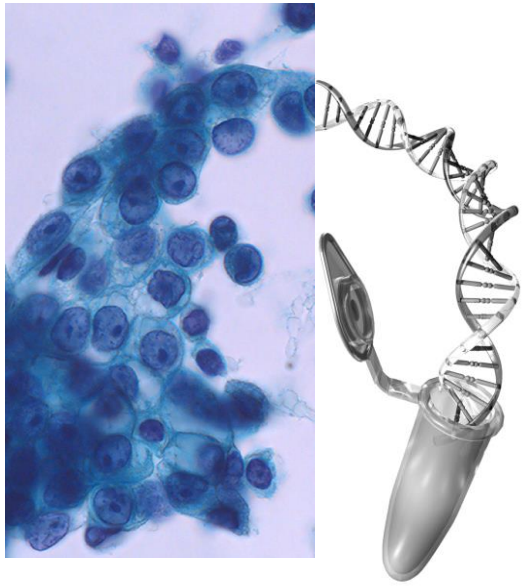
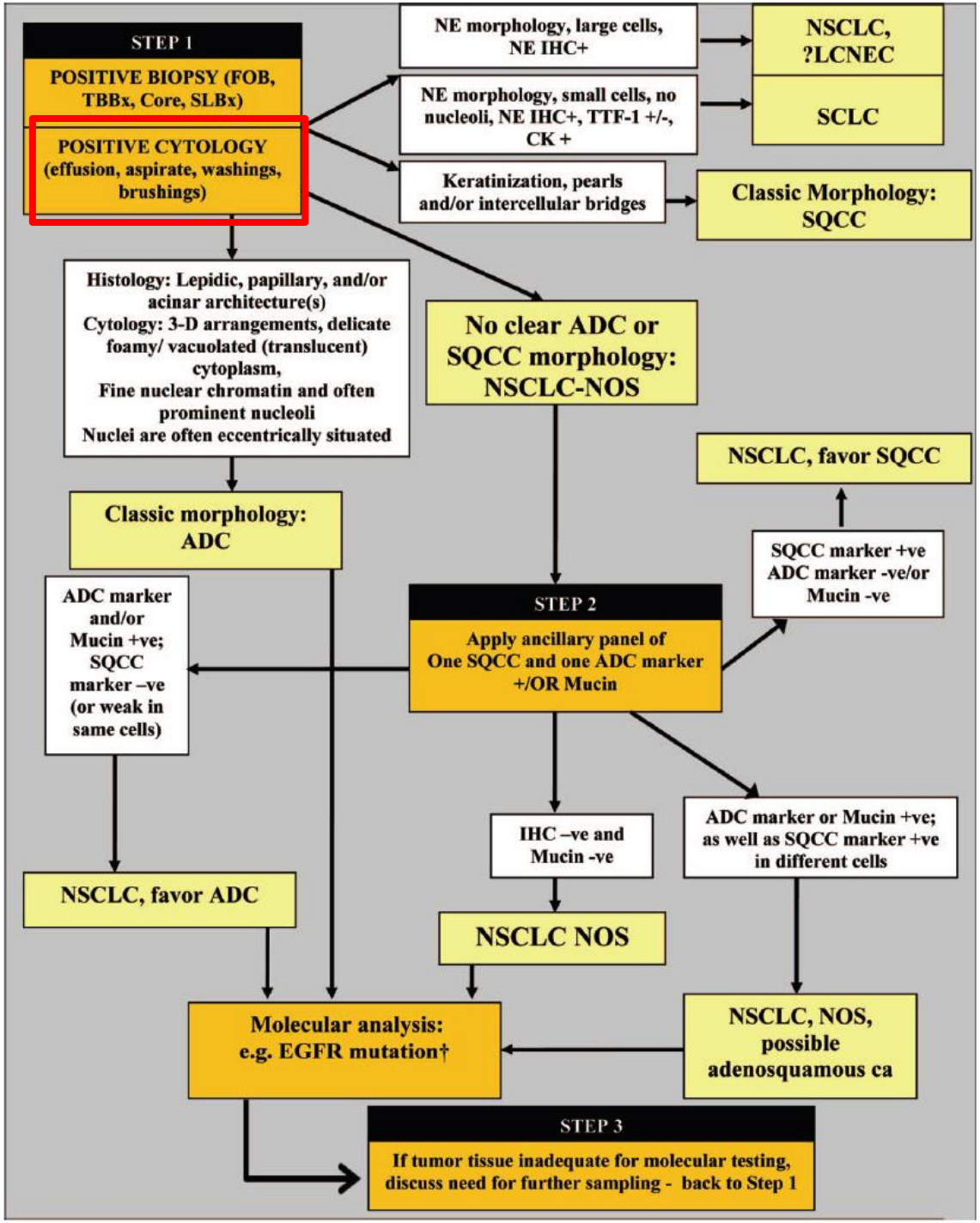
Biomarker testing on cytology samples



