BIOPSY - KEY TO THE DIAGNOSTICS INITIALLY AND AT PROGRESSION

Erik Thunnissen MD PhD

Consultant Pathologist
Department of Pathology,
VU University Amsterdam, Amsterdam, NL
e.thunnissen@vumc.nl
Disclosures

- I have acted as consultant for MSD, Pfizer, Clovis, BMS, AZ
- I have received honoraria for speaker AZ, Pfizer, Roche trainer SP142
- The VUmc received Grants: IIR Pfizer, Astra Zeneca
Diagnosis of lung cancer
- Large cell carcinoma
- Small samples
Predictive analysis
- EGFR
- ALK
- ROS1
- MET
- PD-L1
Tissue management
Lung Cancer Classification and sample type

WHO 1967-2015: intended for, and only applicable to, resected cases

- Small Cell Carcinoma
- Squamous Cell Carcinoma
- Adenocarcinoma
- Large cell carcinomas
- Sarcomatoid carcinomas
- Adenosquamous carcinomas
- Carcinoid tumours
- Salivary-type carcinomas

WHO 2015: a simplified classification intended for small sample diagnosis

- Small Cell Carcinoma
- Squamous Cell Carcinoma
  - Probable Squamous Cell Ca
- Adenocarcinoma
  - Probable Adenocarcinoma
- NSCLC-NOS
  - NSCLC-NOS (null IHC)
- Carcinoid tumour
- Salivary-type (occasionally)
Lung Cancer Classification and sample type

**WHO 1967-2015: intended for, and only applicable to, resected cases**
- Small Cell Carcinoma
- Squamous Cell Carcinoma
- Adenocarcinoma
- Large cell carcinomas
- Sarcomatoid carcinomas
- Adenosquamous carcinomas
- Carcinoid tumours
- Salivary-type carcinomas

**WHO 2015: a simplified classification intended for small sample diagnosis**
- Small Cell Carcinoma
- Squamous Cell Carcinoma
  - Probable Squamous Cell Carcinoma
- Adenocarcinoma
  - Probable Adenocarcinoma
- NSCLC-NOS
  - NSCLC-NOS (null IHC)
- Carcinoid tumour
- Salivary-type (occasionally)
Well Differentiated Squamous Cell Ca
Poorly Differentiated Squamous Cell Ca
Non-keratinising Squamous Cell Ca

Well Differentiated Adenocarcinoma
Poorly Differentiated Adenocarcinoma
Solid-pattern Adenocarcinoma

Morphology & Immunohistochemistry
Large Cell Carcinoma

Tumour progression and de-differentiation

Pathologists will still differ how they call marginal cases !!!!

IHC profile
A Genomics-Based Classification of Human Lung Tumors

The Clinical Lung Cancer Genome Project (CLCGP) and Network Genomic Medicine (NGM)

Definition

Large cell carcinoma is an undifferentiated non-small cell carcinoma (NSCC) that lacks the cytological, architectural, and immunohistochemical features of small cell carcinoma, adenocarcinoma, or squamous cell carcinoma. The diagnosis requires a thoroughly sampled resected tumour, and cannot be made on non-resection or cytology specimens.

The diagnosis of large cell carcinoma is only made on resection specimen when additional staining (immunohistochemistry and/or mucin stains) is negative, unclear, or not available.
Adenocarcinoma: Solid subtype

Large cell carcinoma becomes

Non-keratinizing Squamous cell carcinoma
Clinical impact

- Large cell category gets smaller
- Classification shift may reduce post-operative 5 year survival for squamous cell and adenocarcinomas
- No impact on molecular testing practice
- New nomenclature
WHO classification of lung cancer
- Large cell carcinoma
- **Small samples**

Predictive analysis
- EGFR
- ALK
- ROS1
- MET
- PD-L1

Tissue management
Uncertainty of H&E in NSCLC

Resection specimen

Biopsy / Cytology

Large cell carcinoma
1%

NOS
34%

Travis et al. Multidisciplinary classification of lung adenocarcinoma, JTO 201, 6(244-285)
Adenocarcinoma

