Workshop in Diagnostic Immunohistochemistry

Lung Cancer: IHC and Molecular Classification

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Lung Cancer

In the workflow of LC materials technicians play fundamental role!

One key point is tissue handling!
Lung Cancer

Molecular assays to be done in NSCLC

**Essential:** EGFR, EML4-Alk, ROS1,

**Addiitonal**
BRAF, MET, PDGFR, MSI, KRAS, and others → next generation sequencing

**All assays should be controlled continuously in repeated ring trials**
*(round Robin tests)*
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Just one example: ALK
Comprehensive diagnosis of lung cancer requires:

- 1 or 2 $4\mu$m slides for H&E / PAS
- 4 to 6 $4\mu$m slides for IHC (syn, chrom A, CK5/6, CK7/8/18, p63, ERCC1, TTF1, ALK [IHC/FISH] ….controls)
- 2 to 3 $10\mu$m slides for EGFR-mut testing or NGS
- ? for controls and other assays.

For the work-up of (small) biopsies this means in general:

- Very careful handling of the tissue
- Neatly embedding with all biopsies in on a similar level
- Cutting as precise as possible to enable all assays necessary
- Spare slides in advance to avoid re-cutting.

Thunnissen E, et al. Virchows Arch 2012;
NSCLC - Macroscopy

For these types of tumors a therapy with TKIs should be considered if the molecular prerequisites are proven.
NSCLC: Past and Current Landscape

1999 Histology-driven selection¹

- Adenocarcinoma
- Squamous-cell carcinoma
- Large cell carcinoma

2012 Targeting oncogenic drivers

- NO MUTATION DETECTED
- KRAS 22%
- EGFR 17%
- EML4-ALK 7%
- NO DOUBLE MUTANTS
- BRAF 2%
- PIK3CA
- HER2
- MET AMP
- MEK1
- NRAS
- AKT1
- KRAS 22%
- EGFR 17%
- EML4-ALK 7%

Kris MG, et al. Presented at ASCO 2011; CRA7506

Actionable driver mutations identified in 54% of lung adenocarcinoma tumors
Lung Cancer

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*(round Robin tests)*
Currently, Two Approved Personalised Treatment Options:

**EGFR-TKIs in EGFR-mut NSCLC**
- Gefitinib, Erlotinib (US, EU)
  - Afatinib filed in EU
  - RR 60–80%, PFS 10–13 months, OS 19–30 months

**Crizotinib in ALK-positive NSCLC**
- (US, EU filed)
- RR 60%, PFS 8 months, OS 14–16

**Chemotherapy in unselected patients**
- RR 20%, OS <12 mths

Substantial Benefit for ~15 – 20 % of Patients


Dacomitinib (PF-00299804; Pfizer Inc.) is an investigational compound not currently licensed for use in any market; Crizotinib (PF-02341066; Pfizer Inc.) is not yet approved in member states of the European Union. Crizotinib is currently licensed for use in Argentina, Canada, Israel, India, Japan, South Korea, Macau, Mexico, Switzerland, and the USA.
Tumour Response to Crizotinib by Alk+ Patients

Study A8081001
N=116¹

PROFILE 1005
N=240²*

Best objective response according to RECIST:

PD  SD  PR  CR

2. Kim DW, et al. Presented at ASCO 2012; Ab. 7533

*Mature population, excluding those with early death, indeterminate response and non-measurable disease
Rapid Responses Seen In Some Patients

Almost all tumors become resistant to targeting drugs. Novel approaches that have already proven successful include the development of second-generation and third-generation inhibitors and the combination of some of these inhibitors with antibodies directed against the same target or other targets (check points).