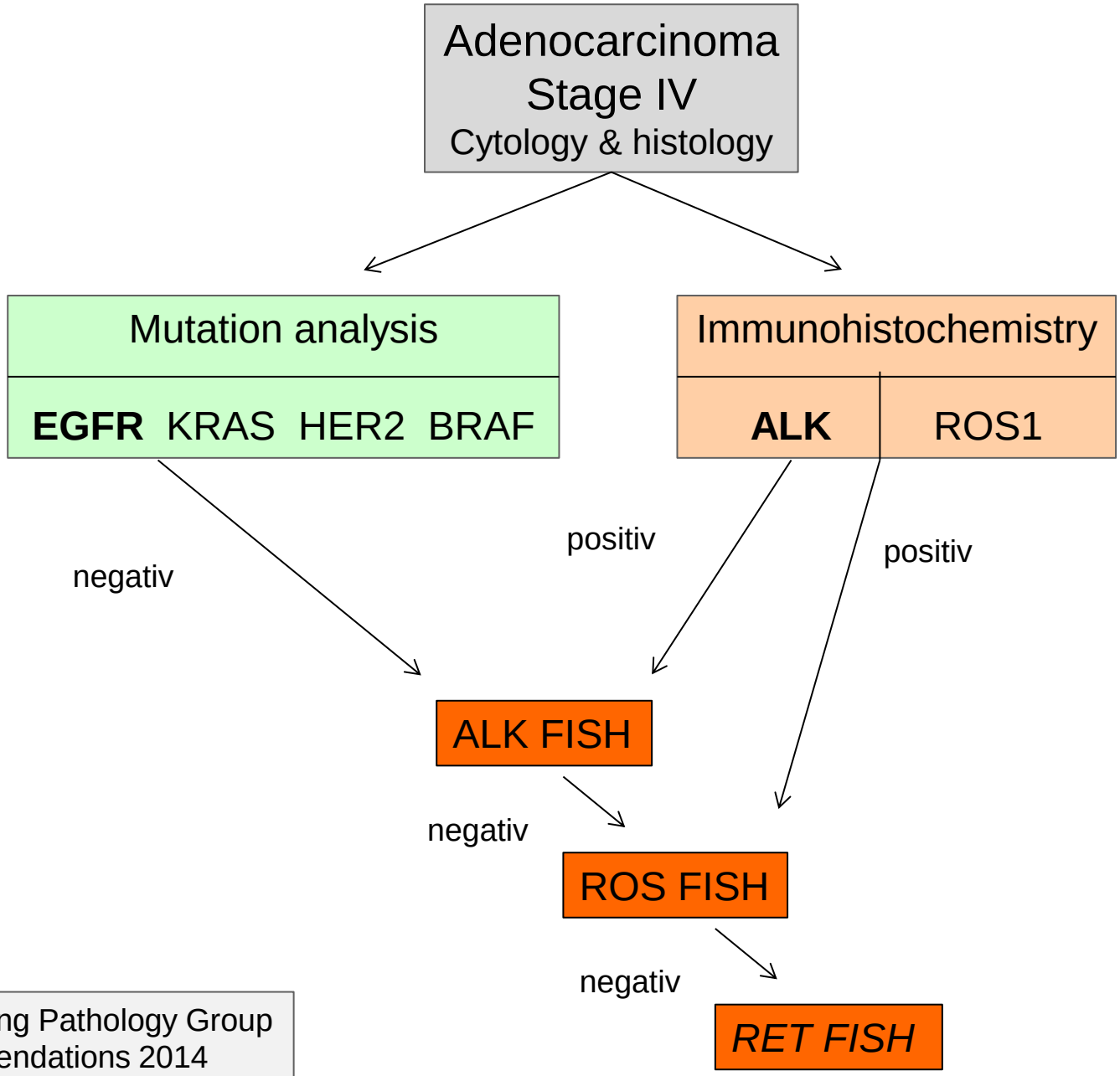
Lukas Bubendorf
Pathologie

Universitätsspital Basel



1/3 NSCLC diagnosed by cytology alone

Travis WD et al,
JTO 2011;6:244-85



Swiss Lung Pathology Group
Recommendations 2014

Starting material

- FNA (TBNA, distant metastases)
- Effusions

- Smears
- Cytospins
- Liquid-based preparation
- Cell blocks

Smear

Cell block

Biopsy

Almost equal….

Same tumor cells – different format & fixation

Cytology

- Ethanol / air-dried
- Cell-blocks
- Retained nuclei
- Adapted protocols

Histology

- Paraffin
- Stroma
- Truncated nuclei
- Histo protocols

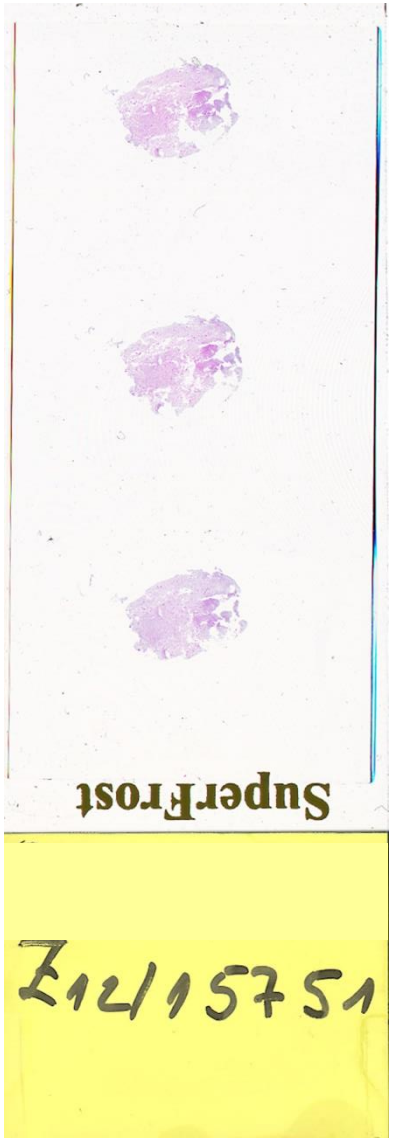
- Many surgical pathologists feel uneasy with cytology
- Specialized cytopathologists do not see histology

Cell block

Cell block

Coagulation (Thrombin)

