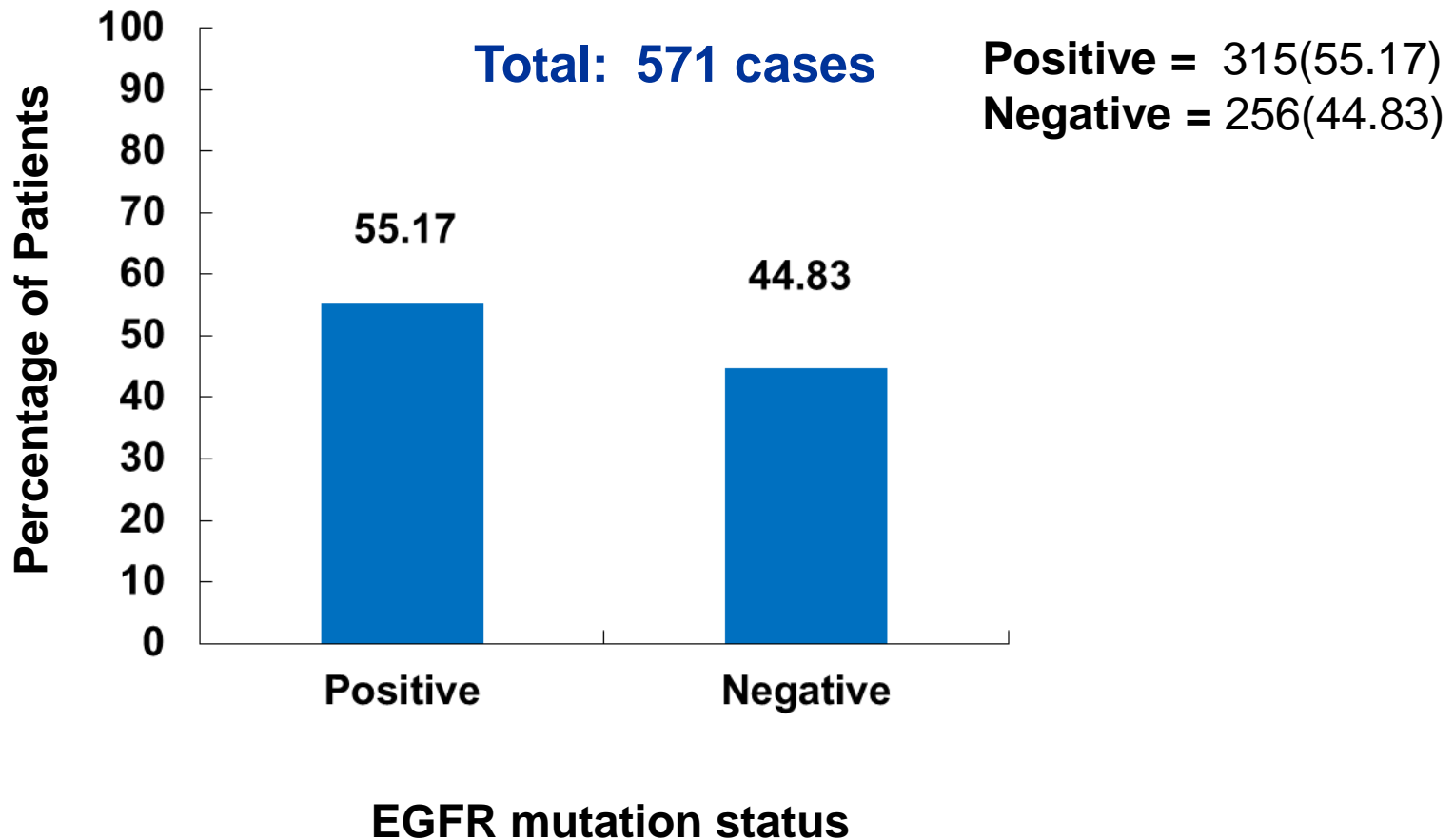
Primary endpoint:
• EGFR mutation status

Secondary endpoints:
• Adjuvant therapy setting
• Clinical outcomes（DFS）
• Risk factors of recurrence

Results

Primary endpoint
Overall EGFR mutation status

Total: 571 cases

Positive = 315(55.17)
Negative = 256(44.83)

Results

EGFR mutation frequency according to gender
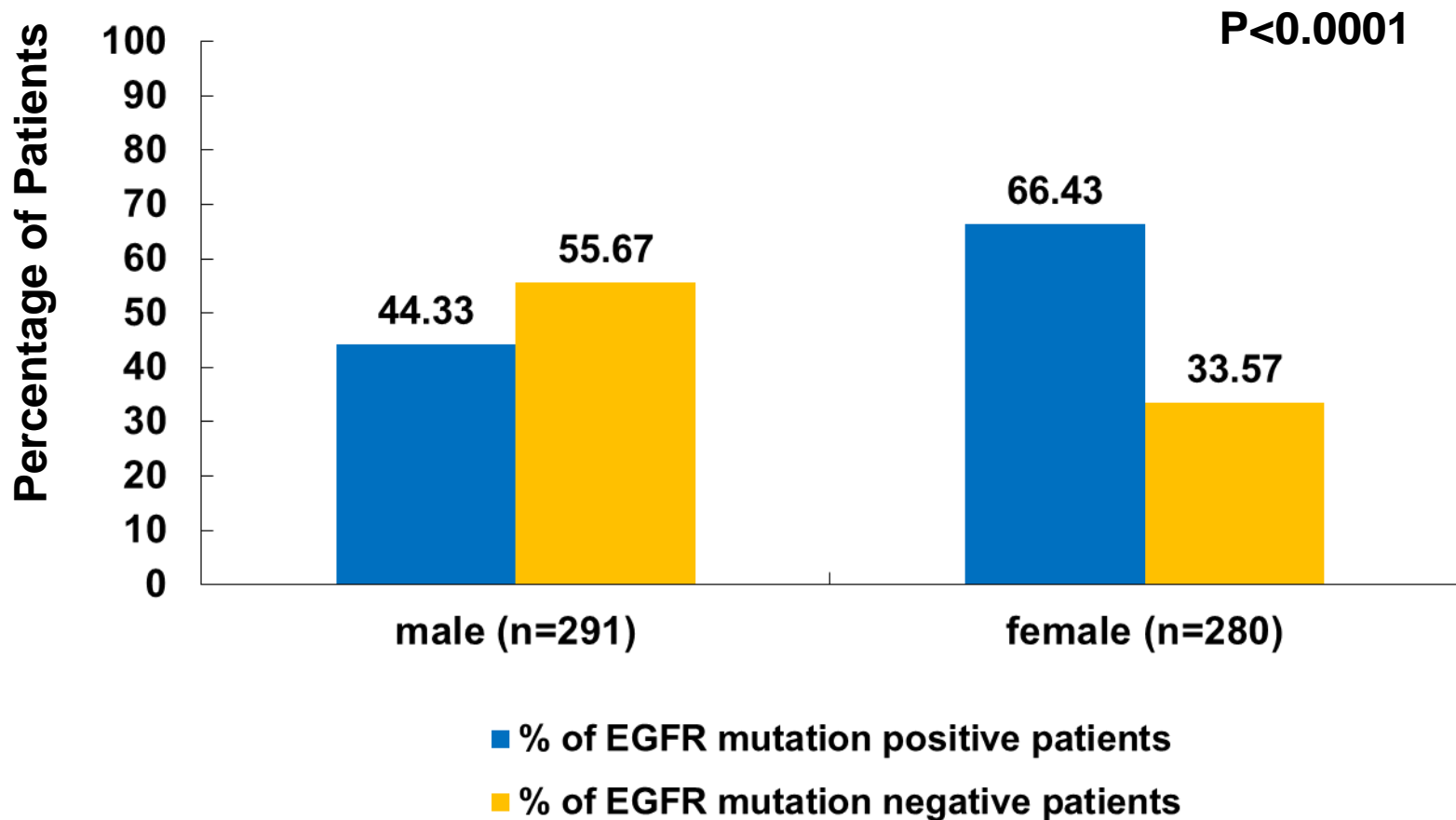
Results
EGFR mutation frequency by smoking status

P<0.0001

Percentage of Patients

- 61.73
- 38.27
- 44.26
- 55.74
- 41.03
- 58.97

Never smoked (n=371) Ex-smoker (n=122) Current smoker (n=78)

■ % of EGFR mutation positive patients
■ % of EGFR mutation negative patients
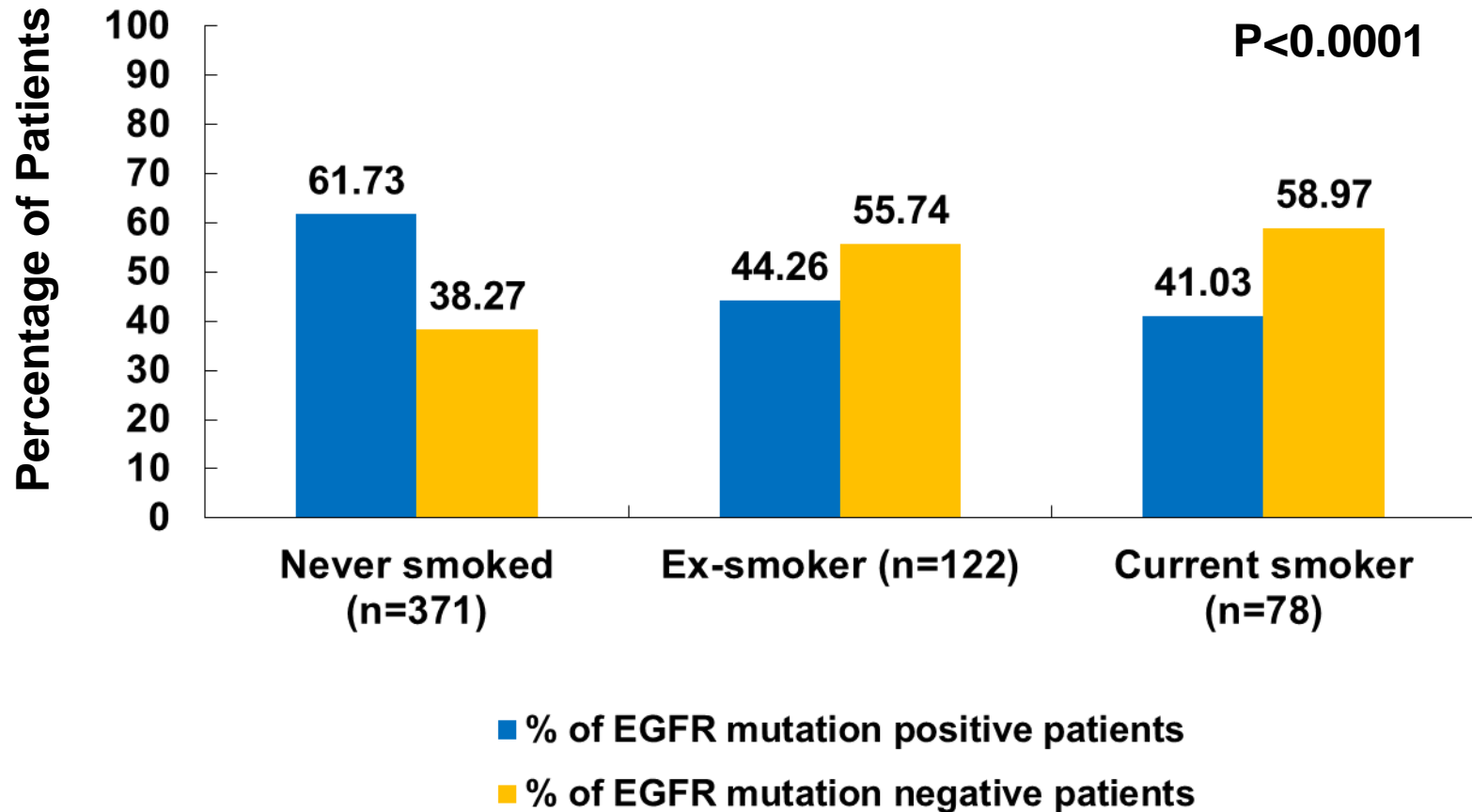
Clinicopathologic implication of *ALK* rearrangement in surgically resected lung cancer
A proposal of diagnostic algorithm for *ALK*-rearranged adenocarcinoma

