Biomarker testing on cytology samples
1/3 NSCLC diagnosed by cytology alone

Travis WD et al, JTO 2011;6:244-85
Adenocarcinoma Stage IV
Cytology & histology

Mutation analysis
EGFR, KRAS, HER2, BRAF
negativ

Immunohistochemistry
ALK, ROS1
positiv

ALK FISH
negativ

ROS FISH
negativ

RET FISH
negativ

Swiss Lung Pathology Group Recommendations 2014
Starting material

- FNA (TBNA, distant metastases)
- Effusions
- Smears
- Cytospins
- Liquid-based preparation
- Cell blocks
Smear  |  Cell block  |  Biopsy

[Images of smear, cell block, and biopsy samples]

- Smear: A stained smear sample.
- Cell block: A sectioned cell block with visible cells.
- Biopsy: A biopsy sample with labeled sections.

Labels:
- Smear: Z12.15751
- Cell block: Superfrost Z12/15751
- Biopsy: Superfrost B12/31525
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

Coagulation (Thrombin)

FFPE protocols for FISH and IHC
Conservation of DNA and proteins

DNA quality & morphology↓
Not always available

Cell block

Smear
Methods for biomarker analyses
(cytology & histology)

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

ALK

ROS1

5A4 antibody, Novocastra
Savic et al, J Thorac Oncol 2013

D4D6 Antibody, Cell Signaling

Very accurate (sensitivity -100%, variable specificity)
ALK FISH
(Break-apart Probe)

No Rearrangement (ALK-)

ALK+ examples

„Single red“

„Break-apart“
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
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)

Diagnostic biopsies (FFPE) & Cytology
10ng DNA

Cancer Hot Spot Panel (50 Genes)

Ion Proton

PGM Ion Torrent (Life Technol.)
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


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!