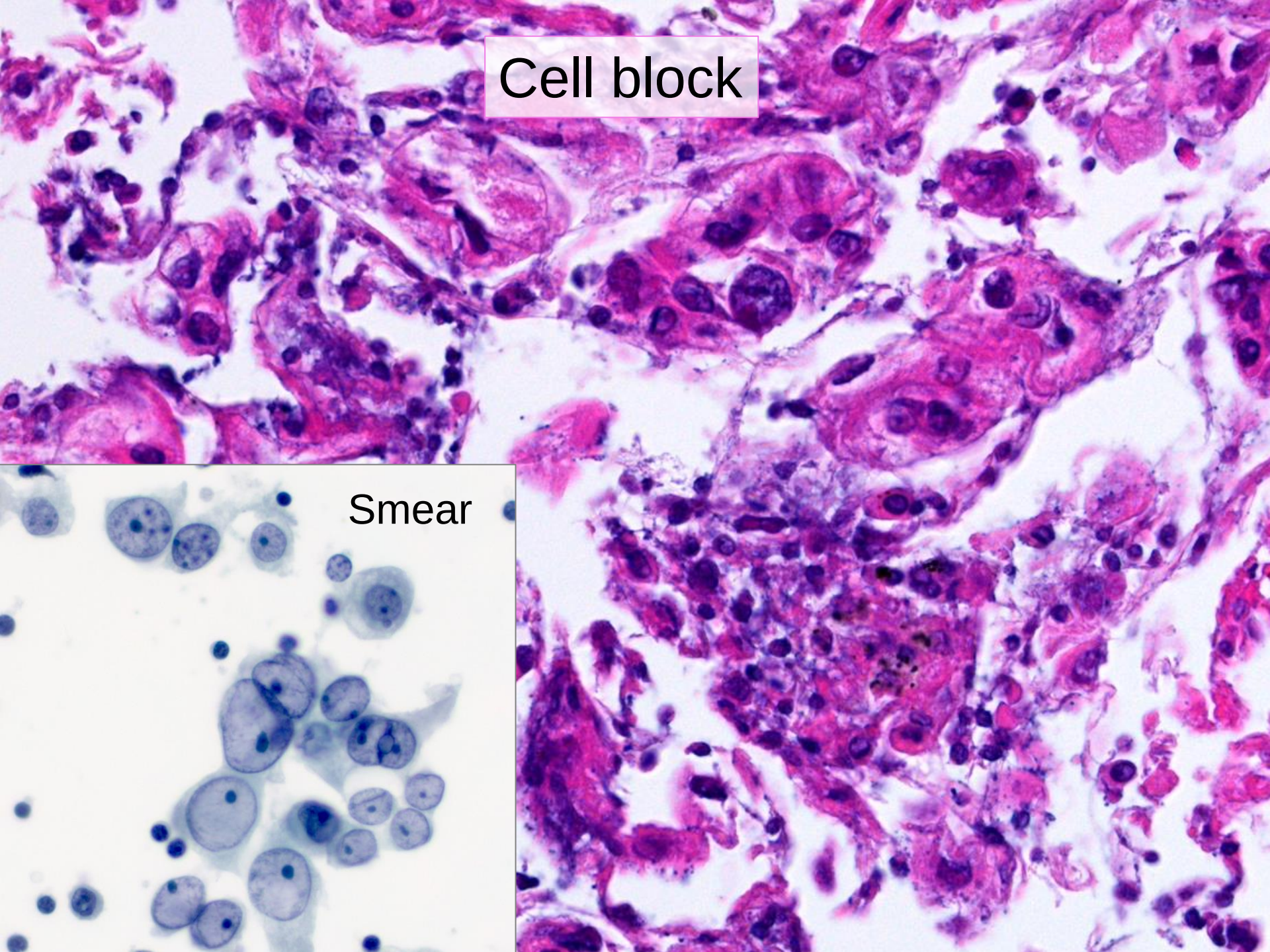
FFPE cell block



FFPE protocols for FISH und IHC
Conservation of DNA und proteins

DNA quality & morphology↓
Not always available

Jain D et al, Cytopathol 2014;25:356-71

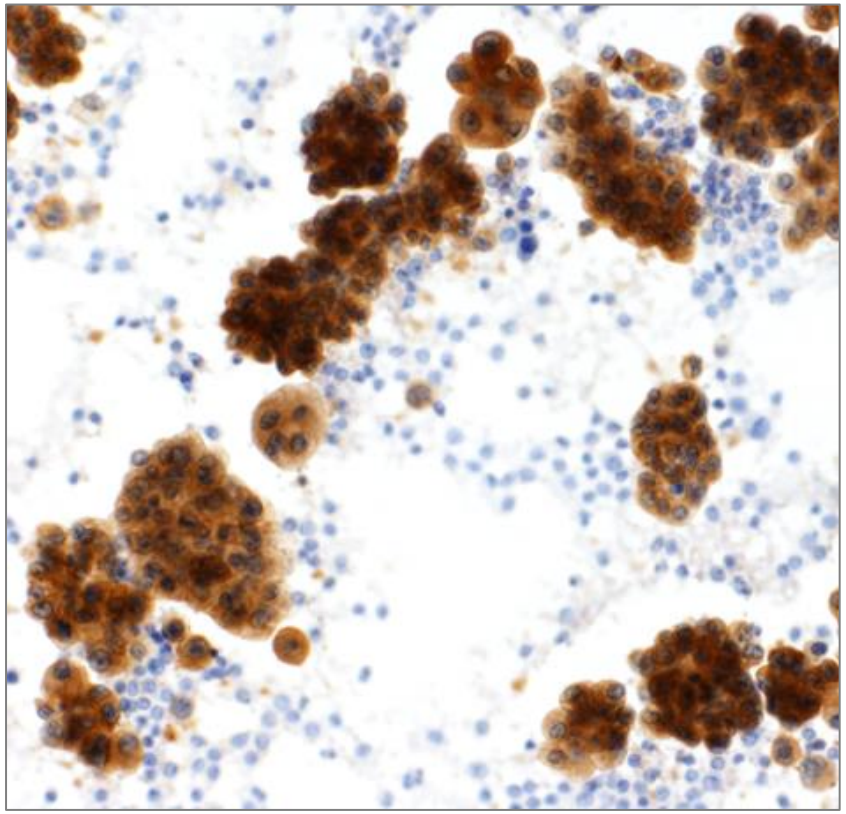

Cell block
Smear

Methods for biomarker analyses
(cytology & histology)

- Immunocytochemistry
- Fluorescence *in-situ* Hybridization (FISH)
- Mutation analysis (e.g. NGS)

