Molecular Testing for Clinical Practice

Prof Keith M Kerr
Department of Pathology
Aberdeen University Medical School
Aberdeen Royal Infirmary
Foresterhill, Aberdeen, UK

Disclosures

- I have acted as Consultant to and/or given sponsored lectures for
- Astra Zeneca, Roche/Genetech, Eli Lilly, Pfizer, Novartis, Boehringer Ingelheim, Glaxo Smith Klein, Merck Serono, Abbott Diagnostics

REVIEW ARTICLE

The pivotal role of pathology in the management of lung cancer

Morgan R. Davidson[1,2], Adi F. Gazdar[3,4], Belinda E. Clarke[1,5]

Revolution in Lung Cancer

New Challenges for the Surgical Pathologist

Philip T. Cagle, MD; Timothy C. Allen, MD, JD; Sanja Dacic, MD, PhD; Mary Beth Beasley, MD; Alain C. Borczuk, MD; Lucian R. Chirieac, MD; Rodolfo Laucirica, MD; Jae Y. Ro, MD, PhD; Keith M. Kerr, MD

Targ Oncol (2013) 8:1–2
DOI 10.1007/s11523-013-0265-x

EDITORIAL

Lung cancer: how to face the revolution?

Jean-François Morère · Frédérique Penault-Llorca

Histopathology 2012, **60**, 531–546. DOI: 10.1111/j.1365-2559.2011.03854.x

REVIEW

Personalized medicine for lung cancer: new challenges for pathology

Keith M Kerr

REVIEW ARTICLE

The pivotal role of pathology in the management of lung cancer

Morgan R. Davidson[1,2], Adi F. Gazdar[3,4], Belinda E. Clarke[1,5]

Revolution in Lung Cancer

New Challenges for the Surgical Pathologist

Philip T. Cagle, MD; Timothy C. Allen, MD, JD; Sanja Dacic, MD, PhD; Mary Beth Beasley, MD; Alain C. Borczuk, MD; Lucian R. Chirieac, MD; Rodolfo Laucirica, MD; Jae Y. Ro, MD, PhD; Keith M. Kerr, MD

Targ Oncol (2013) 8:1–2
DOI 10.1007/s11523-013-0265-x

EDITORIAL

Lung cancer: how to face the revolution?

Jean-François Morère · Frédérique Penault-Llorca

Histopathology 2012, **60**, 531–546. DOI: 10.1111/j.1365-2559.2011.03854.x

REVIEW

Personalized medicine for lung cancer: new challenges for pathology

Keith M Kerr

REVIEW ARTICLE

The pivotal role of pathology in the management of lung cancer

Morgan R. Davidson[1,2], Adi F. Gazdar[3,4], Belinda E. Clarke[1,5]

Revolution in Lung Cancer

New Challenges for the Surgical Pathologist

Philip T. Cagle, MD; Timothy C. Allen, MD, JD; Sanja Dacic, MD, PhD; Mary Beth Beasley, MD; Alain C. Borczuk, MD; Lucian R. Chirieac, MD; Rodolfo Laucirica, MD; Jae Y. Ro, MD, PhD; Keith M. Kerr, MD

Targ Oncol (2013) 8:1–2
DOI 10.1007/s11523-013-0265-x

EDITORIAL

Lung cancer: how to face the revolution?

Jean-François Morère · Frédérique Penault-Llorca

Histopathology 2012, **60**, 531–546. DOI: 10.1111/j.1365-2559.2011.03854.x

REVIEW

Personalized medicine for lung cancer: new challenges for pathology

Keith M Kerr

REVIEW ARTICLE

The pivotal role of pathology in the management of lung cancer

Morgan R. Davidson[1,2], Adi F. Gazdar[3,4], Belinda E. Clarke[1,5]

Revolution in Lung Cancer

New Challenges for the Surgical Pathologist

Philip T. Cagle, MD; Timothy C. Allen, MD, JD; Sanja Dacic, MD, PhD; Mary Beth Beasley, MD; Alain C. Borczuk, MD; Lucian R. Chirieac, MD; Rodolfo Laucirica, MD; Jae Y. Ro, MD, PhD; Keith M. Kerr, MD

Targ Oncol (2013) 8:1–2
DOI 10.1007/s11523-013-0265-x

EDITORIAL

Lung cancer: how to face the revolution?

Jean-François Morère · Frédérique Penault-Llorca

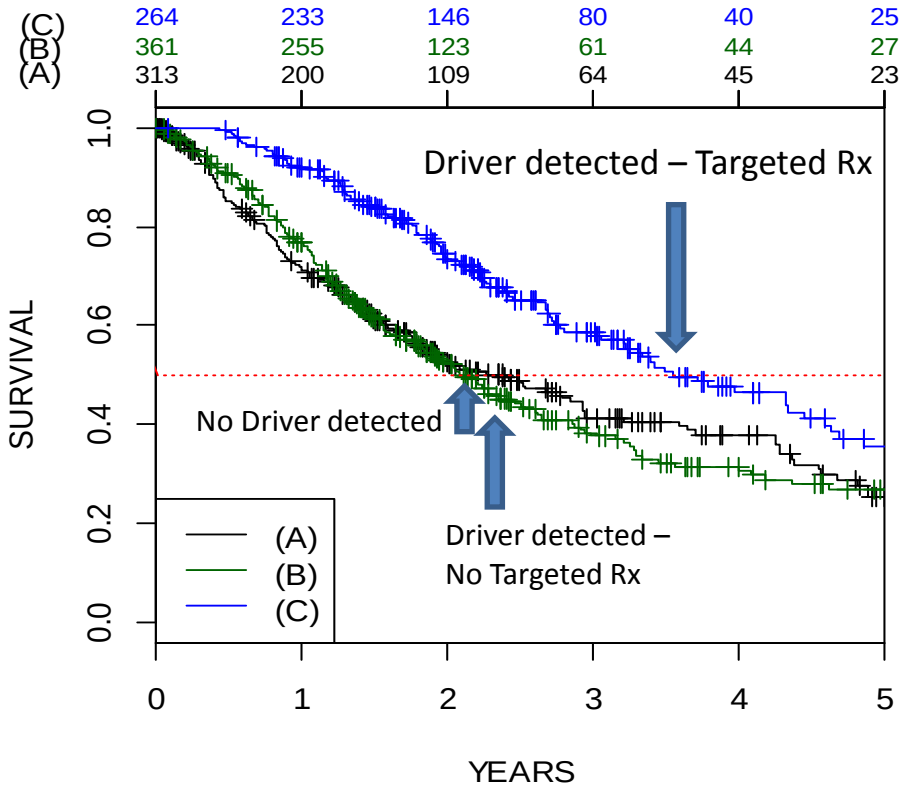
Histopathology 2012, **60**, 531–546. DOI: 10.1111/j.1365-2559.2011.03854.x

REVIEW

Personalized medicine for lung cancer: new challenges for pathology

Keith M Kerr

It is worthwhile finding an actionable genetic alteration in Lung cancer



PRESIDENTIAL SYMPOSIUM INCLUDING TOP RATED ABSTRACTS
TUESDAY, OCTOBER 29, 2013 - 08:15-09:45 WCLC, Sydney

PL03.07 TREATMENT WITH THERAPIES MATCHED TO ONCOGENIC DRIVERS IMPROVES SURVIVAL IN PATIENTS WITH LUNG CANCERS: RESULTS FROM THE LUNG CANCER MUTATION CONSORTIUM (LCMC)

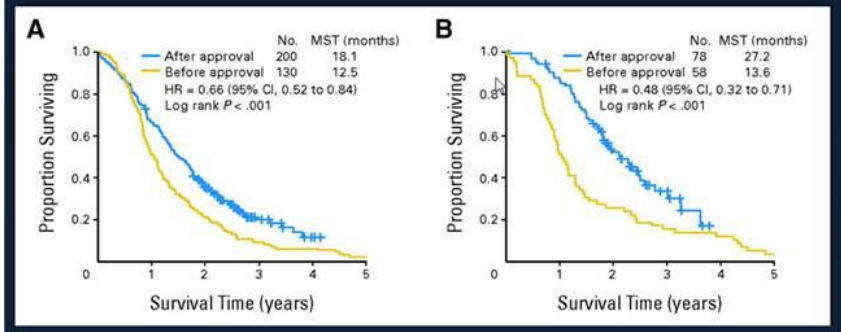
Mark G. Kris[1], Bruce Johnson[2], Lynne Berry[3], David Kwiatkowski[4], et al

Comparison of survival for patients with lung adenocarcinoma in Japan before and after gefitinib approval

EGFR

All patients

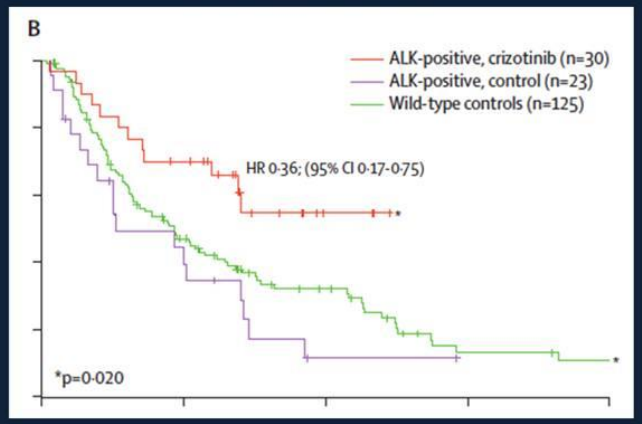
EGFR mut+ patients



Takano, JCO 2008

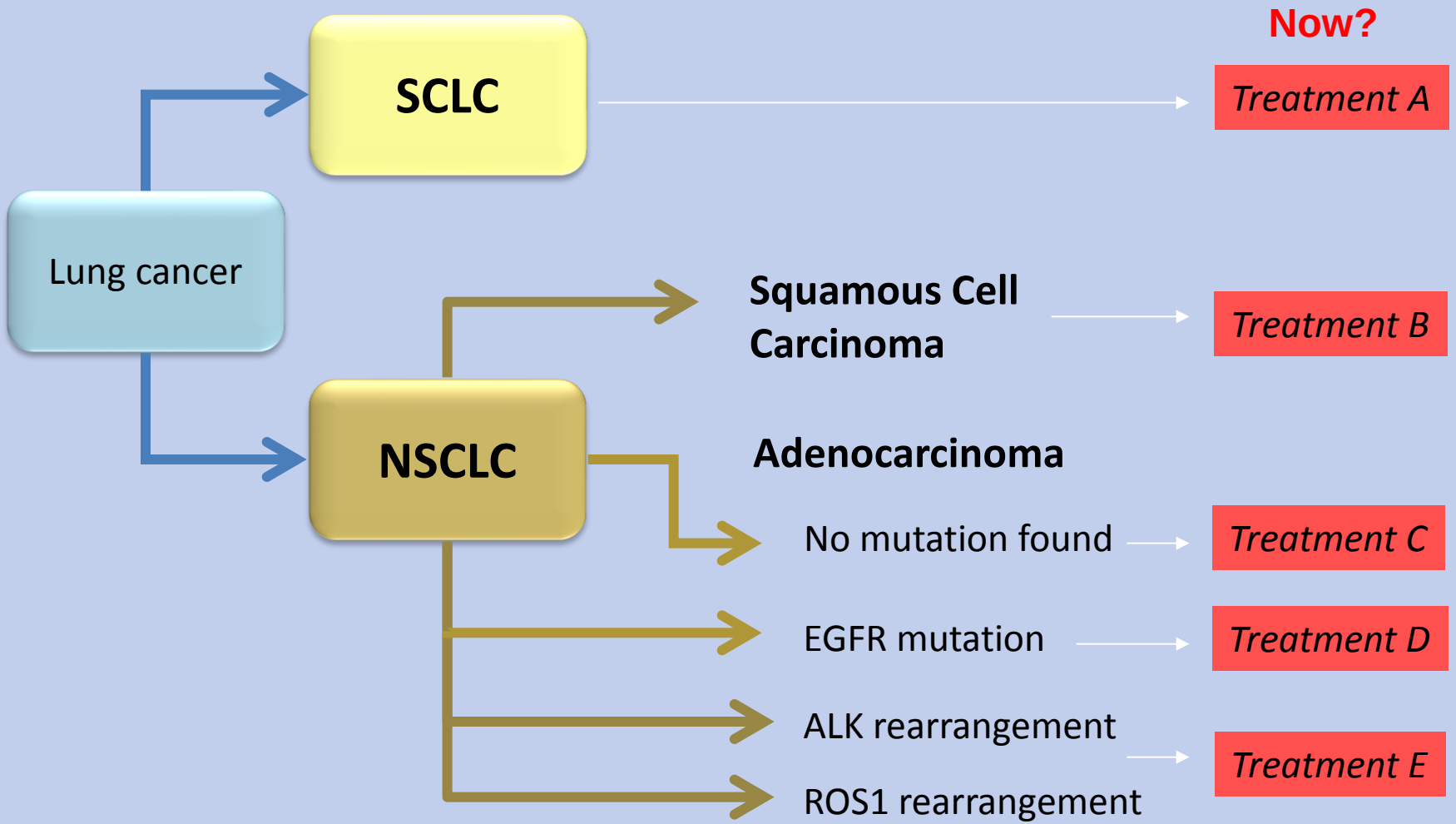
ALK

Comparison of survival for patients with lung adenocarcinoma in second line before and after crizotinib approval

