Role of the pathologist in the diagnosis and mutational analysis of lung cancer

Professor J R Gosney

Consultant Thoracic Pathologist
Royal Liverpool University Hospital

Disclosure

JRG is a paid advisor to and speaker for *AstraZeneca, Boehringer-Ingelheim, Bristol-Myers Squibb, Dako, Lilly & Co, Merck, Merck Sharp and Dohme, Novartis, Pfizer, Roche and Ventana*

Classification of carcinoma of the lung

- **small cell lung cancer (SCC)**
- **non-small cell lung cancer (NSCLC)**
  - ***Strictly*, all malignant epithelial neoplasms other than SCC**
  - ***In* practice, squamous or adenocarcinoma**

Pathology report 2006

SPECIMEN

LEFT UPPER LOBE BRONCHIAL BIOPSY

MACROSCOPIC DESCRIPTION

Fragments of white tissue measuring up to 4mm

MICROSCOPY

This bronchial mucosa and submucosa are infiltrated by poorly differentiated carcinoma with a ‘squamoid’ growth pattern, although desmosomes and keratin are not evident. Some cells may contain vacuoles, possibly of mucin.

The features are those of a poorly differentiated non-small cell carcinoma, probably adenocarcinoma.

Pathology report 2016

SPECIMEN

LEFT UPPER LOBE BRONCHIAL BIOPSY

MACROSCOPIC DESCRIPTION

Fragments of white tissue measuring up to 4mm

MICROSCOPY

This bronchial mucosa and submucosa are infiltrated by poorly differentiated non-small cell carcinoma. The growth pattern is 'squamoid', but desmosomes and keratin are not evident. Occasional cells contain vacuoles, possibly mucin.

Immunochemistry reveals expression of cytokeratins of class 7 as well as of TTF-1. There is no expression of p63. These features support a diagnosis of **adenocarcinoma.**

PREDICTIVE PROFILING

EGFR GENE MUTATIONS

Epidermal growth factor (EGFR) mutation analysis has been performed to determine suitability of this patient's non-small cell lung cancer (NSCLC) for treatment with tyrosine kinase inhibitors (TKIs).

Analysis was done using RT-PCR (Scorpions/ARMS methodology, Qiagen Therascreen EGFR kit). The Therascreen kit detects the following 29 somatic mutations:

Exon 18: G719X
The kit detects, but does not distinguish between, any from the following 3 mutations;
G719A (c.21567G>A p.G719A)
G719S (c. 2155G>S p.G719S)
G719C (c.2155G>C p.G719C)
Exon 19: deletions
The kit detects, but does not distinguish between, any from a total of 19 deletions;
Exon 20: T790M
Exon 20: S768I
Exon 20 : Insertions
The kit detects, but does not distinguish between, any from the following 3 mutations;
c.2307_2308ins9 p.V769_D770insASV
c.2319_2320insCAC p.H773_V774insH
c.2319_2311insGGT p.D770_N771insG
Exon 21: L858R (c. 2573T>G p.L858R)
Exon 21: L861Q (c.2582T>A p.L861Q)

Test sensitivity
The limit of sensitivity is stated to be 1% mutated EGFR alleles in a wild-type background

Results
NO MUTATIONS IN THE EGFR GENE HAVE BEEN DETECTED

Conclusion
This patient is unlikely to respond to TKIs active against NSCLCs with sensitising mutations in the EGFR gene.

Any remaining DNA from this patient's sample will be stored in the laboratory archives.

ALK GENE REARRANGEMENT

Immunochemistry using the Ventana system and Roche D5F3 antibody has been performed to detect the **ALK fusion protein**.

STRONG, GRANULAR EXPRESSION OF THE PROTEIN IS DETECTED. Such expression correlates with rearrangement of the ALK gene and indicates that this patient is likely to respond to TKIs active against NSCLCs with this genetic abnormality

PD-L1 EXPRESSION

Expression of PD-L1 has been sought by immunochemistry using the Dako 22C3 antibody as a guide to the likely sensitivity of the tumour to pembrolizumab.

Expression of PD-L1 on cell membranes is as follows:
Neoplastic cells: overall expression 60% (strong 30%; moderate 30%
Immune cells: overall expression 10% (weak 10%)
In terms of guiding the use of pembrolizumab, this specimen should be considered **POSITIVE** for PD-L1 expression

Tailored therapy for NSCLC

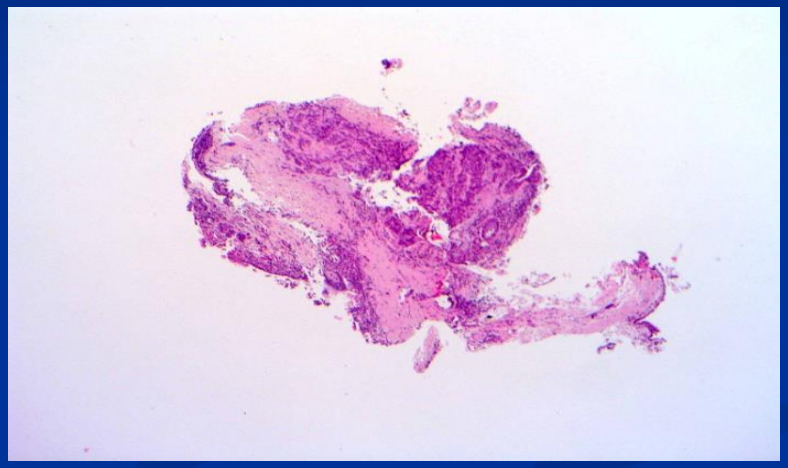
- Therapies dependent on morphology
  - Antifolate
    - pemetrexed (Alimta)
  - Anti-VEGFA monoclonal antibody
    - bevacizumab (Avastin)
- Therapies dependent on genetic aberrations
  - Small molecule tyrosine kinase inhibitors (TKIs)
    - erlotinib (Tarceva), gefitinib (Iressa), afatinib (Giotrif), osimertinib (Tagrisso) crizotinib (Xalkori), ceritinib (Zykadia), alectinib (Alecensa)
- Therapies dependent on protein expression
  - Anti-PD-1 and PD-L1 monoclonal antibodies
    - nivolumab (Opdivo), pembrolizumab (Keytruda), atezolizumab (Tecentriq), durvalumab, avelumab
  - Anti-EGFR monoclonal antibody
    - necitumumab (Portrazza)