Immunocytochemistry

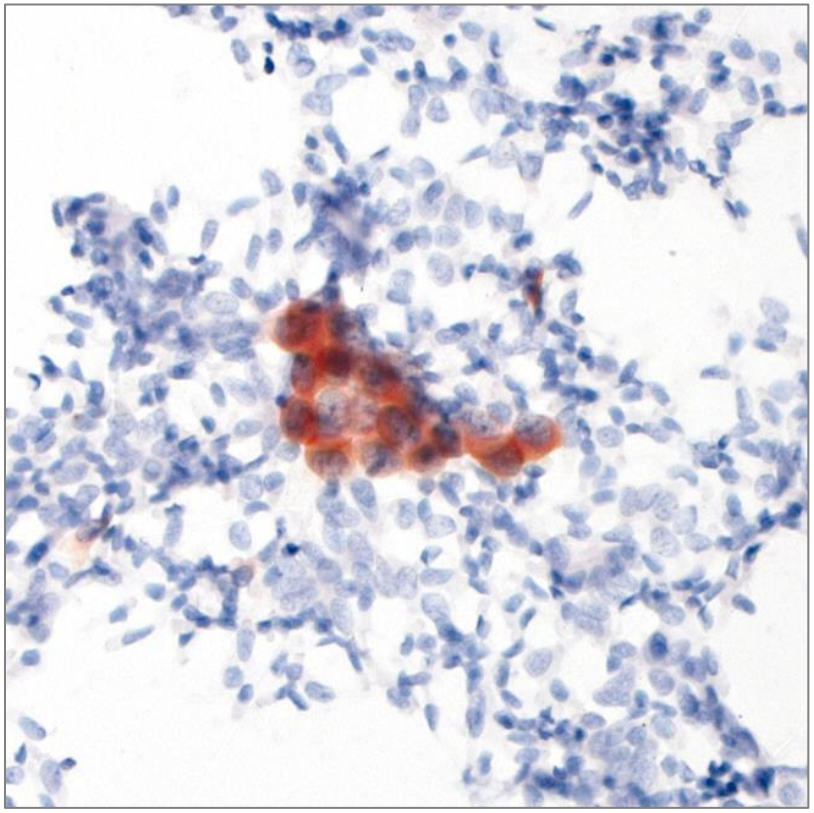
ALK



5A4 antibody, Novocastra

Savic et al, *J Thorac Oncol* 2013

ROS1



D4D6 Antibody, Cell Signaling

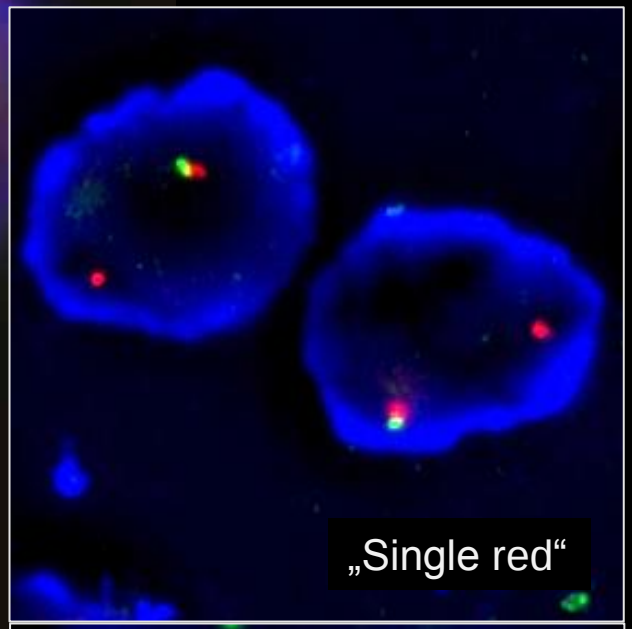
⇨ Very accurate (sensitivity -100%, variable specificity)

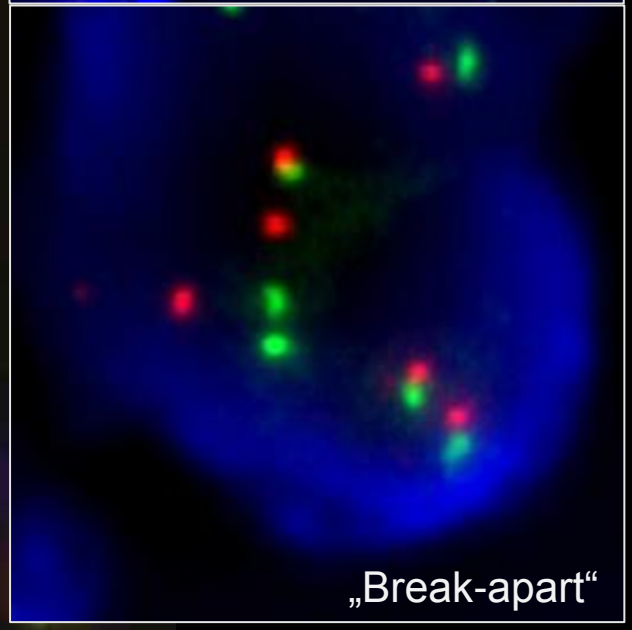
ALK FISH
(Break-apart Probe)

1.33

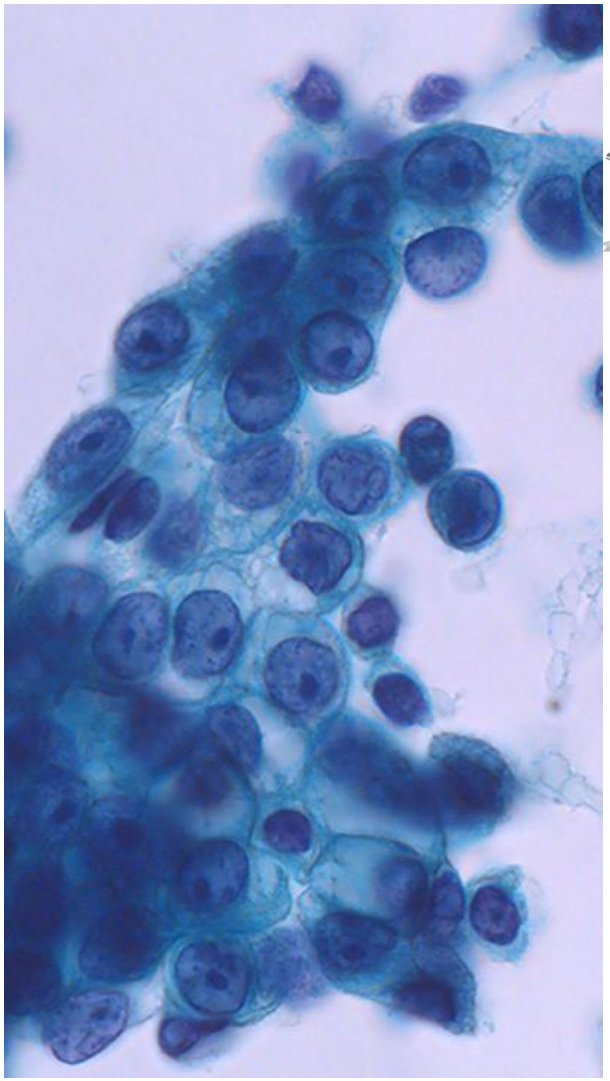
No Rearrangement (ALK-)