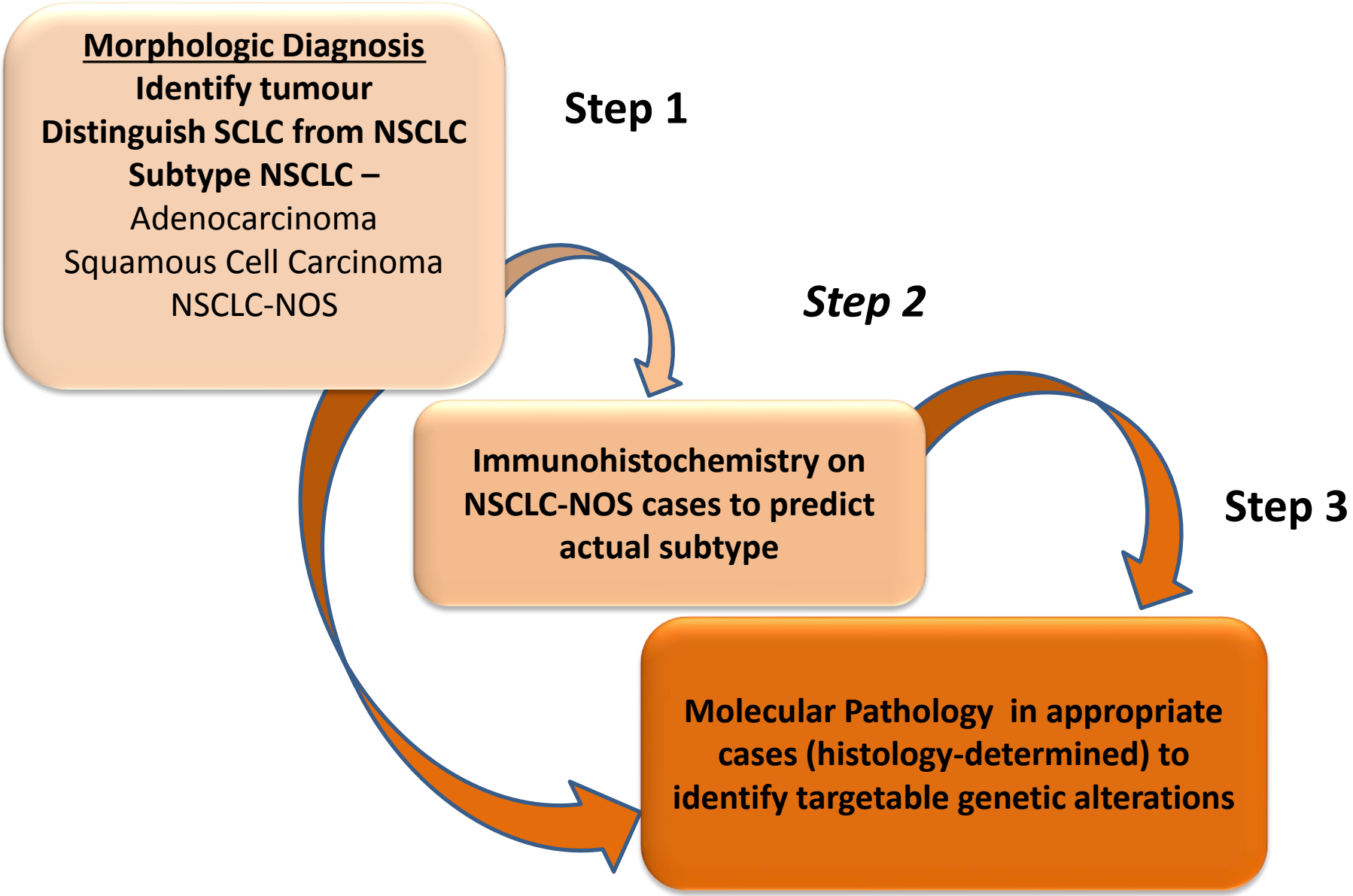


Shaw, Lancet Oncol 2011

Tumour histology and genotype influences treatment in Lung Cancer

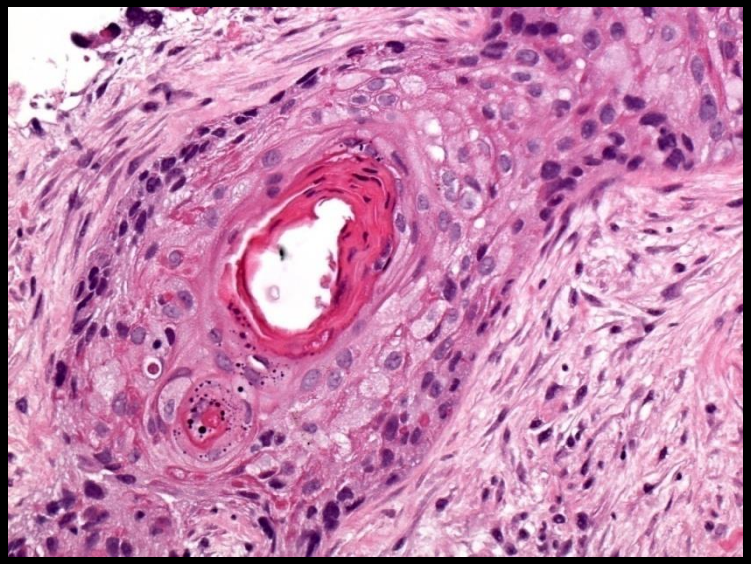


Lung Cancer Diagnosis

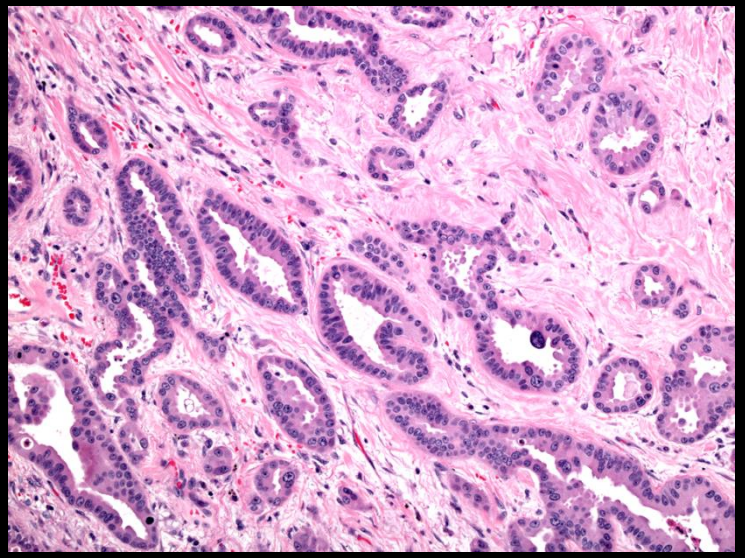


Histological Subtyping of NSCLC: Small sample – biopsy/cytology

Squamous Cell Carcinoma

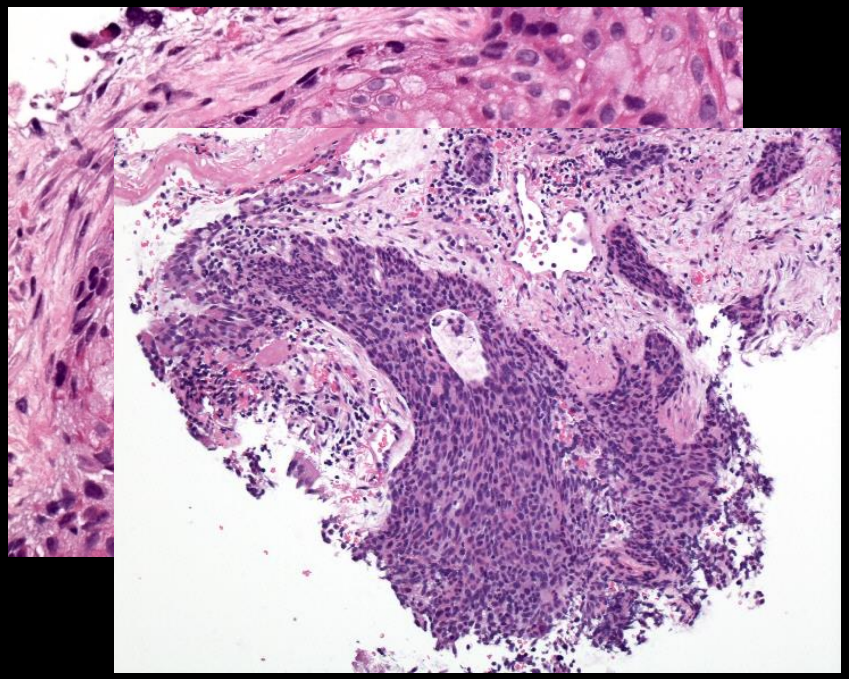


Adenocarcinoma

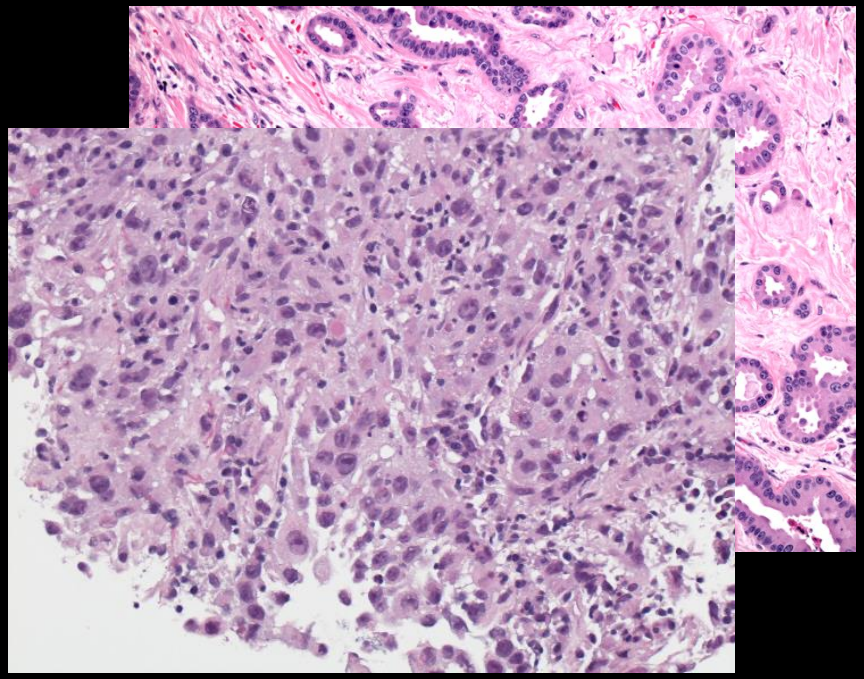


Histological Subtyping of NSCLC: Small sample – biopsy/cytology

Squamous Cell Carcinoma



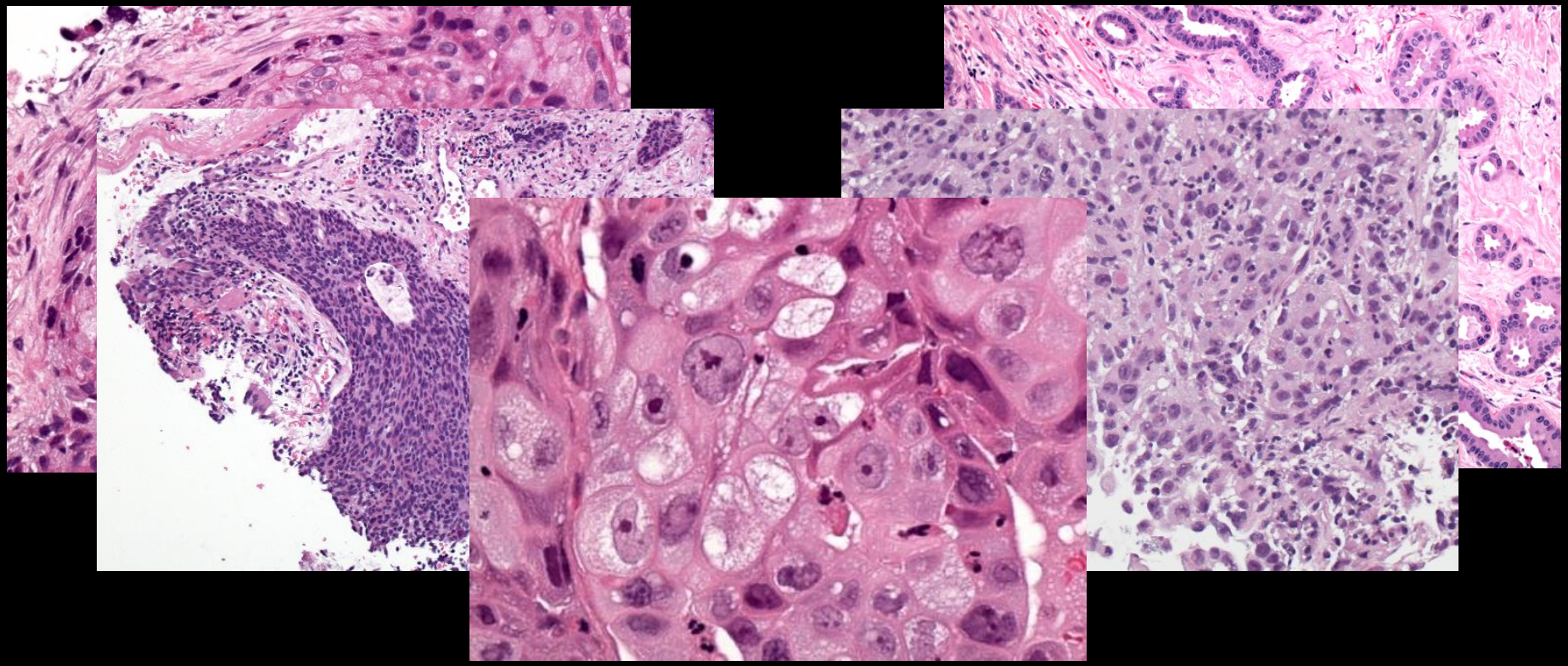
Adenocarcinoma



Histological Subtyping of NSCLC: Small sample – biopsy/cytology

Squamous Cell Carcinoma

Adenocarcinoma

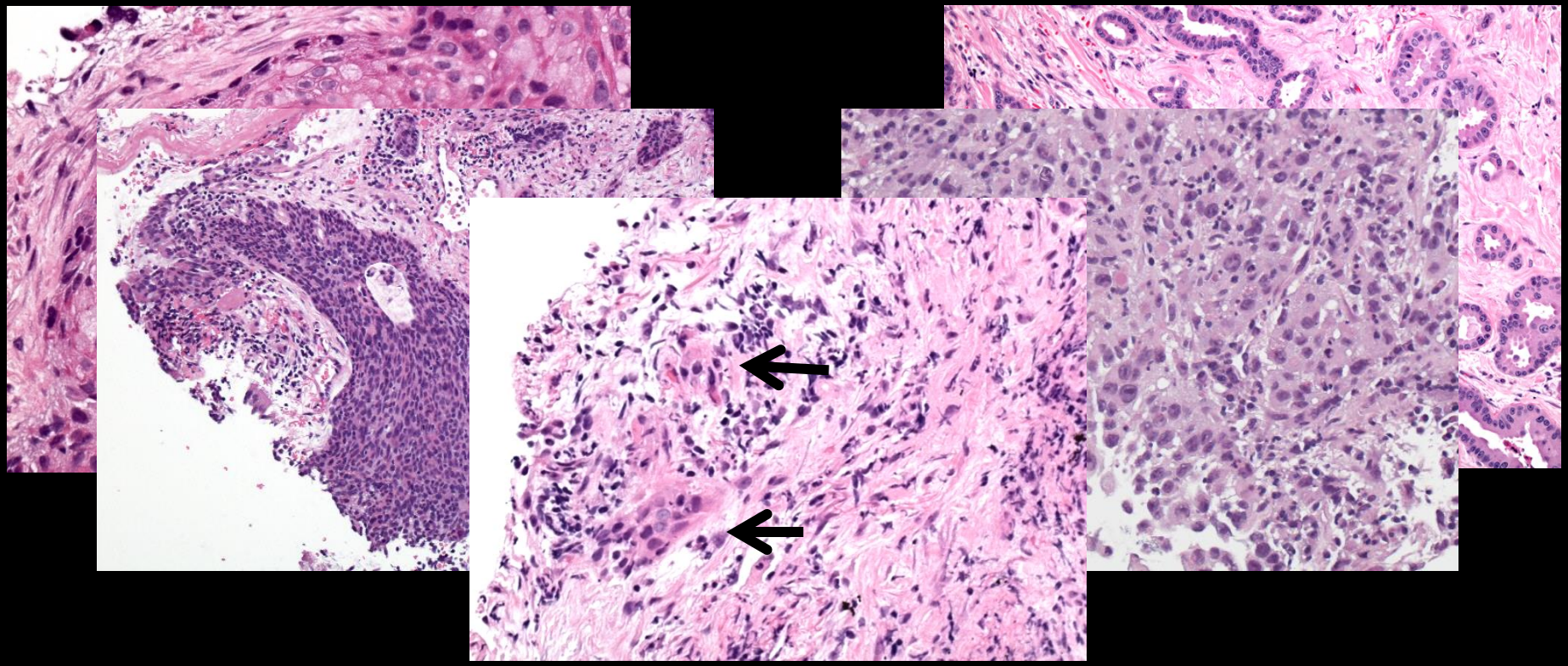


NSCLC-NOS

Histological Subtyping of NSCLC: Small sample – biopsy/cytology

Squamous Cell Carcinoma

Adenocarcinoma

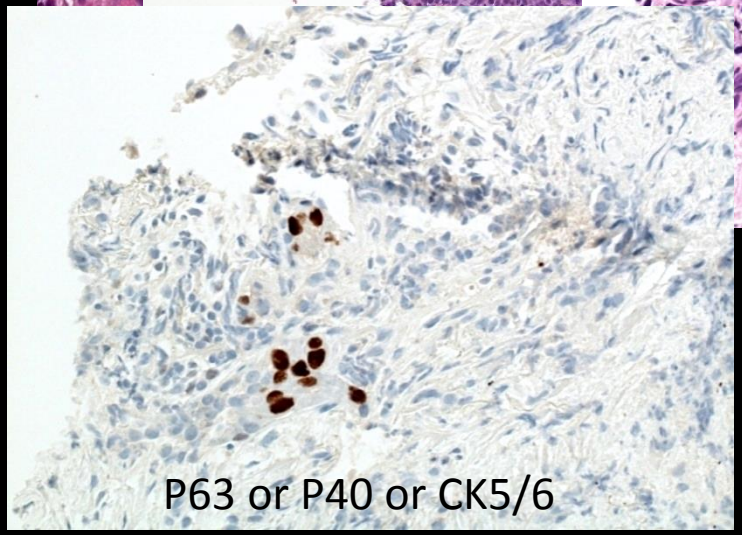


NSCLC-NOS