Jin Ho Paik[a,1], Chang-Min Choi[b,1], Hyojin Kim[a], Se Jin Jang[c], Gheeyoung Choe[a], Dong Kwan Kim[d], Hwa Jung Kim[e], Hoil Yoon[f], Choon-Taek Lee[f], Sanghoon Jheon[g], Ji-Young Choe[a], Jin-Haeng Chung[a,*]

Chung's SNUBH molecular test protocol

Small biopsy or Cytology — Bronchoscopic biopsy, needle biopsy, cytology etc.

Adenocarcinoma — First, 3μm tissue or cell block sections(X2) for ALK test. Second, DNA extraction for *EGFR/KRAS* mutation study

Simultaneous study

EGFR or KRAS mutation — 2-5 working days

Use the first unstained slide — ALK IHC — 1 working day

EGFR mutation+

negative — positive

EGFR-TKI

Use the 2nd unstained slide

ALK FISH test (+) — 2 working days

ALK rearrangement (+)

ALK-TKI

- **The results of ALK IHC and FISH obtained from tissue microarray /biopsy specimens and whole sections after resection were concordant.**

- **Simultaneous tests for ALK IHC and EGFR ,which has important implications for the storage and use of small biopsy or cytology samples for genetic analysis.**

J.H. Paik et al. / Lung Cancer *76 (2012) 403– 409*

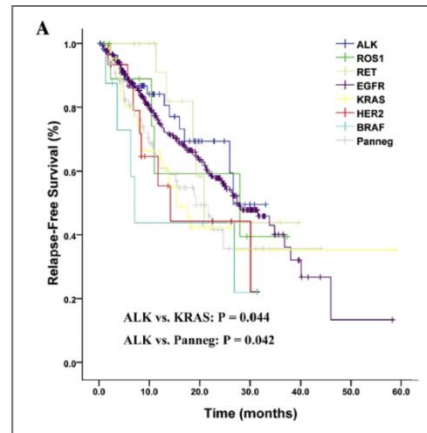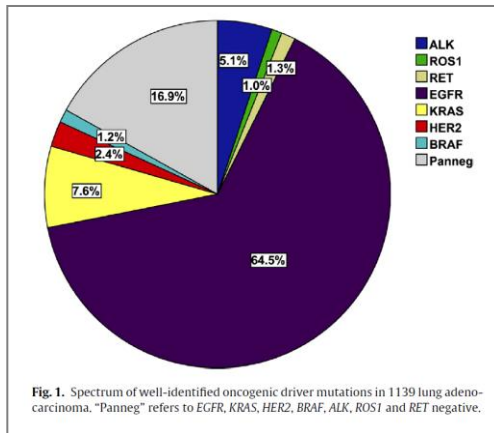# Clinicopathologic, histologic and cytologic features

ALK, *ROS1* and *RET* fusions in 1139 lung adenocarcinomas: A comprehensive study of common and fusion pattern–specific clinicopathologic, histologic and cytologic features

Yunjian Pan [a,b,1], Yang Zhang [a,b,1], Yuan Li [b,c], Haichuan Hu [a,b], Lei Wang [a,b], Hang Li [a,b], Rui Wang [a,b], Ting Ye [a,b], Xiaoyang Luo [a,b], Yiliang Zhang [a,b], Bin Li [a,b], Deng Cai [a,b], Lei Shen [b,c], Yihua Sun [a,b,**], Haiquan Chen [a,b,*]

[a] Department of Thoracic Surgery, Fudan University Shanghai Cancer Center, Shanghai 200032, China
[b] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China
[c] Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai 200032, China

Fig. 1. Spectrum of well-identified oncogenic driver mutations in 1139 lung adenocarcinoma. "Panneg" refers to *EGFR*, *KRAS*, *HER2*, *BRAF*, *ALK*, *ROS1* and *RET* negative.
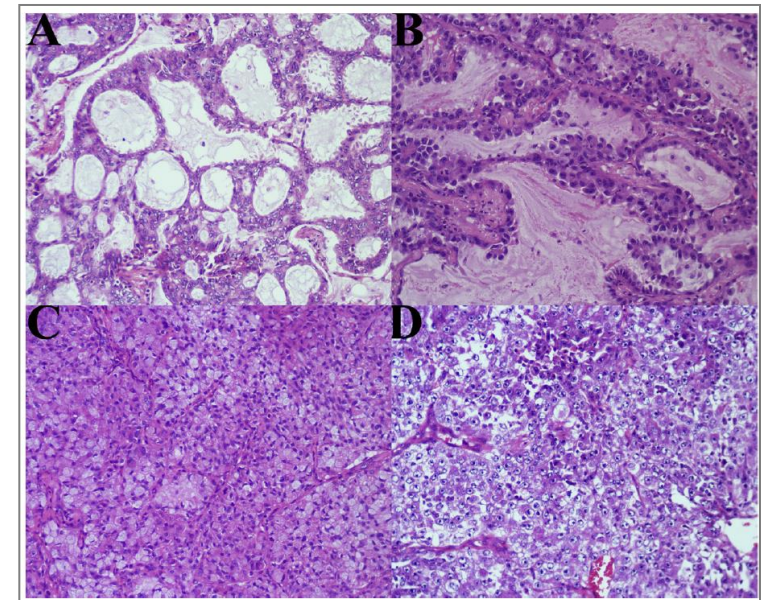
> solid-predominant adenocarcinoma
> extracellular mucin (P < 0.001)
> cribriform pattern (P < 0.001)
> signet ring cells (P < 0.001)
> hepatoid cytology (P < 0.001）

Pan, wr al. Lung Cancer 2014;84:121-6.

Prevalence and Clinical Outcomes for Patients With ALK-Positive Resected Stage I to III Adenocarcinoma: Results From the European Thoracic Oncology Platform Lungscape Project

Fiona H. Blackhall, Solange Peters, Lukas Bubendorf, Urania Dafni, Keith M. Kerr, Henrik Hager, Alex Soltermann, Kenneth J. O'Byrne, Christoph Dooms, Aleksandra Sejda, Javier Hernández-Losa, Antonio Marchetti, Spasenija Savic, Qiang Tan, Erik Thunnissen, Ernst-Jan M. Speel, Richard Cheney, Daisuke Nonaka, Jeroen de Jong, Miguel Martorell, Igor Letovanec, Rafael Rosell, and Rolf A. Stahel
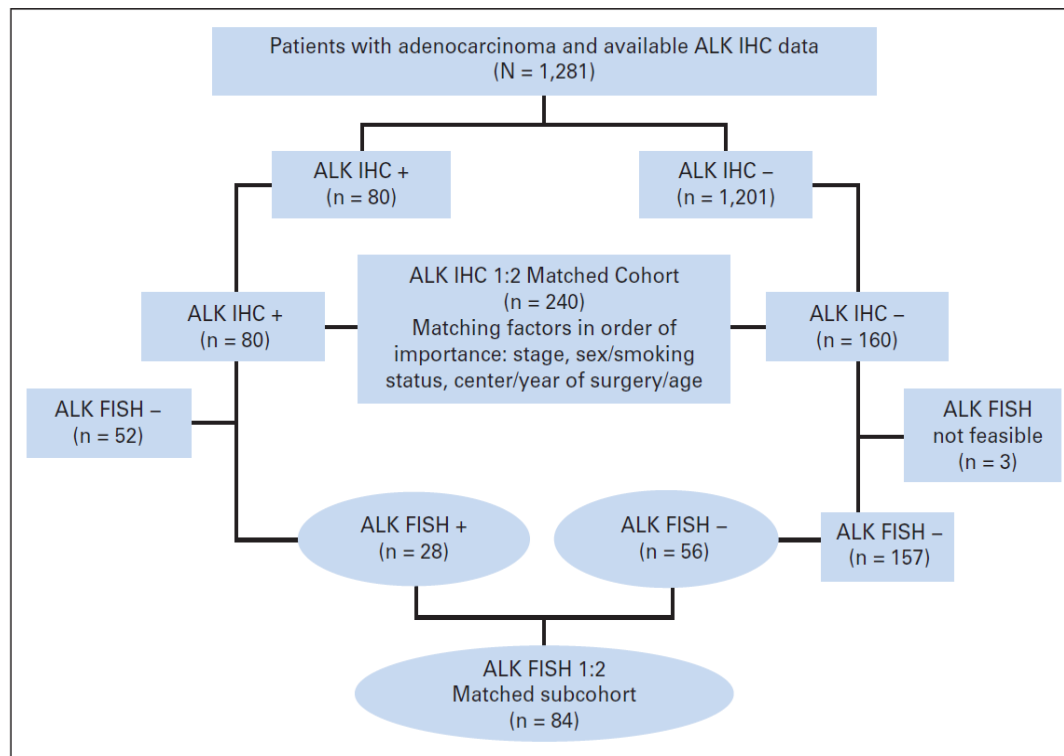
FISH and IHC in ALK+
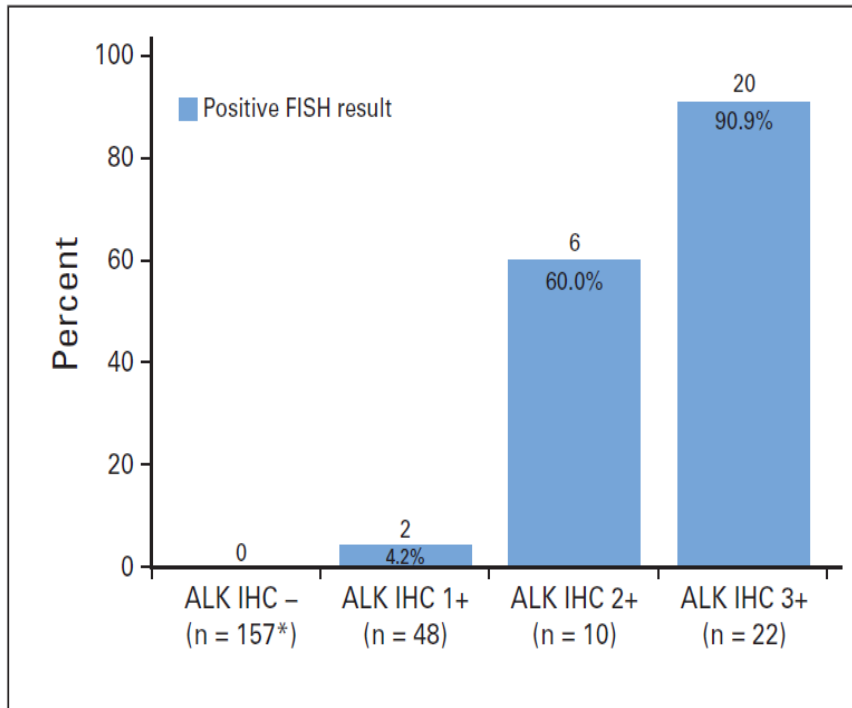
Fig 2. Agreement between fluorescent in situ hybridization (FISH) and immunohistochemistry (IHC) results in anaplastic lymphoma kinase (ALK) status determination (n = 237). (*) Note: FISH was not feasible for three of 160 ALK IHC-negative samples.
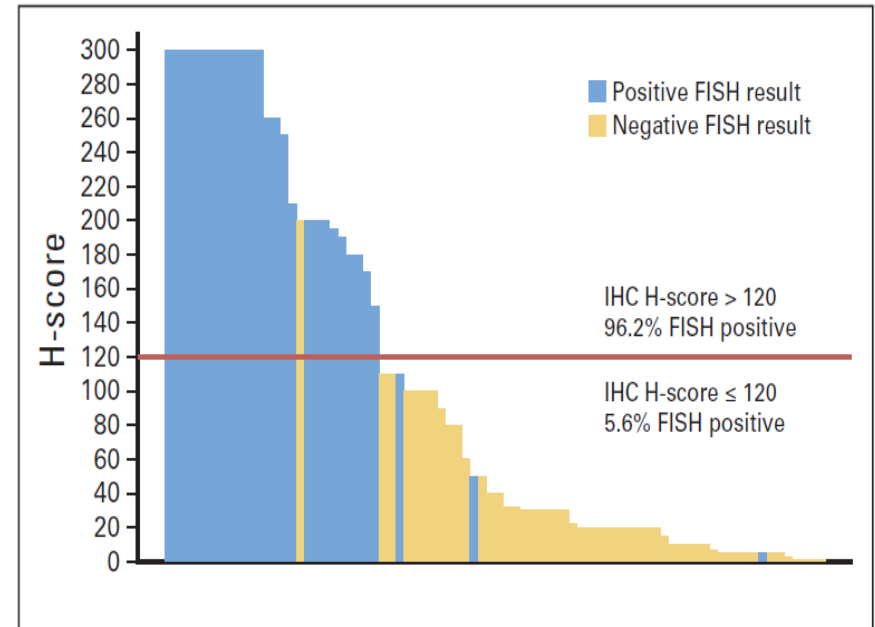
Fig 3. Plot of H-score and fluorescent in situ hybridization (FISH) status for anaplastic lymphoma kinase (ALK) immunohistochemistry (IHC) -positive patients (n = 80).