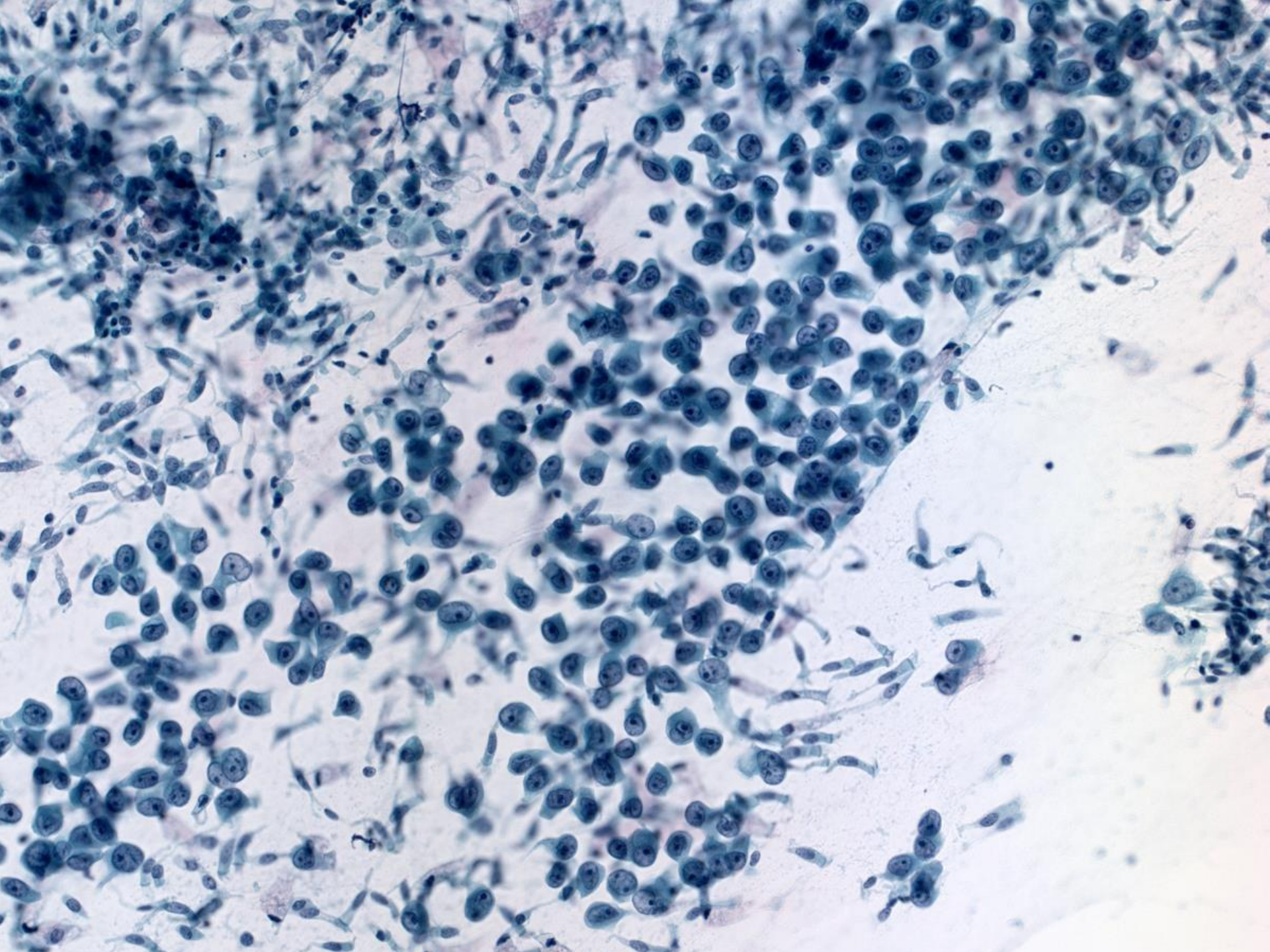
ALK+ examples





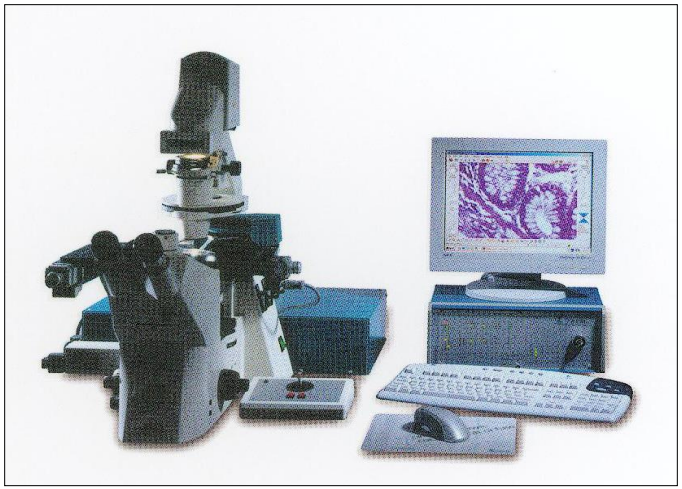
DNA extraction for mutation analysis

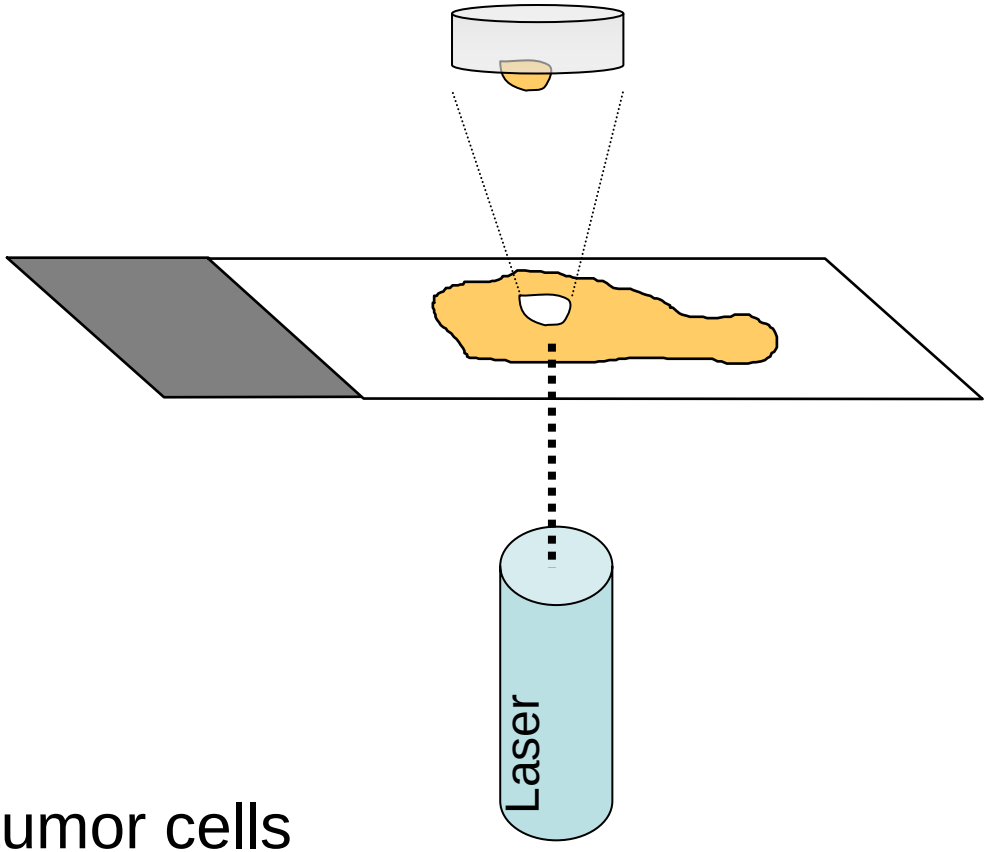