Histological Subtyping of NSCLC: Small sample – biopsy/cytology

Squamous Cell Carcinoma

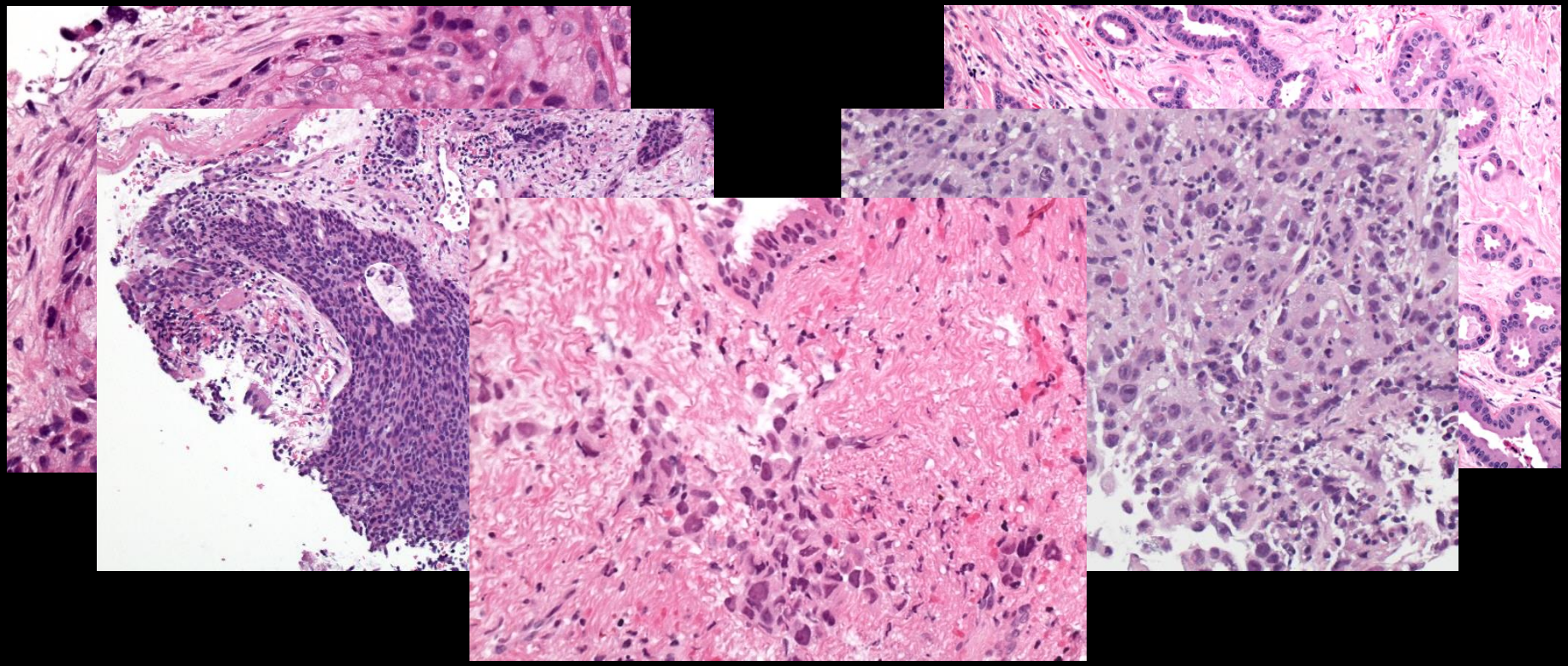
Adenocarcinoma



Histological Subtyping of NSCLC: Small sample – biopsy/cytology

Squamous Cell Carcinoma

Adenocarcinoma

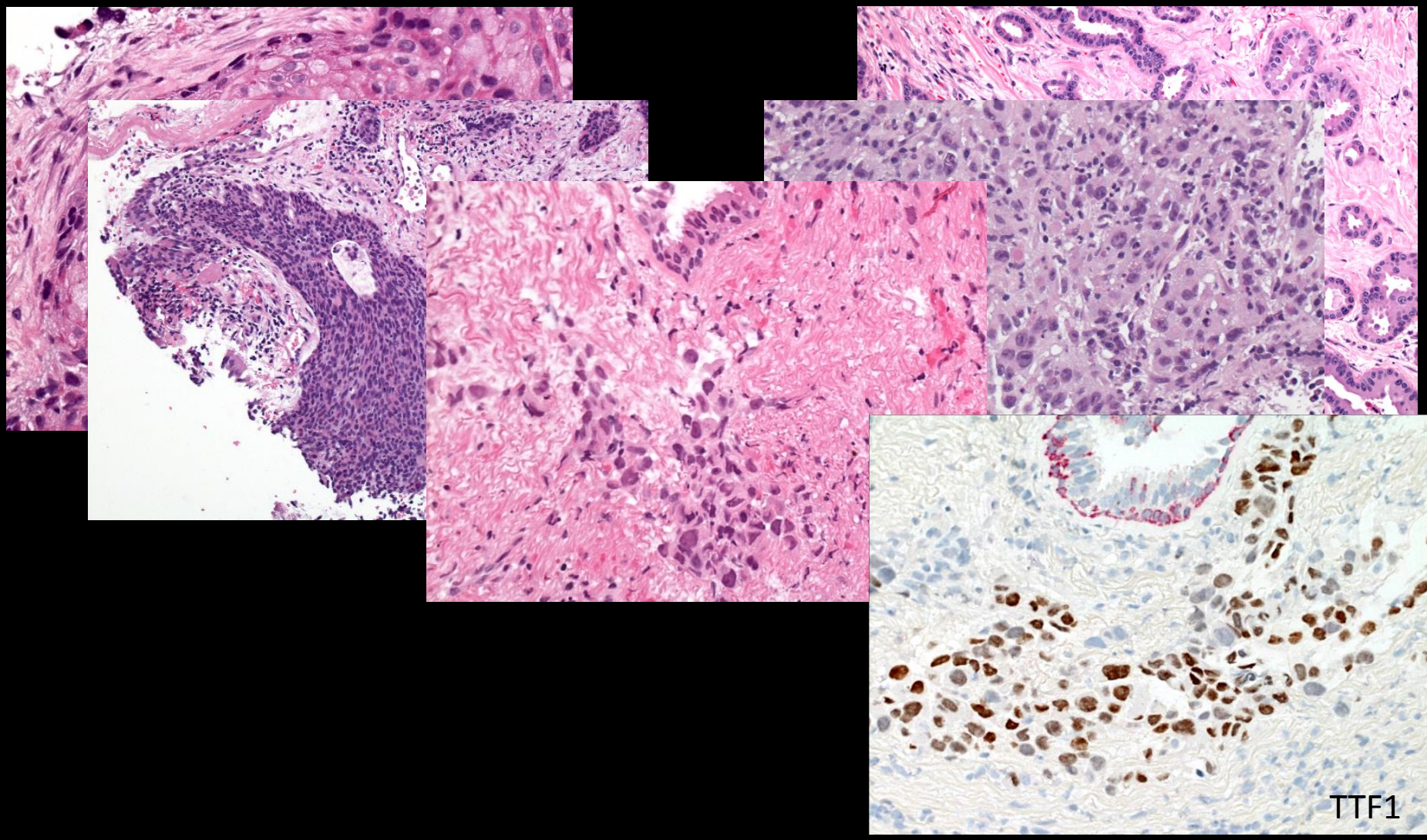


NSCLC-NOS

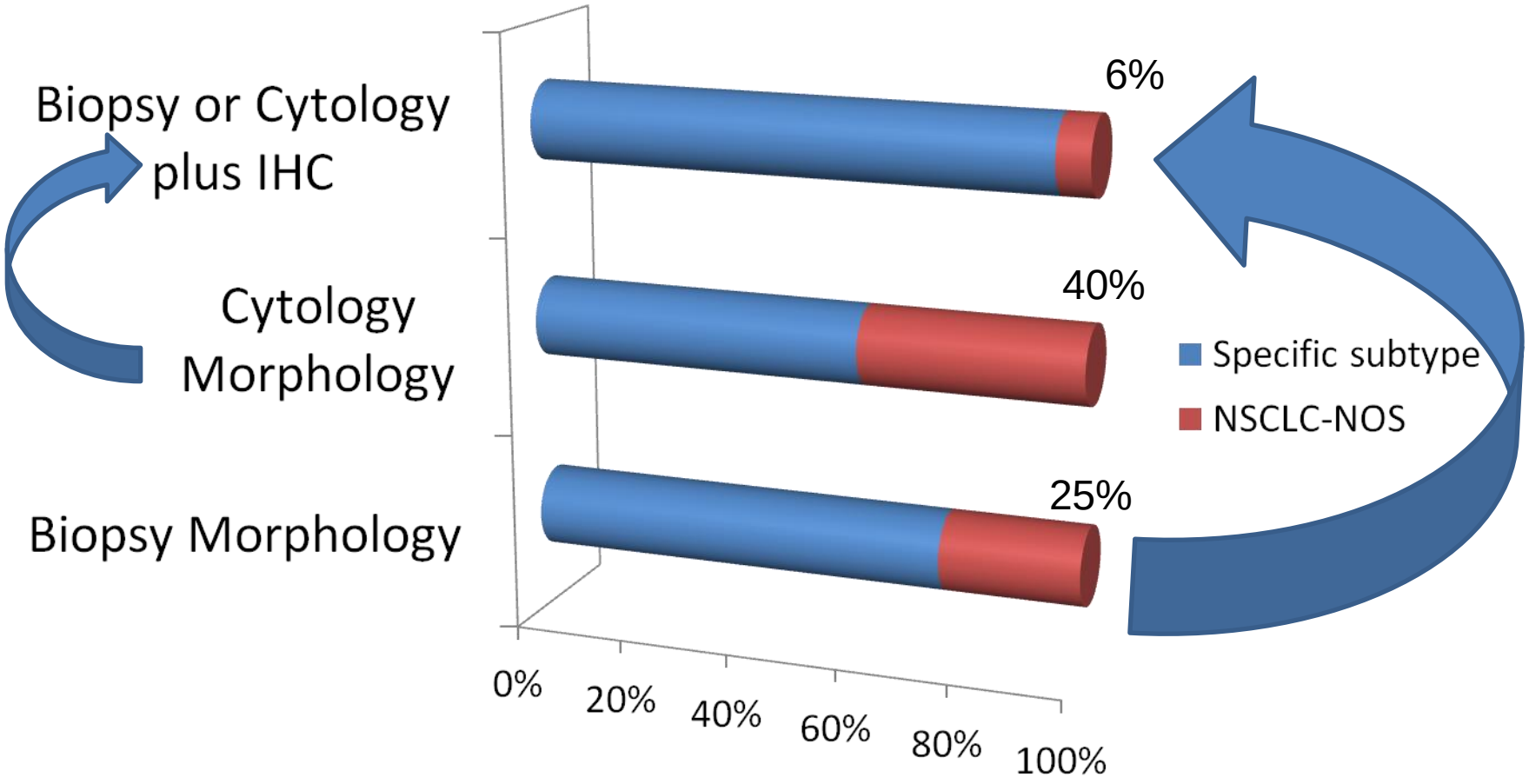
Histological Subtyping of NSCLC: Small sample – biopsy/cytology

Squamous Cell Carcinoma

Adenocarcinoma



Subtyping NSCLC: How good?



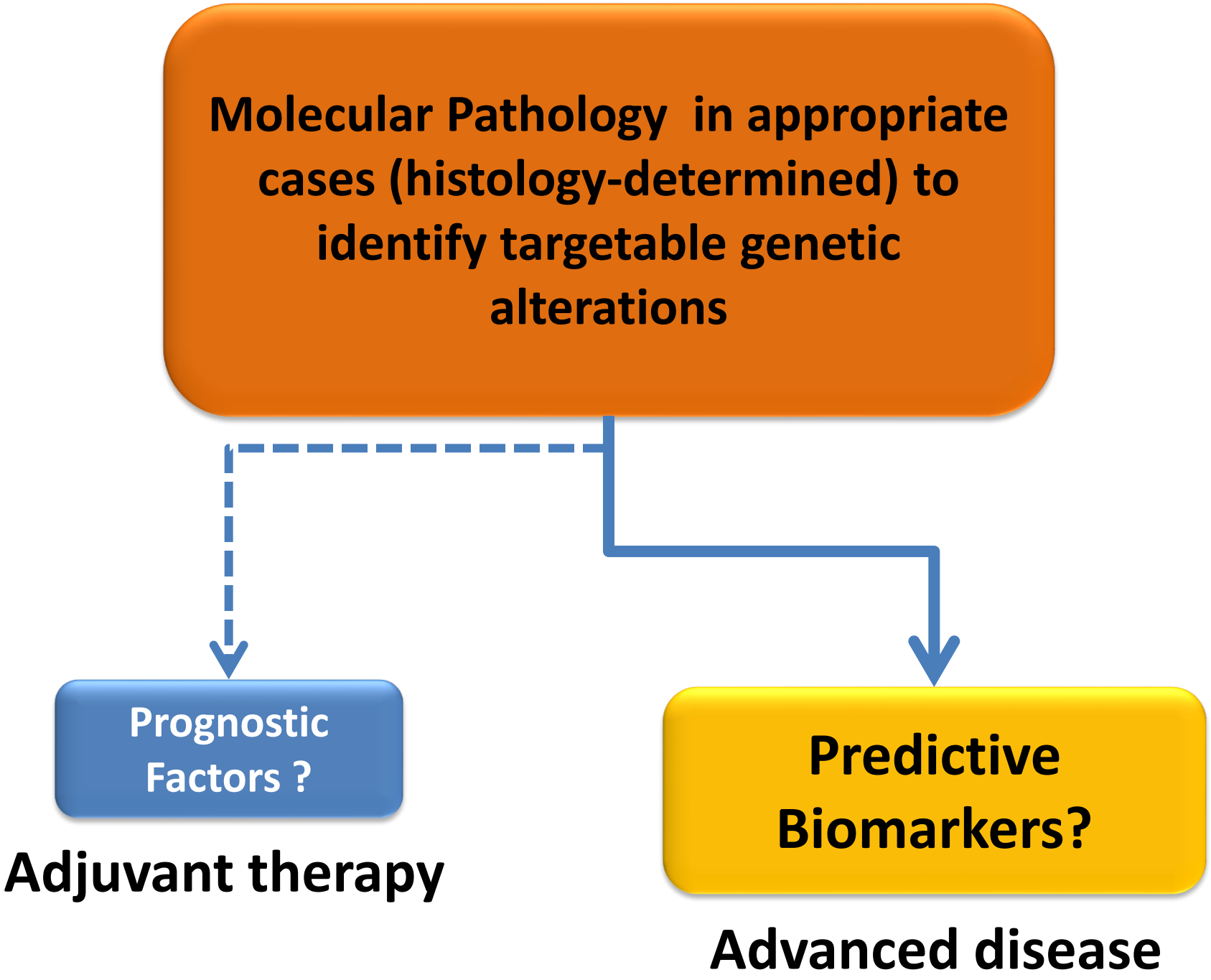
- Predictive IHC has ‘levelled the playing field’
- Better diagnosis possible on poorer specimens

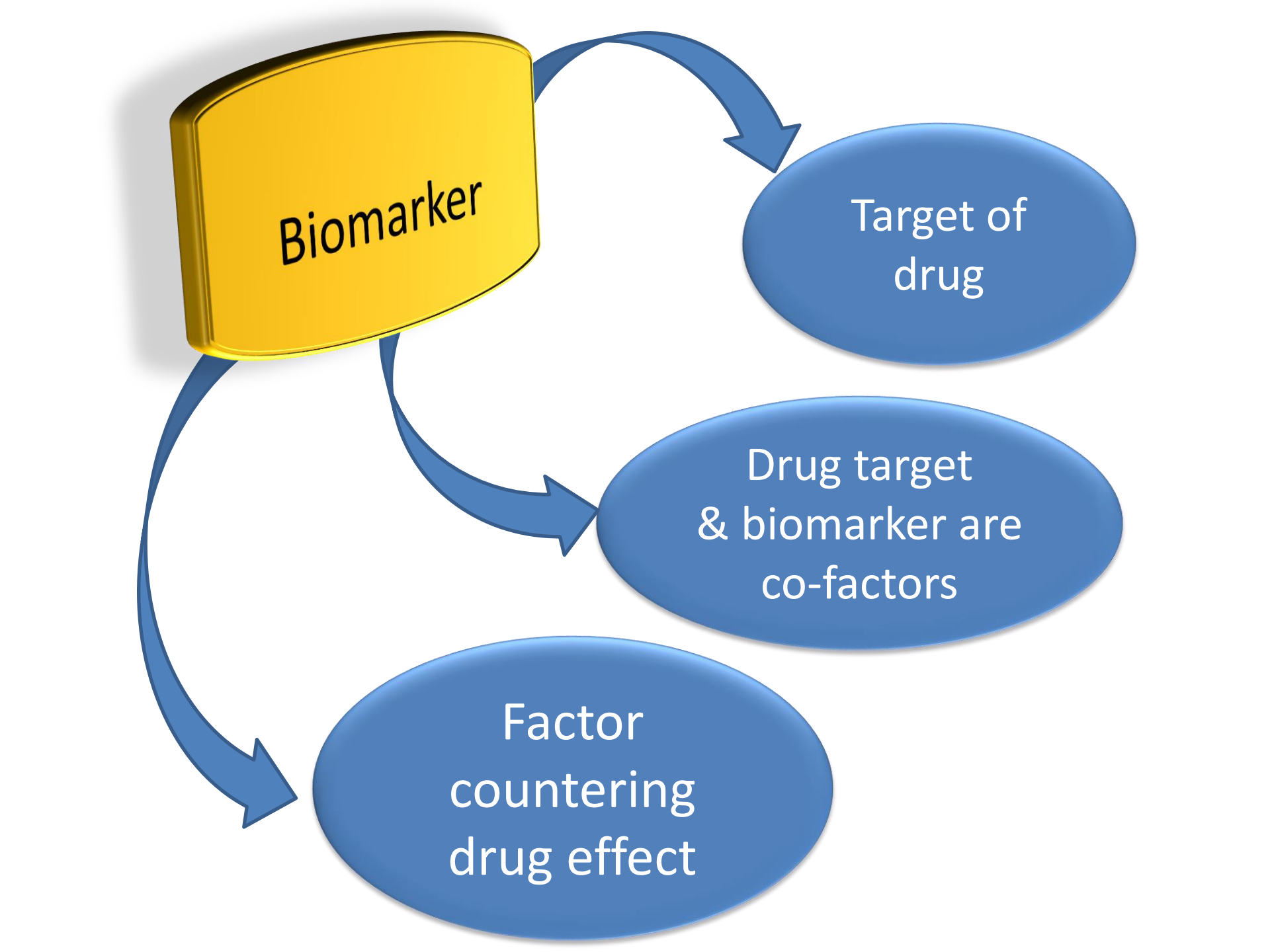
Morphological diagnosis in advanced NSCLC

- Squamous cell carcinoma
- NSCLC, probably squamous cell (IHC)

- Adenocarcinoma
- NSCLC, probably adenocarcinoma (IHC)

- NSCLC-NOS – cannot be resolved (null IHC)
  - Sarcomatoid features?
- Other specific type (carcinoid tumour, etc.)