Blackhall et al. J Clin Oncol 32:2780-2787.

R0 resected NSCLC

TEST with the GOAL in Mind

EGFR
ALK

Molecular epidemiology study

Prognosis

Treatment after relapse

Tumor heterogeneity

Adjuvant TKI therapy
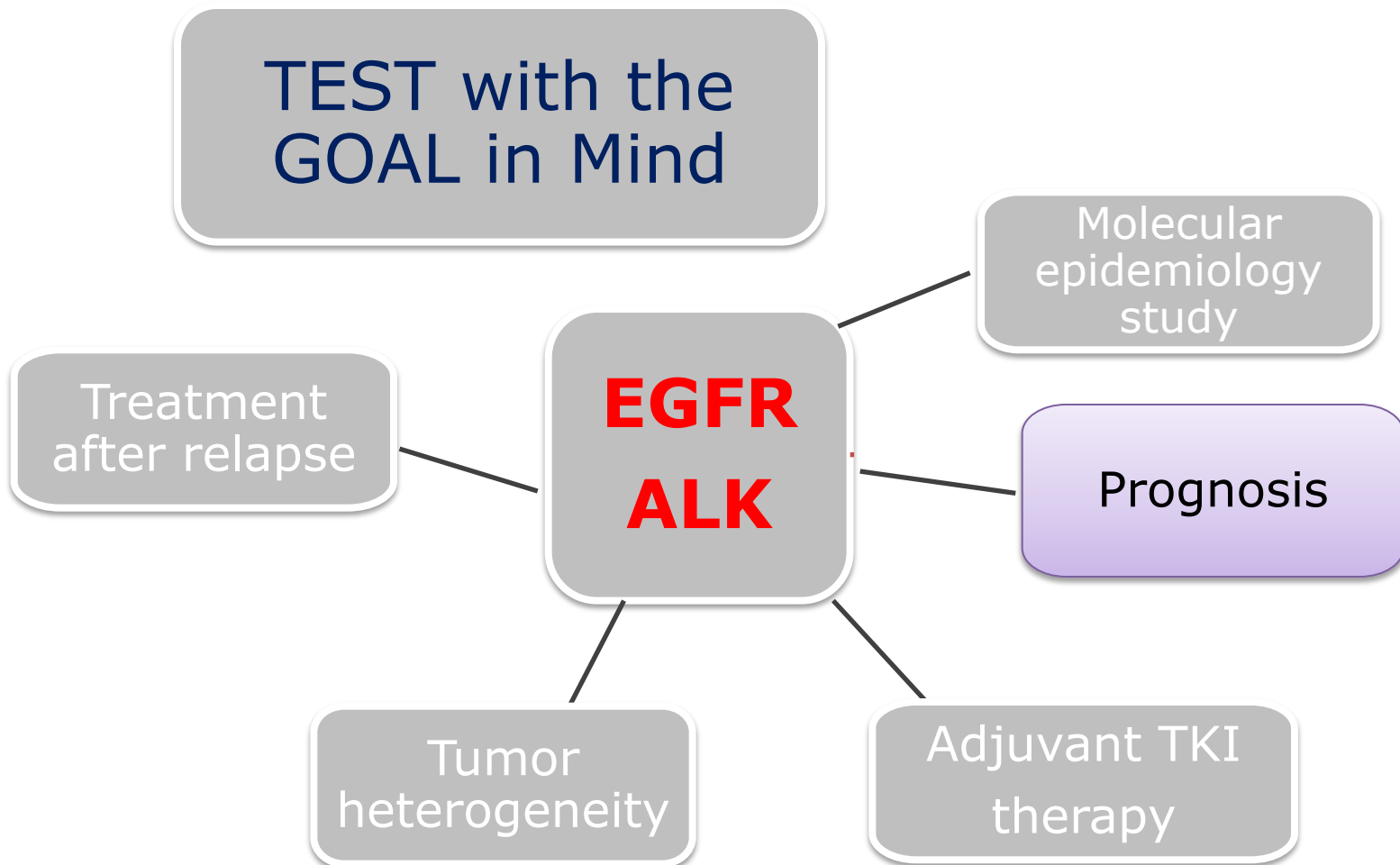
Patient desire

What is my genetic Profiles?

How long could I still live?

ICAN Results

2nd endpoint

2-year DFS rate by EGFR mutation status

Survival n (%)	EGFR mutation status			Statistic	P
	Positive	Negative	Total		
Survival with no evidence of recurrence	198(72.79)	170(63.67)	368(68.27)	4.1620	**0.1248**
Disease recurrence or death	74(27.21)	97(36.33)	171(31.73)		
Total (missing)	272(12)	267(20)	539(32)		
2-year DFS rate (95% CI)	72.89% (67.17%, 77.78%)	64.83% (58.85%, 70.16%)	68.83% (64.75%, 72.54%)		

NSCLC with *EGFR* mutations without TKI therapy had better survival than wild type?
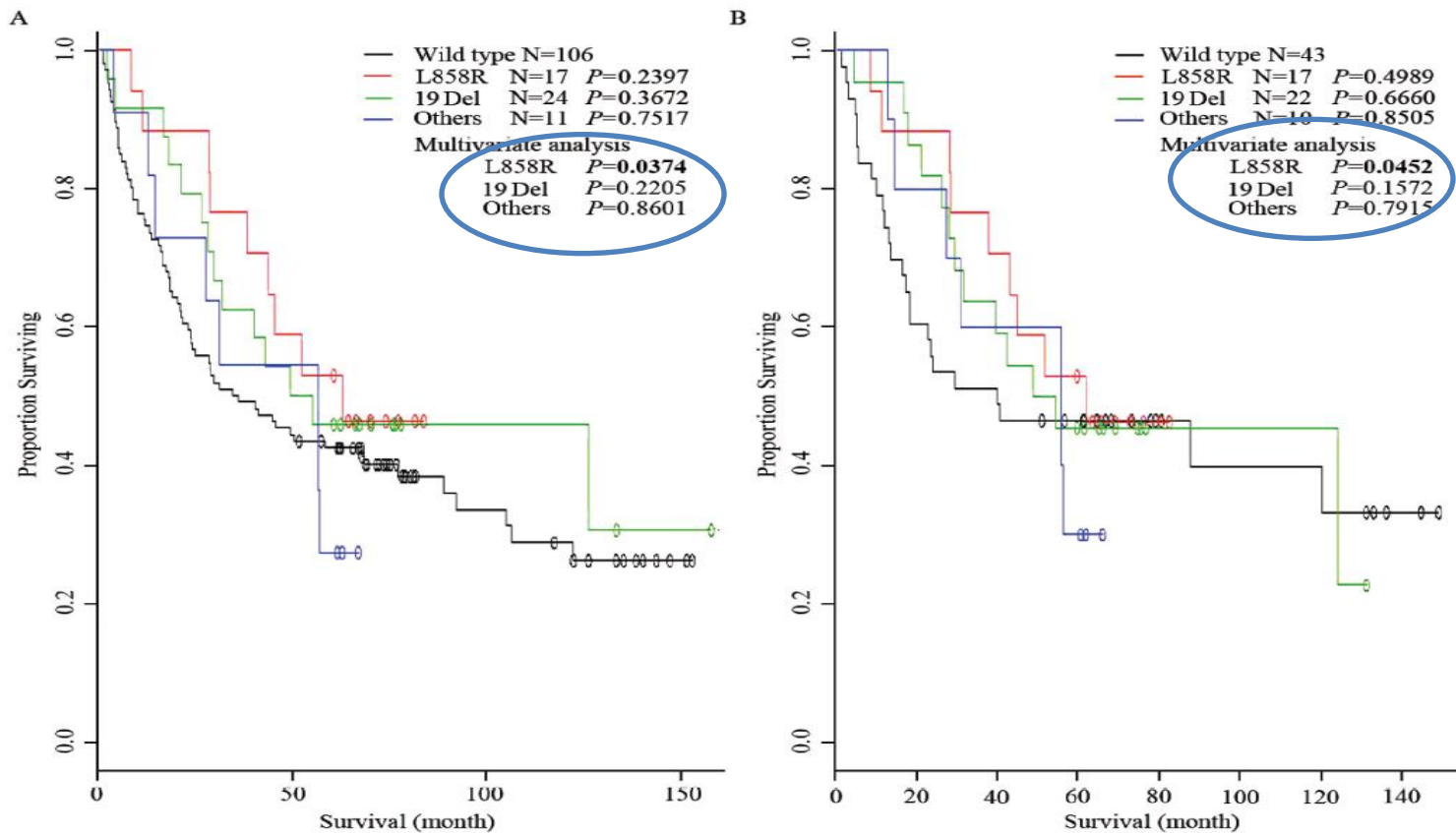
FIGURE 2. Kaplan-Meier curves for overall survival of wild type versus three groups of EGFR mutations: L858R, exon 19 deletion, and others, respectively. The *p* values of multivariate analyses were shown. *A*, A total of 158 patients with non-small cell lung cancer. *B*, Ninety-two patients with adenocarcinoma. Only L858R group was significantly better than wild type by multivariate analysis.