Laser Mikrodissection „Laser Pressure Catapulting“

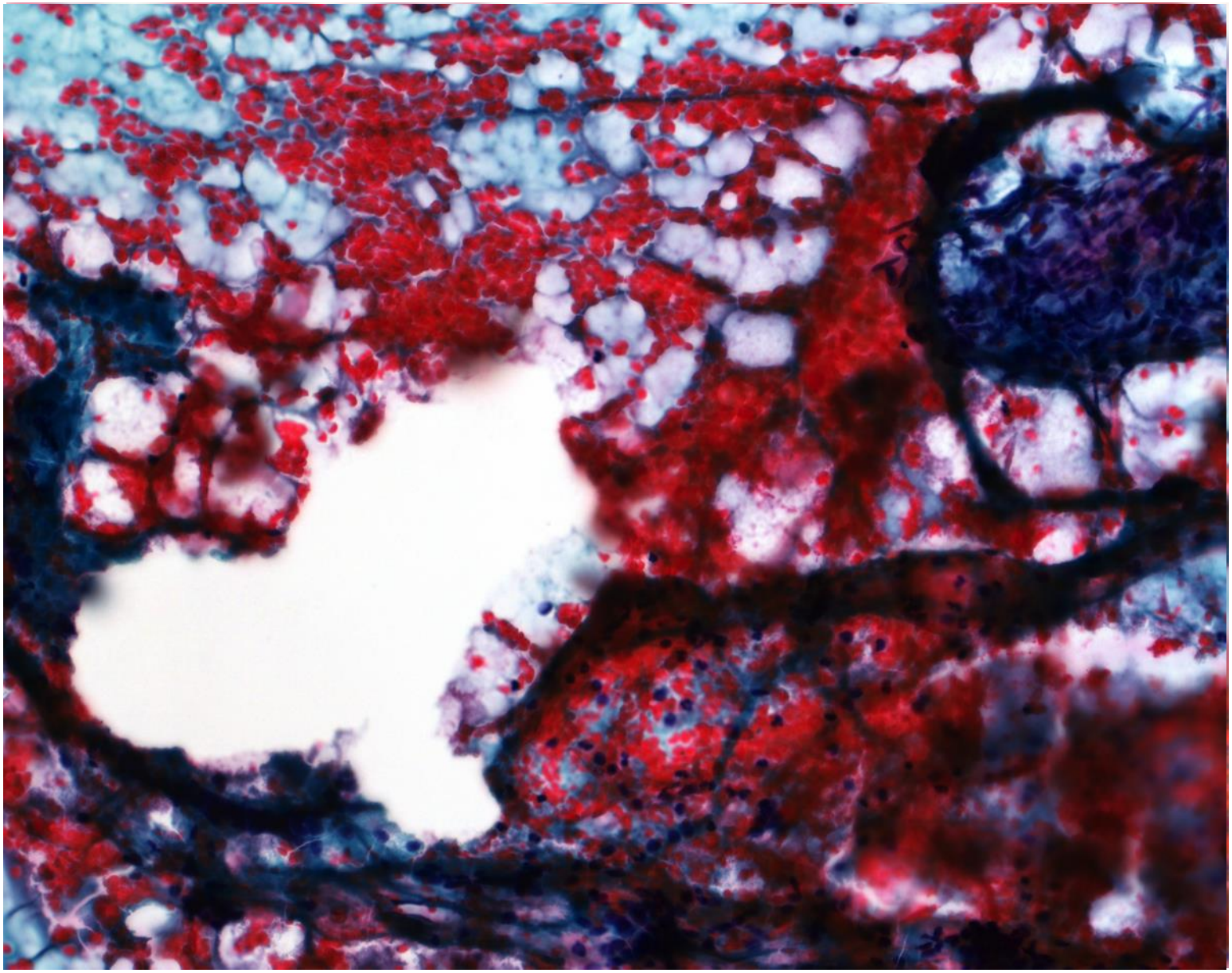
<20-30% tumor cell content





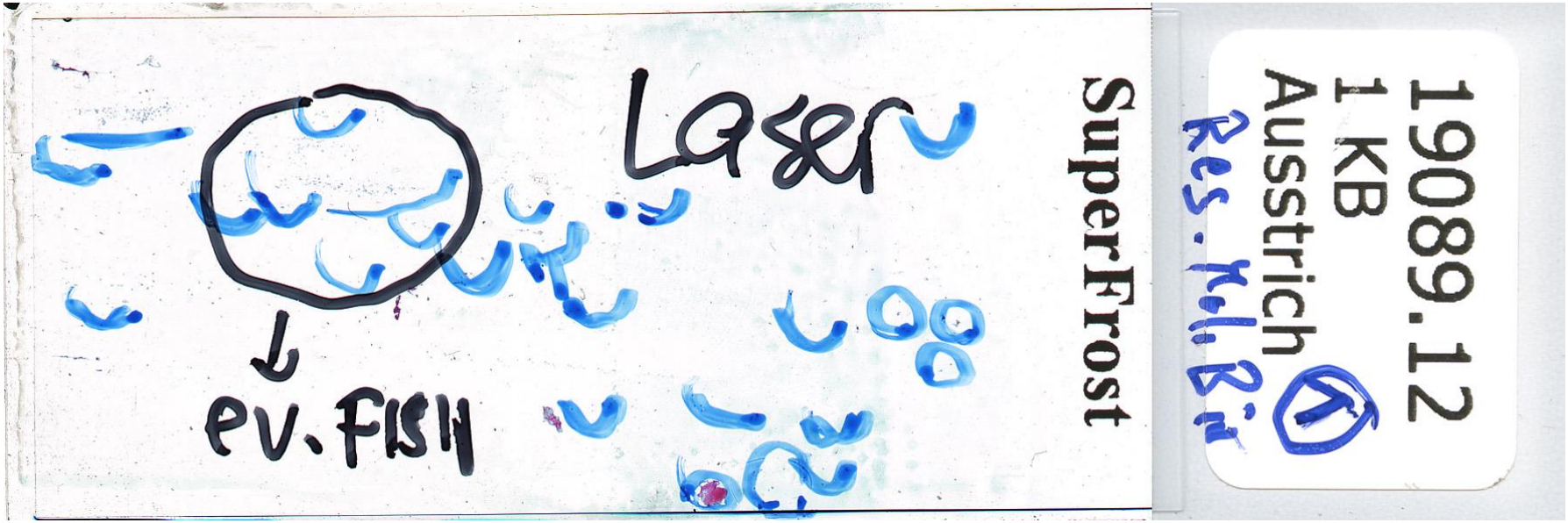
At least 50-100 tumor cells
Ideally >200