Molecular Pathology in appropriate cases (histology-determined) to identify targetable genetic alterations
Prognostic Factors ?
Adjuvant therapy
Predictive Biomarkers?
Advanced disease


Biomarker
Target of drug
Drug target & biomarker are co-factors
Factor countering drug effect

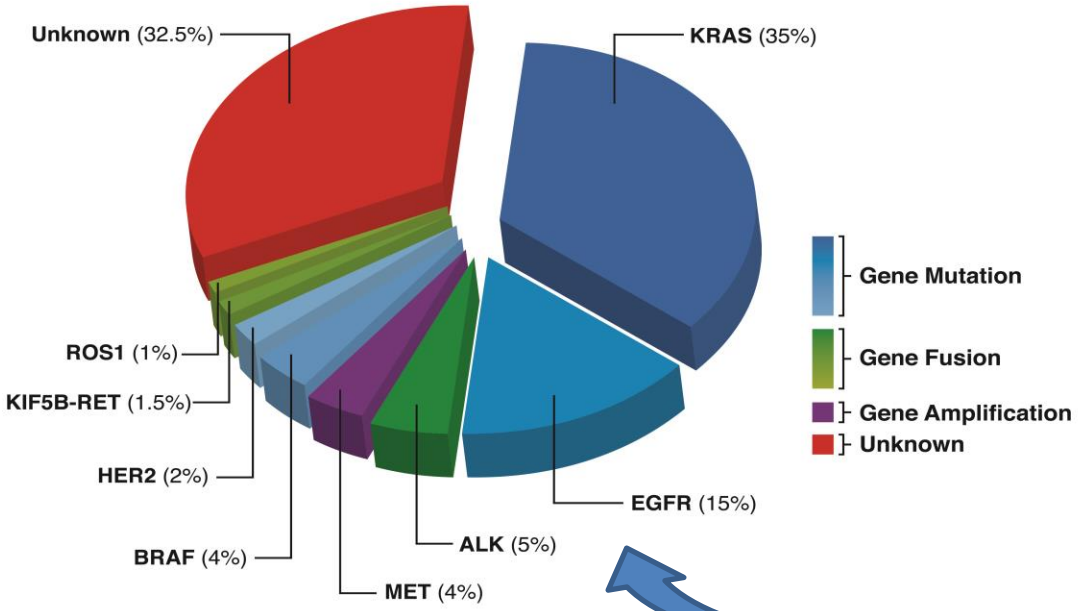
There are MANY **potential** biomarkers in lung cancer but…….

In clinical practice……

- **EGFR mutation testing**
  - EGFR tyrosine kinase inhibitors

- **ALK gene rearrangement testing**
  - ALK tyrosine kinase inhibitors

Oncogene ‘drivers’ in Adenocarcinoma

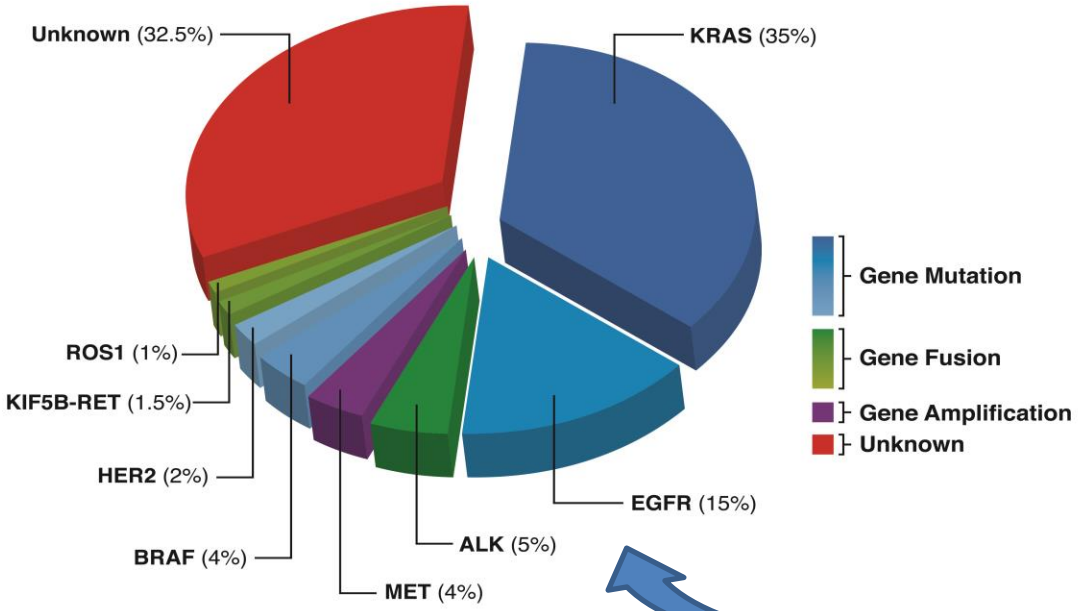


Some are addictive oncogenes
Some are mutually exclusive
Some are good drug targets

EGFR mutation and ALK fusion
20% of adenocarcinomas

Kerr KM. J Clin Pathol 2013;66:832–838

Oncogene ‘drivers’ in Adenocarcinoma



Kerr KM. J Clin Pathol 2013;66:832–838

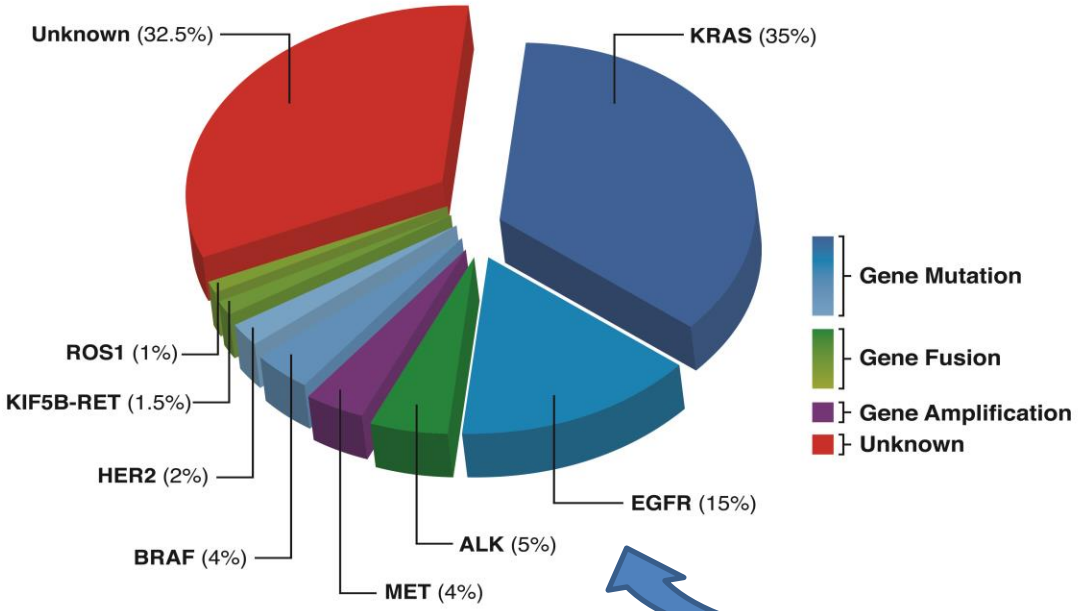
Some are addictive oncogenes
Some are mutually exclusive
Some are good drug targets

EGFR mutation and ALK fusion
20% of adenocarcinomas

So we should test

- **Adenocarcinomas**
- **Probably adenocarcinomas**
- **‘cannot exclude adenocarcinoma’**
- **Partly adenocarcinoma**

Oncogene ‘drivers’ in Adenocarcinoma



Some are addictive oncogenes
Some are mutually exclusive
Some are good drug targets

EGFR mutation and ALK fusion
20% of adenocarcinomas

Kerr KM. J Clin Pathol 2013;66:832–838

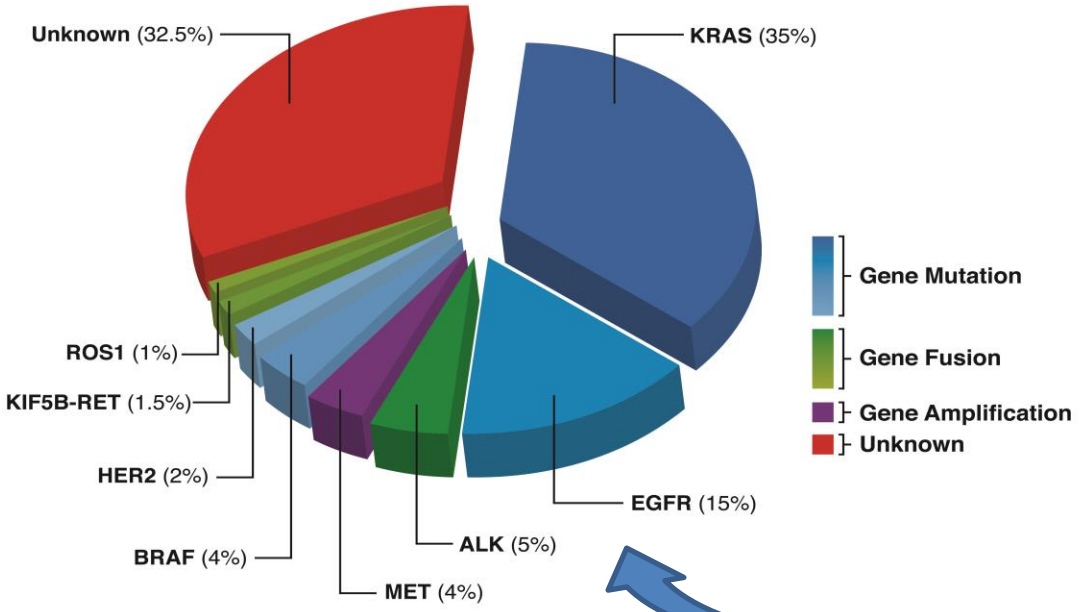
So we should test

- **Adenocarcinomas**
- **Probably adenocarcinomas**
- **‘cannot exclude adenocarcinoma’**
- **Partly adenocarcinoma**

And we should test

- **Smokers**
- **Males**
- **Any ethnic group**

Oncogene ‘drivers’ in Adenocarcinoma



Kerr KM. J Clin Pathol 2013;66:832–838

Some are addictive oncogenes
Some are mutually exclusive
Some are good drug targets

EGFR mutation and ALK fusion
20% of adenocarcinomas

So we should test

- **Adenocarcinomas**
- **Probably adenocarcinomas**
- **‘cannot exclude adenocarcinoma’**
- **Partly adenocarcinoma**

And we should test

- **Smokers**
- **Males**
- **Any ethnic group**
- **Any tumour in a never smoker**
  - **Or long time ex-smoker…….**

Who orders the test?
Reflex versus Bespoke testing

Reflex – pathologist driven

- Fast
- Cases not missed, becomes ‘routine’
- Ready for tumour board decision
- *Potential for waste*
  - *Time*
  - *Tissue*
  - *Money*

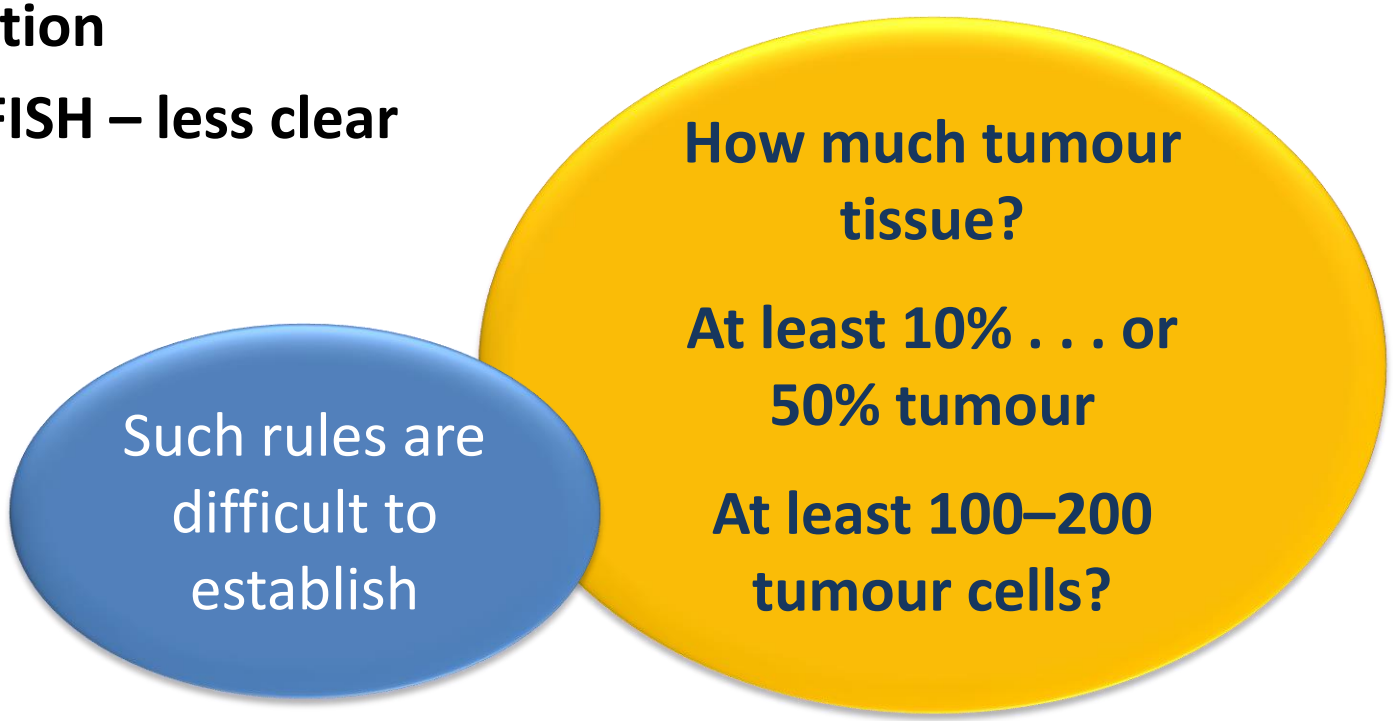
Bespoke – to order from oncologist

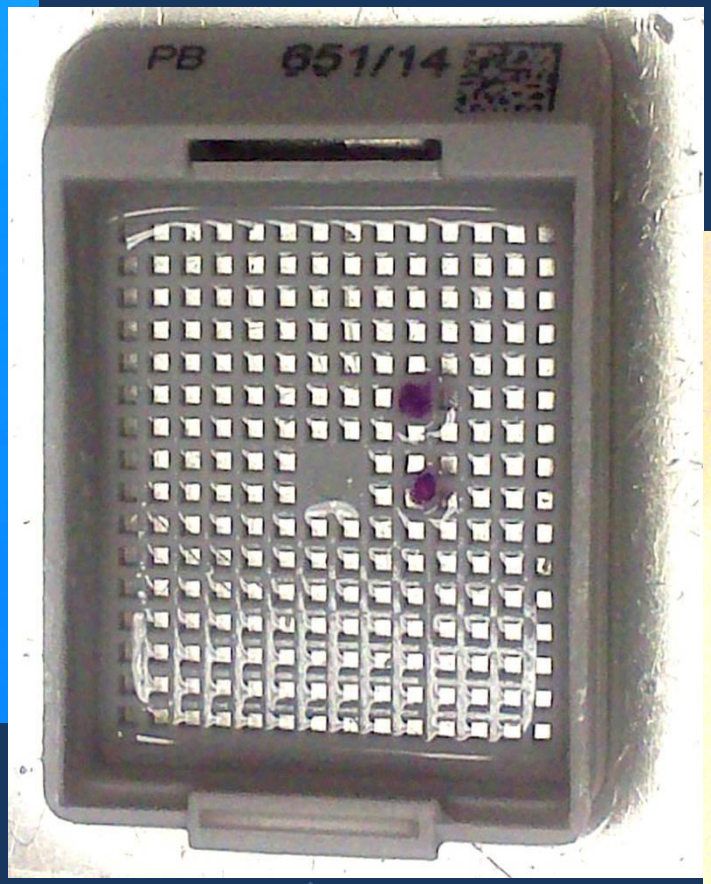
- Only when needed
- Preserves tissue
- Lab time not wasted?