IHC TTF1 (NKX2-1) (clone 8G7G3/1)
Adenocarcinoma

IHC         TTF1

TTF1 Threshold weak is also positive
H&E

IHC p63

Threshold
Strong positivity in all undifferentiated cells

P63/p40
NSCLC – probably adeno-carcinoma

TTF1 positive in tumour cell nuclei

NSCLC-NOS

Tumour cells express Nuclear p63 Or p40

NSCLC-NOS

NSCLC-NOS

NSCLC – probably squamous cell

NSCLC – probably adeno-carcinoma
WHO lung cancer classification 2015

NSCLC H&E + stain

- Resection specimen
  - Large cell carcinoma: 0.2%
  - NOS: 4%
- Biopsy / Cytology
  - NOS: 4%

WHO lung cancer classification 2015
Evolving Diagnostic Algorithm: Small sample diagnosis

Identify lung cancer

Small cell lung cancer

Cisplatin Etoposide

Non-small cell lung cancer

Morphology +/- IHC
Only when needed

Squamous cell carcinoma

Non-squamous cell carcinoma (de facto Adenocarcinoma)

No molecular profiling

Molecular profiling

MDT decision
If patient is never smoker
Evolving Diagnostic Algorithm: Small sample diagnosis

- Identify lung cancer
  - Small cell lung cancer
    - Cisplatin Etoposide
  - Non-small cell lung cancer
    - Morphology +/- IHC
      - Only when needed
- Squamous cell carcinoma
- Non-squamous cell carcinoma (de facto Adenocarcinoma)
  - Predictive analysis
  - Molecular profiling
- No molecular profiling

MDT decision
If patient is never smoker

On top of diagnostic process: additional costs
## WHO 2015: Histology and mutation

<table>
<thead>
<tr>
<th>Cell type</th>
<th>EGFR mutation</th>
<th>KRAS mutation</th>
<th>BRAF mutation</th>
<th>ALK fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>15%</td>
<td>30.9%</td>
<td>2.5%</td>
<td>12.5%</td>
</tr>
<tr>
<td>Probable Adenoca (by IHC)</td>
<td>5.9%</td>
<td>41.8%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Squamous Carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Probable Squamous ca (by IHC)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NSCLC-NOS</td>
<td>7.3%</td>
<td>31%</td>
<td>2.7%</td>
<td>0</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1 case*</td>
</tr>
</tbody>
</table>

Kret A et al. Lung Cancer Jan, 2015; suppl 1
WHO classification of lung cancer
- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis
- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

Tissue management
Frequency of driver oncogene aberrations in lung adenocarcinoma, according to smoking status

Saito, Cancer Sci 2016, 107 (713-20)
<table>
<thead>
<tr>
<th>Biomarker Prediction: treatment related</th>
<th>IHC</th>
<th>FISH</th>
<th>mut/indel/ transl</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR mut</td>
<td>erlo-, gefi-, afatinib</td>
<td>-/+</td>
<td>-</td>
</tr>
<tr>
<td>ALK</td>
<td>crizotinib</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ROS1</td>
<td>crizotinib</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MET ex14/ampl</td>
<td>met mab</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>BRAF</td>
<td>sora/vemurafenib</td>
<td>+?</td>
<td>-</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>ever/temsirolimus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HER2</td>
<td>trastuzumab/lapatinib/ dacomatinib</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EGFR ihc</td>
<td>cetuximab</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>RET</td>
<td>Sunitinib, sorafenib, vandetanib cabozantinib</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>NRAS</td>
<td>vemurafenib</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cKIT</td>
<td>imatinib/sunitinib</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>KRAS</td>
<td>selumetinib, docetaxel</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DDR2</td>
<td>dasatinib</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FGFR1</td>
<td>ponatinib</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Effect of gefitinib in case of present or absent EGFR mutation

Absent

Present

Conclusion:
Since in patients without EGFR mutations more harm is done with EGFR-TKI than with chemo-x, EGFR-TKI treatment only for patients with EGFR mutations: selection required.