Savic et al, Br J Cancer 2008




PS4

Sequential biomarkers analyses on one cytological specimen



ICC
(TTF1, ALK)

→

LCMD

→

FISH
(ALK, ROS1)

Biomarker analysis in cytology

- new tasks -

- Selection of appropriate specimens
- Marking of areas and cell groups
- Estimate percentage of tumor cells
- Fill order form
- Interpretation of results
- Report to clinician

Next-generation sequencing (NGS)

MiSeq Personal Sequencer
(Illumnia)

Ion Proton

Diagnostic biopsies
(FFPE)
& Cytology

10ng DNA

Cancer Hot Spot Panel (50 Genes)

PGM IonTorrent (Life Technol.)

MODERN PATHOLOGY (2014) 27, 314–327

Methods in Pathology

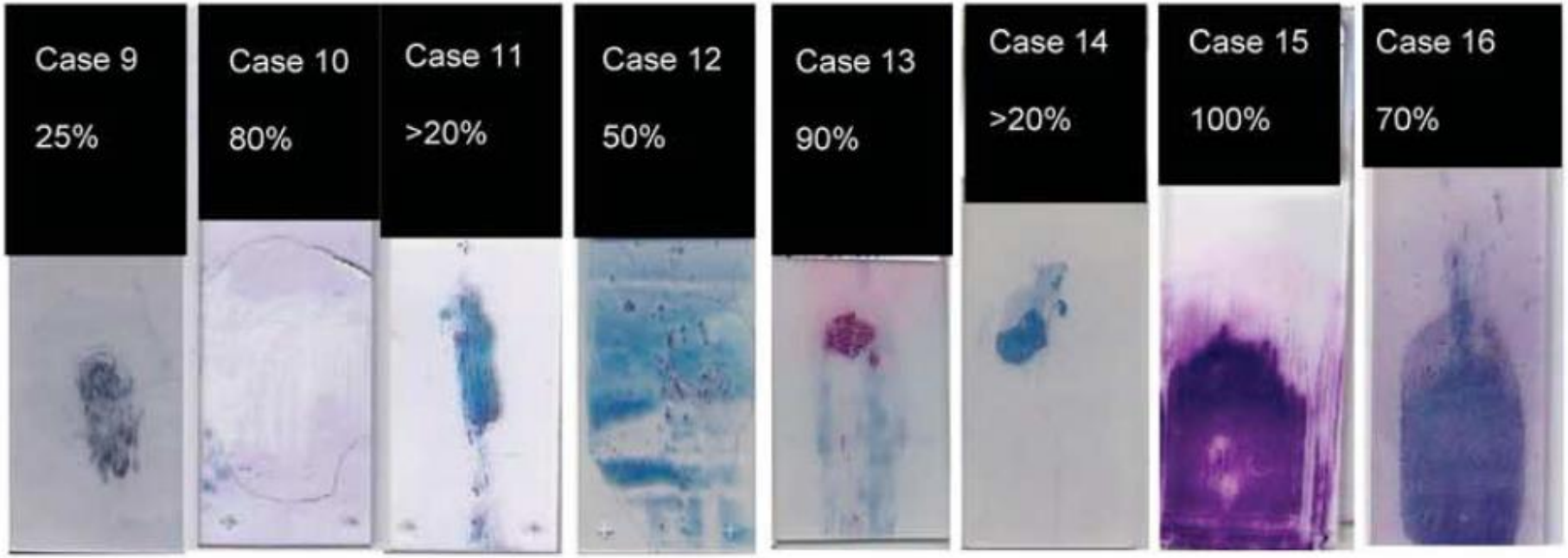
Next-generation sequencing-based multi-gene mutation profiling of solid tumors using fine needle aspiration samples: promises and challenges for routine clinical diagnostics

Rashmi Kanagal-Shamanna, Bryce P Portier, Rajesh R Singh, Mark J Routbort, Kenneth D Aldape, Brian A Handal, Hamed Rahimi, Neelima G Reddy, Bedia A Barkoh, Bal M Mishra, Abhaya V Paladugu, Jawad H Manekia, Neda Kalhor, Sinchita Roy Chowdhuri, Gregg A Staerkel, L Jeffrey Medeiros, Rajyalakshmi Luthra and Keyur P Patel

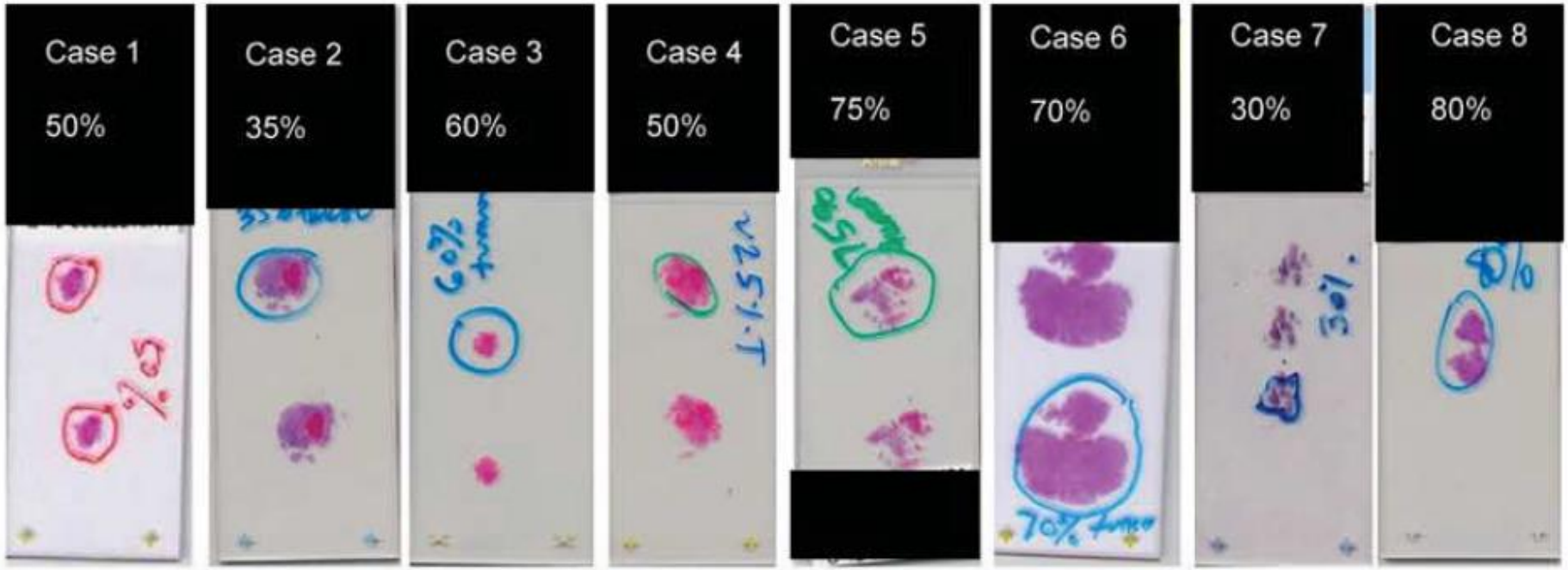
Department of Hematopathology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