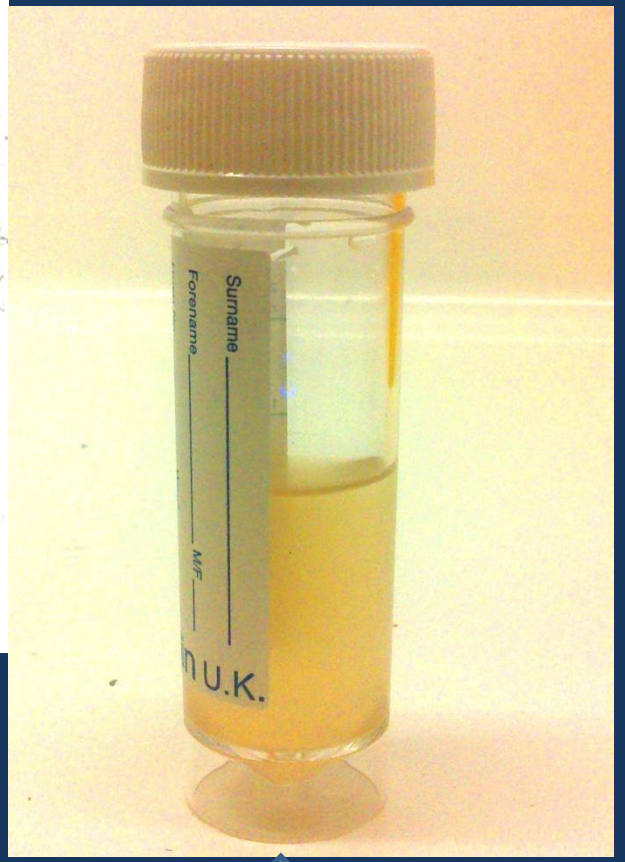
- *‘Cost’ higher per test*
- *Slower turnaround*
- *Could be illogical; cases may be missed*

What do we use for the test?

- Whatever is available – **we need tumour cells!!!**
- Tissue or Cytology Cell block sections
  - **Maximise tumour cells in material submitted for DNA extraction**
  - **Minimise non-tumour cells in material submitted for DNA extraction**
  - **For IHC or FISH – less clear**







Most Lung Cancer **samples** are small biopsies or cytology-type samples

What do we use to test?

	Aberdeen Royal Infirmary, UK*	Aichi Cancer Centre, Nagoya, Japan
Sample type	Percentage of cases submitted	
Surgical Tumor Resection	19%	46%
Lung core biopsy	20%	21%
Bronchial biopsy	19%	11%
Pleural biopsy	7.3%	0.7%
Other biopsy types	22%	4.2%
Total Biopsy samples	**87.3%**	**82.9%**
Aspiration cytology	6.5%	9.3%
Pleural fluid cytology	5.0%	6.6%
Bronchial cytology	1.2%	1.2%
Total Cytology samples	**12.7%**	**17.1%**

2010 data: In Aberdeen Cytology type samples now ~50% of those tested

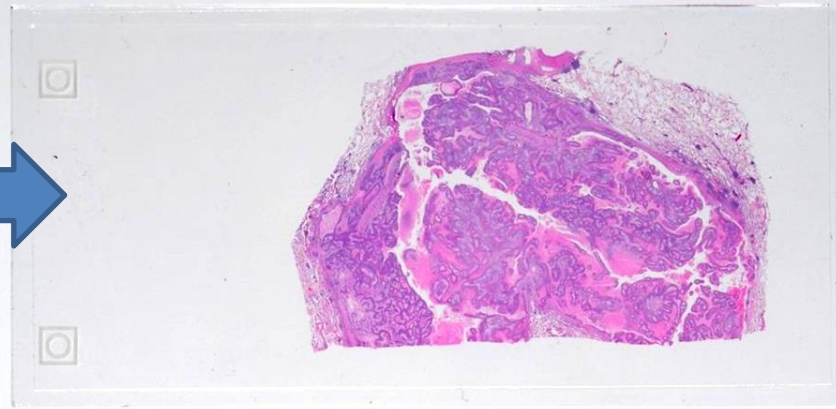
a

b

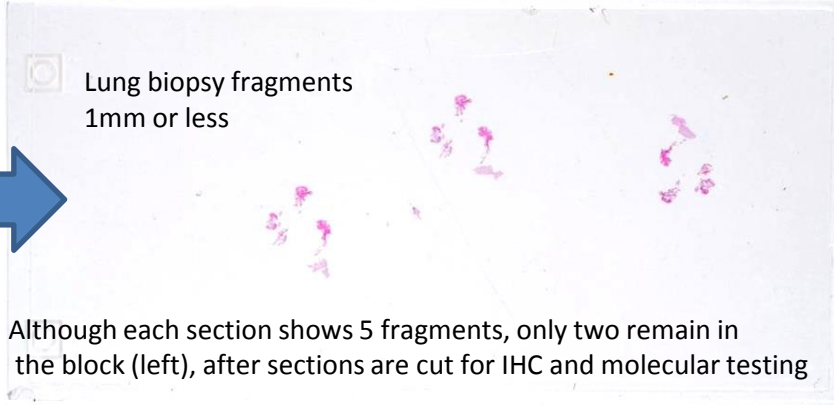
c

The plastic cassettes used for processing tissue are also used to support the paraffin wax embedded block

Abundant tumour tissue in a block taken from a resected tumour

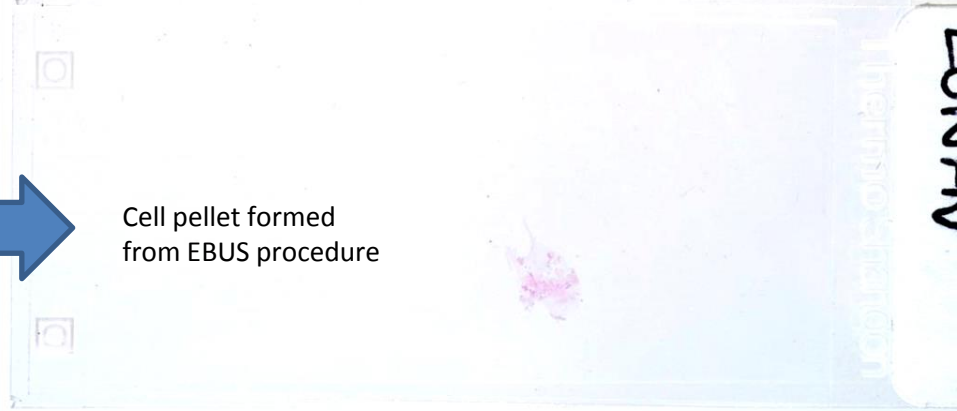


Lung biopsy fragments
1mm or less



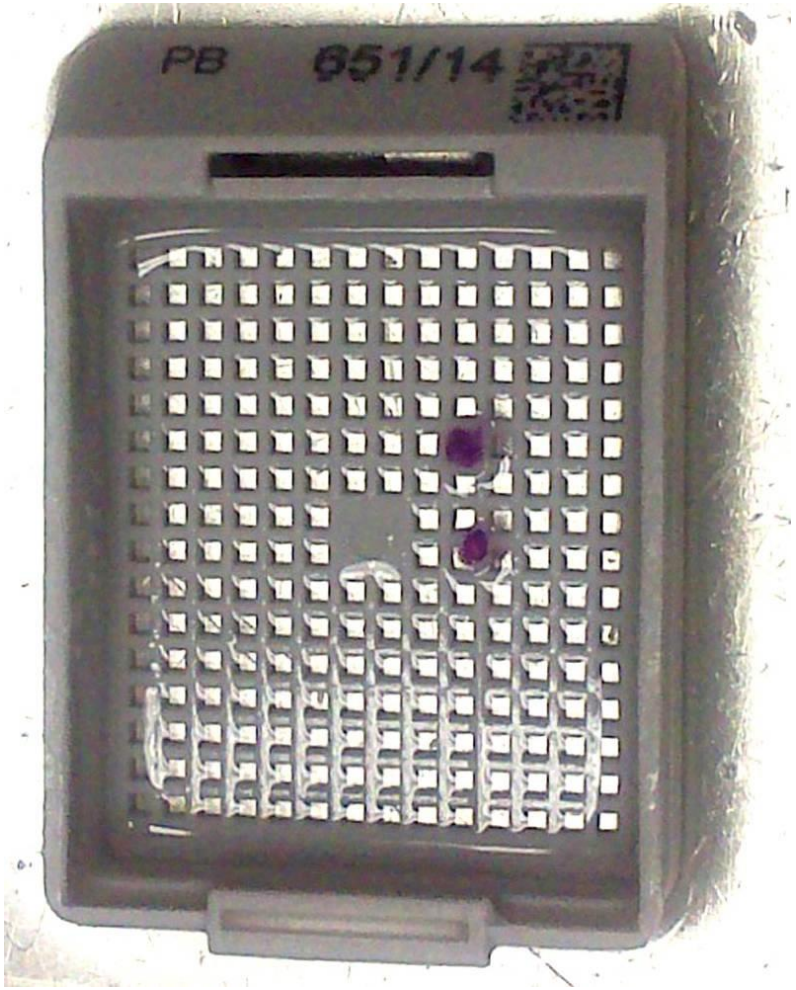
Although each section shows 5 fragments, only two remain in the block (left), after sections are cut for IHC and molecular testing

Cell pellet formed
from EBUS procedure



Sections cut from the block, mounted on glass slide and stained with Haematoxylin and eosin (H&E)

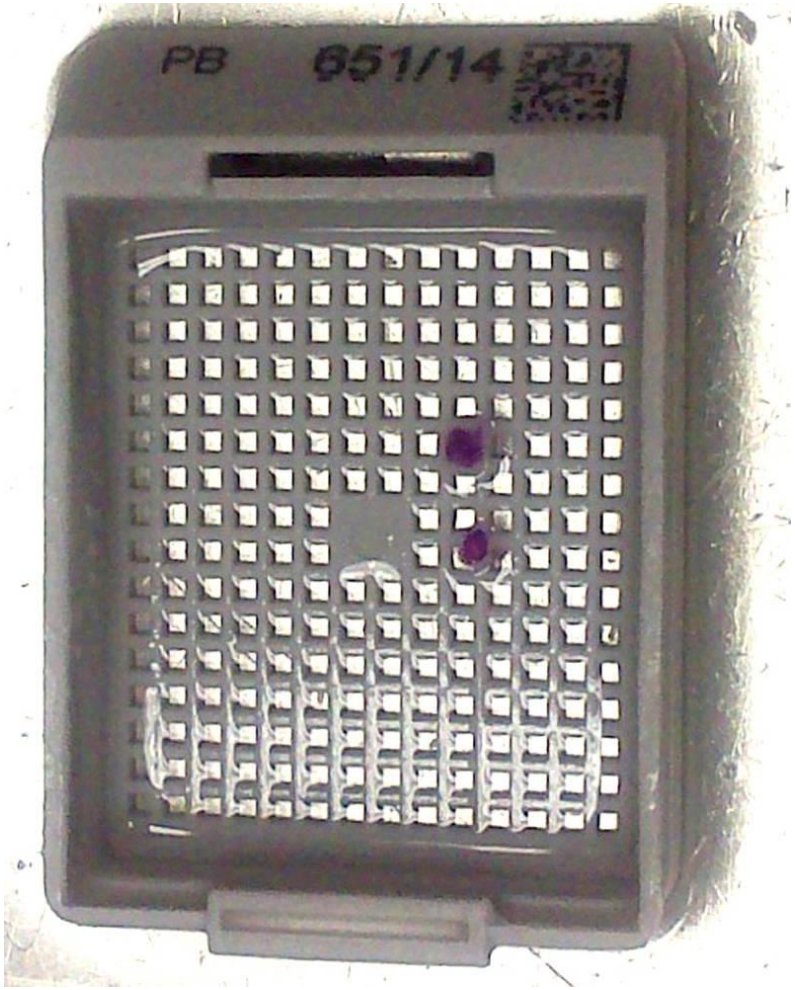
Is there enough material for these studies?



Two biopsy fragments <1mm

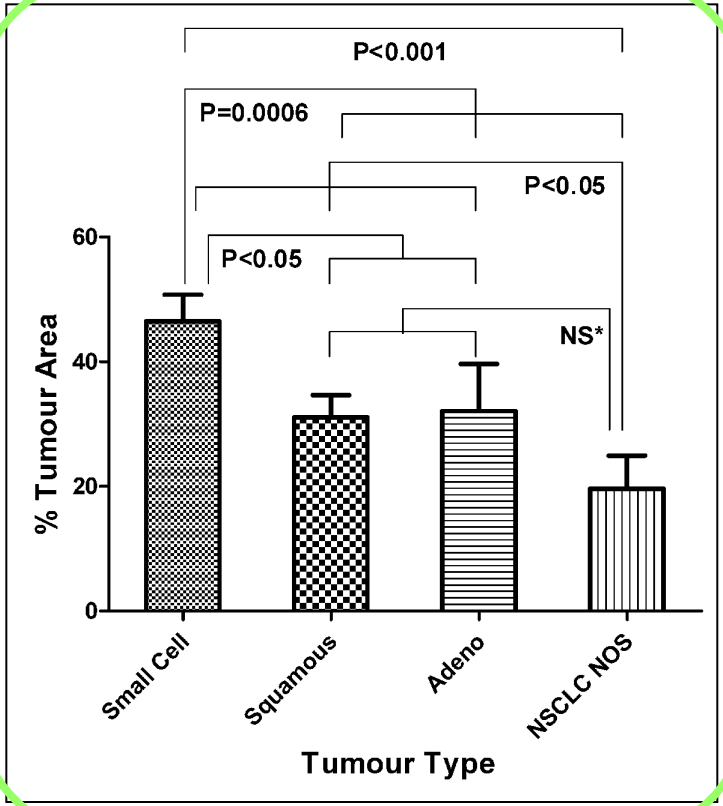
- **Morphologic diagnosis**
- **Immunohistochemistry**
- **Molecular testing**
- Conserve tissue
- Don’t waste

Is there enough material for these studies?