Mok NEJM 2009, 361 (947-957)
Mutations in the TK domain of EGFR: Meta analysis of 5 studies (n=1256)

Resistance to first line EGFR TKI’s

- 37 Bx after recurrence: all retain original EGFR mutation
- T790M (48-63%),
- Part MET amplification (5%)
- Part Her2 amplification (13%)
- Part PIK3CA, EGFR amplification
- Part EMT
- Part SCLC (<2-4%)
- Induction of FGF2 and FGFR1
- Acetylcholine receptor

- Repeatedly assessing cancer throughout the course of disease

- Sequist Sci Transl Med. 2011, 3 (75ra); Wang JTO 20 13,8(719-25); Ware Oncogenesis 2013,2e39;
- Yu CCR 2013, 19(2240-7)
3rd generation EGFR TKI (afatinib): resistance

### Table 1 Patients with NSCLC who developed resistance to third-generation EGFR TKIs

<table>
<thead>
<tr>
<th>Case report</th>
<th>Pretreatment EGFR mutations</th>
<th>Prior treatments</th>
<th>Third generation EGFR TKI</th>
<th>Gene status at resistance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 YF</td>
<td>del.19</td>
<td>Chemo, erlotinib Radiotherapy, afatinib/cetuximab Chemo/erlotinib</td>
<td>AZD9291</td>
<td>del.19 and T790M and C797S and TSC22N480f</td>
<td>[25]</td>
</tr>
<tr>
<td>57 YF</td>
<td>del.19</td>
<td>Gefitinib, chemo Afatinib/nimetuzumab</td>
<td>HM61713</td>
<td>del.19 and T790M and C797S</td>
<td>[27]</td>
</tr>
<tr>
<td>71 YF</td>
<td>L858R</td>
<td>Gefitinib</td>
<td>AZD9291</td>
<td>L858R and T790M and L718Q</td>
<td>[29]</td>
</tr>
<tr>
<td>64 YF</td>
<td>L851Q and T790M and HER2 A</td>
<td>NA</td>
<td>AZD 9291</td>
<td>EGFRL851Q and T790M</td>
<td>[30]</td>
</tr>
<tr>
<td>54 YF</td>
<td>del.19 and T790M and MET amp</td>
<td>Chemo, erlotinib</td>
<td>CO-1686</td>
<td>NA</td>
<td>[30]</td>
</tr>
<tr>
<td>51 YF</td>
<td>del.19 and T790M</td>
<td>Gefitinib, chemo Afatinib, afatinib/cetuximab</td>
<td>AZD9291</td>
<td>del.19 and KRAS G12S</td>
<td>[30]</td>
</tr>
</tbody>
</table>

**Abbreviations:** EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; del. 19, EGFR exon 19 deletion; amp, amplification; chemo, chemotherapy; NA, not available; PFS, progression free survival.

Wang Front Med 2016
WHO classification of lung cancer
- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis
- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

Tissue management
Frequent ALK genetic alterations in cancer

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>ALK alteration</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT</td>
<td>TPM3-ALK, TPM4-ALK</td>
<td>50–60%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50–60%</td>
</tr>
<tr>
<td>Neuroblastoma</td>
<td>Point mutations and/or</td>
<td>6–8% /</td>
</tr>
<tr>
<td></td>
<td>amplification</td>
<td>4.4%</td>
</tr>
<tr>
<td>ALCL</td>
<td>NPM-ALK, TPM3-ALK</td>
<td>60–80%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12–18%</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>EML4-ALK, KIF5B-ALK, TFG-ALK</td>
<td>3–7%</td>
</tr>
<tr>
<td></td>
<td>more</td>
<td>Rare</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rare</td>
</tr>
</tbody>
</table>