→ NGS works well from cytological specimens

Kanagal-Shamanna R eta l, Mod Pathol 2014;27:314-27

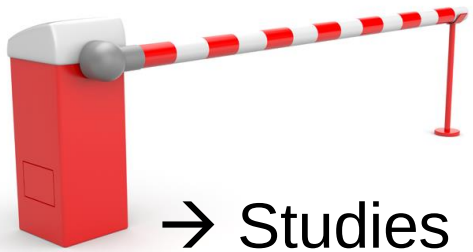


Representative slide images of the FNA smears

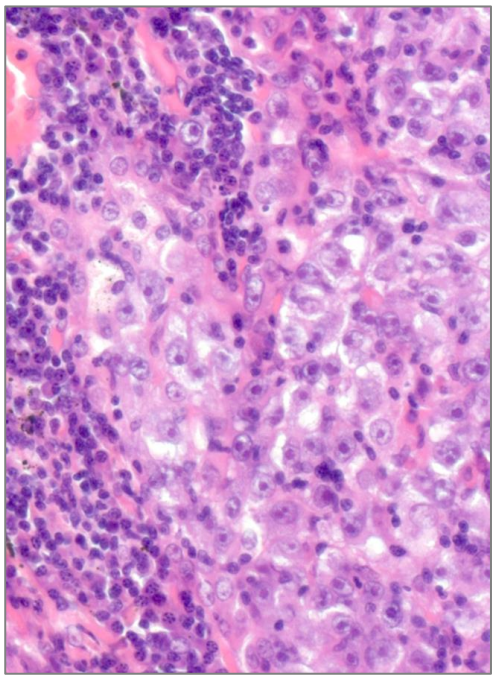


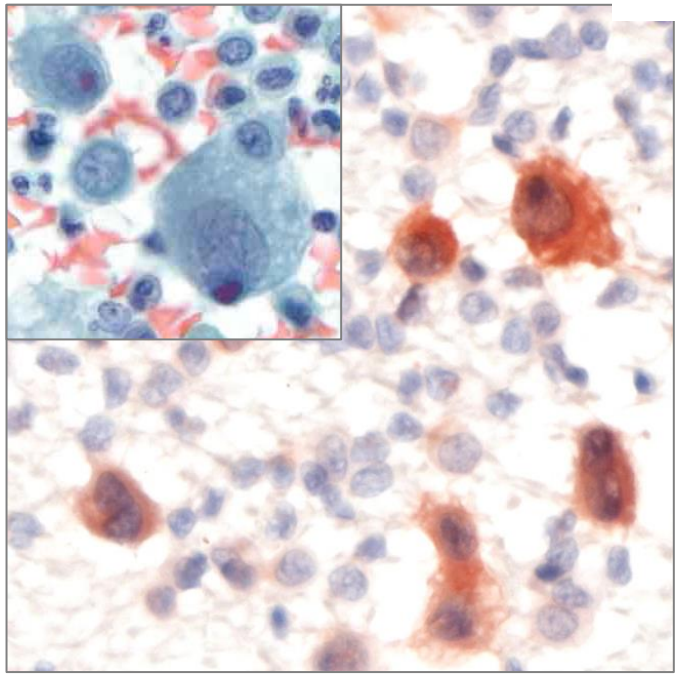
What about PD-L1 in cytology?

- Immune cells
- Tumor cells

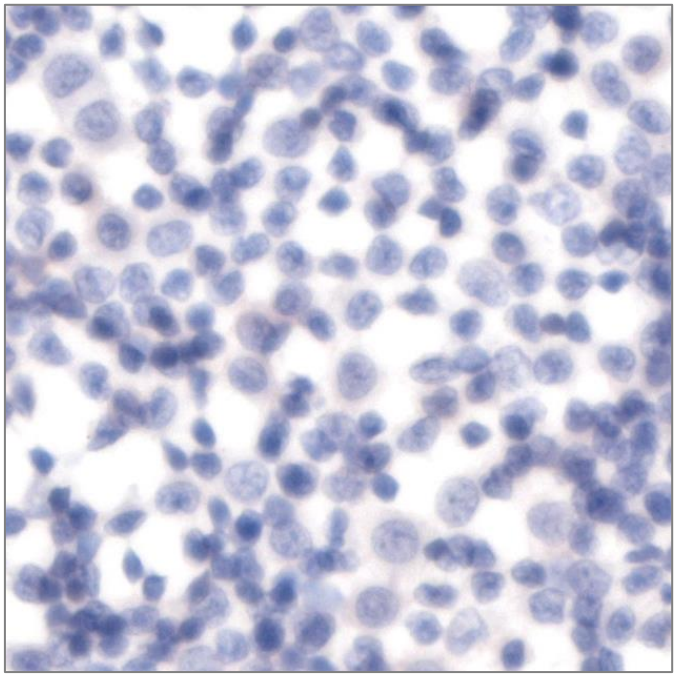


→ Studies needed





PD-L1+ adenocarcinoma



Benign effusion

E1L3N™ XP® (Cell Signaling)

Summary

- Review of all specimens of one patient for prioritization: one pathologist.
- „Management of tumor specimens“
- (Almost) all biomarker analyses are equally applicable to both cytology and histology
- Minor changes in protocols
- Expertise in cytology
- Well-trained cyto-technicians
- EQA mandatory


Thank you!