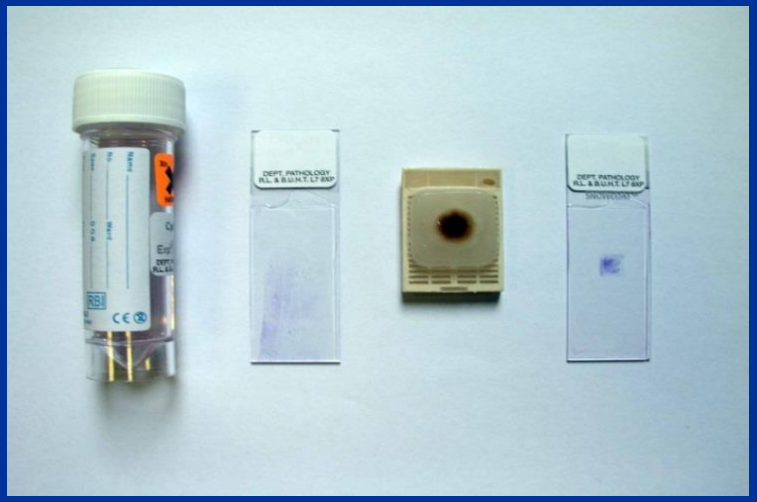
Predictive profiling of NSCLC

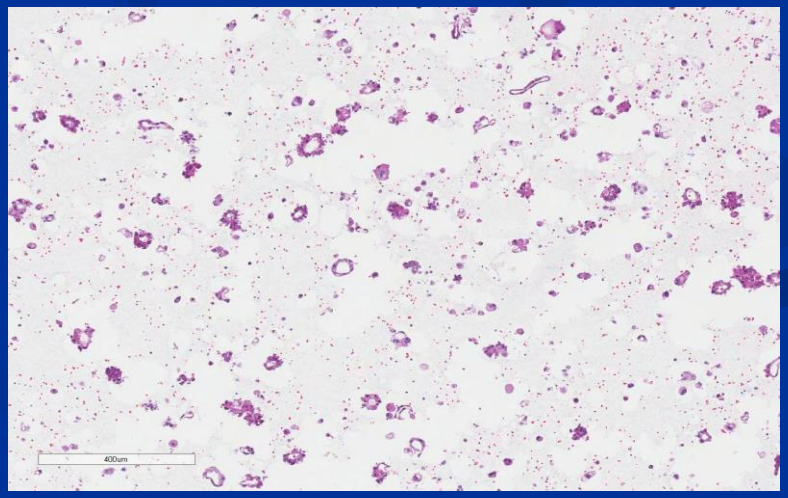
- EGFR gene mutations
  - ~12%
- ALK gene rearrangement
  - <5%
- PD-L1 protein expression
  - depends on ‘cut-off’

A challenge!