Ref: Mol Cancer Ther 2011;10:569-579
ALK Fusions in NSCLC (2007–Present)

20+ Unique ALK Fusion Variants in NSCLC!
Demonstrating ALK

FISH

Immunohistochemistry (IHC)

2–8% in adenocarcinomas

Break apart fluorescence *in situ* hybridisation

- Positive: (typical) split apart / (atypical) single 3’
- Start first 50 nuclei
  - Positive 25 out of 50
  - Negative <5 positive
  - Else equivocal then
- Second step additional 50
  - Positive: ≥ 15%
  - Else not rearranged

- Borderline cases False Positive or False Negative; von Laffert Lung Cancer 2015, 90 (465-71)
ALK IHC cytoplasm

ETOP protocol 5A4 Novocastra
Novolink detection system
IHC H-score

Two validated ALK IHC protocols (A)

- Ventana ALK (D5F3 cell signaling)
- Tyramide enhancement system
- Score yes/no

ALK IHC good screening method

Immunohistochemistry detected/visible

Indirectly labeled
Visible with lower epitope concentration

Directly labeled
Visible with high concentration of epitopes

Second layer
Goat anti-mouse

Indirectly labeled

Directly labeled

mouse
Immunohistochemistry

Indirectly labeled polymer, 5–20x

Indirectly labeled
Directly labeled
Relation signal intensity and epitope concentration

Relation signal intensity and epitope concentration

IHC
- Epitope concentration
- Signal enhancement system
- Intensity plateau
- Small linear dynamic range (factor 2-4)
- Lack of standard for epitope concentration
- Eye vs machine......

Relation between epitope concentration and signal enhancement in IHC

ALK IHC adequate for screening of ALK IHC pos lung cancer
Overview of on-target mechanisms of resistance among ALK-positive specimens obtained from patients progressing

‘First line’ A crizotinib

‘Second line’ B ceritinib

C alectinib

Gainor et al. Cancer Discov 2016;6:1118-1133
WHO classification of lung cancer
- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis
- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

Tissue management
MET Amplification

MET SISH
- Red Chr 7, black MET

Ratio
- Normal 1.8 – 2.2
- Low 2.2 – <5
- High >5

Amplification ~ 10%

Camidge, ASCO 2014 abs 8001

Prevalence 3% high level gains in NSCLC
Equally distributed between squamous cell and adenocarcomas

Schildhaus 2015
**MET exon 14 mutations**

- NGS large panel (282 genes)
- Frequency 1-3% non-squamous NSCLC
- Function exon 14 binding site of CBl E3 ubiquitin ligase
- May be early event in carcinogenesis
- Mutually exclusive with EGFR, ALK, ROS, RET, BRAF, Her2, NRAS
- Slightly older women
- Adenocarcinoma, sarcomatoid carcinoma, SqCC?
- TX with Met inhibitor
- Co-occurrence KRAS rapid recurrence
- Resistance 2nd acquired mutation D1228N TKI domain, Y1230C

Paik, Cancer Disc, 2015, 5(842-9); Frampton, cancer Disc 2015, 5 (850-9); Awad, JCO 2016, 34 (721-30); Li, JTO 2016; Heist JTO 2016, 8 (1242-5); OU, JTO 2016; Liu, JTO 2016, 9(1503-10); Liu, JCO 2016, 34(794-802);
WHO classification of lung cancer
- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis
- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

Tissue management
**ROS1 biology**

- v-ROS1 = avian sarcoma virus (chicken retrovirus) \(^1\)
- Receptor tyrosin kinase family
- Human ROS1 chromosome 6 (6q22) \(^3\)
- Protein: N-terminal domain 1800 amino acids \(^2\)
- Ligand for ROS1 unknown!