Worse disease-free survival in never-smokers with *ALK+ lung* adenocarcinoma
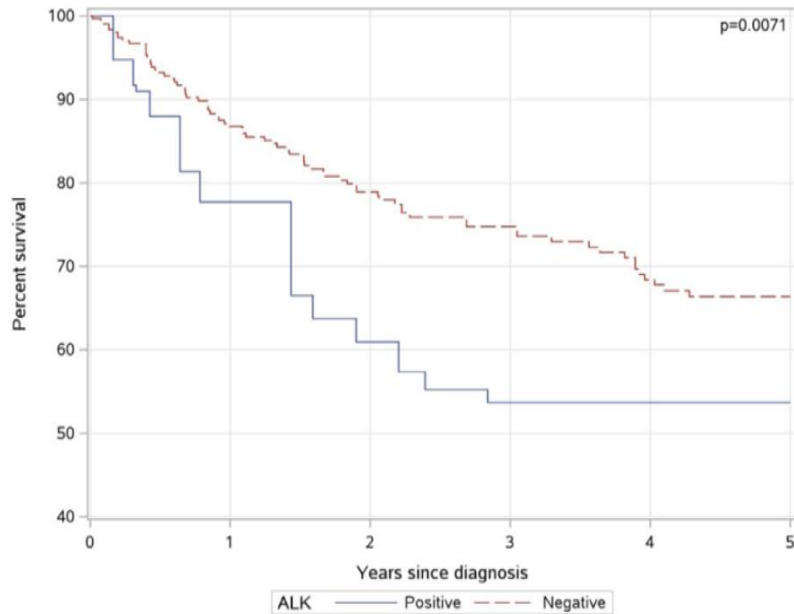
IHC +++ vs. IHC 0/+   FISH + vs. FISH -
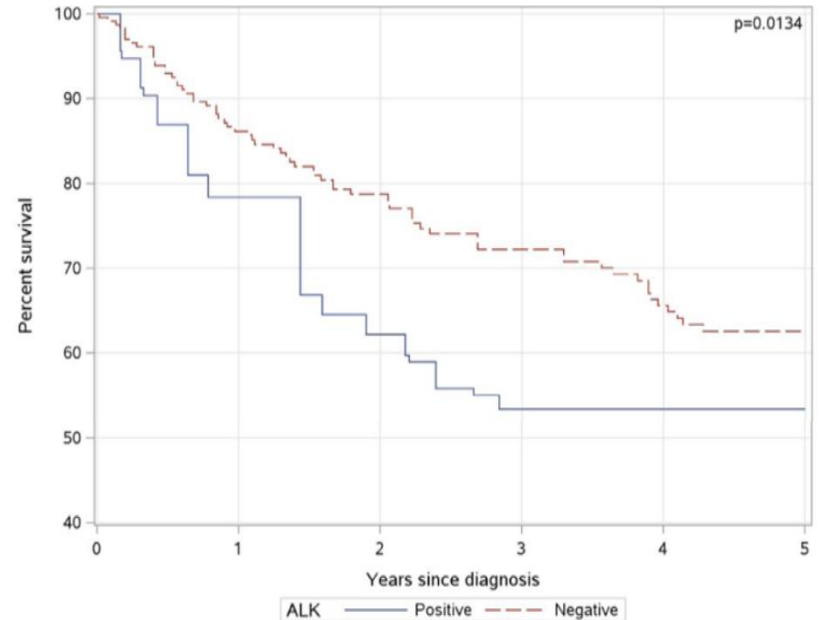
Worse disease-free survival in *ALK+ lung* adenocarcinoma

OS

PFS
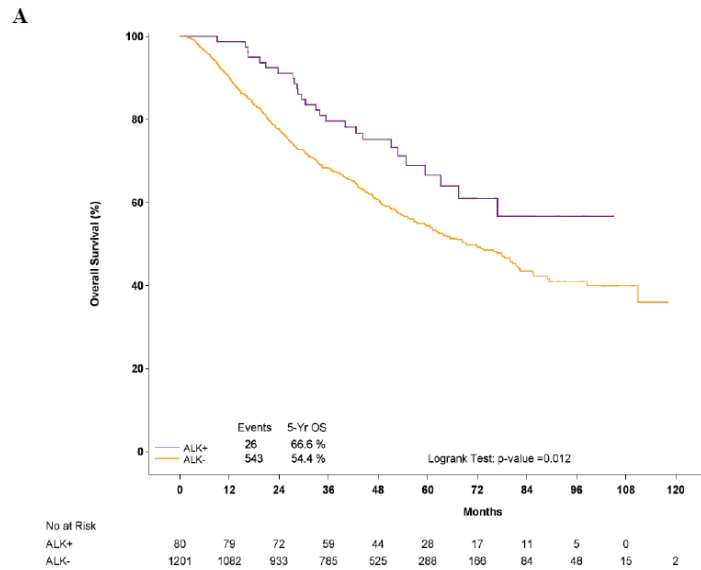
Figure A1 – Panel A: Kaplan – Meier curves showing Overall survival by ALK IHC status (N=1281)

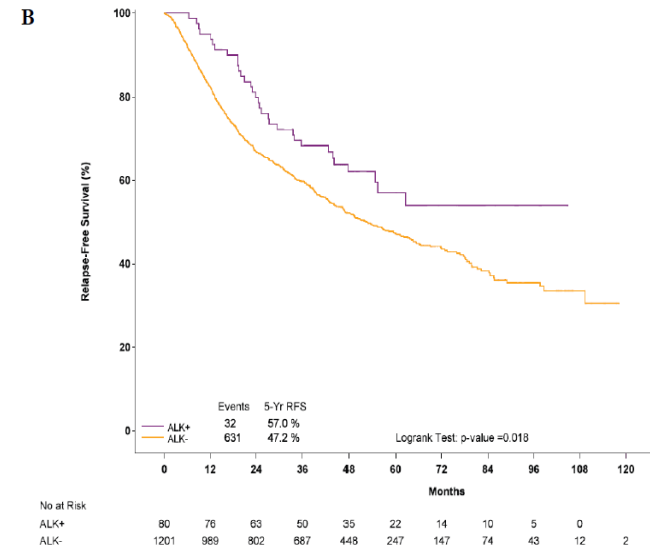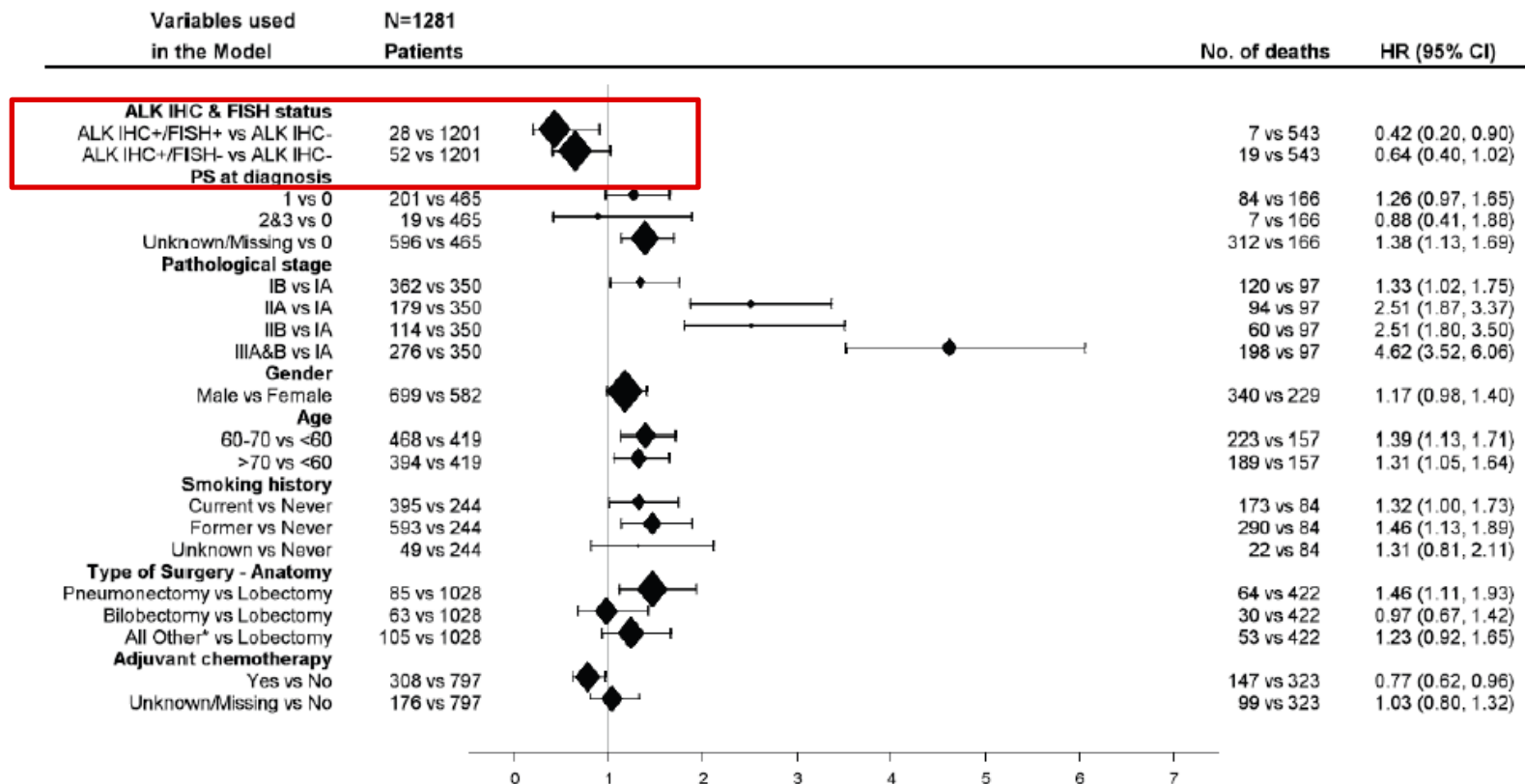ALK: Anaplastic lymphoma kinase; IHC: Immunohistochemistry