Two biopsy fragments <1mm

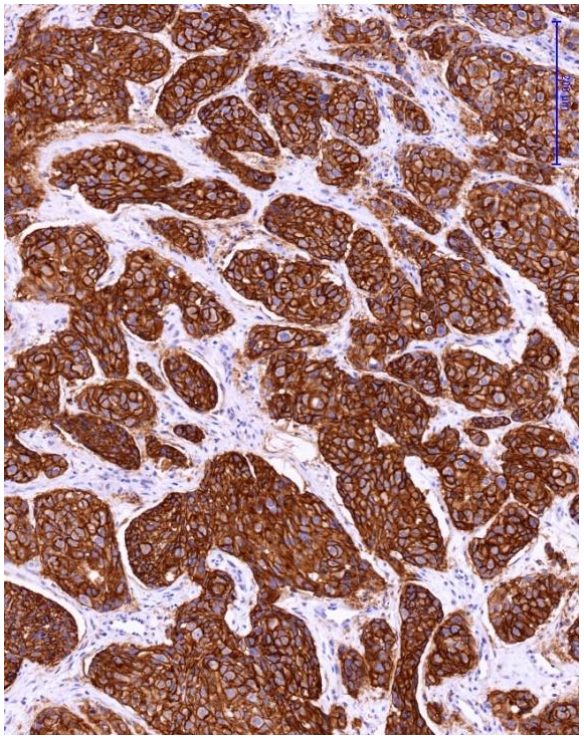
% tumour in bronchial biopsy samples



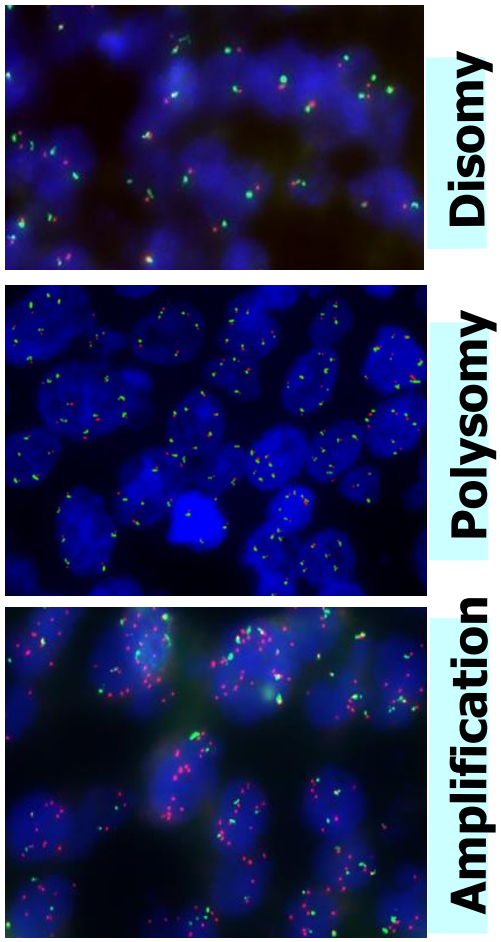
In 'malignant' bronchial biopsy samples
33-50% of fragments do not contain tumour

Coghlin CL et al, JTO 2010, 5:448-452

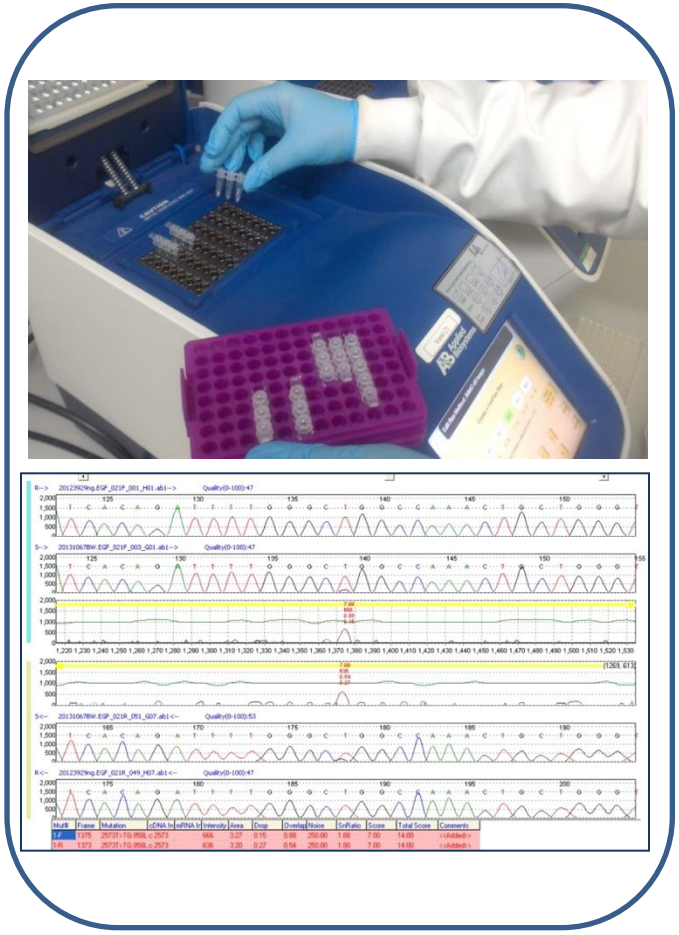
‘Test for EFGR’……



EGFR IHC? Or……..



EGFR FISH test? Or…..

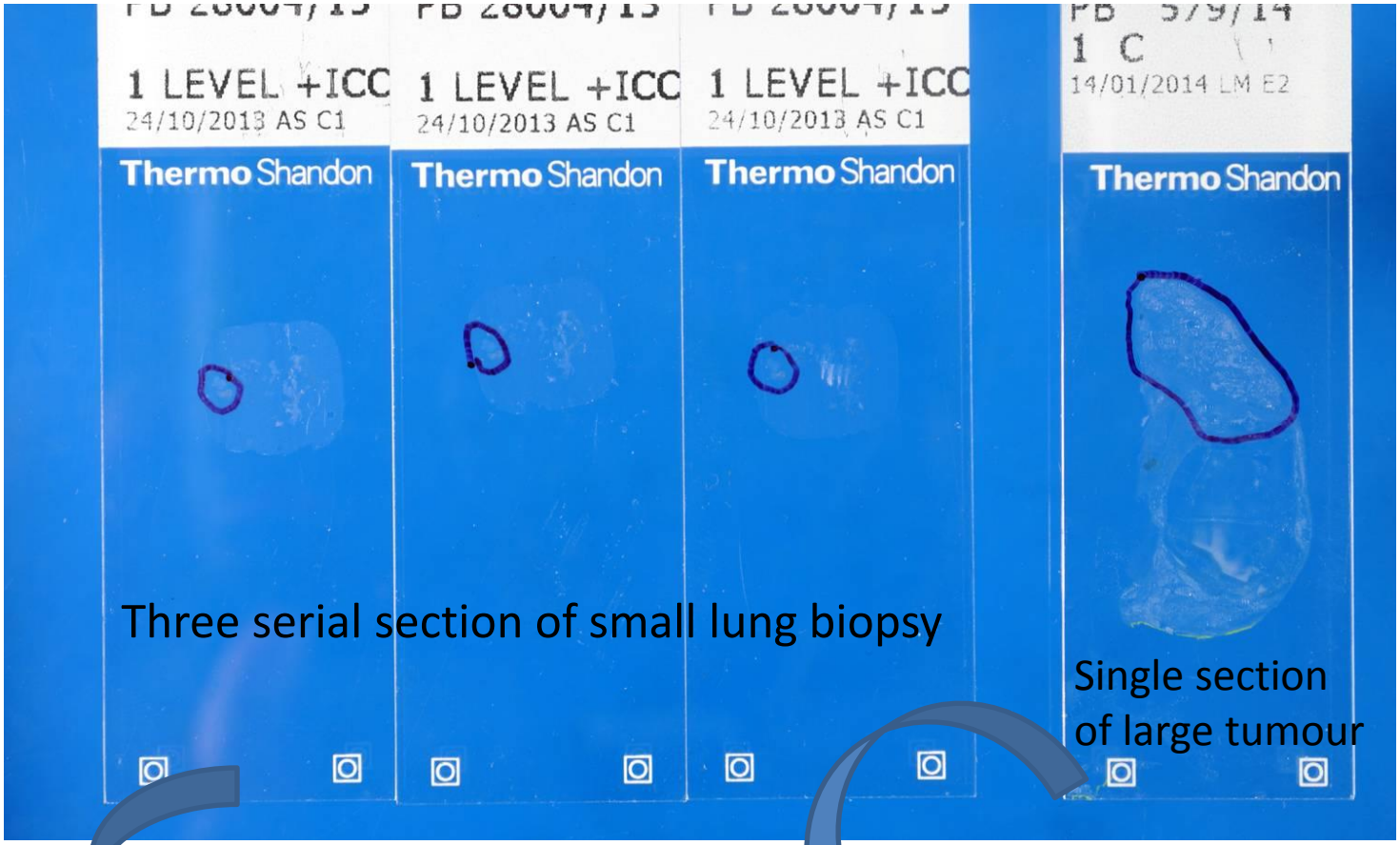


EGFR mutations?

Pathological assessment for molecular testing

- There is tumour present
- It has been prepared in an appropriate way
  - Fixation window 6-48hrs…….

- There is enough tumour?
- The molecular lab knows what it is getting?
  - % tumour in extraction sample
  - Tumour cell number?



Sections are marked to indicate the zone(s) with highest % tumour content.

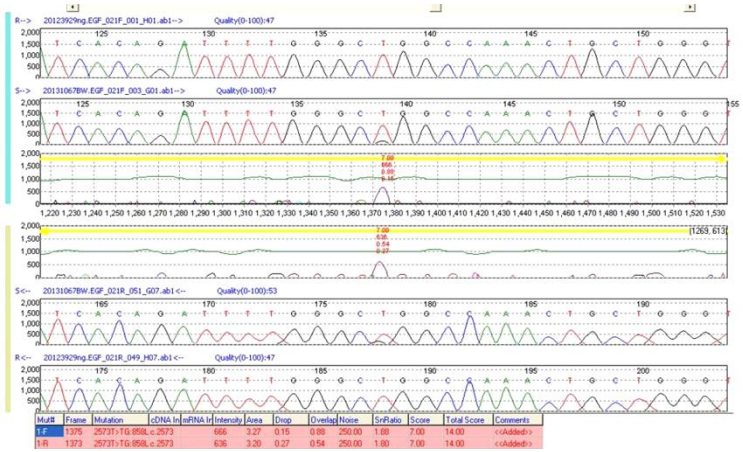
A single section may give enough DNA for Tumour Genotyping.

The marked zone(s) are scraped from the slide and DNA extracted

‘GENETIC STERILITY’

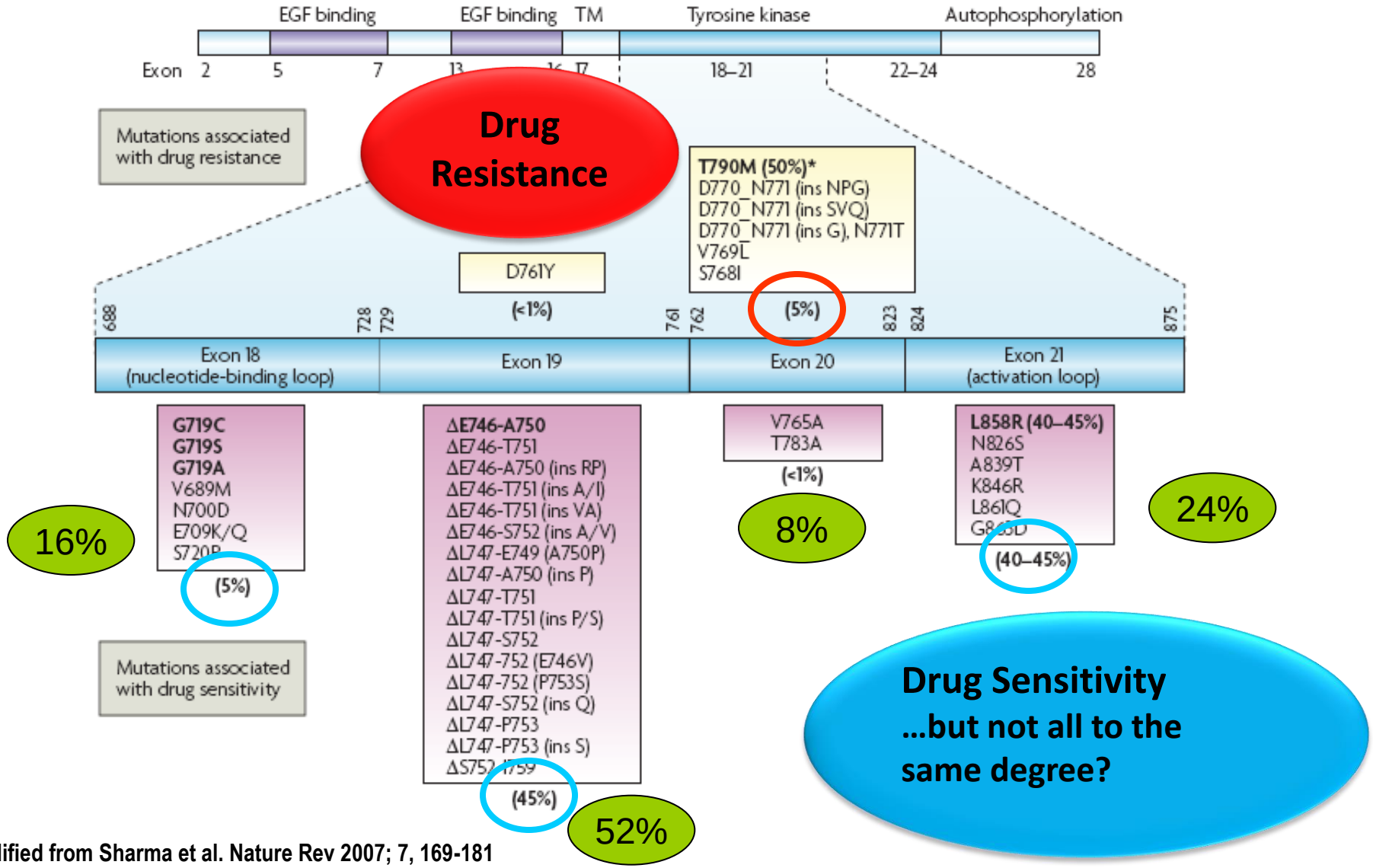
Extracted DNA, mixed with primers for genes of interest, undergoes PCR reaction

Sanger sequencing of PCR products – EGFR mutation



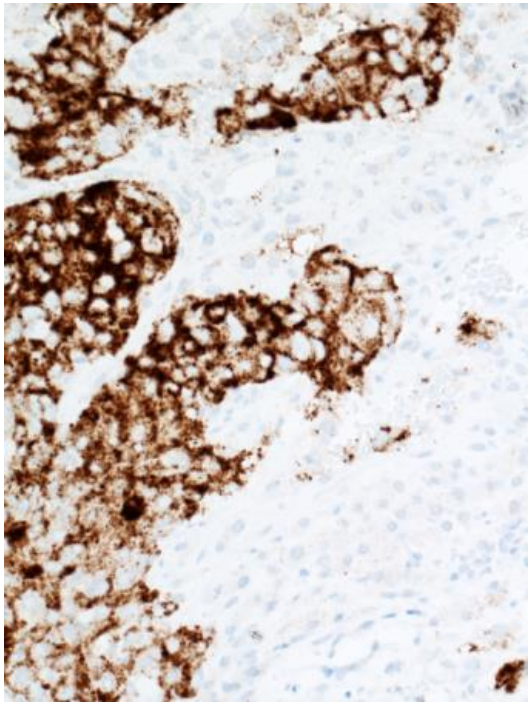
EGFR c.2573T>G; p.Leu858Arg (exon21 L858R)

All EGFR mutations are not equal

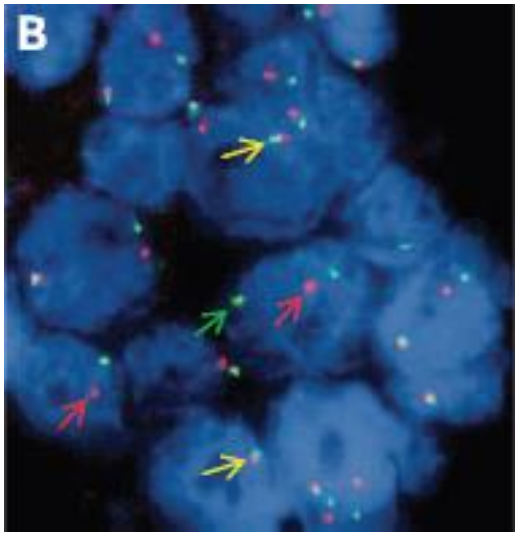


Modified from Sharma et al. Nature Rev 2007; 7, 169-181

‘Test for ALK’……

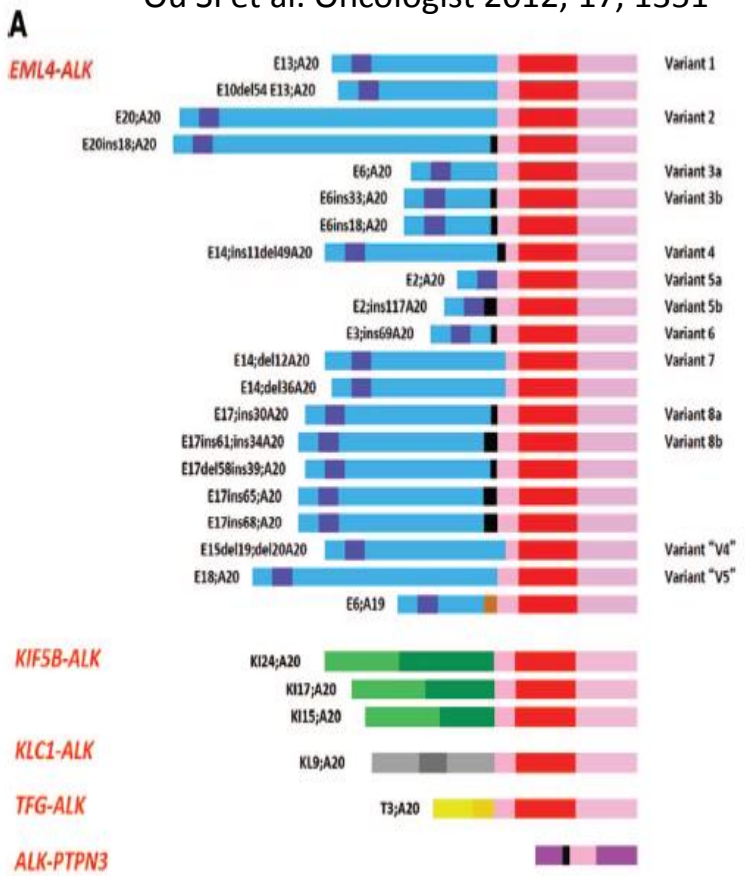


The protein? or …



The break apart FISH test? Or…

Ou SI et al. Oncologist 2012; 17, 1351

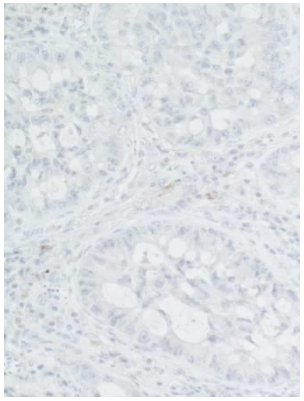


Abnormal gene sequence by multiplex PCR

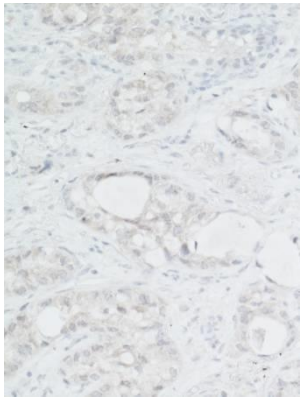
ALK Screening by immunohistochemistry (IHC)

