

**CONVEGNO REGIONALE AIOM EMILIA-ROMAGNA  
I NUMERI DEL CANCRO IN EMILIA ROMAGNA:  
AMBIENTE, STILI DI VITA, SCREENING  
FOCUS SU TUMORI DEL POLMONE E COLON-RETTO**

*III SESSIONE – CANCRO DEL POLMONE: EPIDEMIOLOGIA E  
NUOVI TRATTAMENTI*

**Epidemiologia e profili molecolari: realtà in  
evoluzione ?**

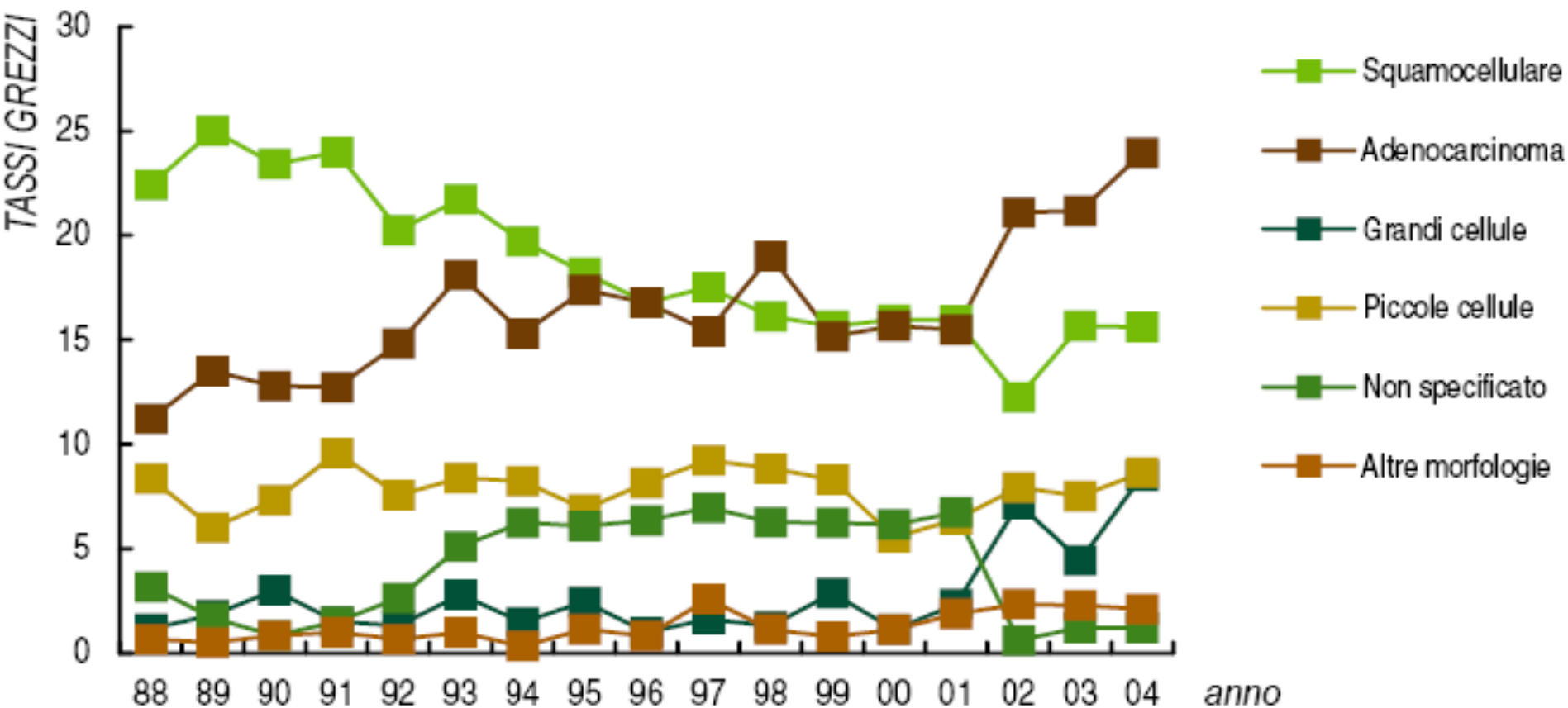
**Giulio Rossi**

**Anatomia Patologica Ravenna, AUSL Romagna**

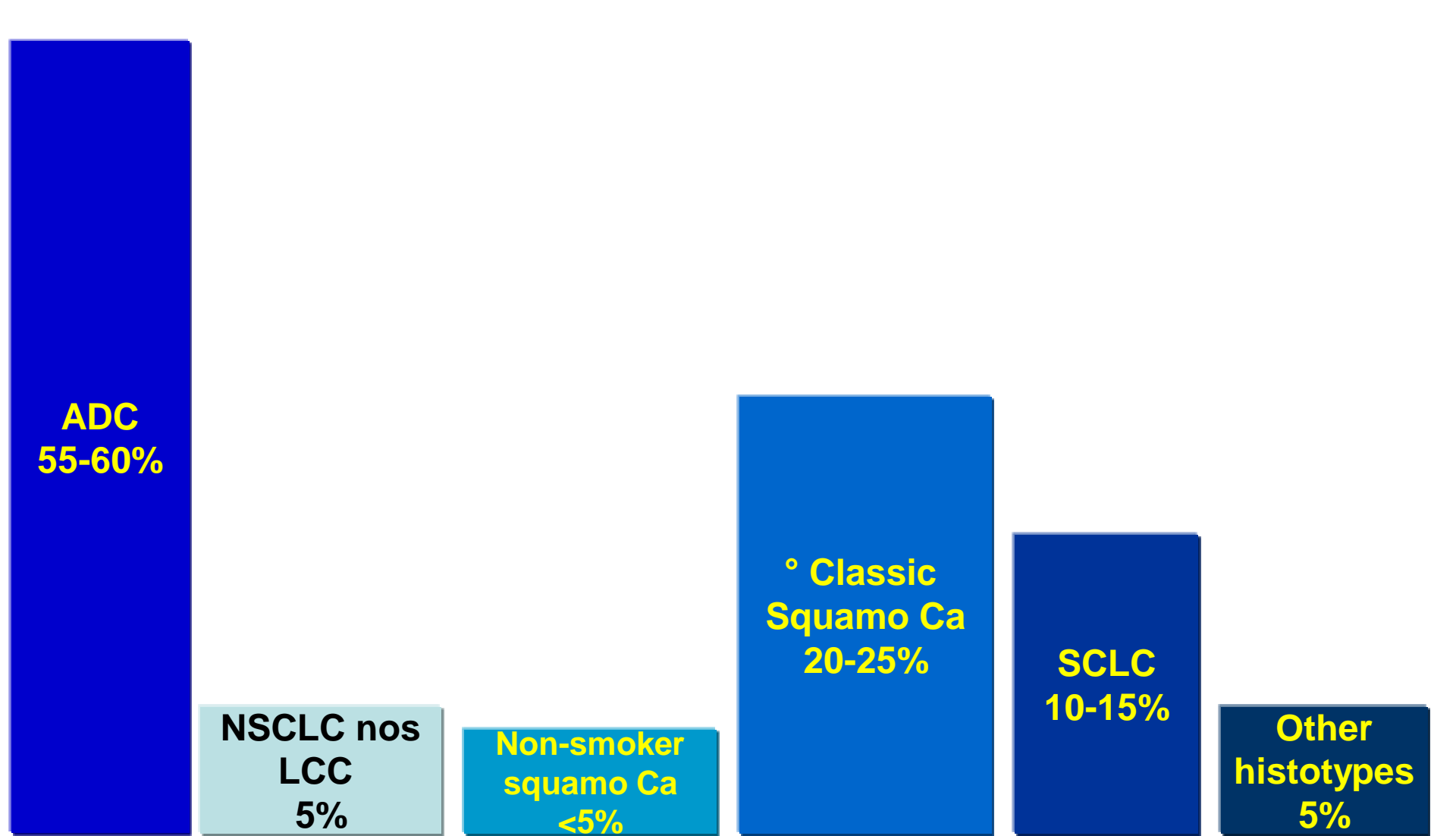
**MODENA, Centro Servizi Università Policlinico  
23 NOVEMBRE 2018**

# Dati Registro Tumori Modena- 2004

Trend del tasso\* di incidenza diviso per morfologia tumorale. Anni 1988-2004



\* Tasso grezzo su 100.000 residenti



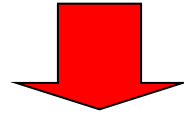
° classic Squamous Ca means active/former smokers and keratinizing squamous ca

# Clinico-radiologic presentation of lung cancer according to histotype

Colby TV, Koss MN, Travis WD. AFIP, 1995

	SQC	ADC	SCLC	LCC
• Peripheral location	29%	70%	26%	65%
• Central location	71%	5%	> 70%	35%
• Hilar/perihilar mass	40%	15%	80%	32%
• Cavitation	5-10%	1%	-	4%
• 5 yrs OS	15.4%	16.6%	4.6%	11%
• Distant mets	<10%	25%	>50%	25%
• Intrathoracic spread	25%	<10%	> 50%	15%