A high quality specimen

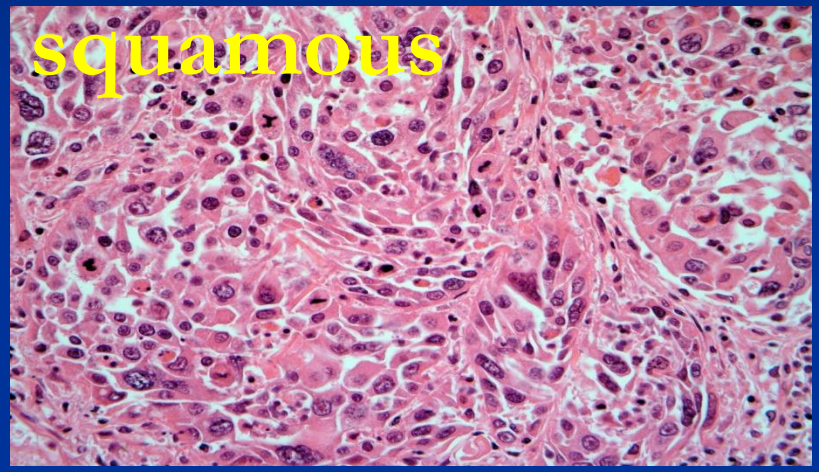


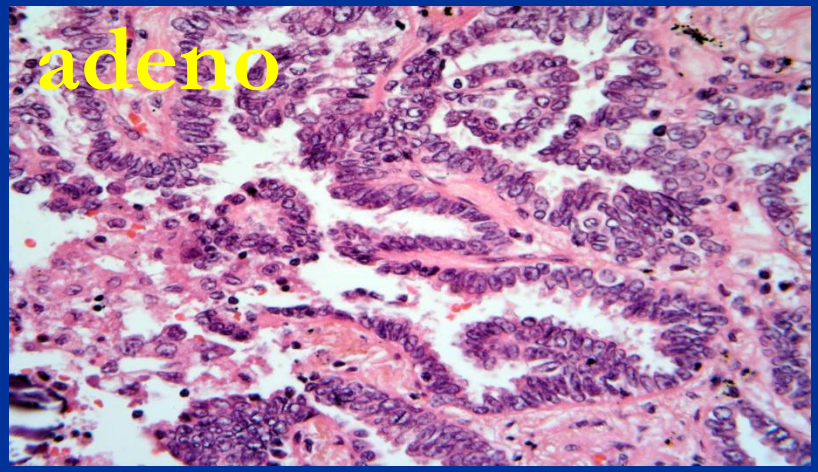


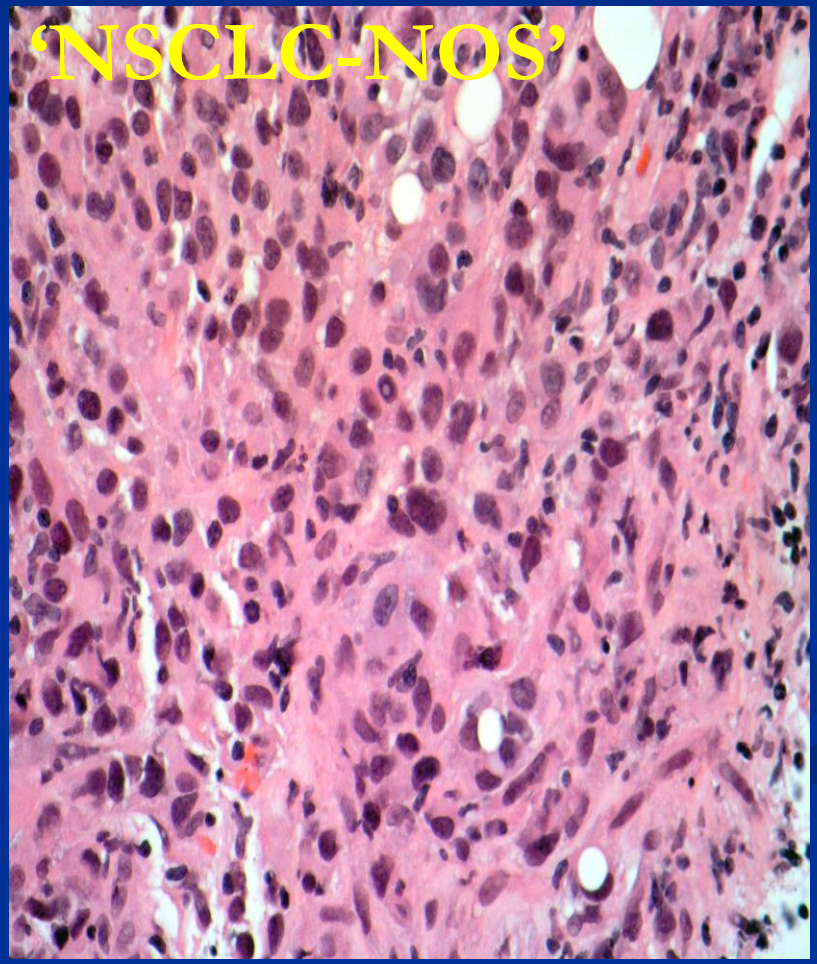


There's no
such thing
as a biopsy that's
too big

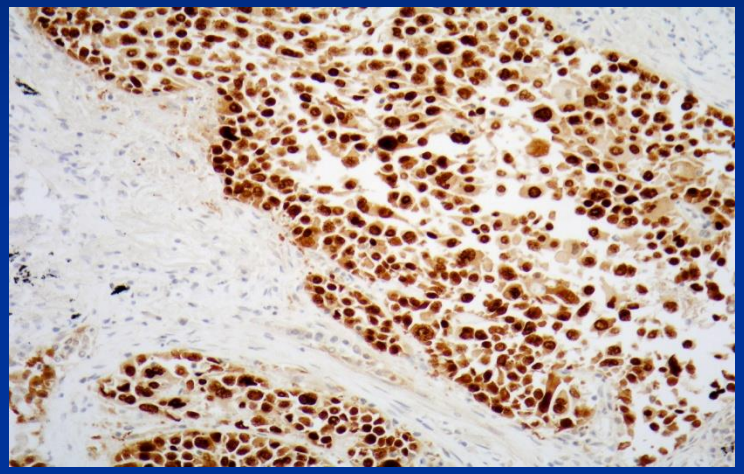
Accurate diagnosis

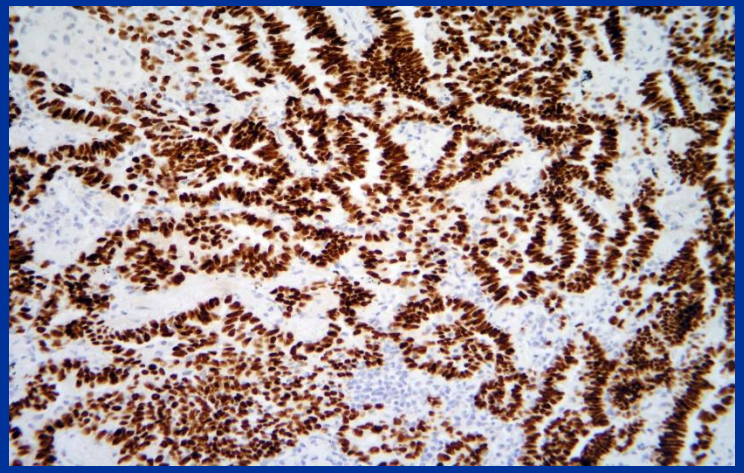






Accurate diagnosis

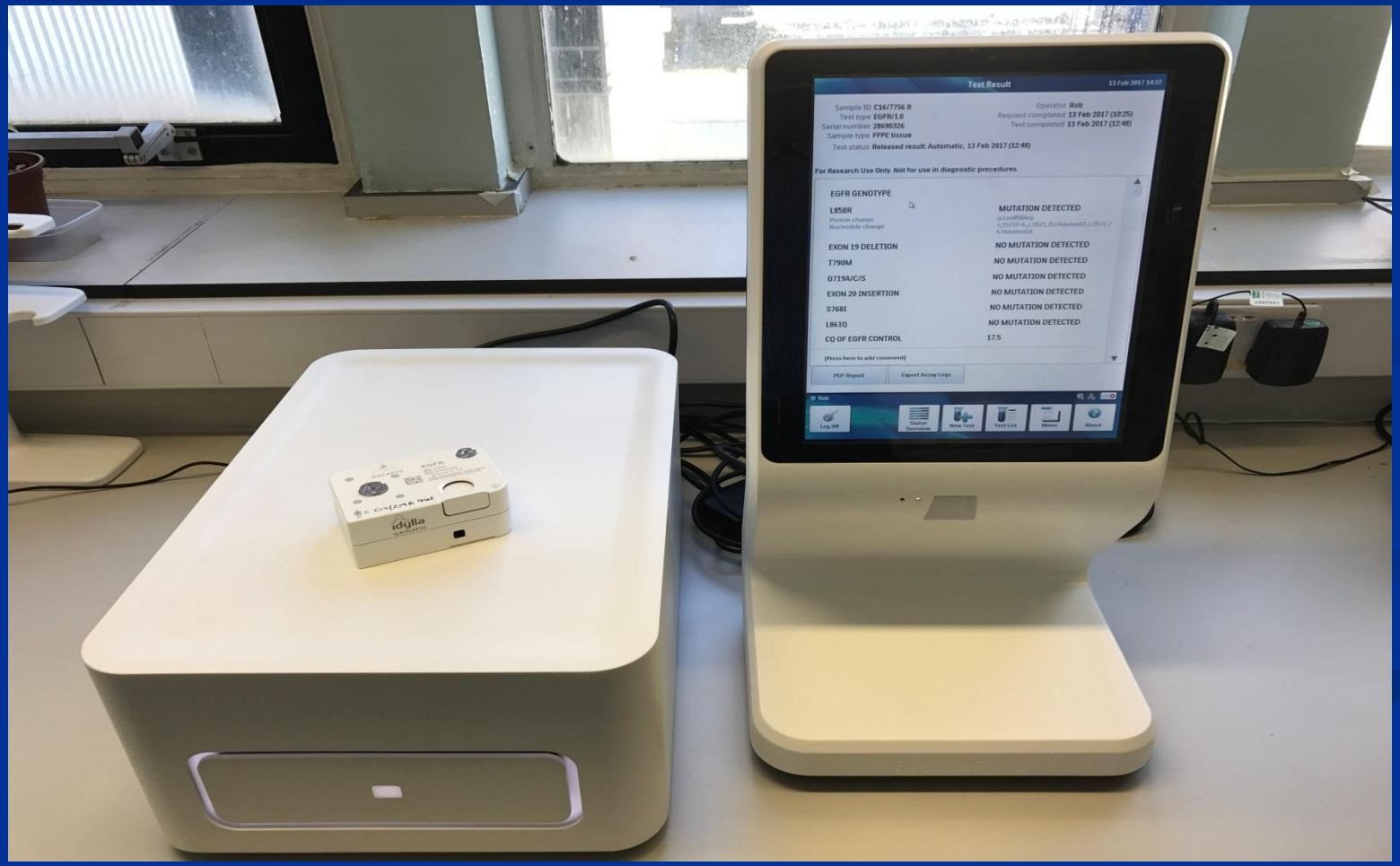




- p40/p63 for squamous differentiation
- TTF-1 for glandular differentiation