---

1) Shibuya, J. Virol 1982, 42 (143-52);
2) Acquaviva, Biochem Biophys Acta 2009, 1795 (37-52);
3) Bergethon, JCO 2012, 30 (863-70)
ROS1 fusion partners

Chin JTO 2012, 7 (1625-30)
ROS1 IHC clone D4D6

- In NSCLC
  - fine granular- globular;
  - intensity varies between tumor cells
- Reactive type II pneumocytes
- Alveolar macrophages
- Osteoclast type giant cells
- Mucin = negative

tumor cells negative
WHO classification of lung cancer
- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis
- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

Tissue management
<table>
<thead>
<tr>
<th>Drug</th>
<th>Company</th>
<th>Ab clone</th>
<th>PD-L1 Binding domain</th>
<th>Platform</th>
<th>2nd line Criteria for PDL1 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab</td>
<td>Bristol-Myers Squibb</td>
<td>28-8 (Dako)</td>
<td>Extra-cellular</td>
<td>Link Autostainer</td>
<td>≥ 1% tumor cells</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Merck</td>
<td>22C3 (Dako)</td>
<td>Extra-cellular</td>
<td>Link Autostainer</td>
<td>≥ 50% tumor cells</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>Genentech/Roche</td>
<td>SP142 (Ventana)</td>
<td>Cytoplasmic</td>
<td>Ventana</td>
<td>Tumor cells / tumor infiltrating immune cells</td>
</tr>
<tr>
<td>Durvalumab</td>
<td>Astra-Zeneca/ MedImmune</td>
<td>SP263 (Ventana)</td>
<td>Extra-cellular 1)</td>
<td>Ventana</td>
<td>≥ 25% tumor cells</td>
</tr>
<tr>
<td>Avelumab</td>
<td>Pfizer/ Merck Serono</td>
<td>73-10 (Dako)</td>
<td></td>
<td></td>
<td>≥ 1% tumor cells</td>
</tr>
</tbody>
</table>
Example of PD-L1 Tumor Expression

Not only technical validation, also clinical validation required
Not all animals are created equal
<table>
<thead>
<tr>
<th>IHC</th>
<th>Technical validation</th>
<th>Clinical validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-analytical steps within limits</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Analytical steps robust</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Minimal number of positive cases for validation 1)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Minimal number of negative cases for validation 1)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Minimal number covering linear dynamic range 2)</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>Requires treatment outcome in study group for a certain drug</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Test needs to be constant in time to maintain predictive value</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>

1) According to CAP guidelines [Fitzgibbon, 2014, 24646069]

2) For clinical validation the samples covering the linear dynamic range (see figure1) are the most relevant, as the threshold for positivity is within this range.
Analytical Evaluation Results: Mean Tumor Proportion Score (TPS) per case based on three readers

- Analytical comparison of % tumor cell staining (Tumor Proportion Score), by case, for each assay
- Data points represent the mean score from three pathologists for each assay on each case
- Superimposed lines / points indicate identical TPS values
- No clinical diagnostic cut-off applied
- **Conclusion:** 3 of 4 assays are analytically similar for tumor cell staining.
Concordance based on PD-L1 expression using matched assay / algorithm (N=38 cases)

- Cases are rank ordered from lowest to highest PD-L1 expression (above and below respective cut-offs)

![Graph showing intensity vs epitope concentration](image)

- Cases in blue boxes (N=24, 63.1%) indicate agreement regardless of assay / scoring method combination
- Cases in black box (N=14, 36.9%) indicate discrepant cases of PD-L1 expression across the four assays

![Table showing PD-L1 expression](image)
POPLAR Phase 2 RCT Atezolizumab vs Docetaxel

Fehrenbacher, Lancet 2016
PD-L1 scores compared by 2 pathologists/ methods

After calibration

Variation around threshold:
~ 8-9%

Scheel Modern Pathology 2016
Field in development

- PD-L1 protein heterogeneous
- 8-9% (biopsy) negative react on immunotherapy

- Biomarker PD-L1 IHC never higher “predictive” than 90%.
• On 18 October, 2016, the US Food and Drug Administration (FDA) approved atezolizumab (TECENTRIQ, Genentech Oncology) for the treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose disease progressed during or following platinum-containing chemotherapy.