Figure A1 – Panel B: Kaplan – Meier curves showing Relapse – free survival by ALK IHC status (N=1281)

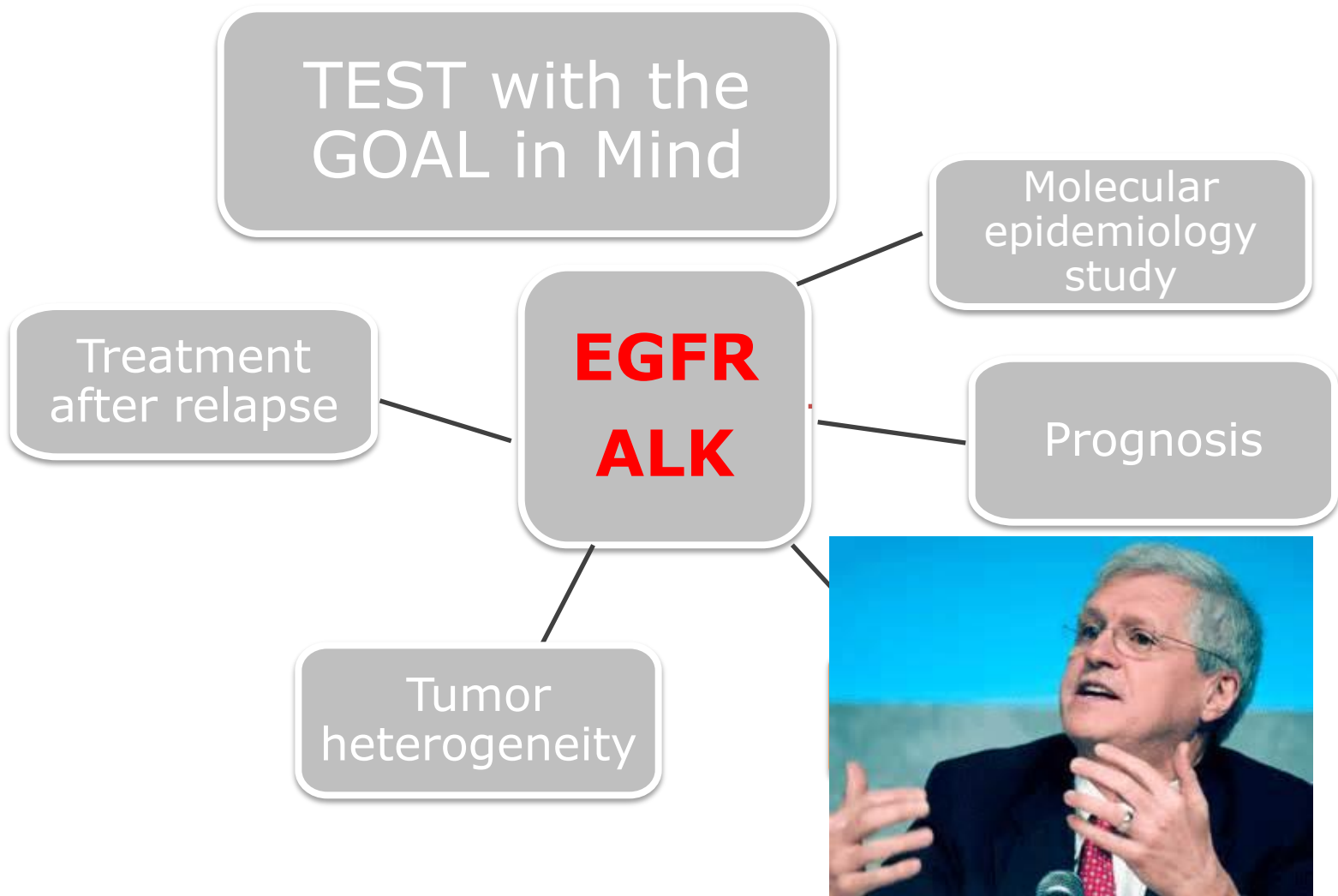ALK: Anaplastic lymphoma kinase; IHC: Immunohistochemistry

Blackhall et al. J Clin Oncol 32:2780-2787.

Figure A2 –Panel A: Forest plots for the multivariate overall survival Cox model (N=1281)
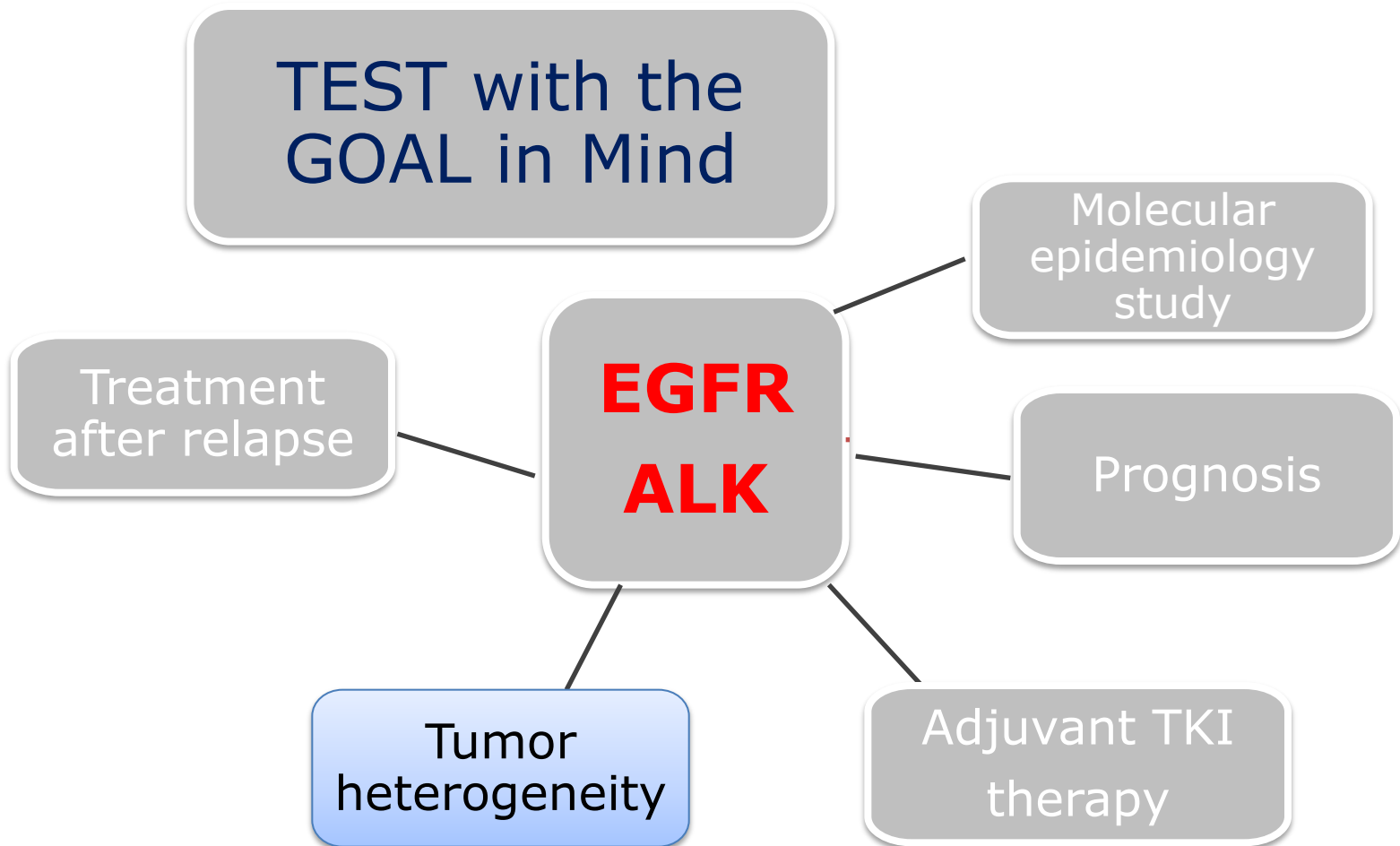
ALK: Anaplastic lymphoma kinase; IHC: Immunohistochemistry; FISH: Fluorescence in situ hybridization; PS: Performance status;
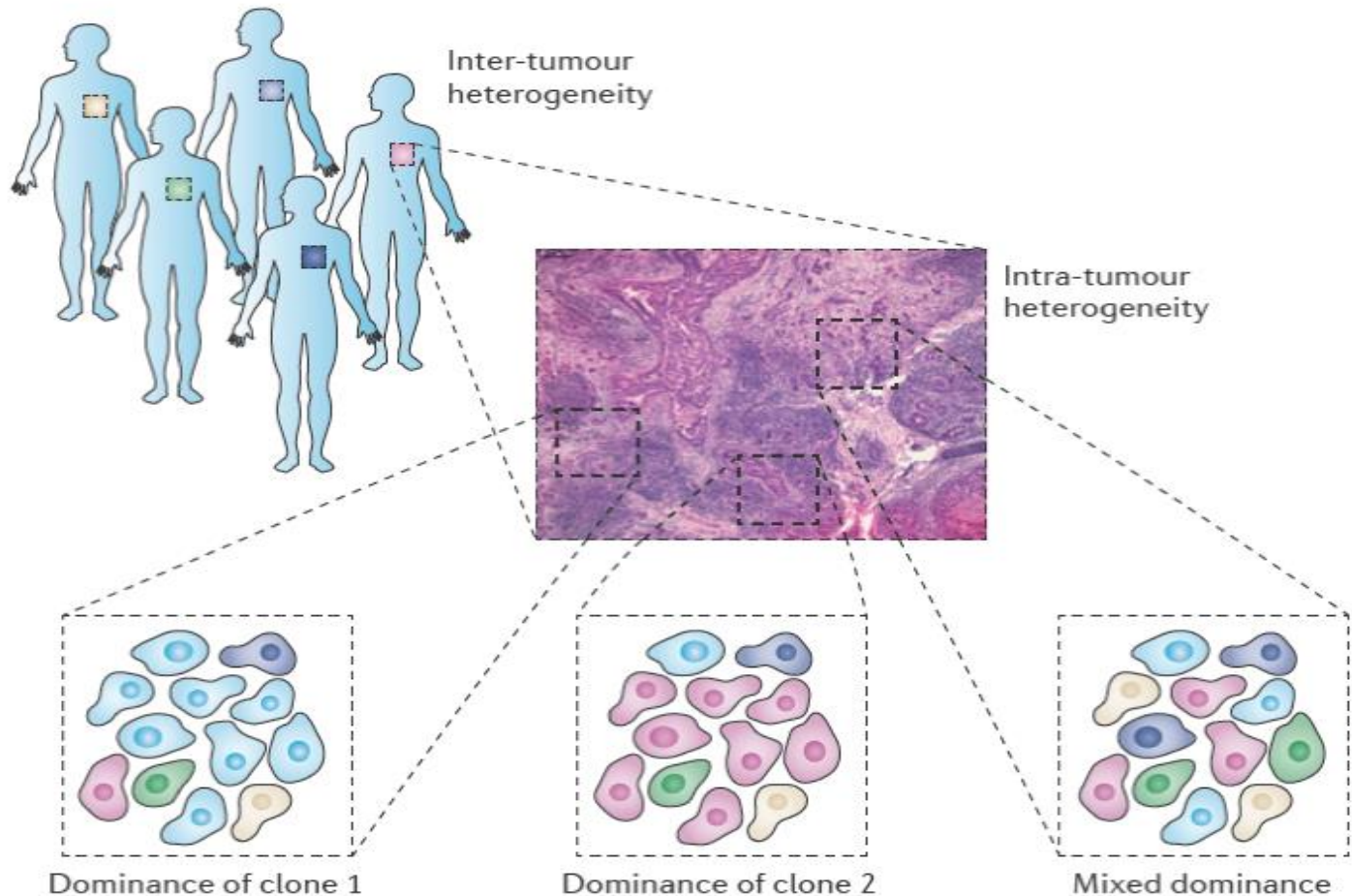
HR: Hazard ratio; CI: Confidence interval