# Adenocarcinoma histotype influences sampling procedures



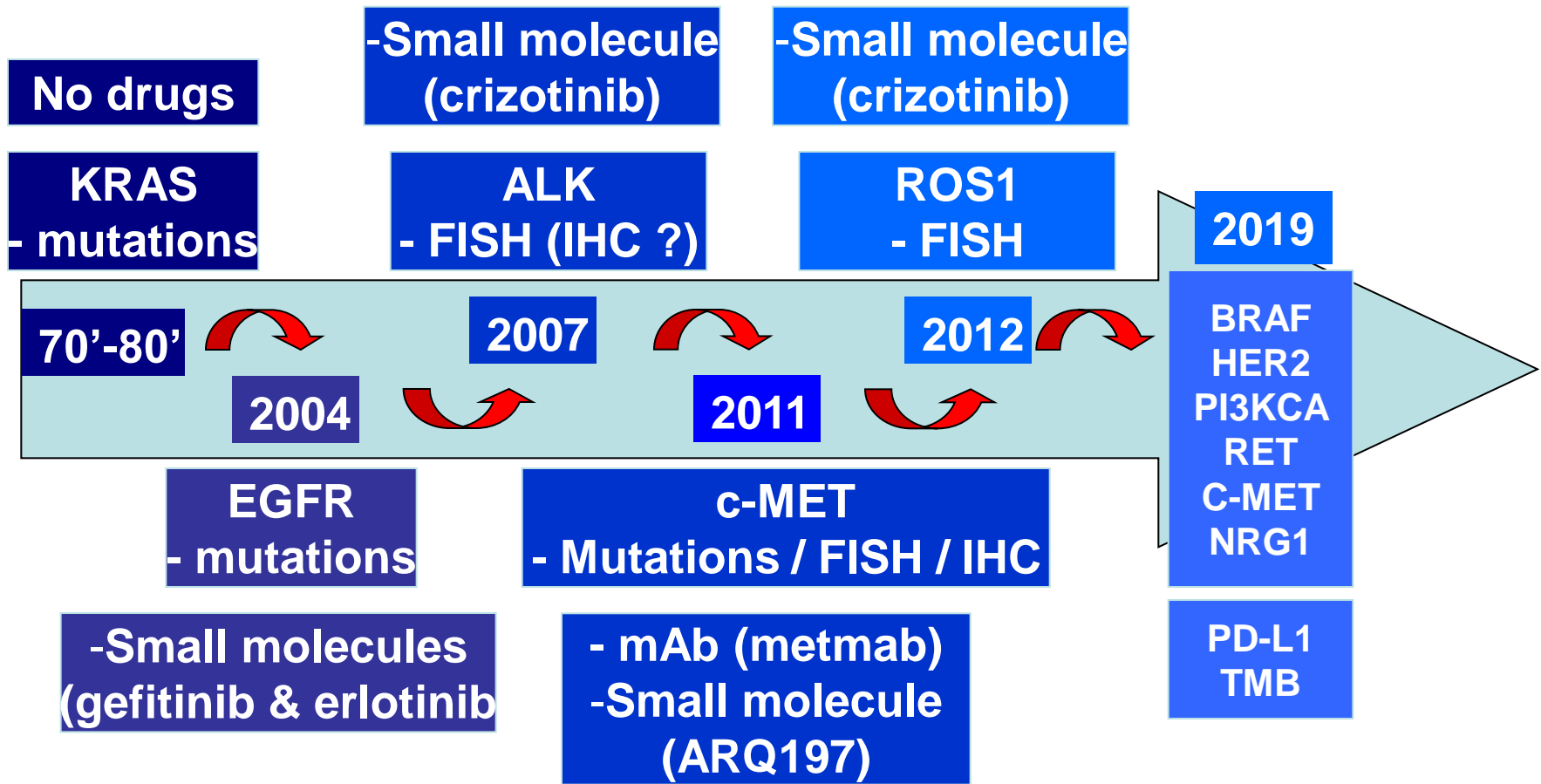
## Peripheral lesions & high frequency of lymph node involvement

1. Bronchoscopy over-rides radiology
  - More TBNA on lymph nodes or primary site
  - Cytology >>> Histology
2. Radiology over-rides bronchoscopy
  - More transthoracic biopsies
  - Histology >>> Cytology

# Type of material & analytical method

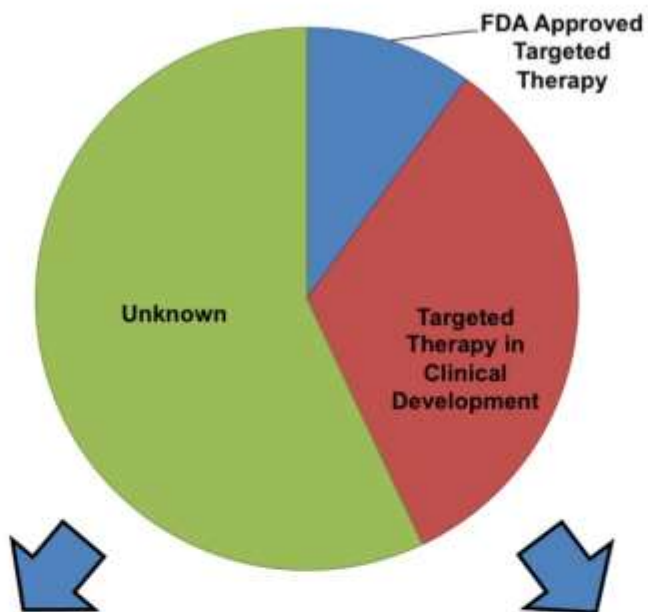
	<b>IHC (i.e. EGFR)</b>	<b>FISH (i.e. ALK)</b>	<b>DNA (i.e. EGFR/KRAS)</b>	<b>RNA</b>
<b>Cytology</b>	<b>++ Cell block 😊</b>	<b>++</b>	<b>+++ Stained slides do no compromise DNA quality 😊</b>	<b>+++</b>
<b>Biopsy</b>	<b>+++</b>	<b>++</b>	<b>++</b>	<b>+</b>
<b>Surgery</b>	<b>+++</b>	<b>+++</b>	<b>+++</b>	<b>+++</b>

# Lung Cancer alterations after EGFR & KRAS

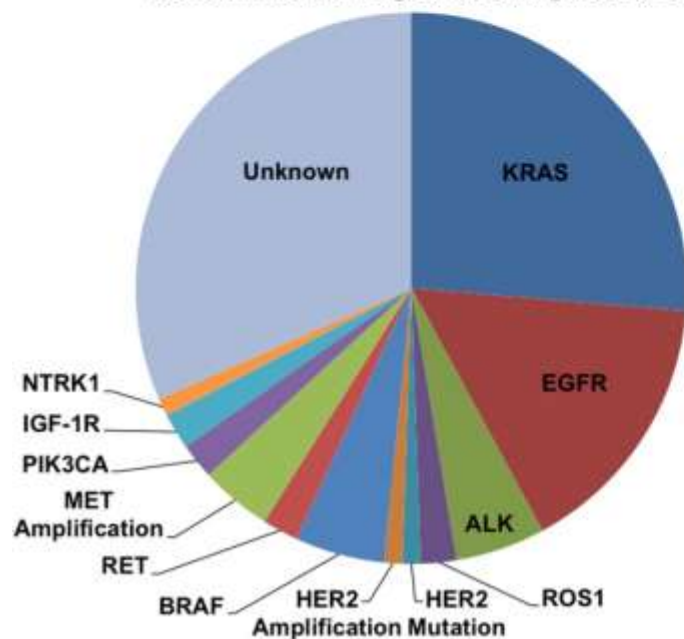


to be continued .....

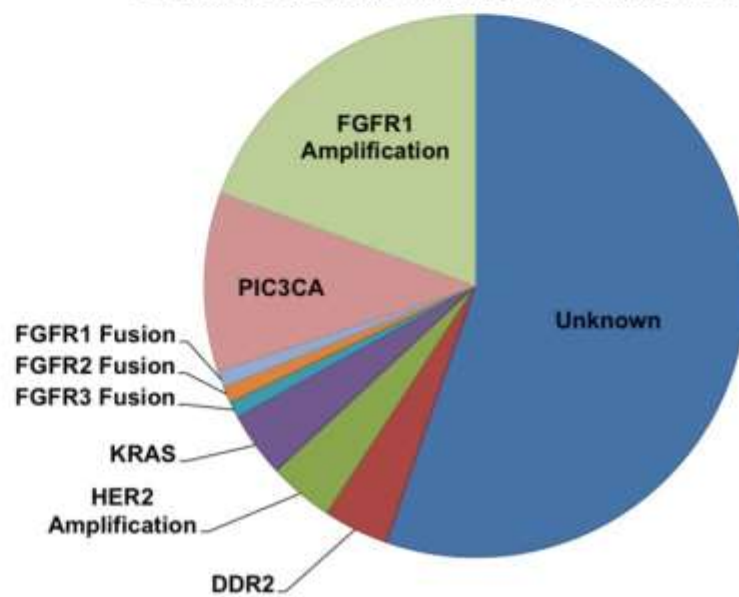
Non-Small Cell Lung Cancer: Targeted Therapies 2013