5A4 clone (Leica)

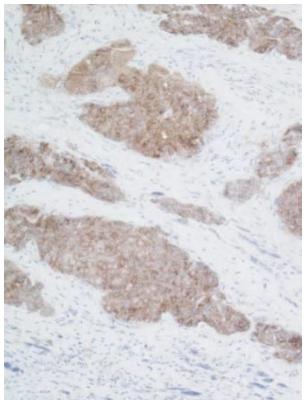
Neg



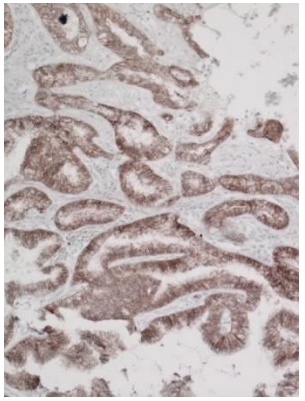
1+



2+

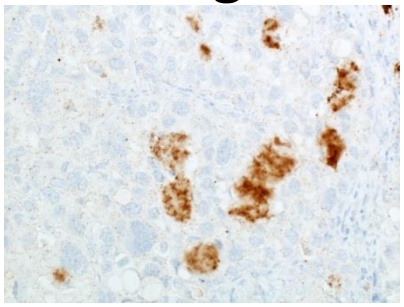


3+

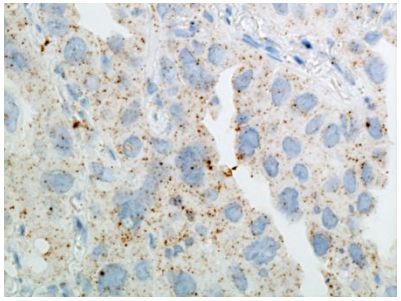


D5F3 clone Ventana 'Kit'

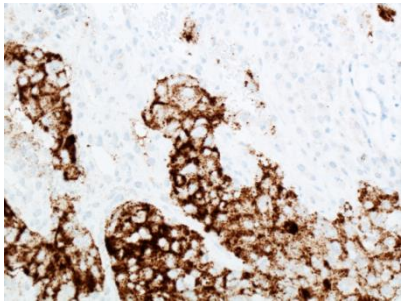
Neg



Borderline



Positive



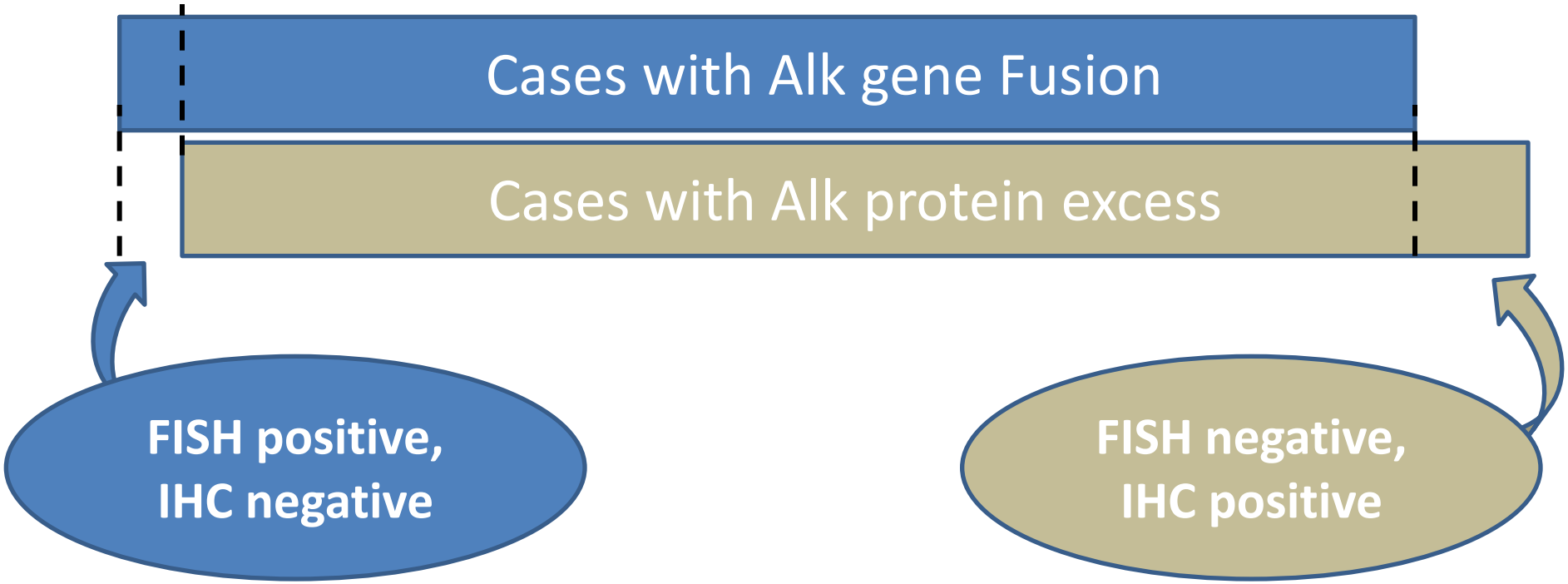
FISHTest?

Almost 100% NEGATIVE

Variable: Majority Negative

Almost 100% POSITIVE

The protein does the job
The protein is the target of the drug

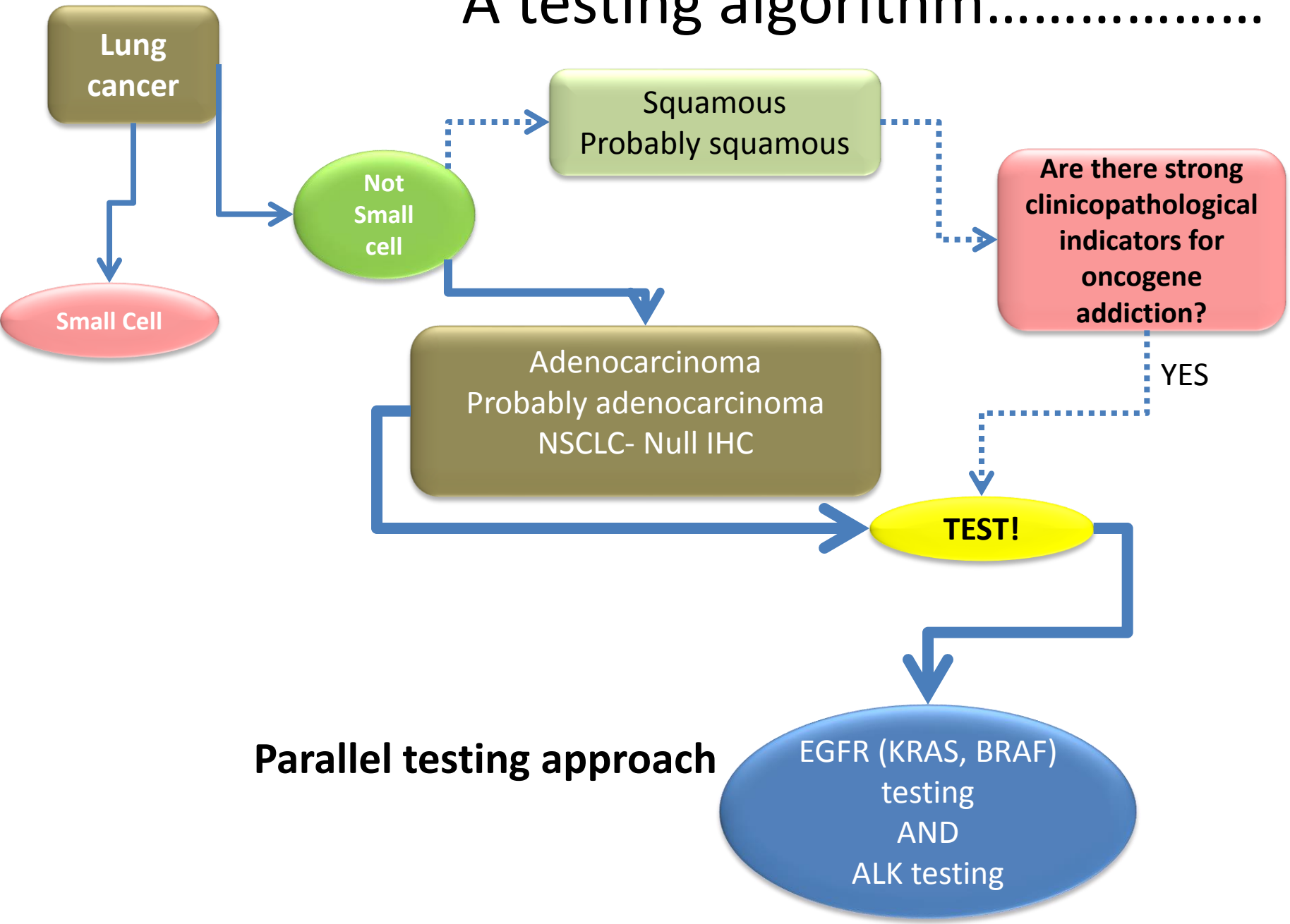


Lower Response Rate ?

Reports of Response to ALK TKI

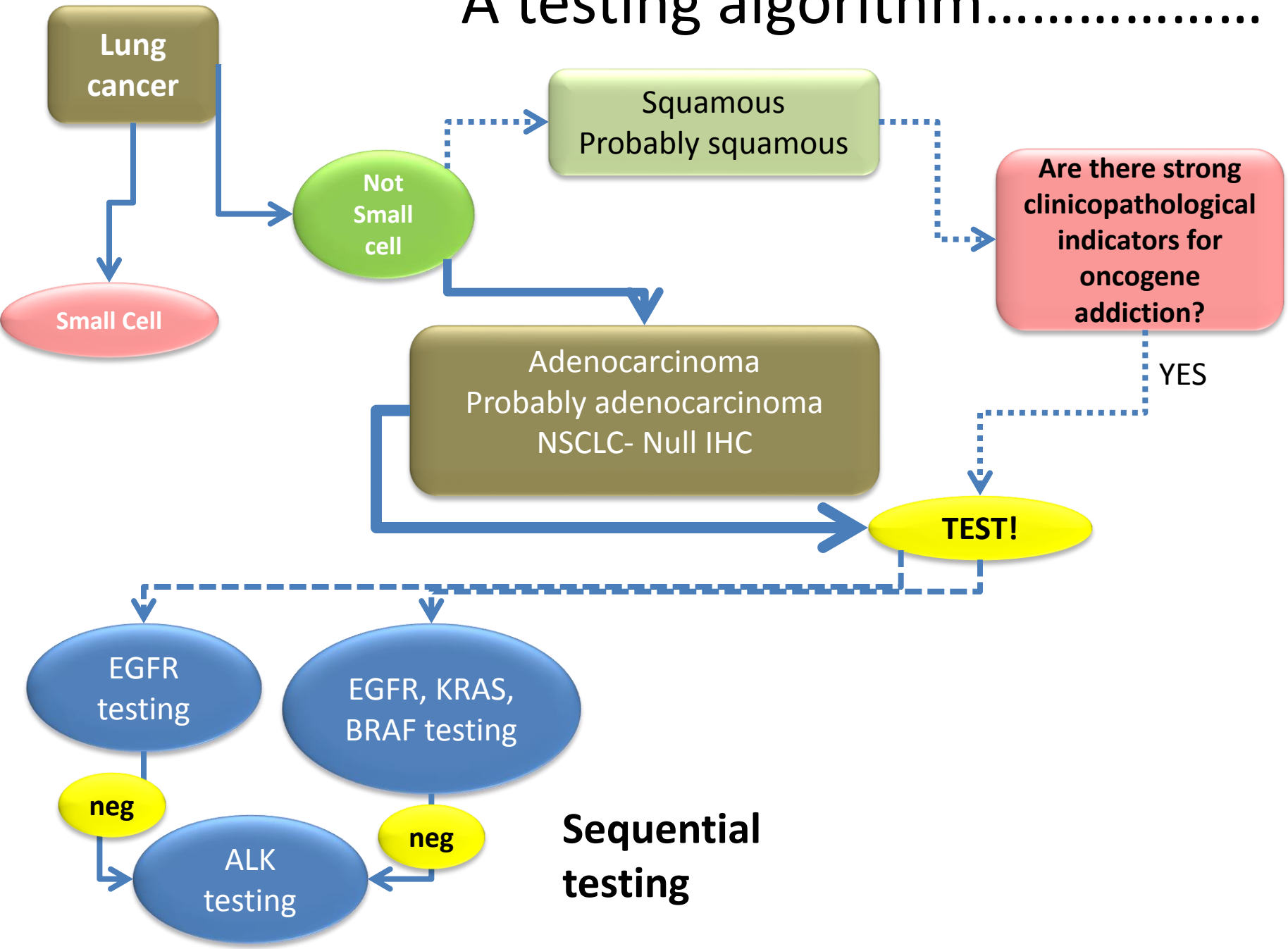
Role of Multiplex PCR in 'discrepant cases'?

A testing algorithm……

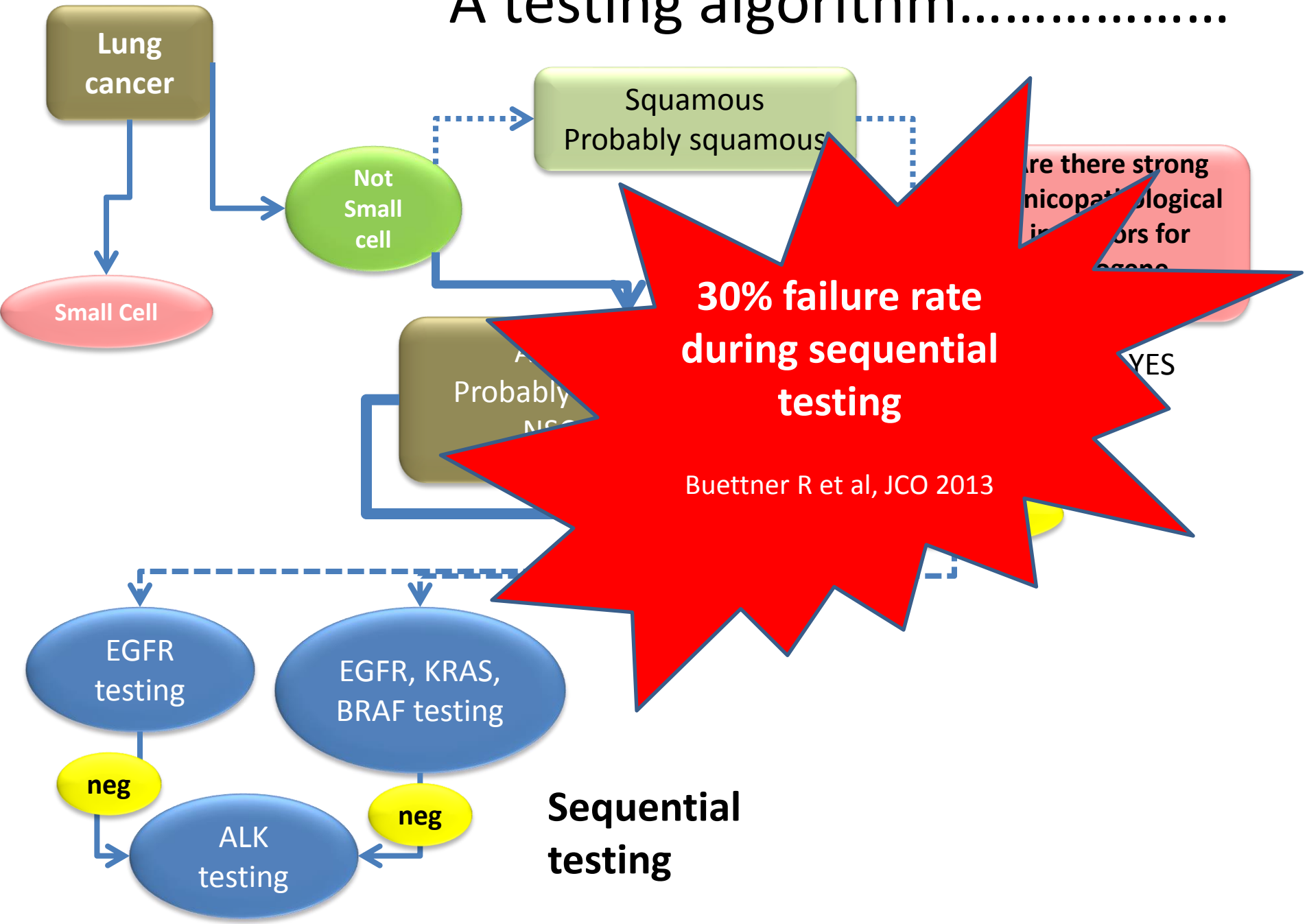


Parallel testing approach

A testing algorithm……



A testing algorithm……



Do we always succeed?