Blackhall et al. J Clin Oncol 32:2780-2787.

R0 resected NSCLC

TEST with the GOAL in Mind

Molecular epidemiology study

Treatment after relapse

EGFR

ALK

Prognosis

Tumor heterogeneity

R0 resected NSCLC

TEST with the GOAL in Mind

EGFR ALK

Molecular epidemiology study

Treatment after relapse

Prognosis

Tumor heterogeneity

Adjuvant TKI therapy

Tailed therapy：Inter-tumor heterogeneity
MDT： intra-tumor heterogeneity

2012 NATURE REVIEWS
2012 NEJM

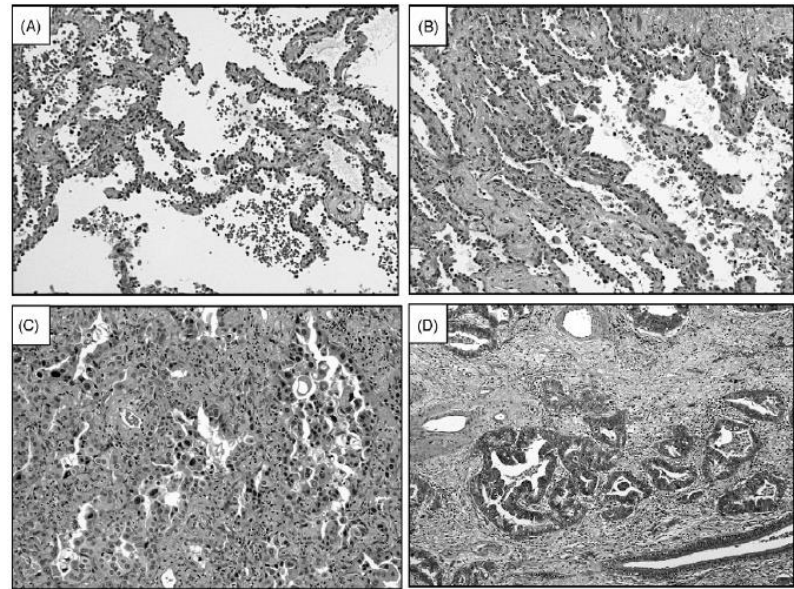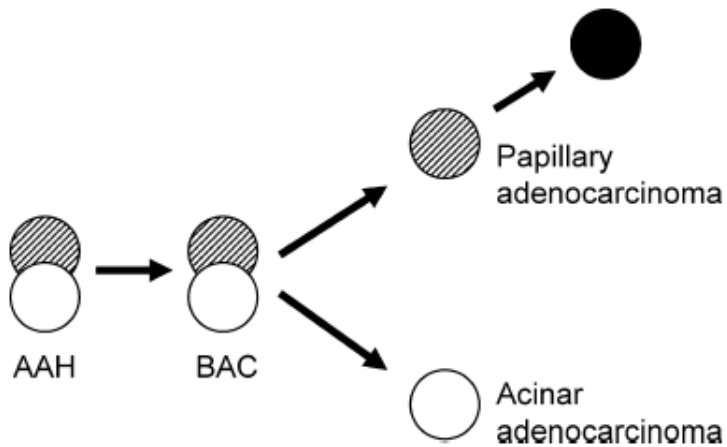# Heterogeneity of EGFR mutations within a mixed adenocarcinoma : Case report

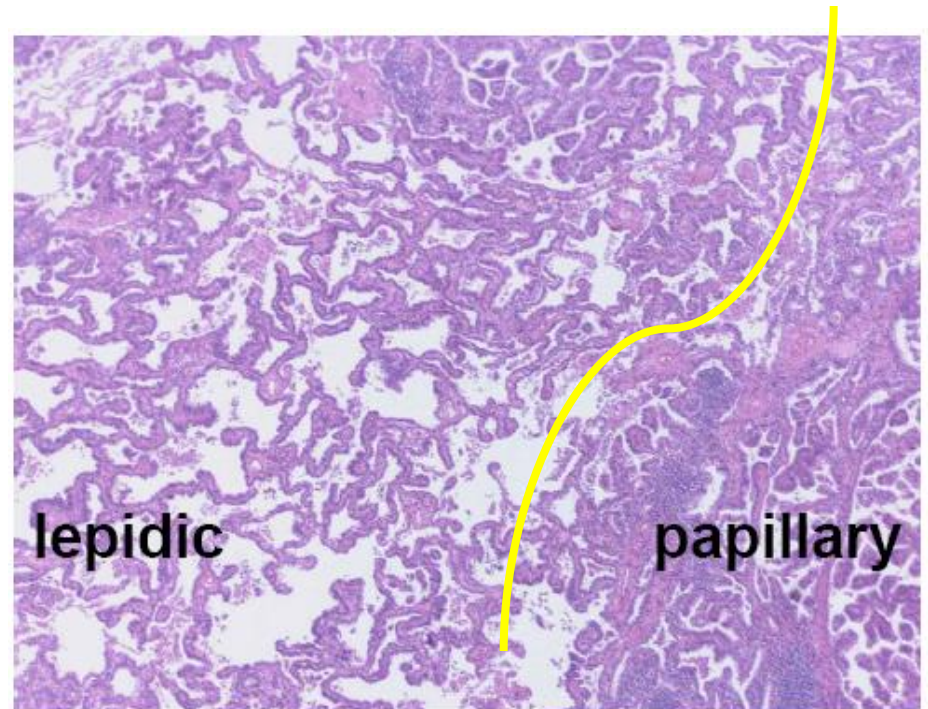Table 1 *EGFR* mutations within a single tumor by histological subtype

Histological subtype (number of samples)	Homozygous deletion in exon 19	Heterozygous deletion in exon 19	No deletion
AAH areas (*n* = 4)	0	2	2
BAC areas (*n* = 4)	0	2	2
Papillary AD (*n* = 4)	3	1	0
Acinar AD (*n* = 4)	0	0	4

AAH: atypical adenomatous hyperplasia, BAC: bronchioloalveolar carcinoma, AD: adenocarcinoma.

Lung cancer 2008, 60(1):136-140.

Concomitant EGFR Mutations and ALK Rearrangements

Yang JJ & Wu YL et al. Clin Cancer Res. 2014;20(5):1383-92.
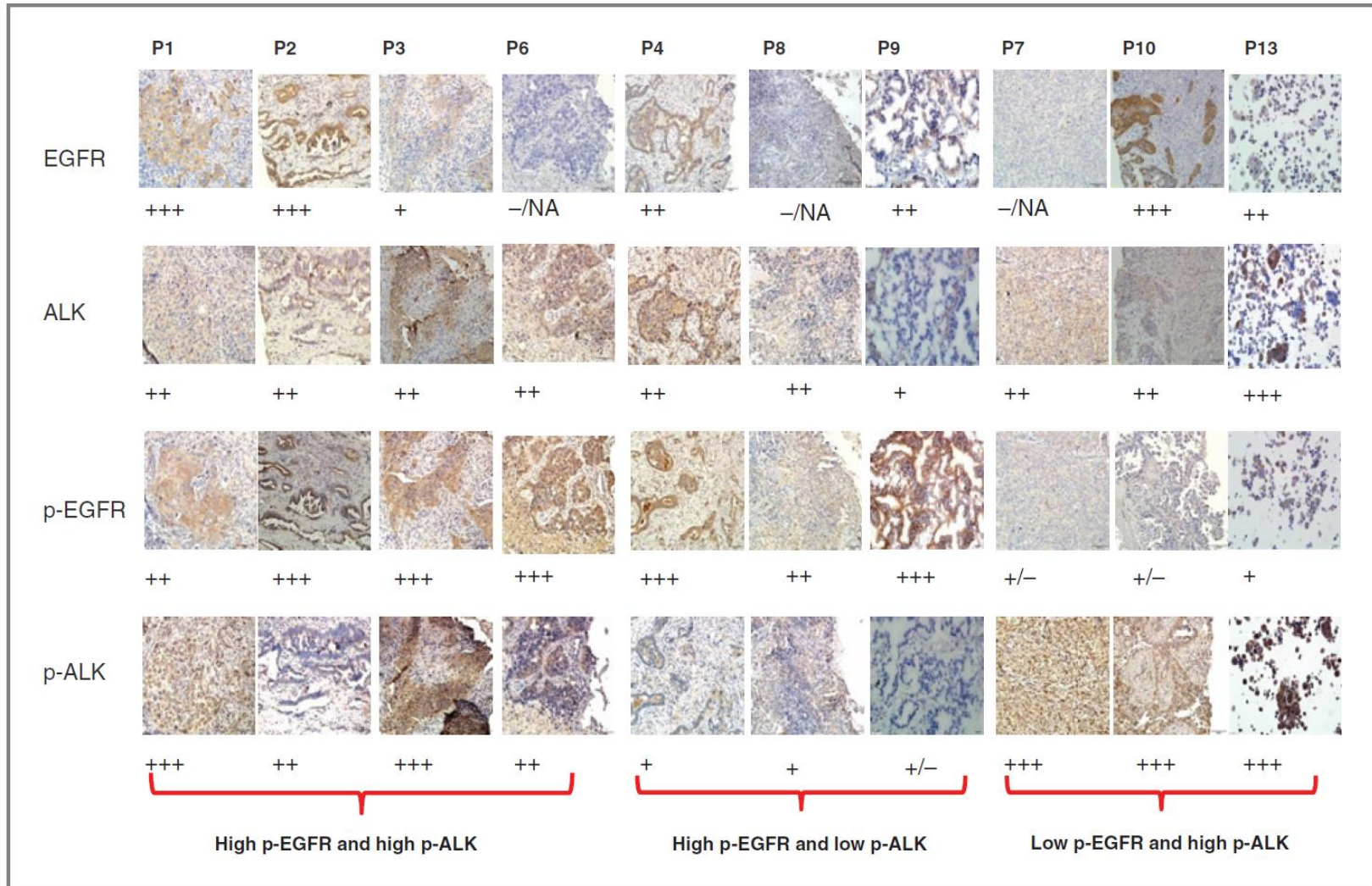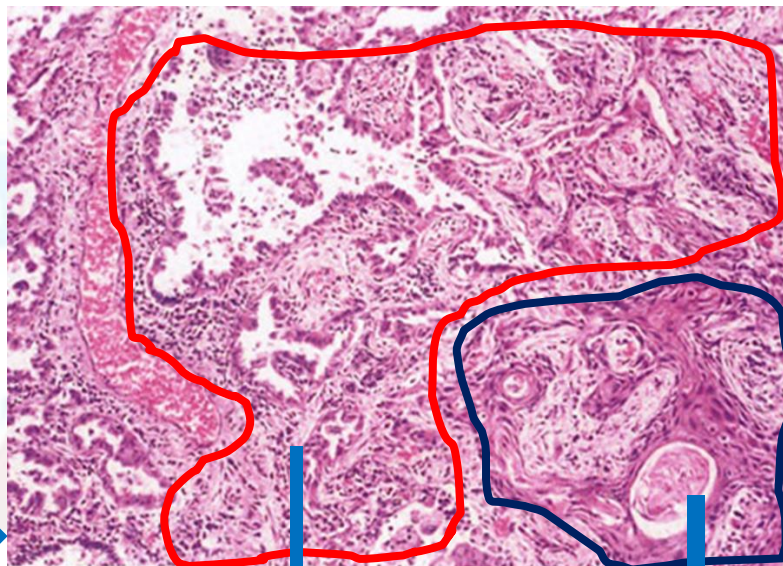
GGO vs solid
lepidic vs invasive