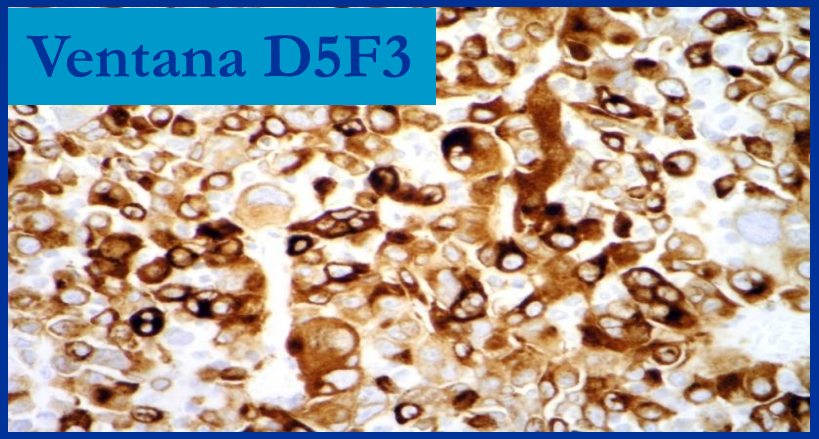
Accurate profiling

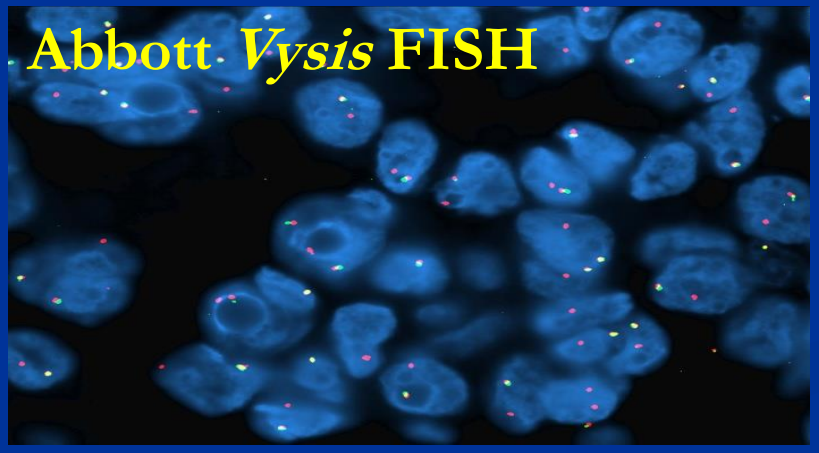
EGFR gene mutation



Accurate profiling

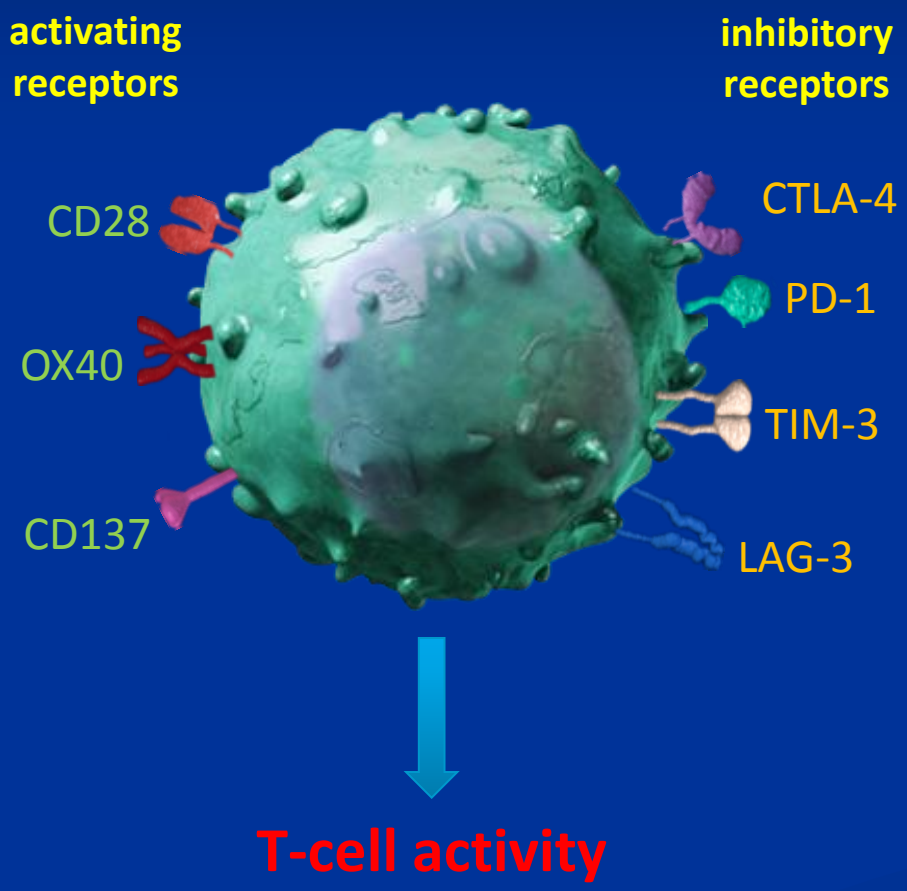
ALK gene rearrangement





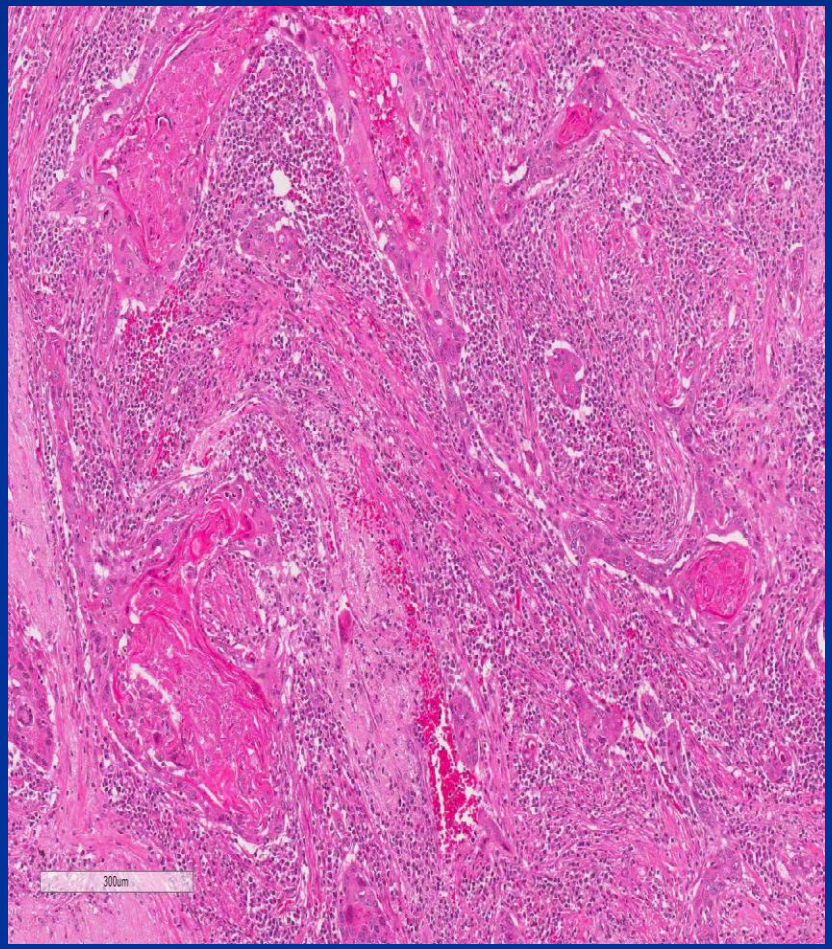
- FISH only
- Immunochemistry only
- Immunochemistry *and* FISH
- Immunochemistry for screening with FISH if immuno-positive
  - CAP/IASLC/AMP; NCCN; JLCS; RCPath UK