Adenocarcinoma: Targetable Oncogenes 2013

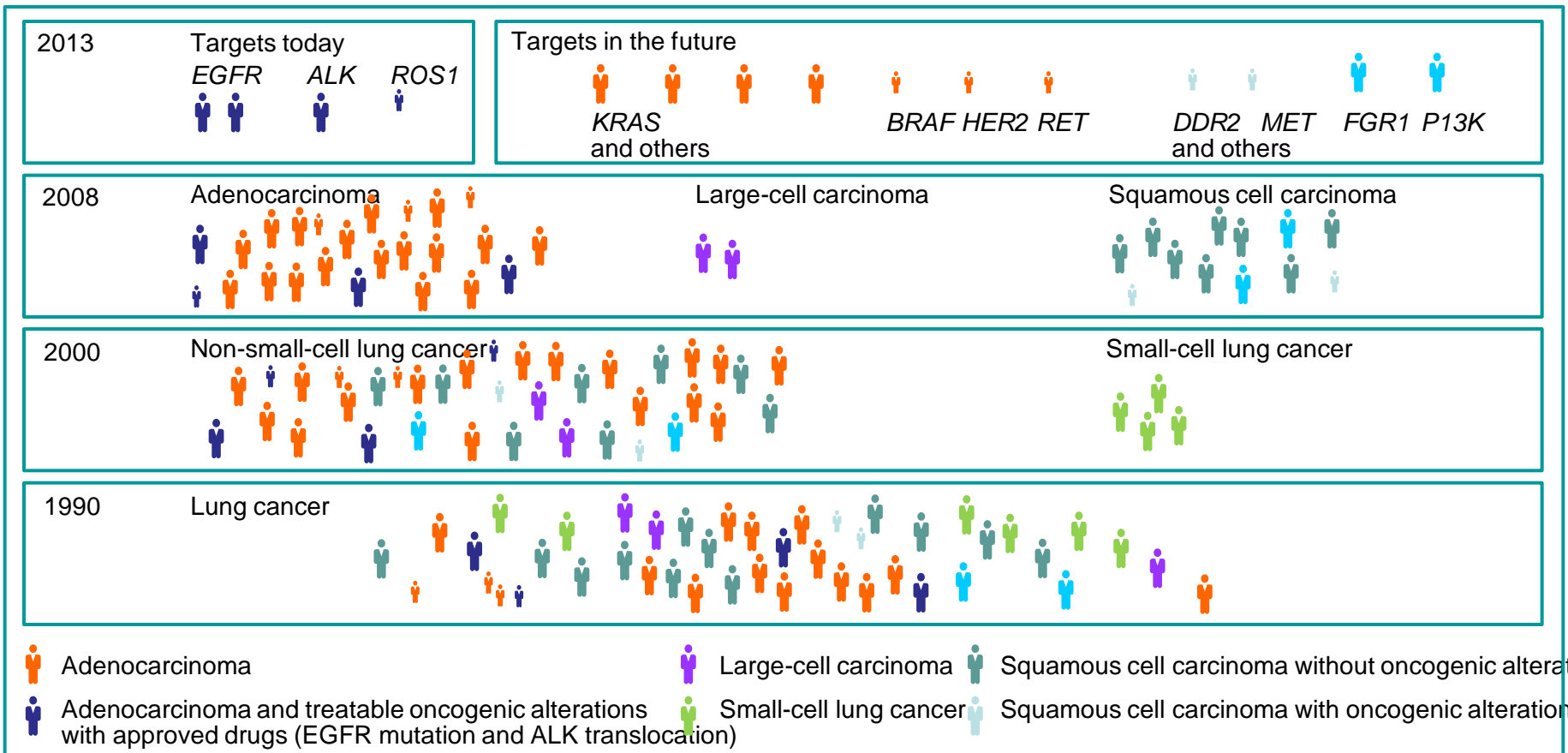


Squamous Cell Carcinoma: Targetable Oncogenes 2013

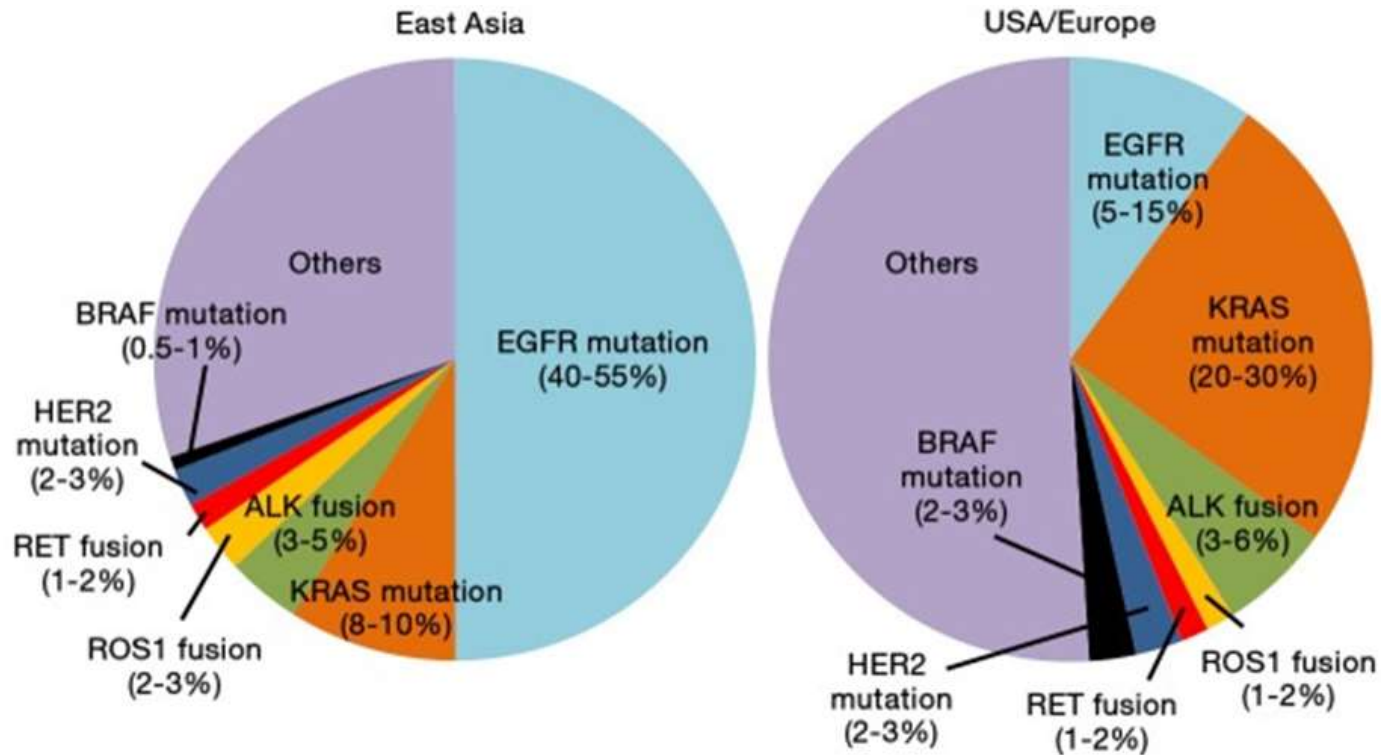




# Evolution and importance of biomarkers in NSCLC



# Geographical variability



# EGFR/KRAS: an ethnicity problem

**Caucasian**

**Asian**

**unselected people**

**EGFR mut**

**10%**

**30-60%**

**KRAS**

**25-35%**

**5%**

# Major NSCLC driver genes and clinico-pathologic features

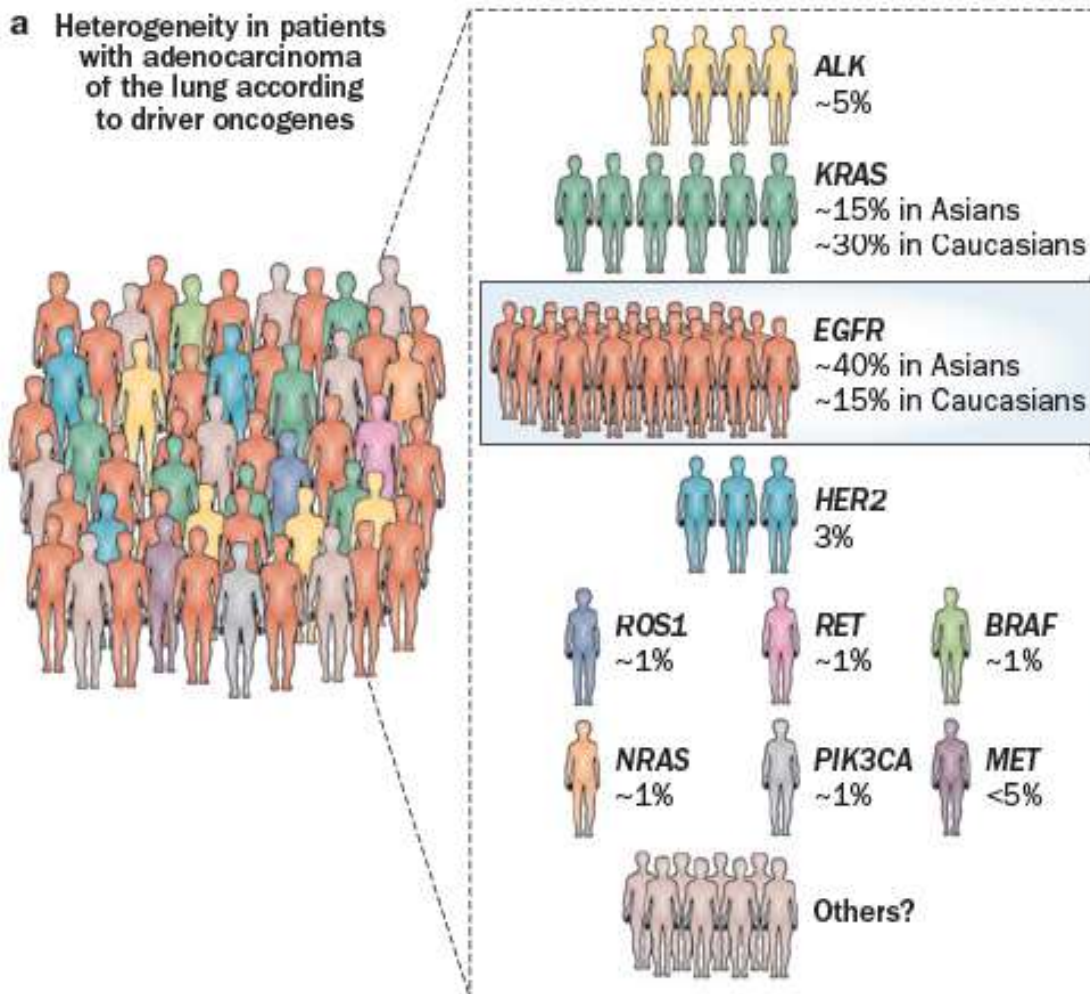
Driver gene	<i>EGFR</i>	<i>KRAS</i>	<i>ALK</i>
• Gender	F>M	M>F	M=F
• Age (years)	>60	>60	<60
• Smoke	No/slight	Active	No/slight
• Histotype	Adc non-muc	Adc muc/acinar	Adc mixed signet
• IHC	TTF-1 + p63 -	TTF-1 -/+ p63 -/+	TTF-1 + p63 +
• Other			PEM +++ (TS low)

# Surgery for NSCLC in the era of personalized medicine

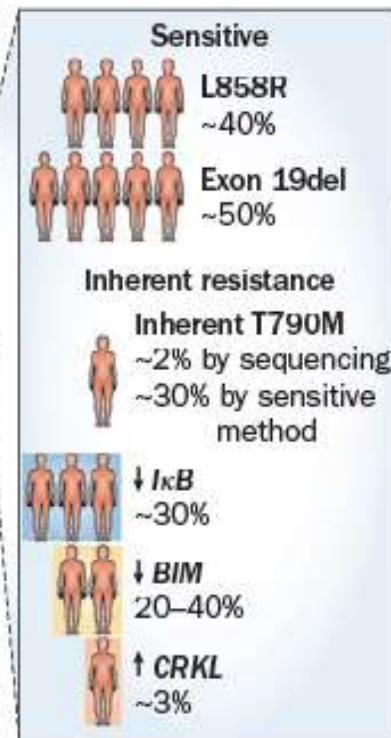
Tetsuya Mitsudomi, Kenichi Suda and Yasushi Yatabe

Nat. Rev. Clin. Oncol. 10, 235–244 (2013)

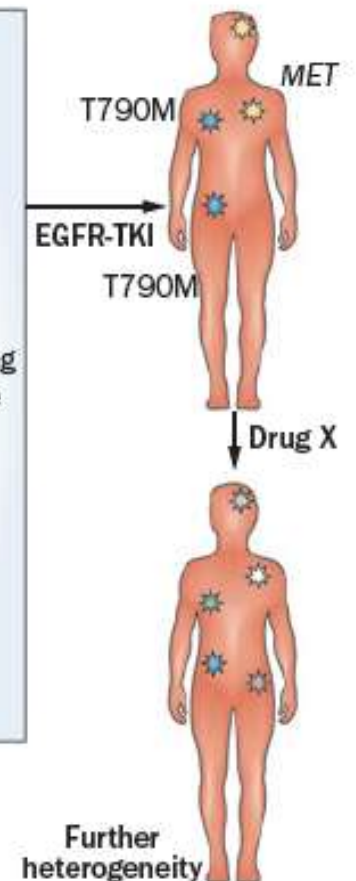
## a Heterogeneity in patients with adenocarcinoma of the lung according to driver oncogenes



## b Heterogeneity within patients with EGFR mutation



## c Heterogeneity in resistance mechanisms in one patient





# Awake VATS biopsy

- 1) Avoid side-effects of intubated general anesthesia with single-lung mechanical ventilation.
- 2) Maintain more physiologic muscular, neurological, and cardiopulmonary status.