Surgery

Section*

* Select the maximum cross-section of the tumor lesion

HE stainning

Pathologic diagnoses of each subtype in a slide

| Lipidic | Acinar | Papillary | Squamous | Solid |

DNA sequencing:
Ten microsections ×10-μm thickness

Detection of mutations/fusions for each pathological subtype in a slide
(DS for EGFR/KRAS, FISH for EML4-AKL)

Validation
(ARMS for EGFR, HRM for KRAS)

GLCI, unpublished dara

Rare Discrepancies in a Driving Gene Alteration within Histologically Heterogeneous Primary Lung Cancers

GLCI, unpublished dara
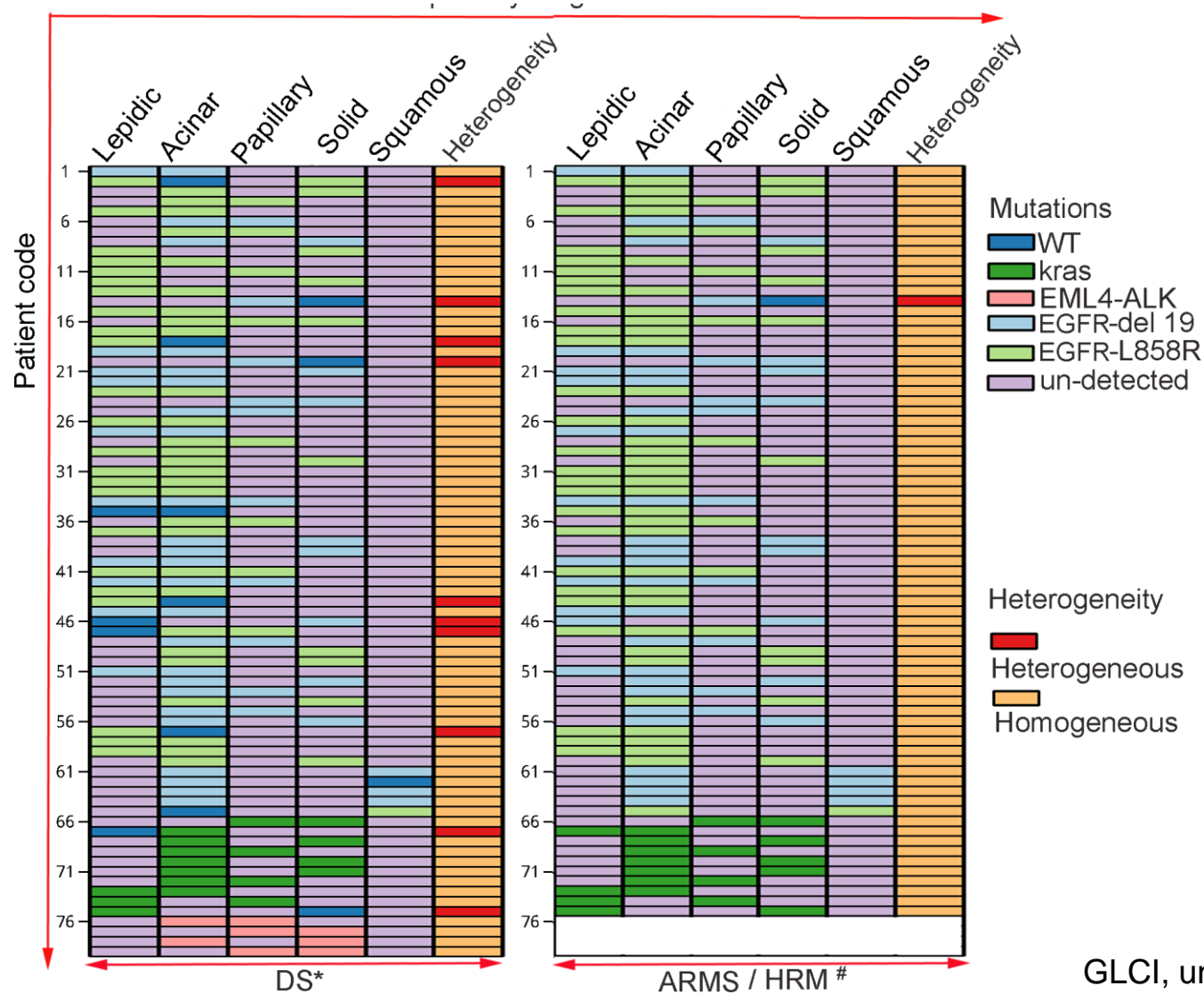
Mutation status between surgery and relapse samples

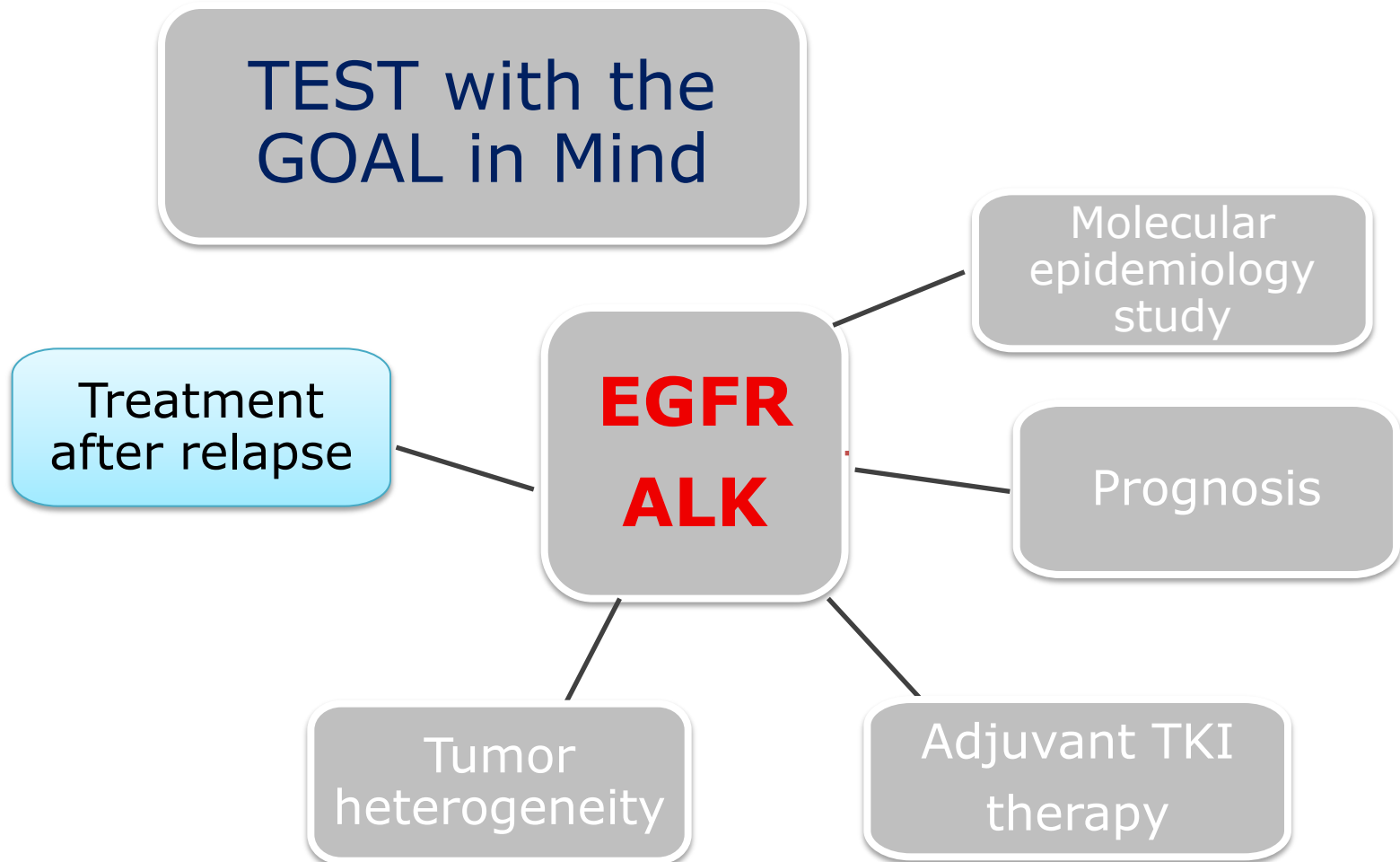## Heterogeneous Distribution of *EGFR* Mutations Is Extremely Rare in Lung Adenocarcinoma