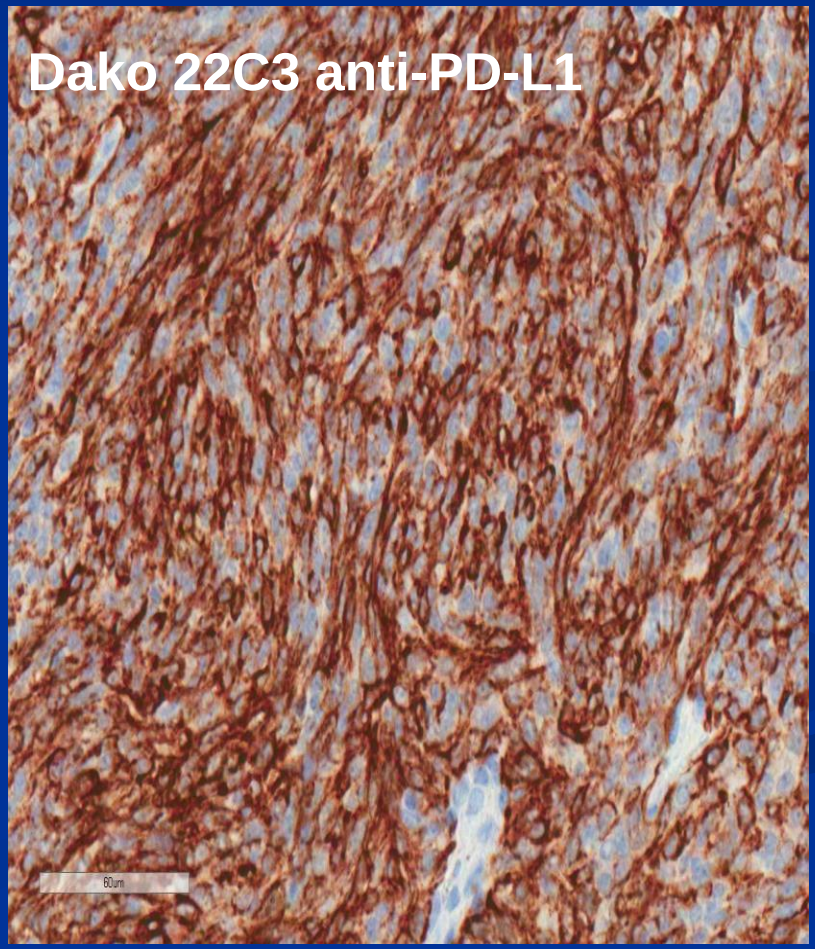
Immune checkpoints



- T-cell responses are regulated by a balance of activating and inhibitory 'checkpoint' signals
- Neoplastic cells protect themselves from immune attack by dysregulating these checkpoints
- Therapeutic targeting of these checkpoints restores the immune response and promote destruction of the neoplastic cells