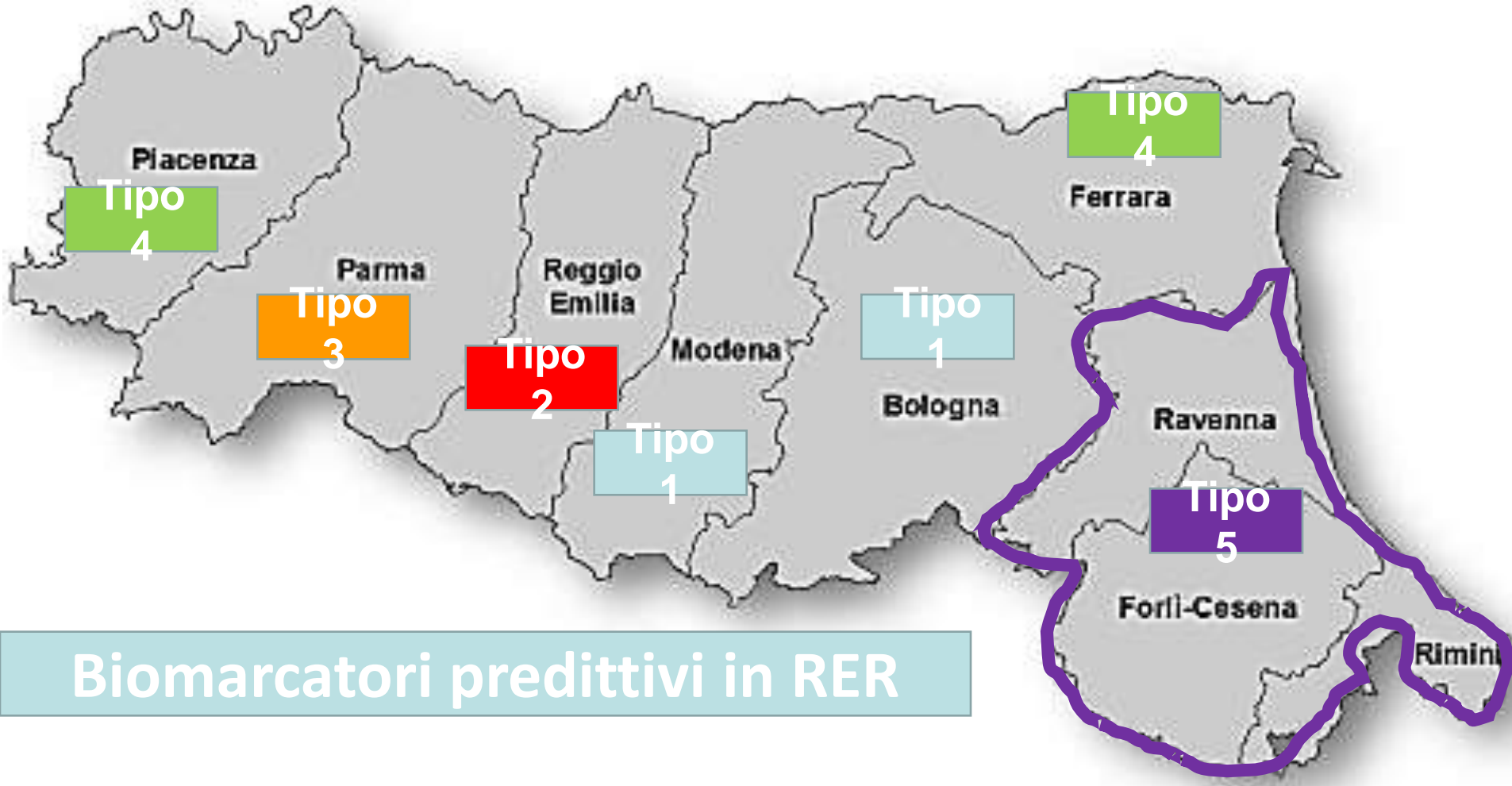
**Minimize the overall surgical  
trauma assuring maximized  
diagnostic accuracy**



# Surgical Approach

Lung biopsy is performed by a single-incision VATS approach, under spontaneous ventilation, through local anesthesia with or without mild sedation.





**Tipo 2: BiolMol estrattiva e BioLiq fuori dall'U.O. Anatomia Patologica**

**Tipo 3: BiolMol estrattiva all'interno dell'U.O. Anatomia Patologica; BioLiq/EGFR in Oncologia**

**Tipo 4: BiolMol estrattiva in U.O. Anatomia Patologica; senza BioLiq**

**Tipo 5: BiolMol estrattiva e BioLiq fuori dall'U.O. Anatomia Patologica (fuori Azienda)**



# Tumore del polmone: diagnosi

## Prevalenza (prevalenza-assi)

- 1. mixing
- 2. intervento chirurgico
- 3. intervento di ablazione
- 4. altro

## ES, fase

- 1. mixing
- 2. non chirurgico
- 3. chirurgico

## Data (data assi)

Scade di assunzione esami

## Esclusione esami

- 1. mixing
- 2. non chirurgico
- 3. chirurgico

## Scade di assunzione esami

- 1. mixing
- 2. Indagini non valutabili
- 3. normale
- 4. alterazioni capistiche
- 5. malignità non piccole cellule
- 6. malignità piccole cellule

## Data accettazione dell'esame

Servizio di Istopatologia

Data del referto

## Altre indagini diagnostiche

- 1. mixing
- 2. mediastinoscopia
- 3. spirometrie transbronchiale
- 4. spirometrie
- 5. biopsie endo broncopolmonari (specif. fibre)

## Scade biopsia sede extrapleuraria

Referto

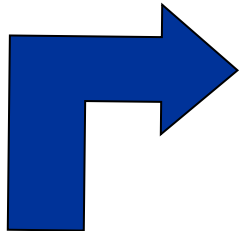
- 1. mixing
- 2. Inadeguato/Non valutabile
- 3. normale
- 4. malignità non a piccole cellule
- 5. malignità a piccole cellule

Servizio di Istopatologia

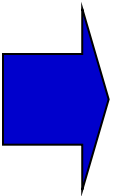
Data del referto



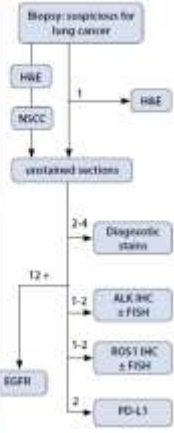
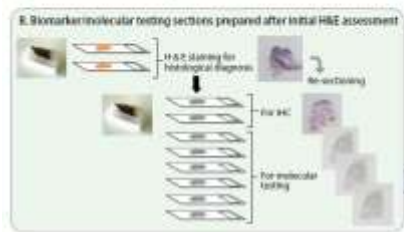
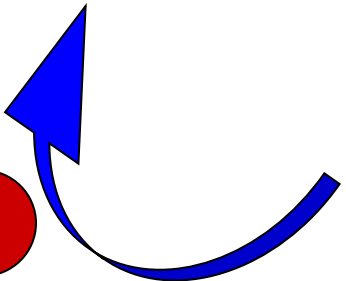
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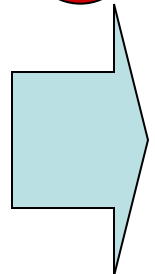
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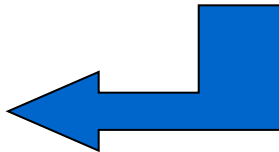


2



Analisi polimorfismi DPYD	Analisi polimorfismi UGT	Analisi di mutazione EGFR su biopsia liquida
Analisi dell'instabilità dei microsatelliti MSI con prelievo ematico	Analisi dell'instabilità dei microsatelliti MSI con tessuto sano	Analisi di mutazione Braf
Analisi di mutazione Kras	Analisi di mutazione Nras	Analisi di mutazione Hras
Analisi di mutazione PIK3CA	Analisi di mutazione KIT	Analisi di mutazione MET
Analisi di mutazione EGFR	Analisi di mutazione ERBB2	Analisi di mutazione IDH2
Analisi stato di metilazione MGMT	Analisi di mutazione IDH1	Analisi hotspot dell'esone 16 RET
Analisi di mutazione PDGFRα	Analisi presenza e genotipizzazione HPV	Analisi di mutazione GNAQ
Analisi di mutazione RET	Analisi di mutazione GNA11	Analisi di mutazione somatica BRCA1 e BRCA2
Profilo NGS tessuto tumorale	Analisi di espressione della proteina ALK mediante immunostochi mica	Analisi di espressione della proteina PDL-1 mediante immunostochi mica
Analisi di espressione della proteina ROS1 mediante immunostochi mica	Analisi traslocazione EML4-ALK mediante FISH/ISH	Analisi riarrangiamento ROS1 mediante FISH/ISH
Analisi traslocazione RET mediante FISH/ISH	Analisi di amplificazione e MET mediante FISH/ISH	