• Patients with EGFR or ALK genomic tumour aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving atezolizumab.
On October 24, 2016, the U.S. Food and Drug Administration (FDA) approved pembrolizumab (Keytruda) for the treatment of patients with metastatic non–small cell lung cancer (NSCLC) whose tumors express programmed cell death ligand 1 (PD-L1) as determined by an FDA-approved test.

This is the first FDA approval of a checkpoint inhibitor for the first-line treatment of lung cancer. This approval also expands the indication in second-line treatment of lung cancer to include all patients with PD-L1–expressing NSCLC.

Patients with metastatic NSCLC whose tumors have high PD-L1 expression (tumor-proportion score ≥ 50%) as determined by an FDA-approved test, with no EGFR or ALK genomic tumor aberrations and no prior systemic chemotherapy treatment for metastatic NSCLC.

Patients with metastatic NSCLC whose tumors express PD-L1 (tumor-proportion score ≥ 1%) as determined by an FDA-approved test, with disease progression on or after platinum-containing chemotherapy. Patients with EGFR or ALK genomic tumor aberrations should have disease progression on FDA-approved therapy for these aberrations prior to receiving pembrolizumab.
WHO classification of lung cancer
- Large cell carcinoma IHC
- Small samples IHC
Predictive analysis
- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy
Tissue management
Concepts of resistance mechanisms

- Resistance due to initially present 2nd acquired mutation?
  - Resistance mutation possibly detectable in initial biopsy
- Resistance mechanism by (induced) stress
  - Resistance mutation not present in initial biopsy
Diverse resistance due to treatment (= stress)

- May derive from a single cell clone (EGFR PC9)
- Crashed with drug (erlotinib)
- Evolving persister cells (0.5%) under same selective pressure
- Diverse drug mechanisms (T790M, MET amplification, MAPK pathway [NRAS mutation], BRAF, ...)

 Ramirez, Nat Comm. 2016, 10690
Concept TKI resistance

- The drug related resistance patterns support the stress hypothesis.
- It is not excluded that by the evolved resistance mechanism the tumor cells get more dependent of the escape route.
- In anticipation of (any) treatment: a recurrence is likely to occur, unless a synthetic lethal drug (combination) is given.
- BIOPSIES of recurrent lesion are needed to uncover the resistance mechanism and give the patient a (still small) chance.
CONSORT diagram

Flow of 24 patients who consented to repeat biopsy.

24 patients with metastatic EGFR mutant NSCLC enrolled

21 patients assessed at time of AR

14 biopsies performed

11 biopsies analyzable

2 pts discontinued prior to PD; 1 pt remains on drug

4 pts with CNS progression only; 3 pts too ill

3 pts with insufficient sample material

pts, patients; pt, patient; PD, progressive disease; AR, acquired resistance; CNS, central nervous system. 
Campo, JTO, 2016 11(2022-26)
Flow of patients who consented to repeat biopsy.

24 patients with metastatic EGFR mutant NSCLC enrolled

Dropout 13/24
- Clinical 10/24 pts no biopsy
- Technical 3/24 biopsy not adequate

11/24 (46%) pts adequate biopsy
11/14 (78%) biopsies adequate

11 biopsies analyzable

pts, patients; pt, patient; PD, progressive disease; AR, acquired resistance; CNS, central nervous system.
Campo, JTO, 2016 11(2022-26)
Algorithm for prediction
(In transition, VUmc Amsterdam)

• Prediction step 1
  • IHC ALK, ROS1, (PD-L1 on request)
  • NGS: EGFR, RAS, BRAF, Her2, MET

• Prediction step 2
  • FISH ALK, ROS1, RET
  • SISH MET
WHO classification of lung cancer
- Large cell carcinoma IHC
- Small samples IHC