Consequently, clinical studies assessing combinations of drugs targeting both the original and the bypass pathways (after resistance) are now being explored in this setting.
Resistance to EGFR Inhibitors

• Primary resistance, e.g. to erlotinib or gefitinib

• Acquired resistance (after treatment)
  • EGFR dominant
    – 2ndary EGFR mutation(s) with steric hindrance of 1st gen. EGFR inhibitors
    – EGFR amplification or point mutation
  • EGFR non-dominant
    – New non-EGFR mutations: Met, HER2, PIK3CA, BRAF, other
Mechanisms of Acquired Resistance in ALK-rearranged NSCLC Resistant to Crizotinib

ALK gene Amplification and Multiple ALK Resistance Mutations in Cancers with Acquired Crizotinib Resistance
One Step Forward: New Drugs to Fight Resistance

For example:
Crizotinib resistant NCSLC showed sensitivity to ceritinib, but became resistant again only many months later.

<table>
<thead>
<tr>
<th>Patient Id</th>
<th>EML4-ALK sequence at Crizotinib Resistance</th>
<th>EML4-ALK sequence at Ceritinib Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGH011</td>
<td>S1206Y</td>
<td>G1202R</td>
</tr>
<tr>
<td>MGH015</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>MGH023</td>
<td>WT</td>
<td>F1174C</td>
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<tr>
<td>MGH034</td>
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<td>MGH049</td>
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<tr>
<td>MGH057</td>
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<tr>
<td>MGH061</td>
<td>WT</td>
<td>WT</td>
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<tr>
<td>JFCR013</td>
<td>N/A</td>
<td>WT</td>
</tr>
<tr>
<td>JFCR021</td>
<td>G1269A (right lung)</td>
<td>F1174V (left lung) and G1202R (right lung)</td>
</tr>
</tbody>
</table>

EML4-ALK sequence: ALK mut

S1206Y

G1202R

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Mechanisms of Acquired Resistance in EGFR-mutant NSCLC Resistant to First Generation EGFR TKIs Erlotinib/Gefitinib

EGFR non-dominant

EGFR dominant

Resistenz gegen EGFR-TKI-Therapie wird in 50% der Fälle durch Austausch der Aminosäure Threonin zu Methionin an Position 790 des EGFR-Proteins vermittelt (T790M)

Second-site mutation frequency following development of acquired resistance to TKI therapy

Gefitinib (yellow)

T790M → threonine to methionine

*AZ9291 binding site
AZD9291 (Tagrisso) – 66% ORR in T790M positive patients*

*as assessed by central tumor tissue testing
cfDNA, circulating free DNA; ctDNA, circulating tumor DNA. 
Crowley, Nat Rev Clin Oncol 10, 472 (2013)
Ring Trial EGFR T790M
Tissue and Blood

26.01.2016
Sabine Merkelbach-Bruse

Institut für Pathologie – Charité Berlin
The German Society of Pathology and the Association of German Pathologists founded the QuiP initiative to perform nationwide quality control (ring trials) on IHC and molecular analyses.

A prerequisite is always a well-organised biobank.
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Mol RTs

BRCA1/2 - ovarian carcinoma
T790M – NSCLC (tissue and blood)
RAS - colon
BRAF - malignant melanoma
Multigene - NSCLC – planned for 1st. quarter of 2017
BRAF - NSCLC – 2nd qu. 2017
ROS1 - NSCLC – planned for 2nd half of 2017
Multidisciplinary Cooperation Enables Precision Oncology

Chemotherapy | Surgery | Targeted therapy | Radiotherapy | Check point inhibitors | experimental

Clinical Suspect of a primary tumor or recurrence

Tissue Sampling

Radiology | Endoscopy

Anatomic and molecular Pathology

Diagnosis | IHC

In situ hybridization | molecular path

Histological tumor typing (TNM, grading etc.) with details on prognosis and prediction of drug efficiency

Interdisciplinary tumour board with oncologists, surgeons, gynecol., (mol.) pathologists, radiologist etc.

Individual treatment decision based e.g. on actionability of mol. alterations, protein pattern resulting in novel therapies

Liquid biopsy

eQA/QC | research
teaching | clinical trials
tumor registry | bio-banking

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Institut für Pathologie, Rudolf-Virchow-Haus, Charité Humboldt-Universität zu Berlin

Berliner Medizin-historisches Museum

Alexander Ufer