PD-L1 expression confers protection

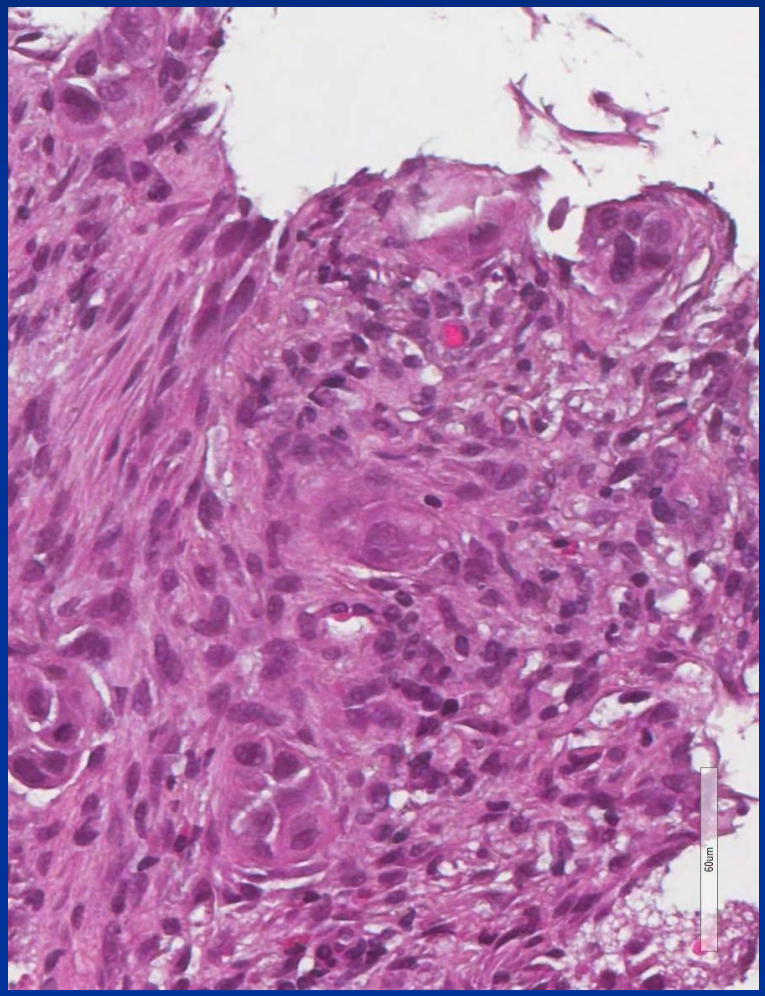


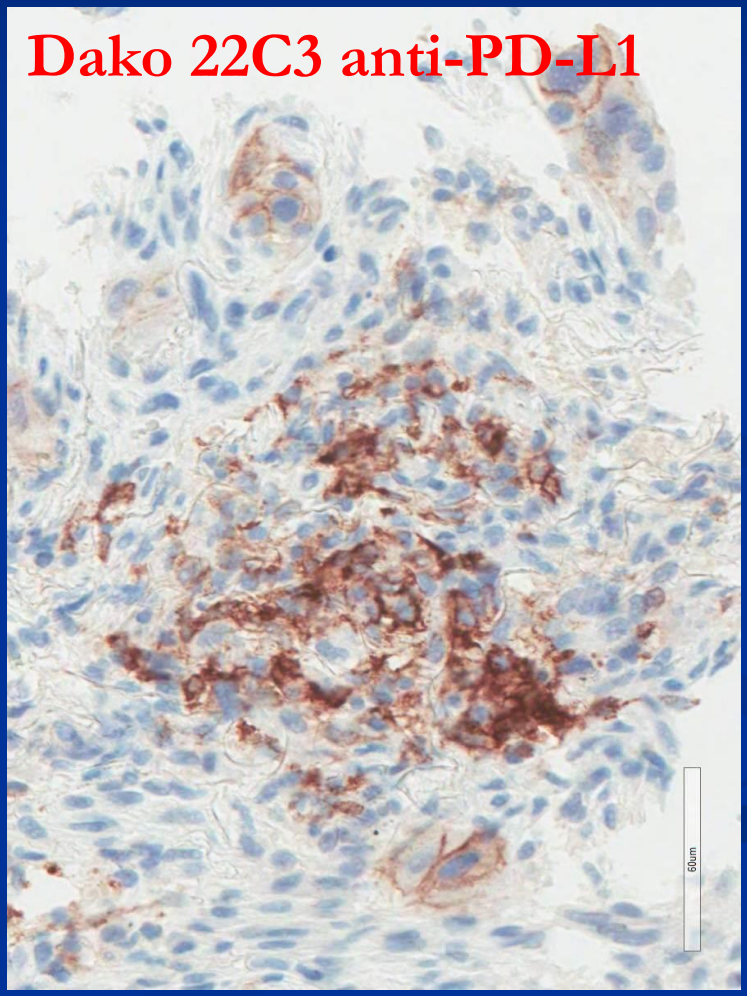


Multiple tests for PD-L1 expression

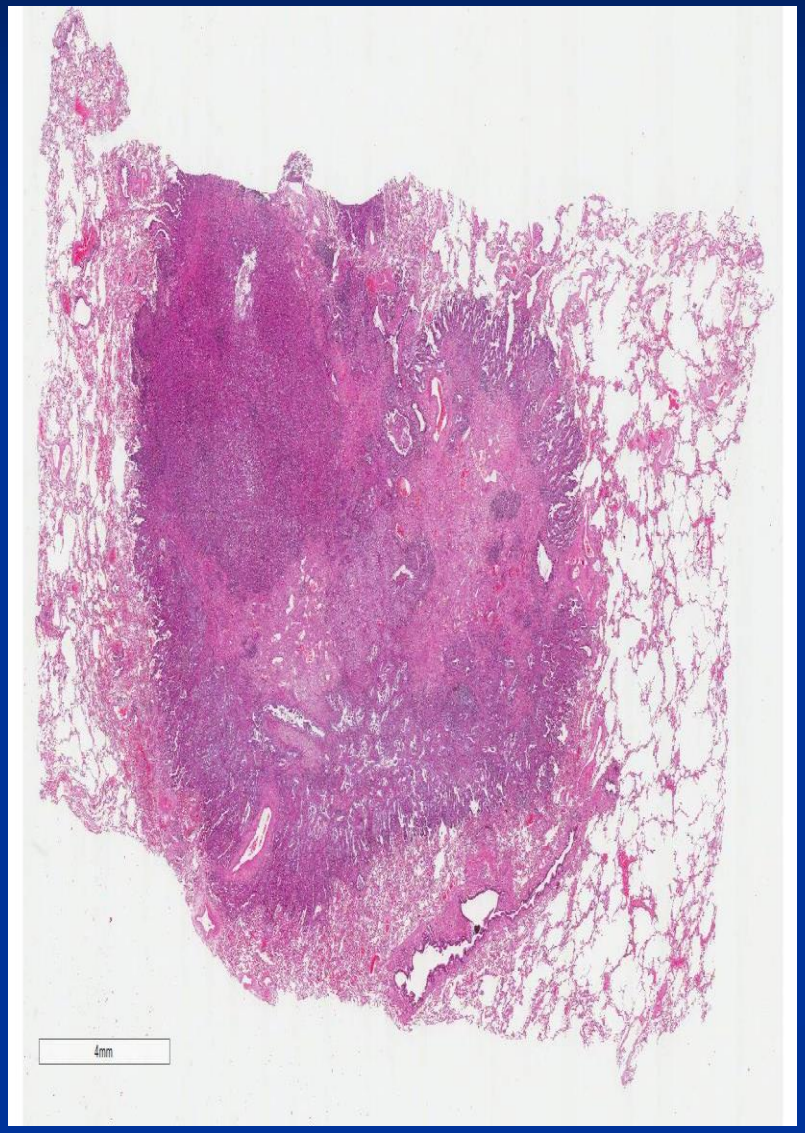
	Pembrolizumab Merck, Sharp & Dohme	Nivolumab Bristol- Myers Squibb	Durvalumab AstraZeneca	Atezolizumab Roche/ Genentech
TARGET	PD-1	PD-1	PD-L1	PD-L1
DETECTION	Dako 22C3	Dako 28-8	Roche SP263	Roche SP142
RELEVANT EXPRESSION	Surface of tumour cells	Surface of tumour cells	Surface of tumour cells	Surface of tumour cells and immune cells
CRITERIA FOR 'POSITIVITY'	≥ 1% or≥ 50% expression	≥1% expression	≥25% expression	TC expression 0-3: <1, 1-4, 5-49, ≥50; % of tumour infiltrated by PD-L1+ve ICs 0-3: <1, 1-4, 5-9, ≥10