Yasushi Yatabe, Keitaro Matsuo, and Tetsuya Mitsudomi

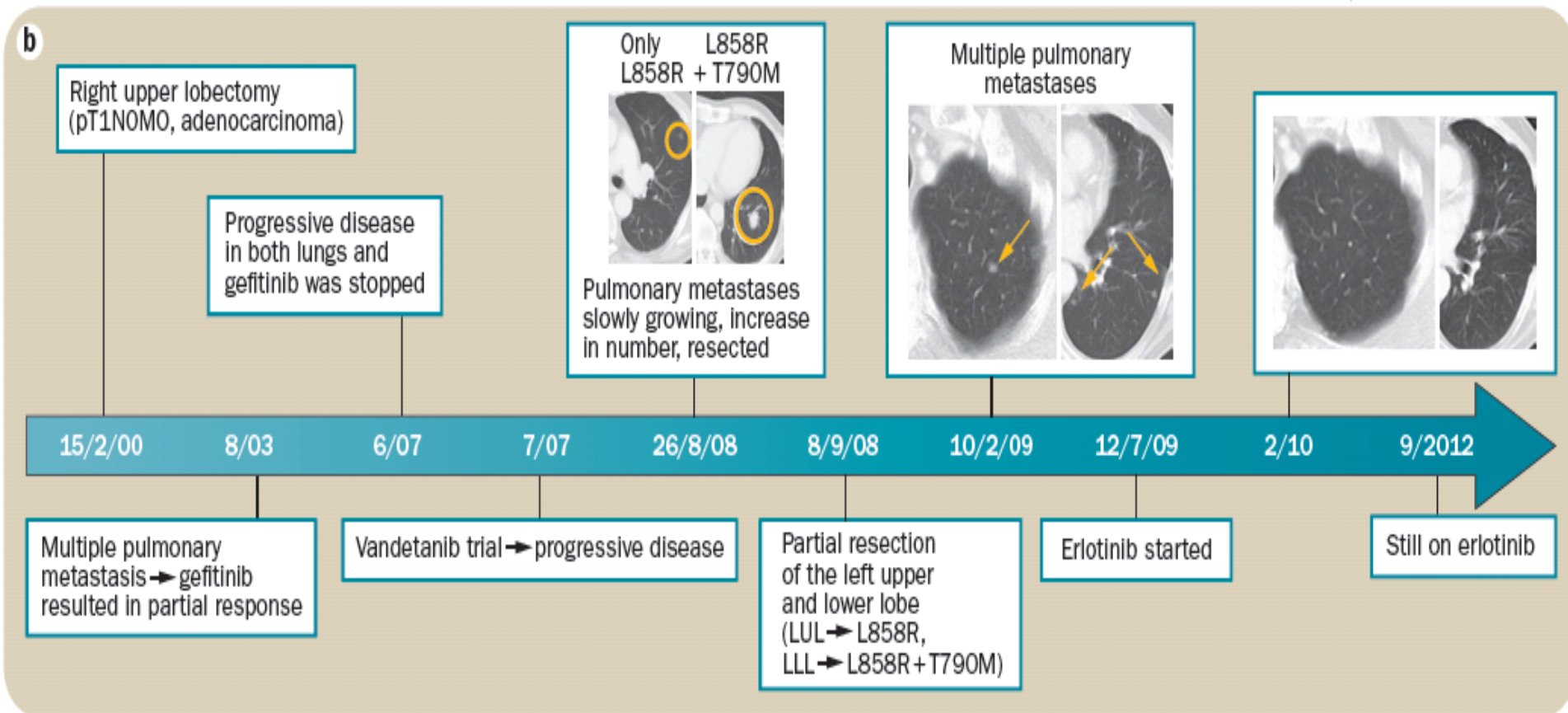**Table 3.** Mutation Patterns in Primary and Recurrent Tumors

Initial Examination Site	Pleural Effusion			Lymph Node			Pericardiac Effusion		Lung Tumor			Central Spinal Fluid		Distant Metastasis	
	No.	Average	Minimal-Maximal	No.	Average	Minimal-Maximal	No.	Average	No.	Average	Minimal-Maximal	No.	Average	No.	Average
Primary tumor	31	30	0-99	7	53	8-212	0		0			1	28	1	34
Distant metastasis	1	1		0			0		0			0		0	
Lymph node	2	17	7-28	3	11	3-20	0		1	2		0		0	
Pleural effusion	2	8	7-9	1	8		1	36	2	3	2-6	1	15	0	
Total	36			11			1		3			2		1	

NOTE. All of the patients demonstrated identical mutation patterns in primary and recurrent tumors.

R0 resected NSCLC

TEST with the GOAL in Mind

Molecular epidemiology study

Treatment after relapse

EGFR ALK

Prognosis

Tumor heterogeneity

Adjuvant TKI therapy

Surgery+TKI in patients with Therapeutic target after relapse

Mitsudomi, T. et al. Nat Rev Clin Oncol, 2013
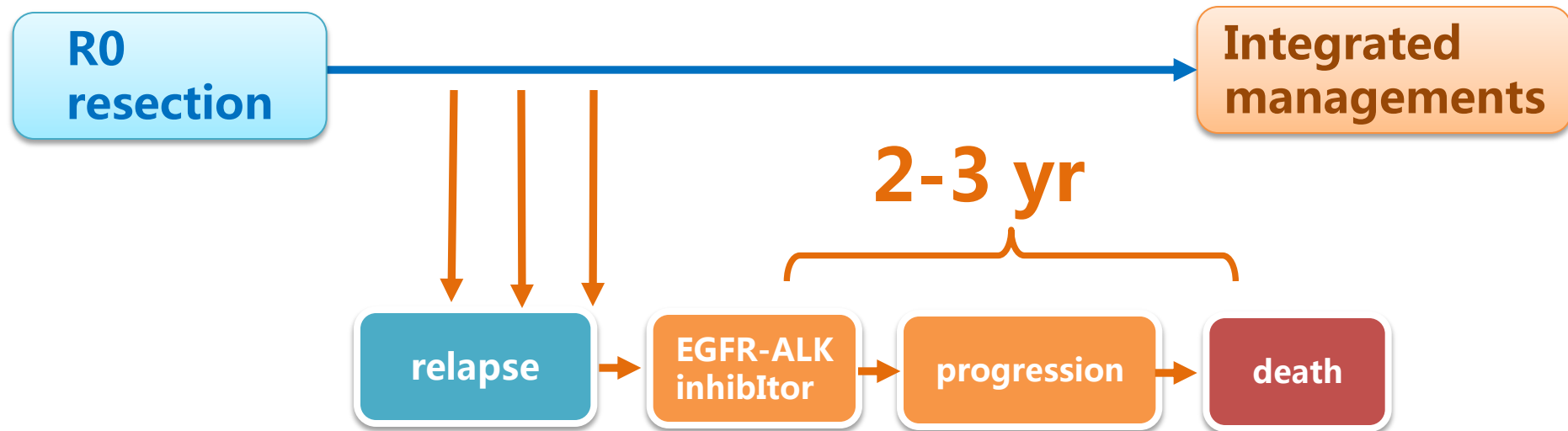
Differential intrapulmonary metastasis from multifocal lung cancer

| Method	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Martini and Melamed	Multiple primary	Multiple primary	Multiple primary	Multiple primary	Metastasis	Multiple primary	Multiple primary
ACCP criteria	Multiple primary	Not classified	Metastasis	Multiple primary	Metastasis[a]	Metastasis	Multiple primary
Histologic subtyping	Different	Different	Different	Different	Different	Different	Different
Molecular analysis	Different	Different	Different	Different	Different	Different	Different
Integrated analysis	Multiple primary	Multiple primary	Multiple primary	Multiple primary	Multiple primary	Multiple primary	Multiple primary
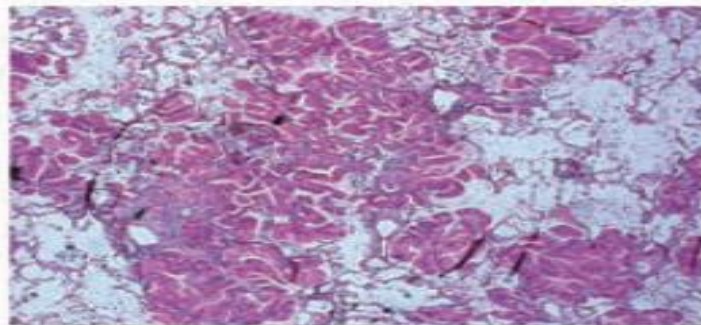
BAC (50%)/acinar adenocarcinoma (50%)
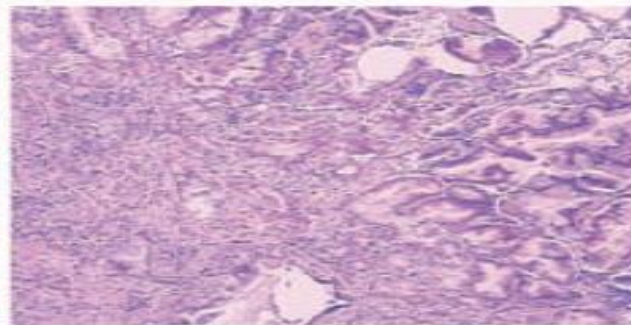
BAC (60%)/acinar adenocarcinoma (40%)

RLL → 19 Months → LL
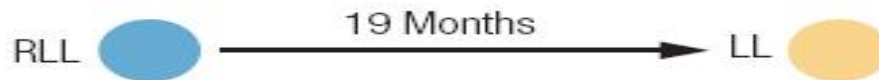
L858R
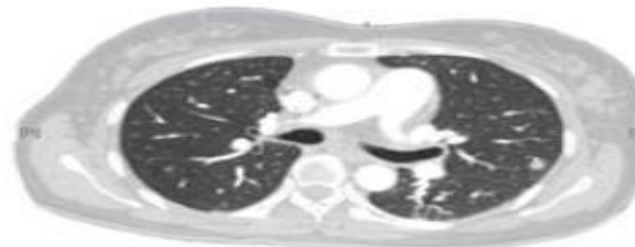
Wild-Type *EGFR*

2010 Chest
2010 CLC

Summary

Should we test for EGFR and ALK in completely resected NSCLC???

GOAL	Importance of TEST	Impact on Clinical Practice
Molecular epidemiology study	High	Two class of disease
Prognosis	Middle	Worse prognosis in NSCLC with ALK+
Adjuvant TKI therapy	High	No enough evidence but ongoing trials.
Tumor heterogeneity	Rare in driving gene	Offer rational for TKI treatment after relapse
Treatment after relapse	High	Local therapy and Integrated management should be considered

16TH WORLD CONFERENCE ON LUNG CANCER

IASLC

INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

WWW.IASLC.ORG

Save the Date!

Abstract Submission Open	January 2015
Registration Open	January 2015
Abstract Submission Deadline	April 24, 2015
Abstract Notifications	June 22, 2015
Early Registration Deadline	June 26, 2015
Late Breaking Abstract Submission Deadline	July 10, 2015
Regular Registration Deadline	July 24, 2015

SEPTEMBER 6–9, 2015

→ DENVER, COLORADO, USA

FIGHTING LUNG CANCER