- Diagnostic IHC – rarely insufficient
  - Occasionally it just doesn't work!
- EGFR mutation
- ALK rearrangement
  - IHC screening
  - Confirmation by FISH

Experience from Clinical Trials

Battle Trial Tam AL et al. J Thorac Oncol 2013;8:436

- **20g needle core biopsies**
- 3 PCR sequencing targets
- Two FISH tests
- 6 IHC markers
- **83% adequate for full set**

EURTAC Benlloch et al. J Clin Oncol 2012;30:s10596

- **70% Bronchial biopsy**
- 15% blocks insufficient for EGFR.
- Additional 3% - PCR failure
- Testing 'beyond EGFR' not possible in 47% as block exhausted

MSKCC Squamous Paik et al. J Clin Oncol 2012;30, s7505

- **72% Core biopsy 11% FNAC**
- **17% Resections**
- Sequenom, 1 FISH, 1 IHC test
- **87% complete full set**
- **8% partial set**

IPASS Yang JC et al Lung Cancer 2014; 83, 174-181

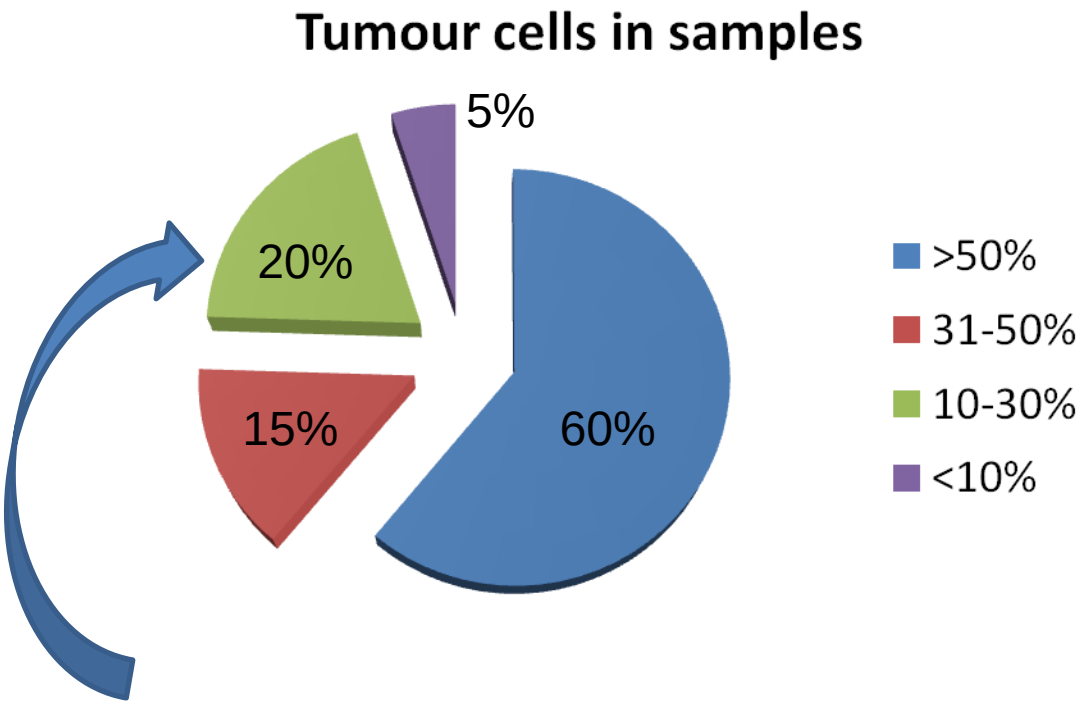
- **Initially rejected samples (<100 cells)**
- 99 histology cases – **80% success**
- 116 cytology cases – **19% success**
- Positives clinically responded

EBUS samples: How do they go?

- 434 malignant samples
  - 70% specific cell type, 30% NSCLC-NOS
    - Navani N et al, AJRCCM 2012

Ref	% EBUS INSUFF for EGFR mutation test	Comment
GarciaOlivia et al 2010	28%	12% for core biopsy
Schuurbiers et al JTO 2010	23%	
Esterbrook et al, Lung Cancer 2013	12%	Cell block based
Navani et al, AJRCCM 2012	10%	
Rekhtman et al, JTO 2011	2%	

Molecular Pathology, Aberdeen Royal Infirmary



Cytology samples
over-represented in this group

- **EGFR, KRAS, BRAF**
- **For EGFR alone**
  - **1.63% total fail rate**
  - **3.3% partial fail**
- **In 'POOR' cases**
  - **6.5% total fail rate**
  - **6.5% partial fail**
- **Huge range with 'outside' cases (up to 35%)**

Alk testing ‘success’??

- In Aberdeen
  - About 4% of cases insufficient for ALK IHC
  - About 10% cases insufficient for ALK FISH
    - 50-60 assessable cells
    - 4 high power fields to assess
- Up to 20% of samples may be ‘insufficient’ for ALK FISH testing
  - Lantuejoul S et al in IASLC ALK Atlas

Strategy to Preserve Tissue

- It is still necessary to make the best diagnosis possible!
- The NSCLC-NOS issue
  - Use the minimum amount of extra material
  - Antibody cocktails – double staining?
- Do not chase ‘phantom’ metastatic disease
- Process all ‘fluids’ if possible
- Reflex section cutting?

EGFR mutation testing: what do the results mean?

Is the result 'real'?

- **False negatives**
  - Real risk
  - Poor samples
  - Pre-analyticals
  - Sample preparation
  - Insensitive analysis
  - Heterogeneity
- **False positives**
  - More dangerous?
  - Poor testing methodology
  - Artefacts
  - Contamination
- **Test failures**
  - Not enough DNA
  - PCR failure
  - Test failure
  - Partial results

Other potential lung cancer biomarkers

Mutation

- KRAS
- BRAF
- MEK
- ERK
- NRAS
- PI3K
- AKT
- STK11
- P53
- DDR2
- FGFR2&3

Other potential lung cancer biomarkers

Mutation

- KRAS
- BRAF
- MEK
- ERK
- NRAS
- PI3K
- AKT
- STK11
- P53
- DDR2
- FGFR2&3

Gene rearrangements – fusion genes

- ROS1 fusion
- RET fusion
- NTRK1 fusion
- CD74-NRG1 fusion
- FGFR3-TAC3 fusion

Gene copy number

- MET
- HER2
- PI3KCA
- FGFR1

Other potential lung cancer biomarkers

Mutation

- KRAS
- BRAF
- MEK
- ERK
- NRAS
- PI3K
- AKT
- STK11
- P53
- DDR2
- FGFR2&3

Gene rearrangements – fusion genes

- ROS1 fusion
- RET fusion
- NTRK1 fusion
- CD74-NRG1 fusion
- FGFR3-TAC3 fusion

Gene copy number

- MET
- HER2
- PI3KCA
- FGFR1

RNA expression

- Expression arrays
- miRNA
- Other individual gene products

Other potential lung cancer biomarkers

Mutation

- KRAS
- BRAF
- MEK
- ERK
- NRAS
- PI3K
- AKT
- STK11
- P53
- DDR2
- FGFR2&3

Gene rearrangements – fusion genes

- ROS1 fusion
- RET fusion
- NTRK1 fusion
- CD74-NRG1 fusion
- FGFR3-TAC3 fusion

Gene copy number

- MET
- HER2
- PI3KCA
- FGFR1

RNA expression

- Expression arrays
- miRNA
- Other individual gene products

IHC

- ROS1
- BRAF
- EGFR
- MET
- PD-1
- PDL-1

Other potential lung cancer biomarkers

Mutation

- KRAS
- BRAF
- MEK
- ERK
- NRAS
- PI3K
- AKT
- STK11
- P53
- DDR2
- FGFR2&3

Gene rearrangements – fusion genes

- ROS1 fusion
- RET fusion
- NTRK1 fusion
- CD74-NRG1 fusion
- FGFR3-TAC3 fusion

Gene copy number

- MET
- HER2
- PI3KCA
- FGFR1

IHC

- ROS1
- BRAF
- EGFR
- MET
- PD-1
- PDL-1

RNA expression

- Expression a
- miRNA
- Other
  gene

Response to Cytotoxic agents

DNA repair mechanisms
Apoptosis pathways
Drug targets – TS
Modifiers – RRM1

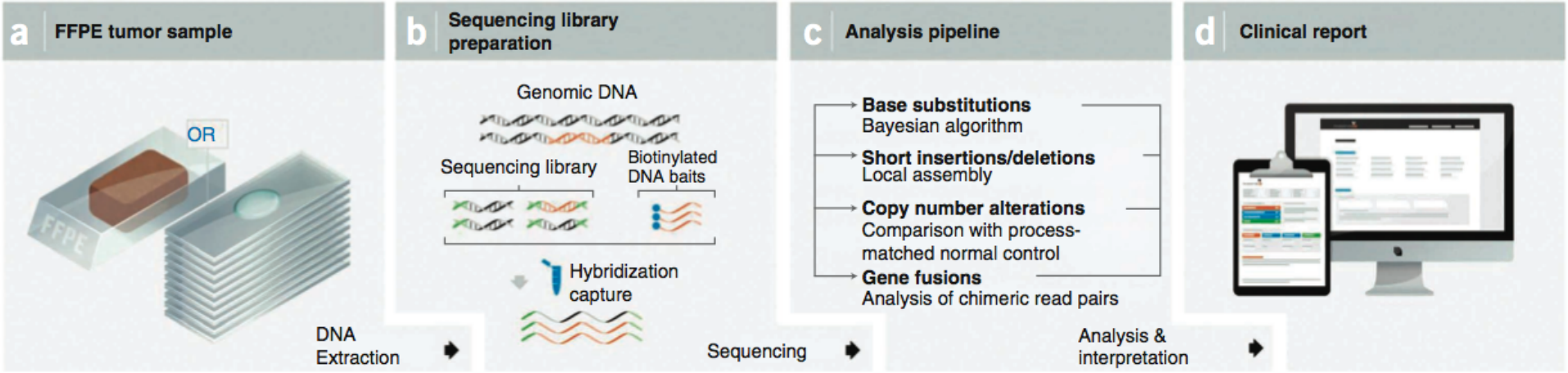
NGS for molecular testing

- Quoted amounts of DNA required rather variable
  - Technology dependant
  - Size of panel
- Mutation > Fusion gene > gene copy number
- Fragmentation of DNA
- Bioinformatic analysis

- 80% samples – complete panel of mutations
- 95% samples – EGFR, KRAS, BRAF, HER2 mutations
- ‘minimum 2000 cells’ - 5 x 10um thick sections

Myerson M et al. Nat Rev Gen 2010

- **Much still to define**



Sample requirements

- Surface area: ≥25 mm^2
- Sample volume: ≥1 mm^3
- Nucleated cellularity: ≥80% or ≥30,000 cells
- Tumor content: ≥20%

Fraction of patients with tissue insufficient for analysis: 10–15%

Laboratory process highlights

- Requires ≥50 ng of dsDNA (quantified by PicoGreen)
- Fragmentation by sonication (Covaris) and 'with-bead' library construction
- Hybridization capture with biotinylated DNA oligonucleotides
- 49 × 49 paired-end sequencing on the Illumina HiSeq platform to >500× average unique coverage, with >100× at >99% of exons

Analysis methods highlights

- Sensitivity to variants present at any mutant allele frequency
- Detection of long (1–40 bp) indel variants using de Bruijn graph–based local assembly
- CGH-like analysis of read-depth for CNAs assessment

Reporting approach

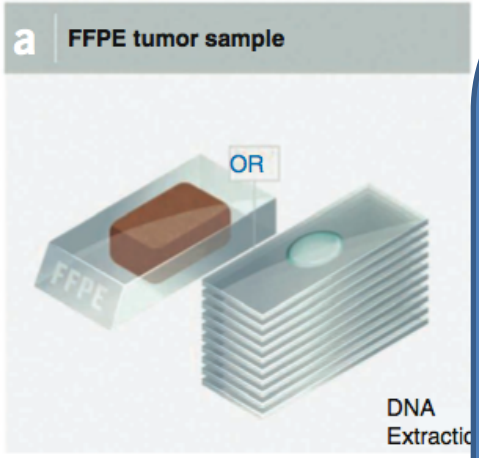
Interpretation without a matched normal

- Germline variants from 1000 Genomes Project (dbSNP135) removed
- Known driver alterations (COSMIC v62) highlighted as biologically significant

A concise summary of the biomedical literature and current clinical trials is provided for each highlighted alteration

- False-negative calls were predominantly low (<10%) mutant allele frequencies substitutions, indels or low-magnitude copy number alterations.
- **Comprehensive genomic profiling was successful for 95% of clinical cases**

Frampton G, *et al.* Nature Biotechnology 2013; Epub ahead of print.



Sample requirements

- Surface area: ≥25 mm^2
- Sample volume: ≥1 mm^3
- Nucleated cellularity: ≥80% or ≥30,000 cells
- Tumor content: ≥20%

Fraction of patients with tissue insufficient for analysis: 10–15%

More than 25mmsq = 5 x 5mm area

1mm cube of tumour

Cellularity >80% or > 30,000 cells

Tumour content > 20% - 1500 tumour cells

10 -15% insufficient for analysis

- False-negative calls were predominantly low (<10%) mutant allele frequencies substitutions, indels or low-magnitude copy number alterations.
- **Comprehensive genomic profiling was successful for 95% of clinical cases**

Frampton G, *et al.* Nature Biotechnology 2013; Epub ahead of print.

Consensus for *EGFR* Mutation Testing in Non-small Cell Lung Cancer

Results from a European Workshop

Pirker R et al. JTO 2010

TABLE 1. Biopsy Techniques

	21-g Needle Aspiration	19-g Needle Aspiration	Transbronchial Biopsy	CT-Guided Needle Biopsy
Total no. of cells per biopsy/ aspiration	≥100	≥150	≥300	≥500
No. of biopsies	4	4	4–5	2–3

400 -600 cells

~1500 cells

PLEASE SIR
CAN I HAVE MORE

PLEASE
Mr Pulmonologist or
Interventional Radiologist
CAN I HAVE MORE

Success in Biomarker testing?

- **Be aware! Anticipate testing.....**
- **Maximize tissue collection – do no harm**
- **Process tissue appropriately**
- **ANY SAMPLE TYPE is *potentially* adequate for biomarker testing**
- **Take steps to 'improve' the test sample**
- **Quality-assured molecular testing**
- **Plan your testing strategy**
  - **This is a MULTIDISCIPLINARY effort**
- **Everyone on the team UNDERSTANDS WHY testing is important**
- **Communication, communication, communication.....**

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

Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology

Quality control by pathologist :

- Fixation : 6-12 hrs 10% neutral-buffered formalin
- Estimate the **cellular tumour content and tumour purity**

Ideally :

high proportion **(>30-50%)** of malignants cells relative to nonneoplastic cells

+

minimal proportion **(<20%)** of substances that may inhibit amplification (e.g. necrosis, mucin)

Number of cells?

Lindeman et al. J Thorac Oncol 2013;8:823-59.