Predictive analysis
- EGFR
- ALK
- MET
- ROS1
- PD-L1
- Resistance biopsy

Tissue management
‘Biopsy’ techniques in lung cancer diagnosis

- Sputum cytology
- Bronchial brushings and washings
- Fluids
- FNA cytology/EBUS-EUS – primary or mets
- Transbronchial biopsy
- Bronchial biopsy
- Pleural biopsy
- Core biopsy – primary or mets
- Mediastinoscopy
- Lymph node excision
- VATS biopsy / resection
- Thoracotomy tumour excision

Increase in Cell number and Tissue architecture

Courtesy of Keith Kerr
‘Biopsy’ techniques in lung cancer diagnosis

- *Sputum cytology*
- *Bronchial brushings and washings*
- *Fluids*
- *FNA cytology/EBUS-EUS – primary or mets*
- Transbronchial biopsy
- Bronchial biopsy
- Pleural biopsy
- Core biopsy – primary or mets
- *Mediastinoscopy*
- Lymph node excision
- *VATS biopsy / resection*
- *Thoracotomy tumour excision*

Increase in Cell number and Tissue architecture

General Tendency Towards less Invasive Techniques To secure A diagnosis

Courtesy of Keith Kerr
Samples getting smaller

Positive proof of global warming.


Courtesy of Keith Kerr
Tissue management

Pulmonologist: Clinical information
Questions: diagnosis, prediction “D+P”, if malignant

Pathology
“D+P” code: specific handling in contrast to regular

NOT performing these additional cuts saves material...
Adequate clinical information is essential: D+P
More analysis from one biopsy?

HE first
- Diagnostic stain TTF1
- Diagnostic stain mucin
- Diagnostic stain P63/p40
- Predictive stain ALK IHC
- Predictive stain ALK FISH
- DNA isolation EGFR
- DNA isolation
- DNA isolation
- HE last

NGS
- miseq 40-250 ng
- Ion torrent 10 ng
Tissue management = interaction between pulmonologist/oncologist/surgeon/radiologist and pathologist

**Sample collector:**
- Sampling more (≥4) biopsies/ tumor tissue
- Clinical request for diagnosis + prediction?
- Clinical suspicion of metastases y/n?

**Pathology:**
- Distribute samples over >1 block
- Careful initial cut
- Spare section for reflex analysis

Lymphangitis carcinomatosa,
- Sufficient for diagnosis/ IHC
- Not enough for FISH/NGS
Cytology Cell Block
Tumor cellularity

2.5x obj

10x obj

20x obj

+  

?  

-
Cytology Cell Block
Tumor cellularity

2.5x obj

10x obj

20x obj

Highly cellular Cell block may be as suitable as tissue
Fraction of neoplastic cells
Related
To test requirements:
e.g. <20% sequencing: negative
10-50 ng input DNA

Molecular tumourboard

- Participants
  - Pulmonologists (treats in NL Lung cancer)
  - Pathologists
  - Molecular biologists
- Special cases (not average)
- Frequency: 2x/ month
STUDY DESIGN:
Prospective Multicenter Registry study of ALK IHC+ NSCLC
CONTACT: Erik Thunnissen (e.thunnissen@vumc.nl)

- Screen NSCLC patients with IHC
  - Confirm with Abbott break apart FISH assay
    - FISH positive
      - Assessment of response to crizotinib
    - FISH negative
      - Assessment of response to crizotinib

- Analysis of the ALK gene locus (centralized sequencing)

- Data collection
  - Collection of local slides and distribution for central
    - ALK IHC confirmation,
    - ALK FISH confirmation, and
    - ALK locus sequencing
Thank you
Questions?
Multiple tumor types are being treated with new medicines launched over the past five years

New Molecular Entity Launches 2010–14 by Indication

Source: IMS-Health WDAIS Dec. 2014
IMS Lifecycle & R&D Focus Dec. 2014

Chart notes: Molecules listed had initial global launch in the period 2010–14. Molecule indications based on approval by one or more regulatory bodies. Excludes sipuleucel-T, an autologous cell product not classified by IMS Health as a fully identifiable substance.
Subclone may be present at time of diagnosis