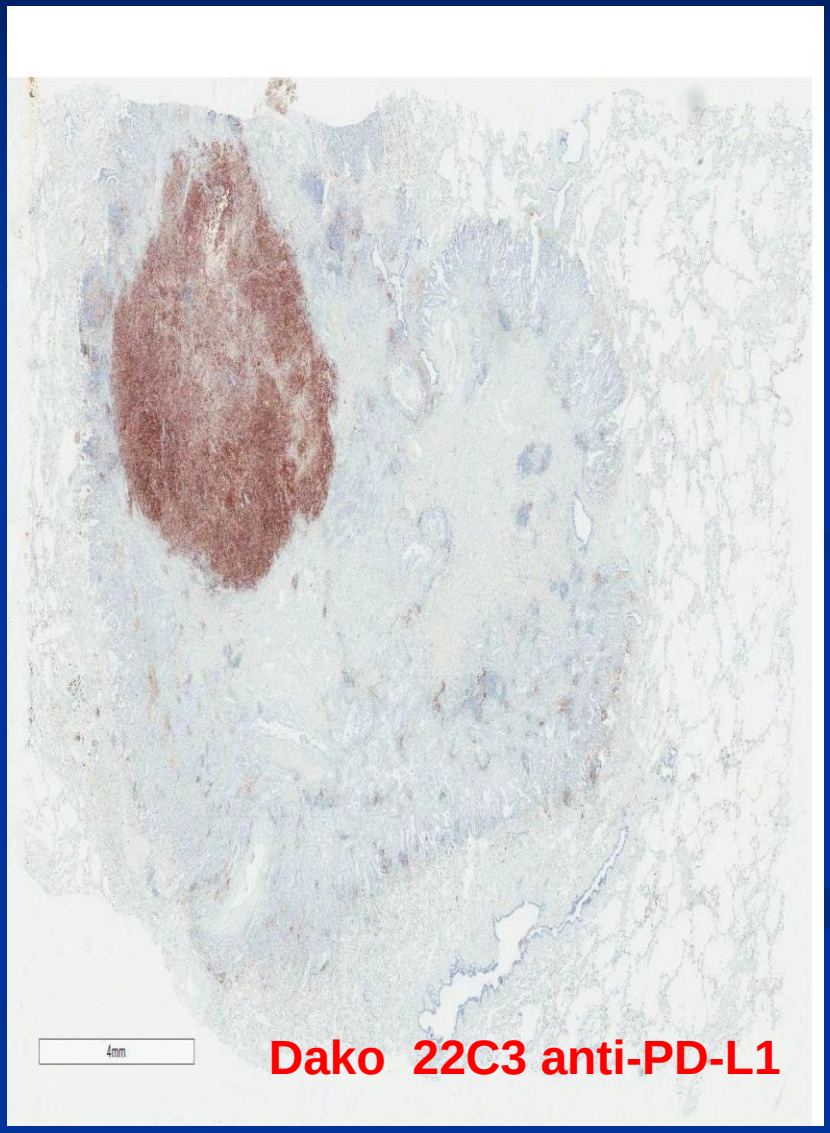
Interpretation



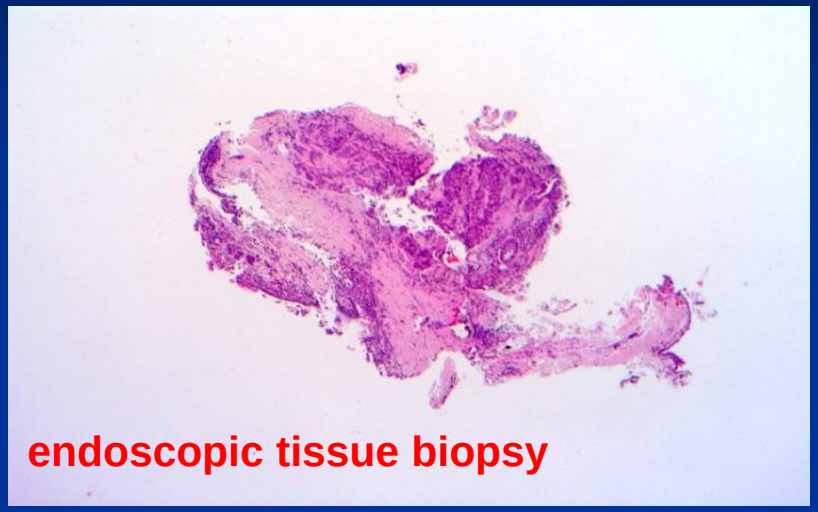


Heterogeneity of PD-L1 expression

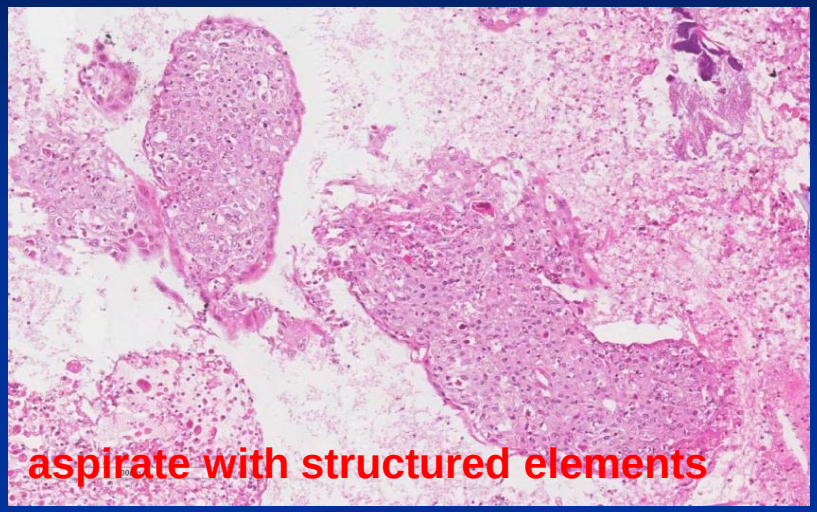




Suitable specimens

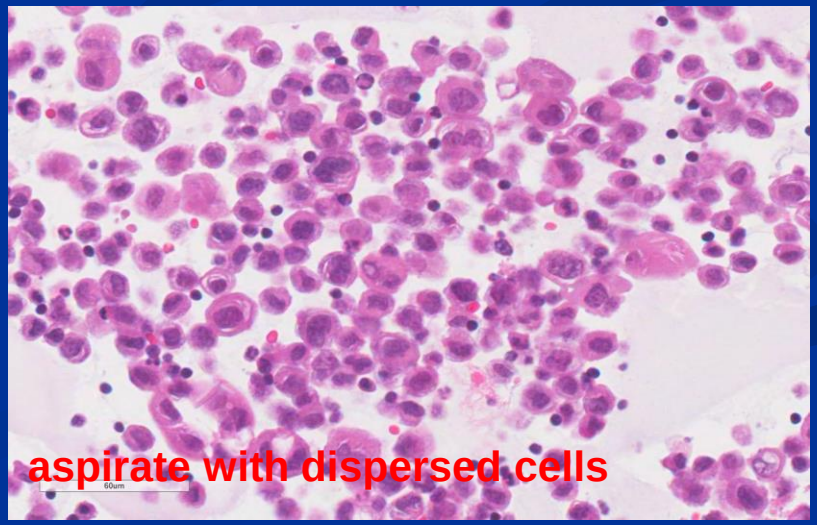


endoscopic tissue biopsy



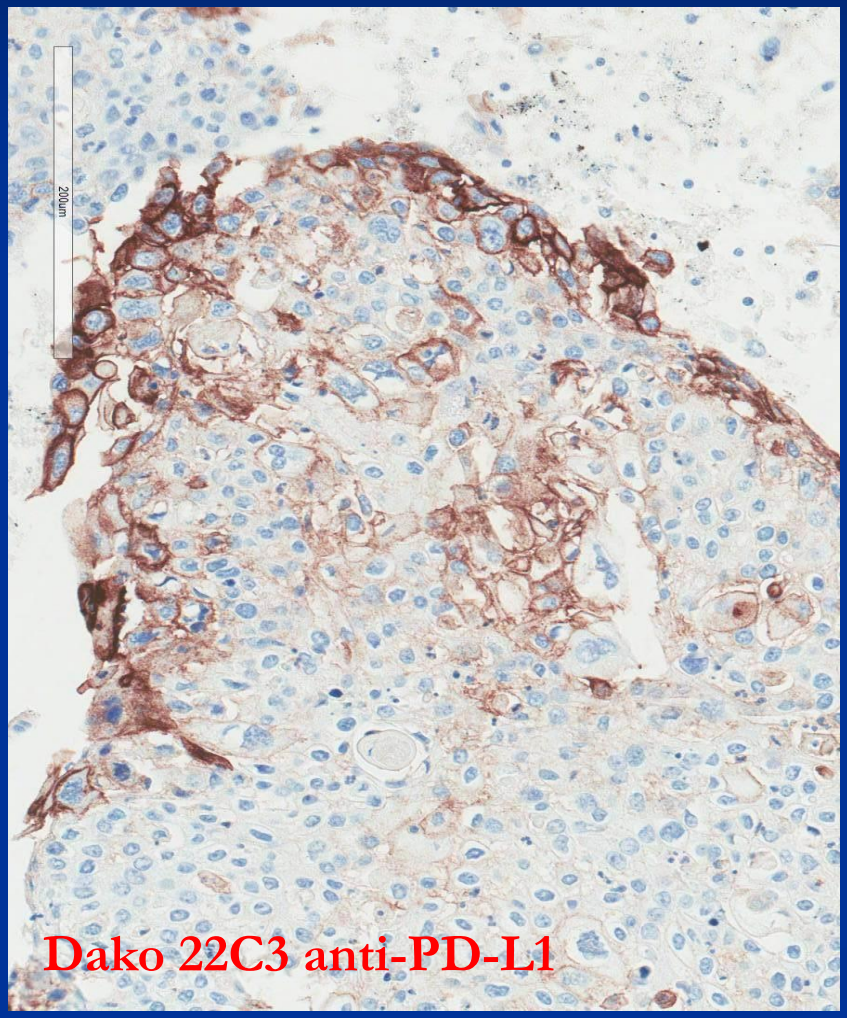
aspirate with structured elements

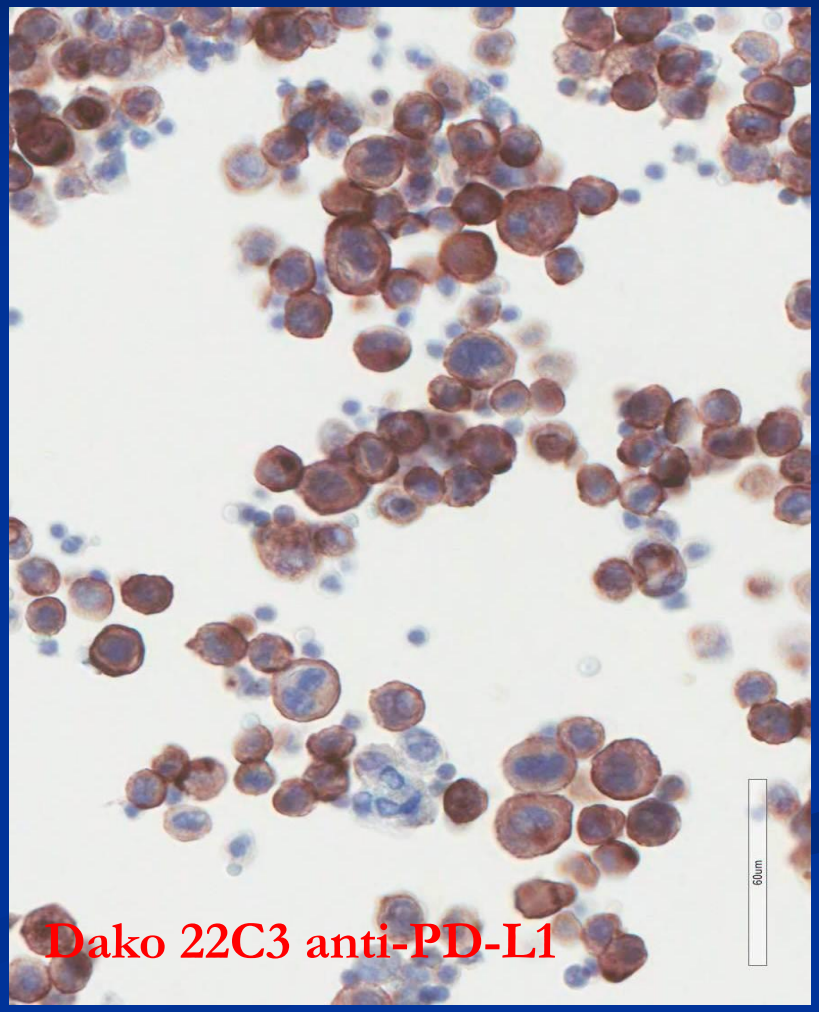
cutting needle tissue biopsy



aspirate with dispersed cells

’Cytology’ specimens





Bringing it together

What and when to test: automatic (reflex) testing?