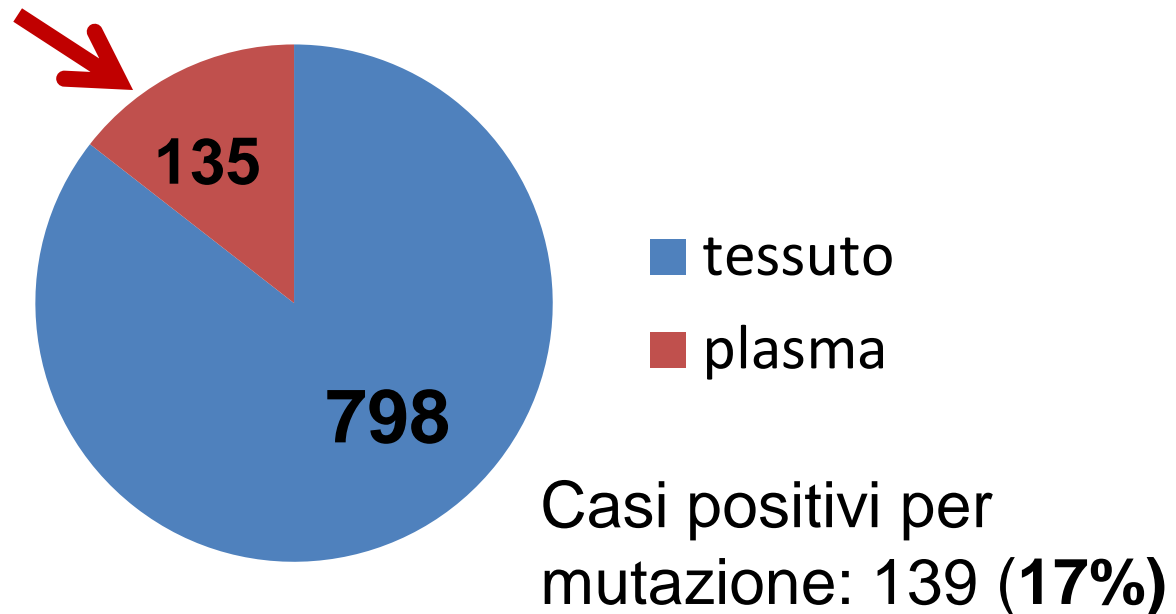
3



# Determinazioni EGFR eseguite da gennaio 2017 a Luglio 2018 presso il Laboratorio di Bioscienze IRST

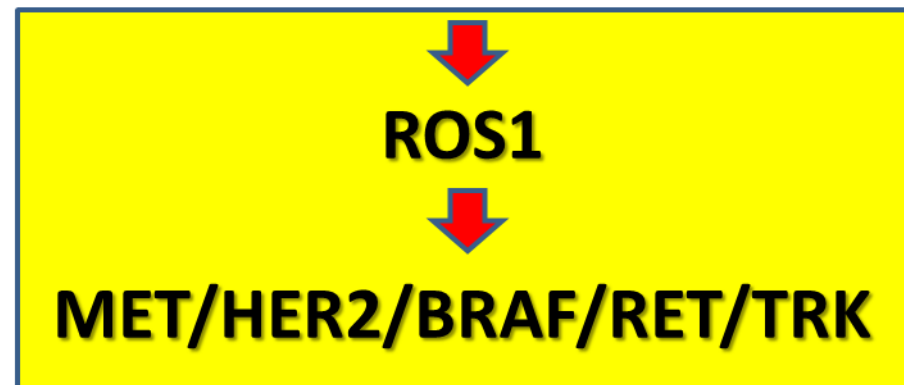
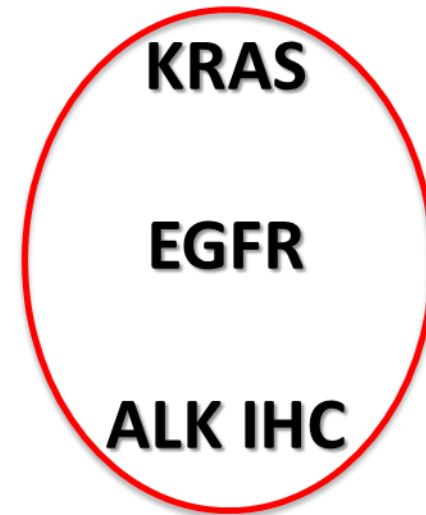
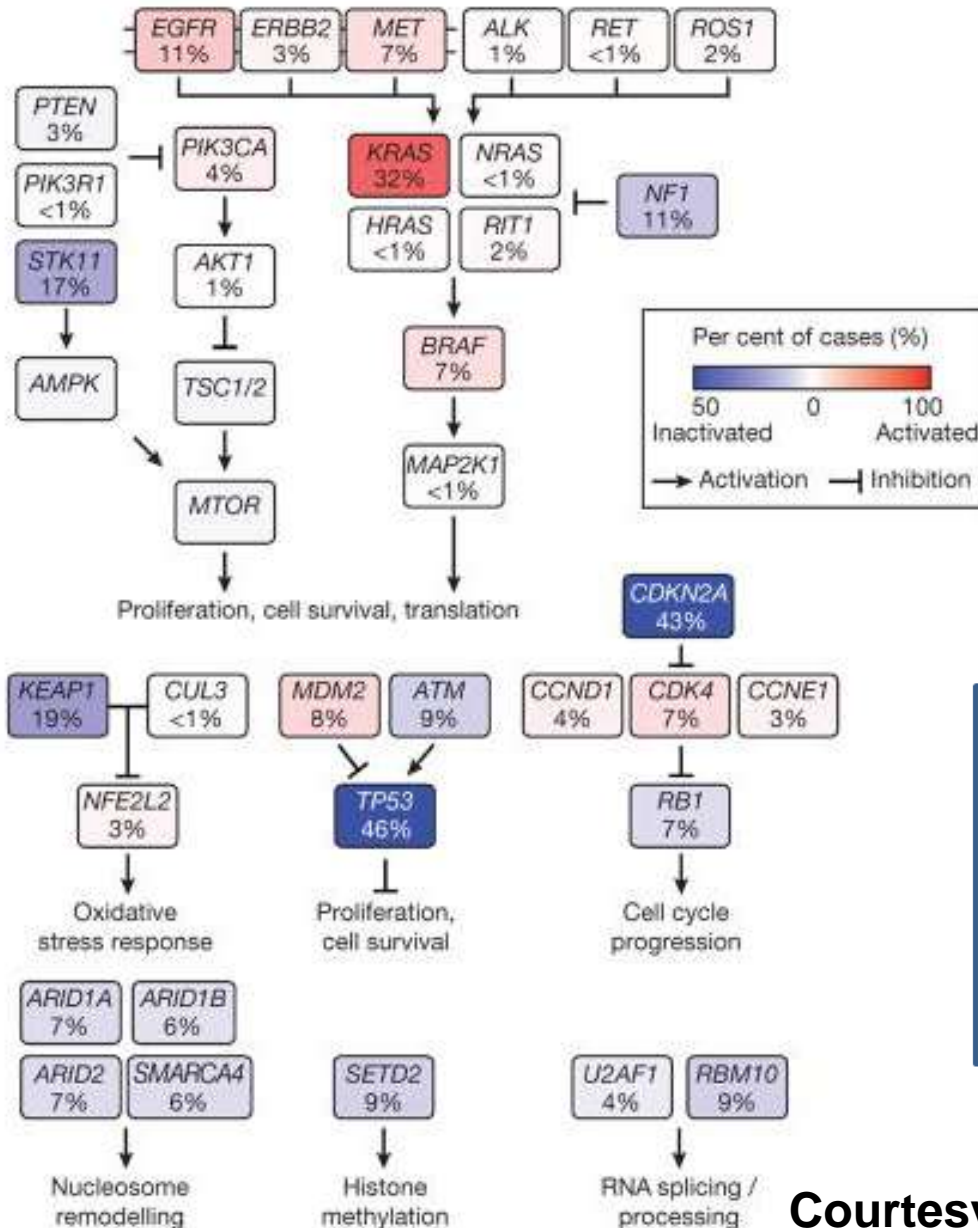
Totale determinazioni: 933

Casi positivi per mutazione: 35 (26%)



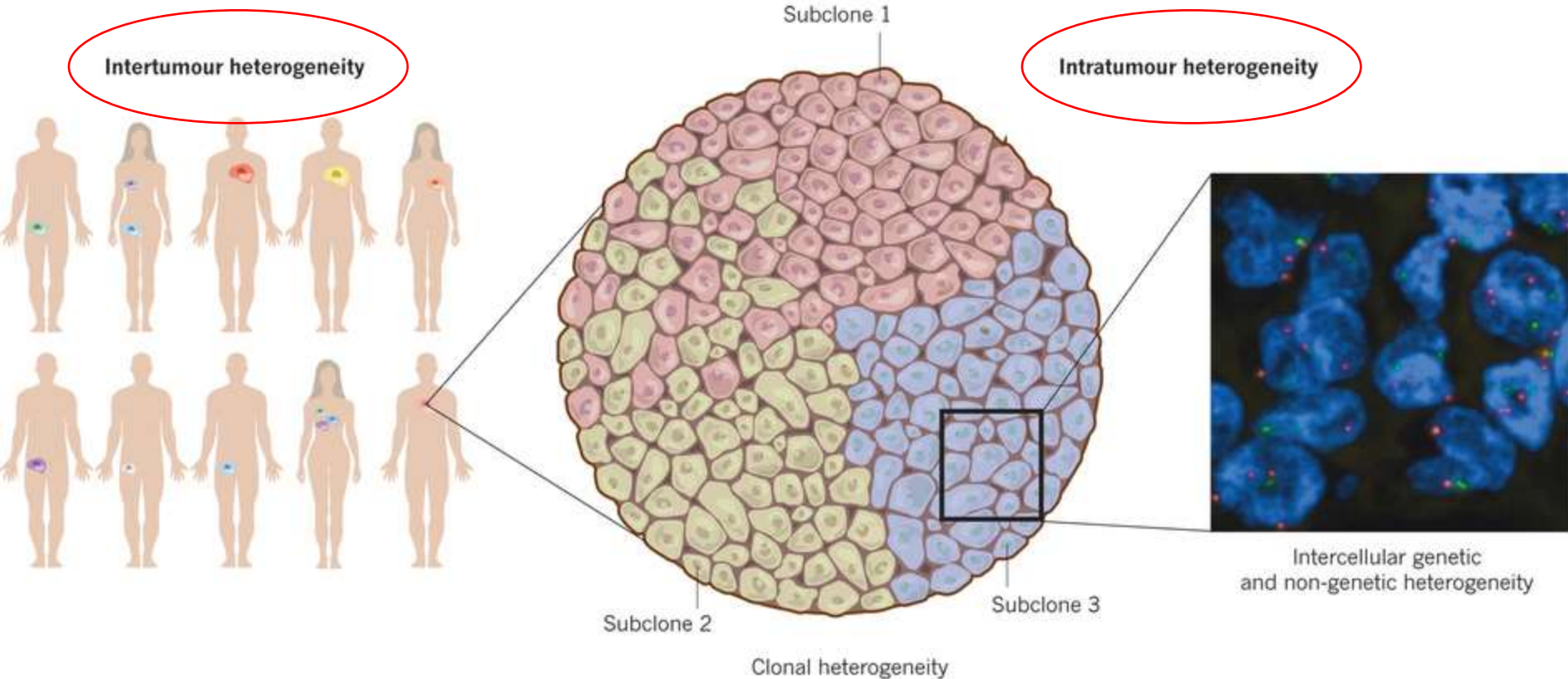
Cortesia: Dott.ssa Paola Ulivi (IRST Meldola)

# Suggested diagnostic flow-chart May 2016

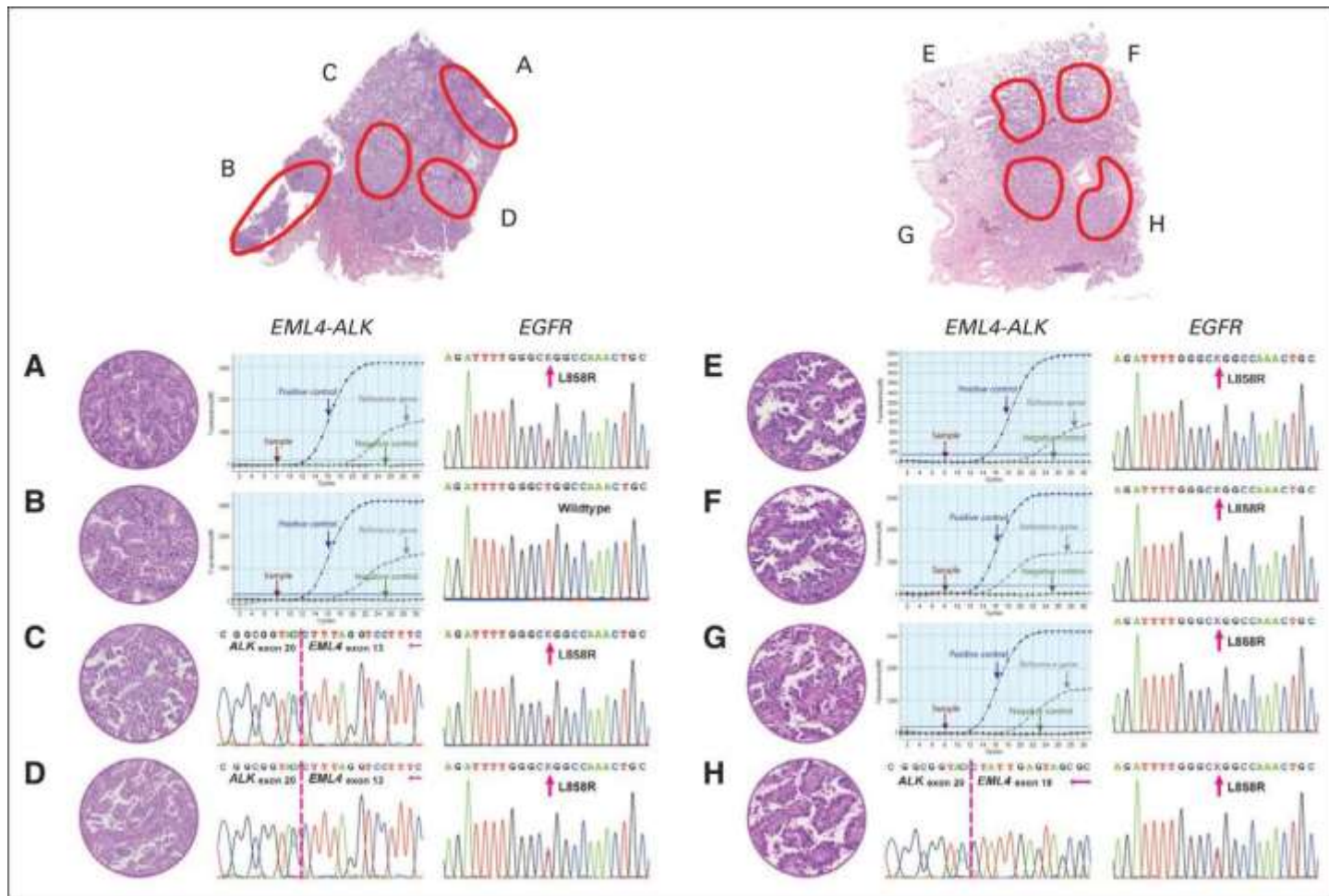


Courtesy: Prof. Fiorentino (S. Orsola – Bologna)

# NSCLS is a paradigm of tumor heterogeneity



# Pathologic and genetic characteristics of two patients with ALK/EGFR coaltered adenocarcinoma by reverse-transcriptase polymerase chain reaction and amplification refractory mutation system assays.