High T790M in FFPE primary tumor: artifact? Ye JTO 2013
Kuiper, Lung cancer 2014
Third generation EGFR TKI: Tagrisso

Janne NEJM 2015, 372(1689-99)
Model Quantitation immunohistochemistry
Indirect tyramide/polymer IHC vs. indirect SABC IHC

DNA concentration = epitope concentration

Semi Q: - - - +/- + ++ ++

Absorption

Linear dynamic range = quantitative range, more signal enhancement: closer to black and white; positive/ negative

Difference between positive and negative: factor 2 in concentration

- = indirect polymer IHC
* = indirect SABC IHC

Linear dynamic range = quantitative range, More signal enhancement: closer to black and white; positive/ negative

ROS1 signaling pathways

Chin JTO 2012, 7 (1625-30)
Cancer in the lung: Primary vs metastases

- Clinical information / PALGA essential:
  - PRIMARY PULMONARY ADENOCARCINOMA: Surfactant prot A, Napsin A, TTF1
  - Metastases:
    - Colorectal: CK7, CK20, CDX2
    - Prostate: PSA, PAP
    - Breast: ER, PR, GCDFP15, GATA3
    - Germ cell: PLAP, AFP, hCG, CD30, OCT3/4, Sox2, Sox17
    - Melanocyte: Melan A, HMB45, Sox 10, MITF
    - Mesothelium: Calretinin, CK5/6, D2-40, WT-1
    - Kidney: RCC, CD10, Pax2, Pax8
    - Ovary: CA125, Pax5, Pax 8

Nonaka, Diagnostic Histopathology 2010, 16 (581-592)
Cancer in the lung: Primary vs metastases

- Clinical information / PALGA essential:
  - PRIMARY PULMONARY ADENOCARCINOMA: Surfactant prot A, Napsin A, TTF1
  - Metastases:
    - Colorectal: CK7, CK20, CDX2
    - Prostate: PSA, PAP
    - Breast: ER, PR, GCDFP15, GATA3
    - Germ cell: PLAP, AFPHcG, CD30, OCT3/4, Sox2, Sox17
    - Melanocyte: Melan A, HMB45, Sox 10, MITF
    - Mesothelium: Calretinin, CK5/6, D2-40, WT-1
    - Kidney: RCC, CD10, Pax2, Pax8
    - Ovary: CA125, Pax5, Pax 8

Discussion between pulmonologist/oncologist and pathologist whether clinical suspicion is present on metastases or not is required.

Only performing these stains when clinically relevant.

Only a few markers have 100% specificity with limited sensitivity.

Nonaka, Diagnostic Histopathology 2010, 16 (581-592)
Screen NSCLC patients with IHC

Confirm with Abbott break apart FISH assay

Assessment of response to crizotinib

Assessment of response to crizotinib

Analysis of the ALK gene locus (centralized sequencing)

STUDY DESIGN: IIR Pfizer
‘European’ Prospective Multicenter Registry study of ALK IHC+ NSCLC

CONTACT: Erik Thunnissen (e.thunnissen@vumc.nl)
STUDY DESIGN:
European Prospective Multicenter Registry study of ALK IHC+ NSCLC

CONTACT: Erik Thunnissen (e.thunnissen@vumc.nl)

- Data collection
- Collection of local slides and distribution for central ALK IHC confirmation, ALK FISH confirmation, and ALK locus sequencing
Loose tumour fragments called STAS: spread through airways
STAS = an assumption

More frequent in micropapillary carcinoma.

Could this be artifact?
Gross cutting

Knife before cutting: clean

Knife after 1st cut: hemorrhagic

Knife after 2nd cut: less hemorrhagic

Clean surface

First cut

2nd cut

Hemorrhagic surface tumor and pleura after 2nd cut