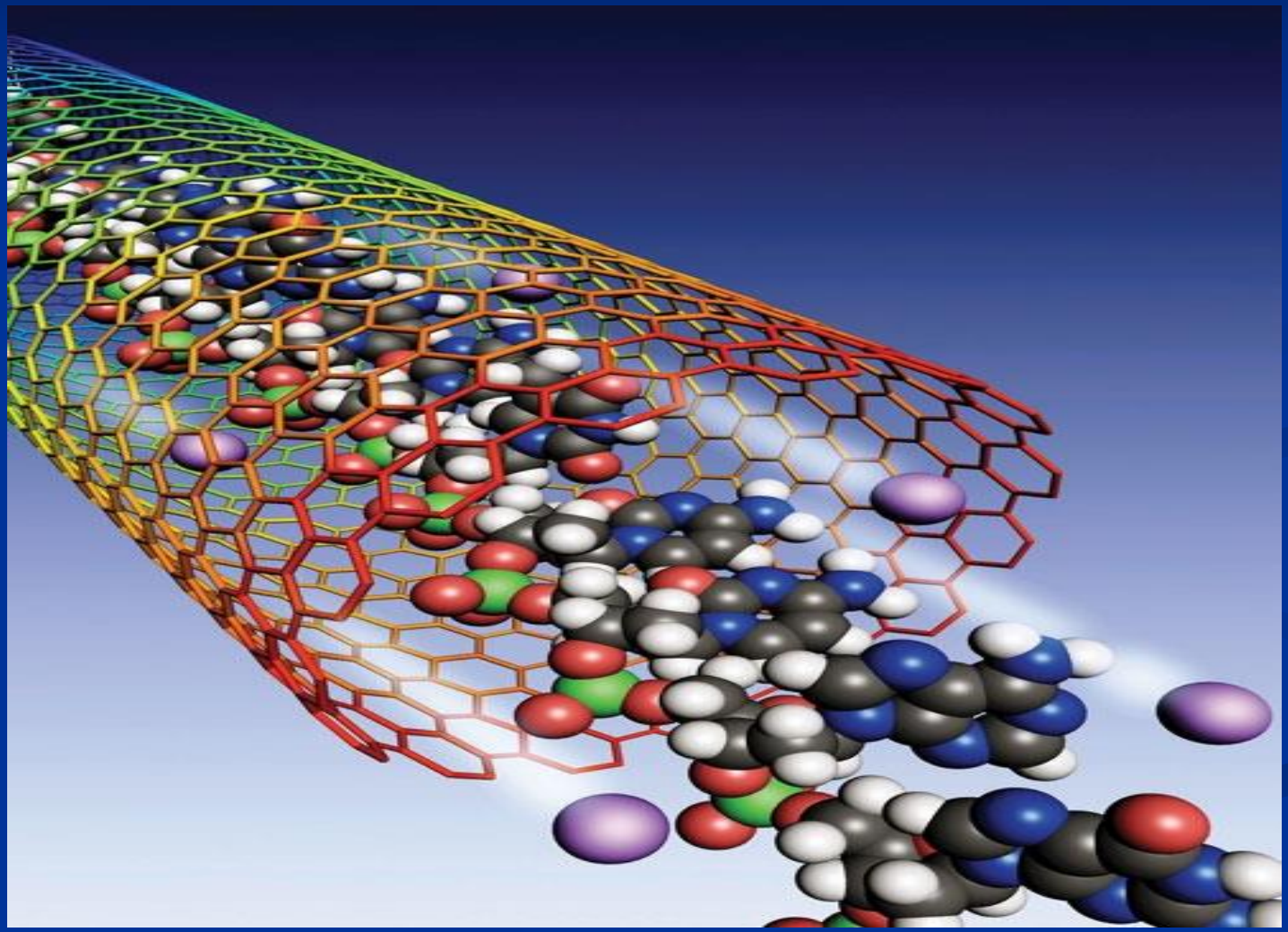
ADVANTAGES

- Saves time
- Provides clinicians with comprehensive information
- Permits ‘forward planning’
- Aids integration of information
- Establishes principle that predictive profiling is routine

DISADVANTAGES

- Costs more
- Information may be inappropriate to immediate management
- Information may be inappropriate when disease relapses

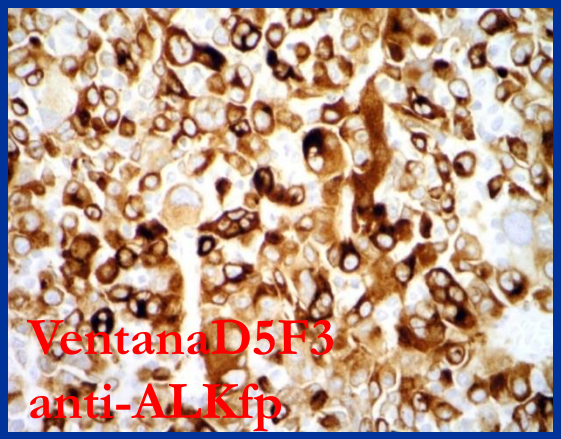
Gene profiling

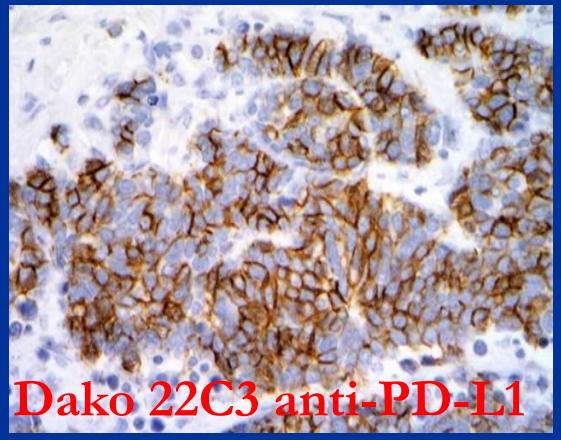


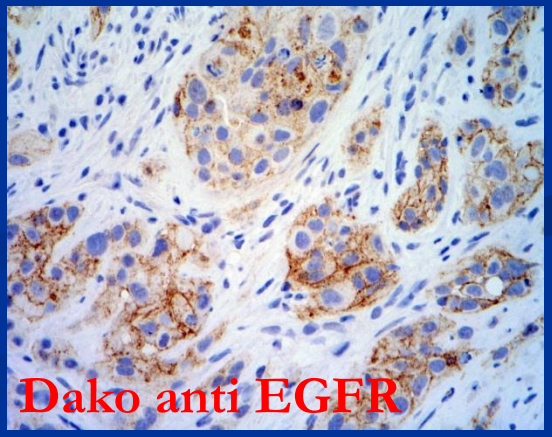
Analysis of blood samples

Protein expression in tissue sections

- ALK fusion protein
  - crizotinib, ceritinib, alectinib
- PD-L1
  - nivolumab, pembrolizumab, atezolizumab, durvalumab, avelumab
- EGFR
  - necitumumab







Have a nice day!