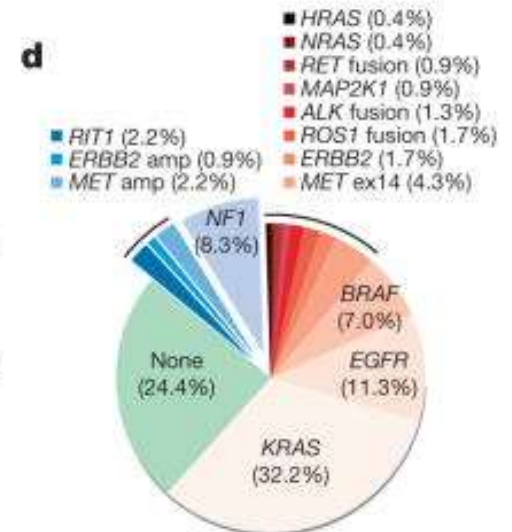
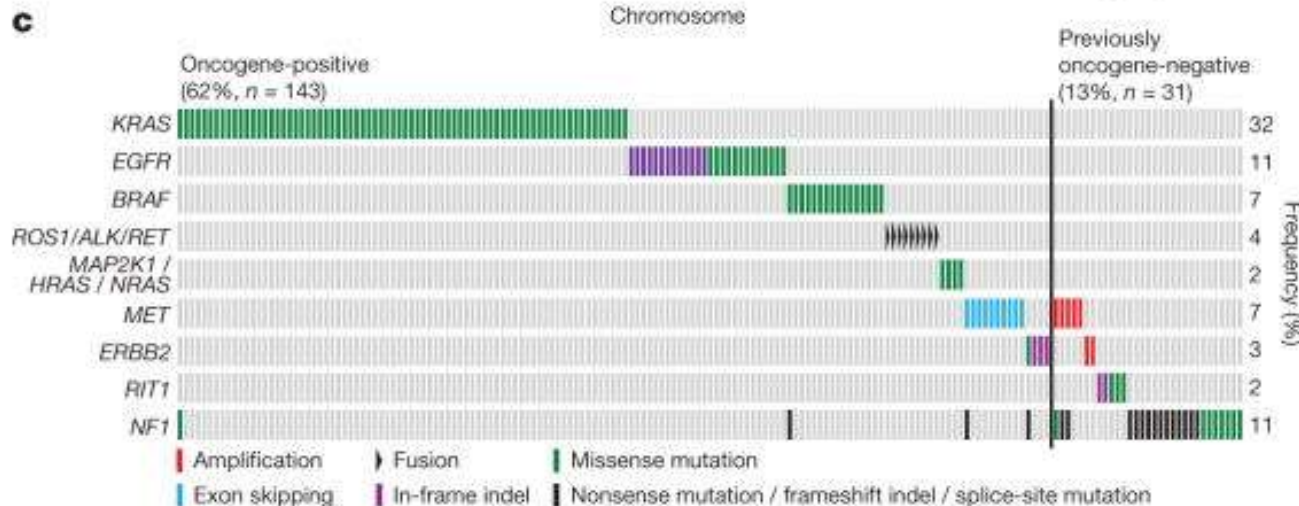
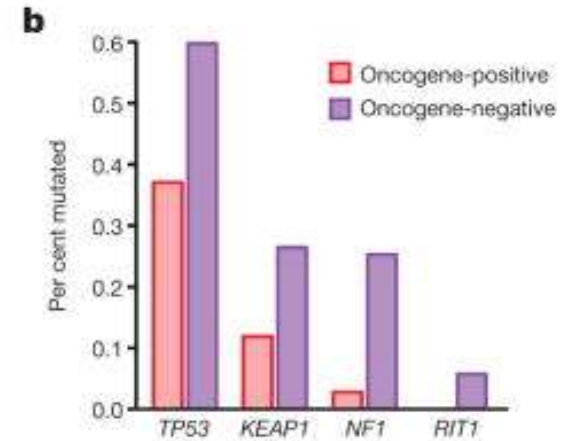
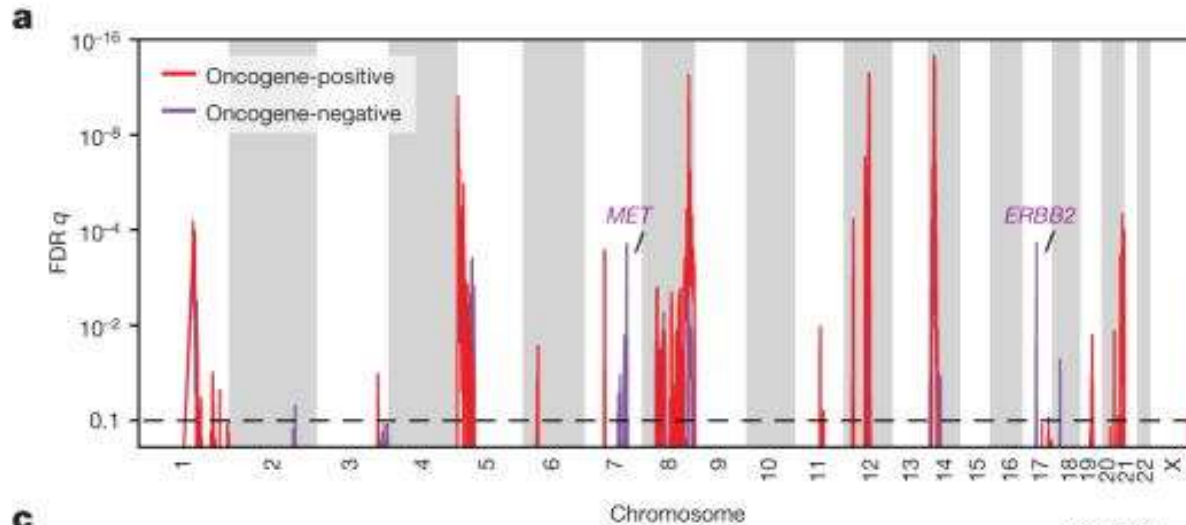


Weijing Cai et al. JCO 2015;33:3701-3709

# The dogma of mutual exclusivity of mutations

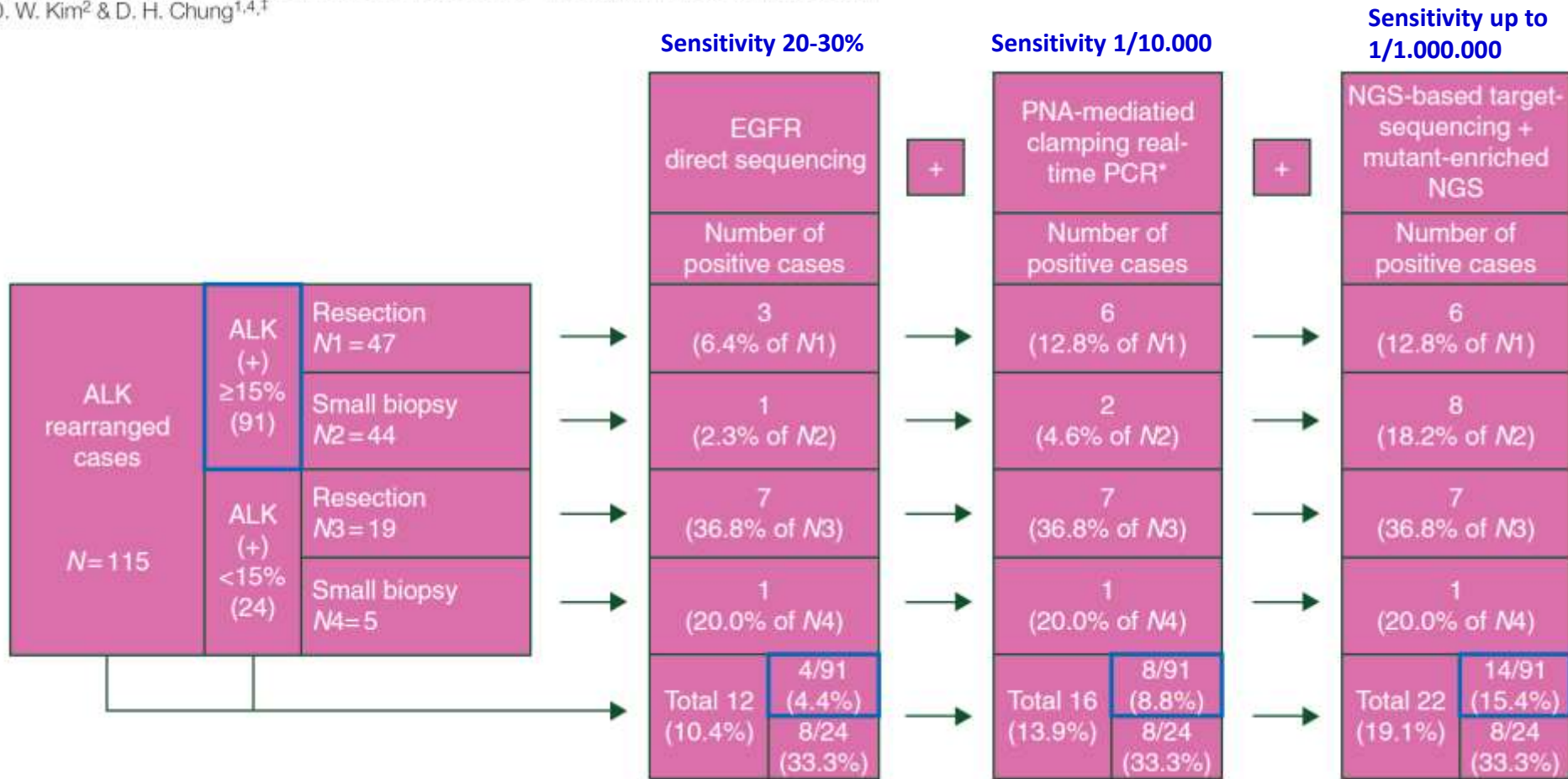
## Comprehensive molecular profiling of lung adenocarcinoma

The Cancer Genome Atlas Research Network\*



# Concomitant **ALK** translocation and **EGFR** mutation in lung cancer: a comparison of direct sequencing and sensitive assays and the impact on responsiveness to tyrosine kinase inhibitor

J. K. Won<sup>1,†</sup>, B. Keam<sup>2,†</sup>, J. Koh<sup>1</sup>, H. J. Cho<sup>1</sup>, Y. K. Jeon<sup>1,†\*</sup>, T. M. Kim<sup>2</sup>, S. H. Lee<sup>2</sup>, D. S. Lee<sup>3</sup>, D. W. Kim<sup>2</sup> & D. H. Chung<sup>1,4,†</sup>



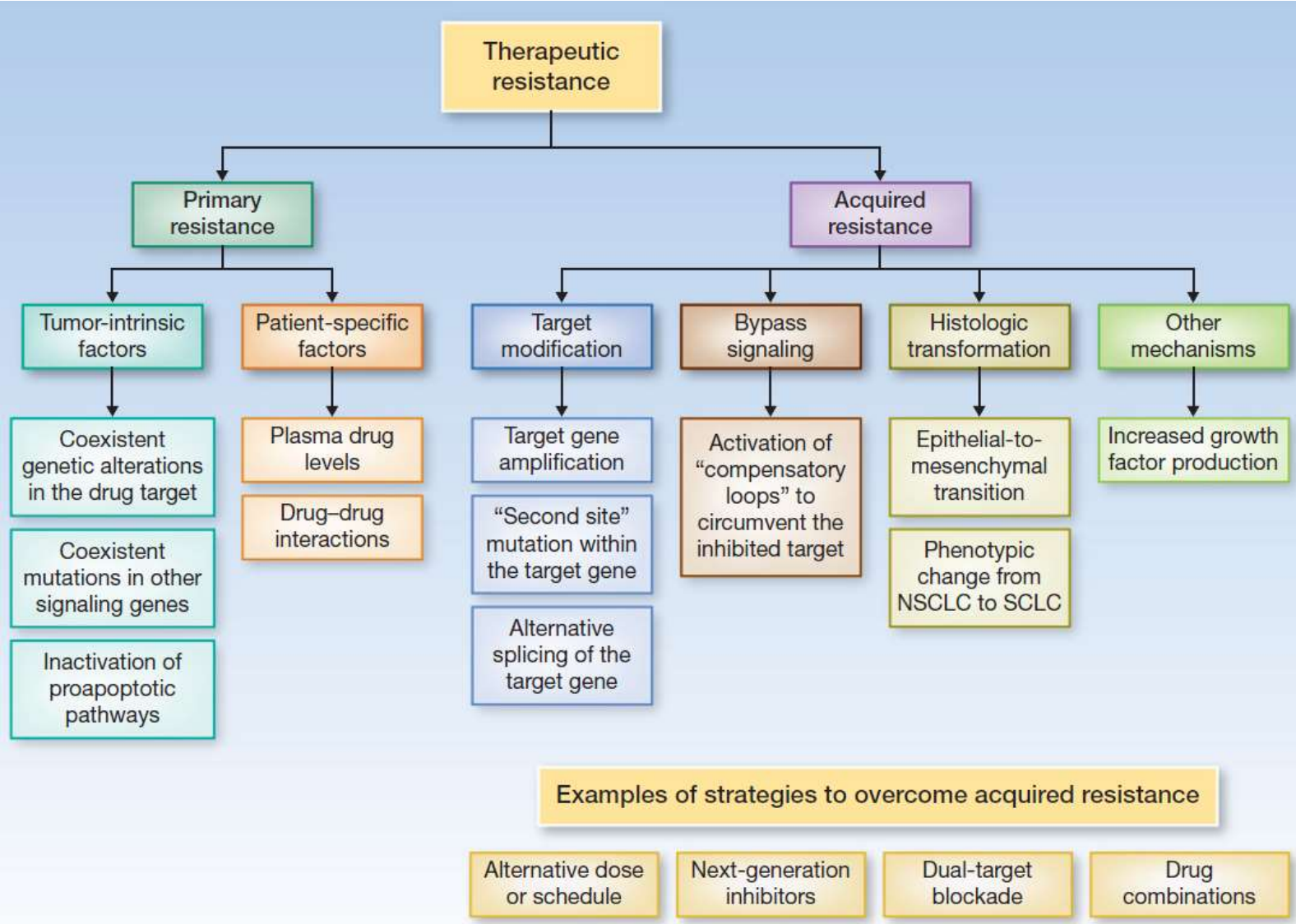
**DOES IT MAKE CLINICAL SENSE TO ASSESS MUTATION IN 1/1,000,000 TUMOR CELLS?**

# Molecular Pathways: Resistance to Kinase Inhibitors and Implications for Therapeutic Strategies



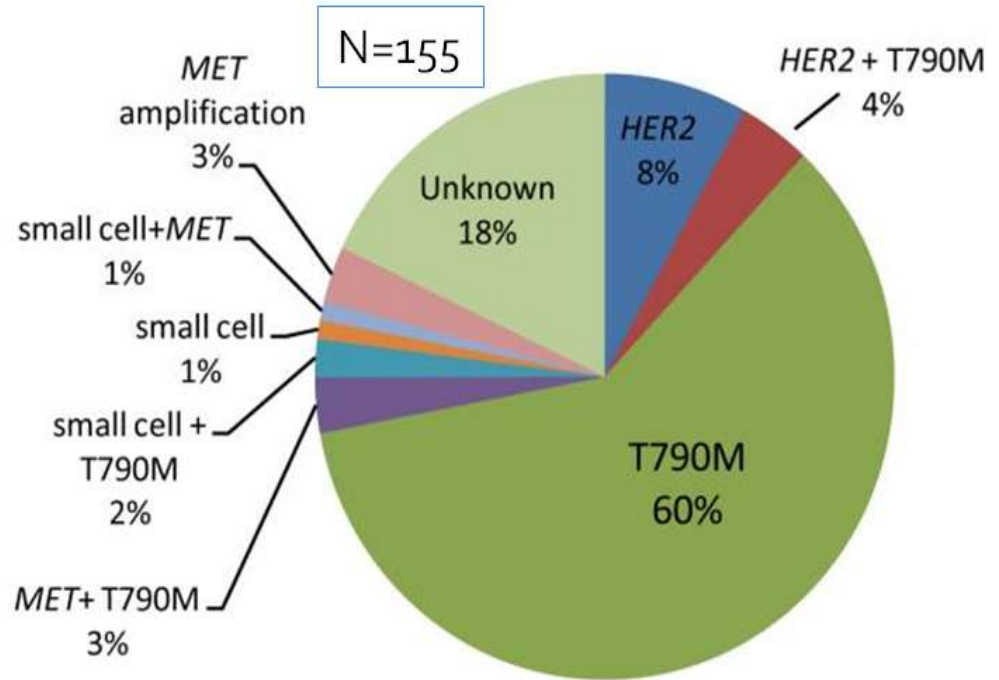
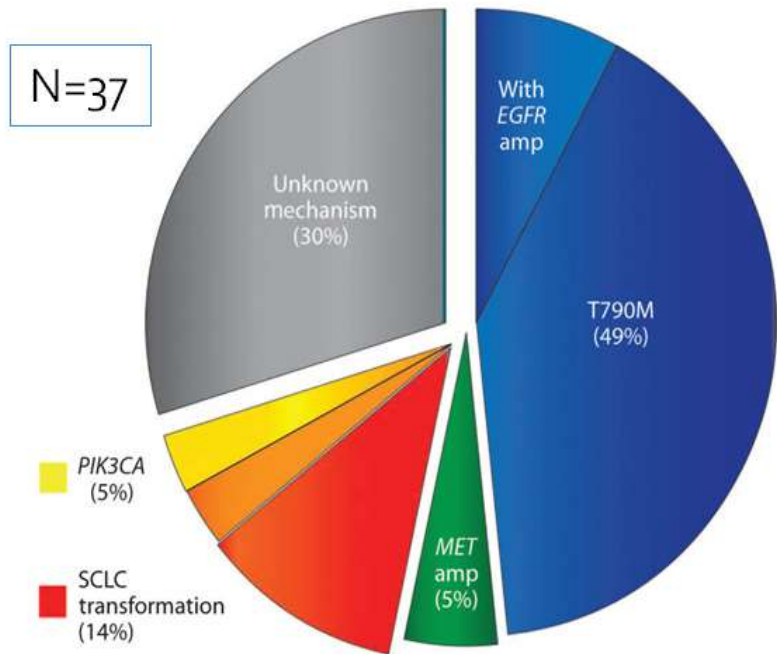
Christine M. Lovly<sup>1</sup> and Alice T. Shaw<sup>2</sup>

*Clin Cancer Res* 2014;20:2249-2256.





# EGFR TKI AR pie charts prior to the clinical NGS era



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

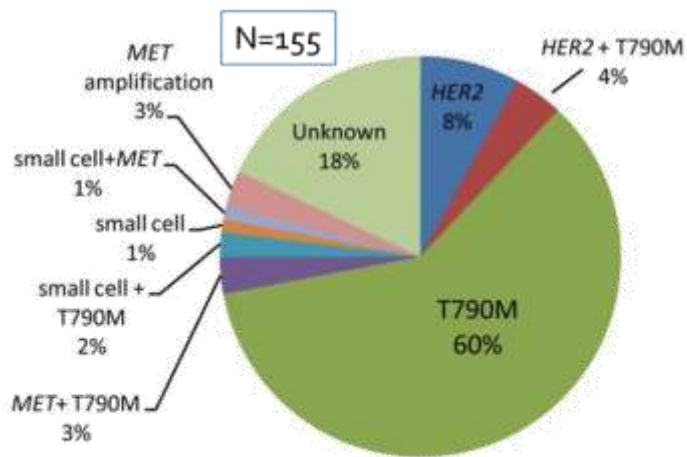
*Science Transl Med* March 2011

Analysis of Tumor Specimens at the Time of Acquired Resistance to EGFR-TKI Therapy in 155 Patients with EGFR-Mutant Lung Cancers

*Clinical Cancer Res* March 2013

# 38 Paired samples (pre-post EGFR TKI): large panel NGS reveals richer landscape of AR mechanisms

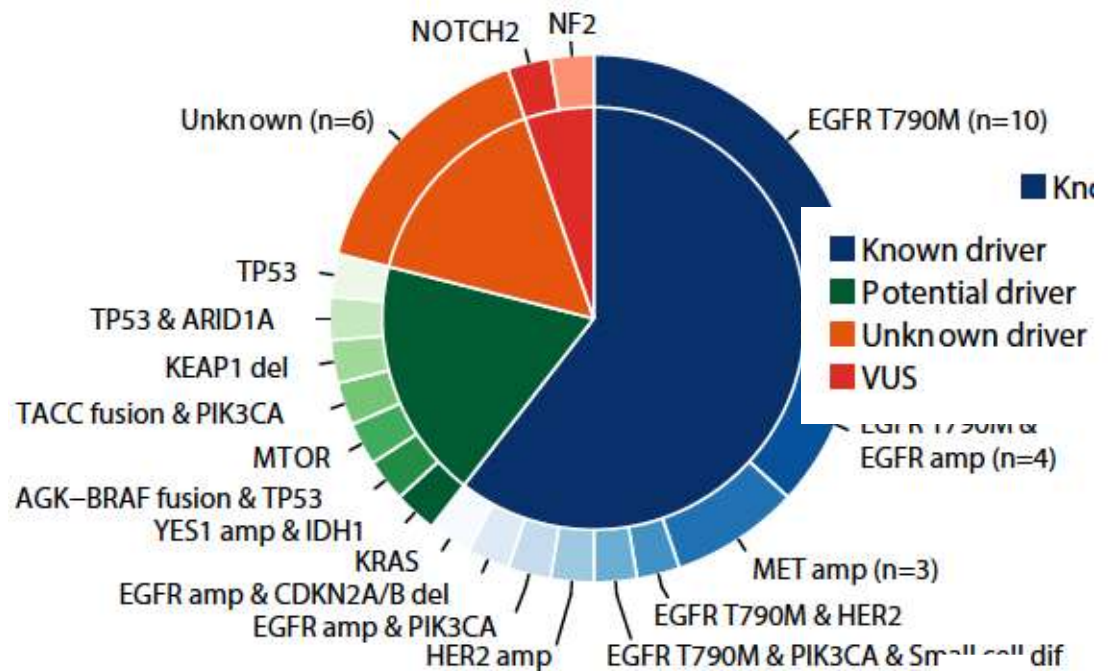
Pre-NGS pie chart → Large panel NGS pie chart:



Analysis of Tumor Specimens at the Time of Acquired Resistance to EGFR-TKI Therapy in 155 Patients with EGFR-Mutant Lung Cancers

*Clinical Cancer Res* March 2013

## Acquired alterations



Helena Yu et al. *CCR* Mar 2018

Tissue Sample

DNA Library Prep

Sequencing

Data

**Current:**

**Illumina HiSeq 2000**



300 – 600 Gigabases  
6 – 11 days

**Illumina MiSeq**



1.5 Gigabases  
1 day

**Ion Torrent PGM**



1 Gigabase  
6 hours

# Which (New) Genes Should Be Tested for Lung Cancer Patients?

**3 categories**

One set as an **absolute minimum**

- EGFR, ALK & ROS1, PD-L1

**A second expanded panel:**

- BRAF, MET, RET, HER2, and KRAS, if adequate material is available

**All other genes are investigational**

# What Is the Role of Testing for Circulating cfDNA for Lung Cancer Patients?

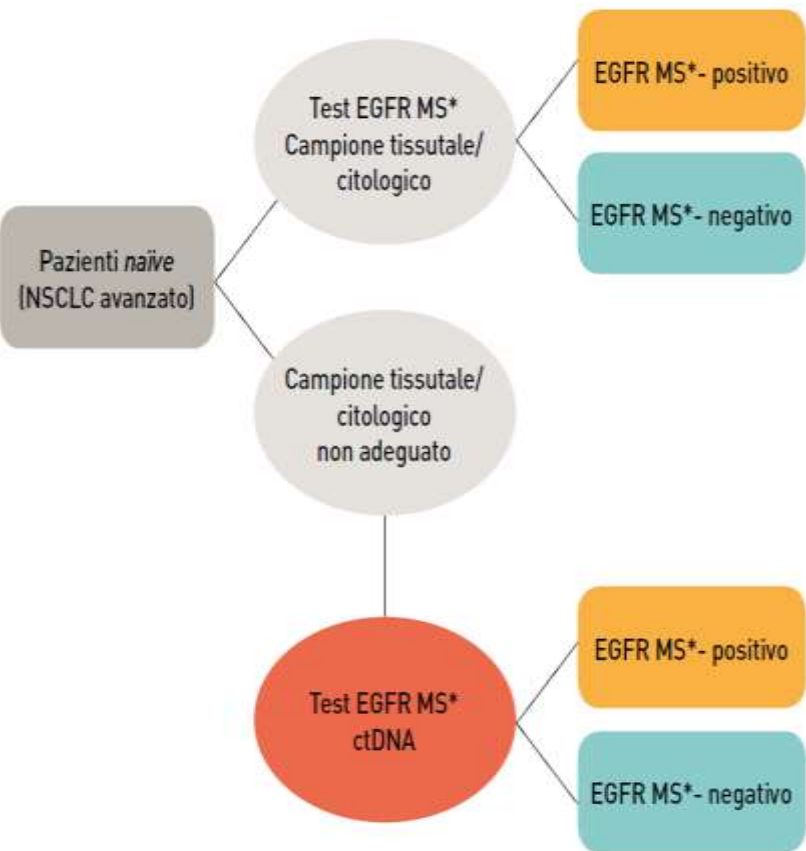
There is currently **insufficient evidence to support** the use of circulating plasma **cfDNA molecular methods for establishing a primary diagnosis** of lung adenocarcinoma.

In some clinical settings in **which tissue is limited and/or insufficient for molecular testing**, physicians may use a **cfDNA** assay to identify EGFR mutations.

Physicians may use plasma **cfDNA methods to identify EGFR T790M mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted TKIs;**

testing of the tumor sample is recommended if the plasma result is negative.

# EGFR



\* *EGFR, ALK, ROS1, and BRAF* at minimum, but a panel if available

# Strongly suggest tissue sparing to facilitate participation in clinical trials

+ While NGS is preferred, based on availability, other validated assays are acceptable

## Patient with advanced treatment naive NSCLC

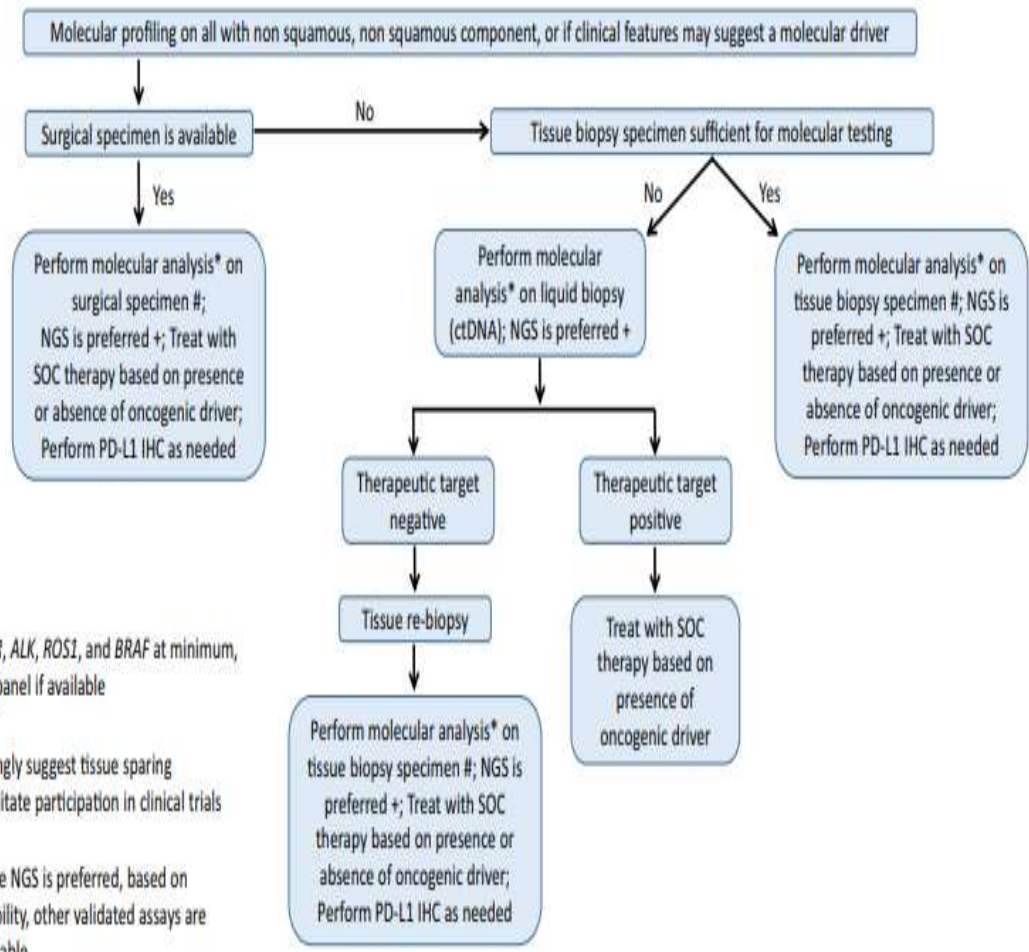


FIGURA 1. Algoritmo del test EGFR in pazienti naive con NSCLC avanzato

**Raccomandazioni AIOM-SIF-SIAPEC 2018**

**Rolfo C et al, J Thor Oncol 2018**

*Natasha Rekhtman, MD, PhD*

The goal of “**no mutation left behind**” must be balanced against the practical realities of resource utilization in the setting of a low probability event. Furthermore, it should be noted that even in resections, there is a possibility that a **biologically combined carcinoma** may be vastly dominated by a single histology suggesting that even resections showing squamous or small cell histology in **younger or never-/light-smokers** should be considered for EGFR/ALK/ROS1 testing

Increasing use of **multigene panels**, especially NGS, encompassing a full set of “**pan-tumor**” genes in a single assay, one can anticipate a **decreasing role of histology-based molecular test selection in the future**

# Summary – Predictive Biomarkers NSCLC

	Tissue	Plasma	Method	Other techno	Approval
<b>EGFR</b>	+++	++ (T790M)	PCR	<b>NGS</b>	Yes
<b>ALK</b>	+++	-	IHC>FISH	<b>NGS</b> Nanostring	Yes
<b>ROS1</b>	+++	-	FISH>IHC	<b>NGS</b> Nanostring	Yes
<b>PD-L1</b>	+++	-	IHC	TMB/ <b>NGS</b>	Yes
<b>BRAF</b>	+++	-	PCR>IHC	<b>NGS</b>	Next
<b>RET</b>	+++	-	FISH	<b>NGS</b> Nanostring	Next
<b>c-MET ex14 skip</b>	+++	-	PCR	<b>NGS</b>	Next



Algorithm for Predictive Biomarkers in **Advanced**  
**(stage IIIB/IV) NSCLC** Routine Practice

**NSCLC**  
**(+/- diagnostic immunostains)**

Concurrently

TISSUE

LIQUID

Mutations

Gene fusions/amplifications

TMB

**NGS**