Lung cancer and Companion Diagnostics

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Disclosures

- Participation in several industry-sponsored advisory boards for which honoraria were received.

- Travel costs have also been refunded.
Structure of the talk on NSCLC Testing

- General introduction on molecular pathology
- What to test: sampling / tissue / cytology
- How to test
  - Methods / quality control
    - FISH
    - IHC
    - Sanger-, pyro- or NG Sequencing
- How to report
- Who to test
- When to test
- Future development
Today’s Challenges in Anatomic and Molecular Pathology

The goal of diagnostic pathology was to provide a correct diagnosis, but today the task is greatly extended to extract from the patient’s tissue as many information as possible by applying classical, immunological (proteomic) and molecular techniques.

This is the prerequisite for personalized medicine.

The capability to predict pre-therapeutically the response of infections or individual tumors to certain (targeted) drug(s) is based on reliable and reproducible biomarker and predictive assays.

But it should not be forgotten that the methodological results have to be interpreted by an experienced tumor board including pathologists. Only then the numerous diagnostic, prognostic and predictive information can be interpreted adequately to assign the optimal treatment to individual patients.
Multidisciplinary cooperation enables personalised oncology

Clinical Trials
Tumour Registries
BioBanks

Predictive tissue-based biomarkers for targeted therapies

FDA / EMA-approved drugs associated with eligibility tests* (selection)

- Trastuzumab/Pertuzumab → metastatic breast cancer, overexpression/amplification of HER-2
- Tamoxifen+/−chemo → ER+/HER2- breast cancer,– Endopredict® - multigene assay
- Cetuximab → metastatic colorectal cancer, overexpressing EGFR/wild-type KRAS
- Panitumumab → colorectal cancer with wild-type KRAS (mutation excluded)
- Gefitinib → non-small cell lung cancer with mutated EGFR
- Erlotinib → non-small cell lung cancer with mutated EGFR
- Crizotinib → non-small cell lung cancer with mutated EML4-ALK
- Nimotuzumab → metastatic colorectal cancer (still experimental)
- Lapatinib → metastatic breast cancer overexpression HER-2/neu(?)
- Vemurafenib → malignant melanoma with mutated B-RAF
- Imatinib → CML, bcr/abl–positive (activated PK),
- Imatinib → GIST with activated c-kit receptor tyrosine kinase/CD117, exon 9 mut
- Rituximab (+ CHOP), Y90-Ibritumomab, I131-Tositumomab → NHLymphoma with CD20
- Olaparib → ovarian carcinoma with BRCA 1/2 mutation and genetic variants

*Strongly suggested by FDA’s Drug-Diagnostic Co-Development Initiative

Already now, in 35% of all tumors a predictive molecular test is appropriate. Notably, prediction of tumour response is exclusively tissue-based.

All these substances have been developed on the basis of histologically characterised human tissue. This underlines the importance of biobanks.

*Strongly suggested by FDA’s Drug-Diagnostic Co-Development Initiative

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For these types of tumors a therapy with TKIs and ALKI should be considered if the histological and molecular prerequisites are proven.
Currently, Two Approved Personalised Treatment Options: Substantial Benefit for ~15 – 20 % of Patients

Crizotinib in ALK-positive NSCLC (US, EU filed)\(^1\)
RR 60%, PFS 8 months, OS 14 -16

EGFR-TKIs in EGFR-mut NSCLC
Gefitinib, Erlotinib (US, EU) (Afatinib filed in EU)\(^3\text{-}^5\)
RR 60–80%, PFS 10–13 months, OS 19–30 months

Chemotherapy in unselected patients\(^2\)
RR 20%, OS <12 mths

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.

EMA/FDA: kinase inhibitors can be applied only in combination with a diagnostic eligibility test.

Example:
- therapeutic anti-EGFR
- kinase inhibitors

**Gefitinib**
(Iressa, Astra Zeneca)

Mok et al., N Eng J Med., 2009
EGFR-mutations and EGFR tyrosine-kinase-inhibitors

EGFR-mutations of NSCLC (10-15%)

Tyrosine-kinase-inhibitors interfere with activated receptors and the corresponding pathway

Stimulation of:
- growth
- angiogenesis
- malignant phenotype

EGFR-mutations and EGFR tyrosine-kinase-inhibitors

EGFR
Kinase-Domäne
activating mutation of EGFR

KRAS (wild type)

Tyrosine-kinase-inhibitors interfere with activated receptors and the corresponding pathway

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## Summary

577 cases* included, 

174 cases no sufficient PCR product for exon 20 
35 cases have less than 30% tumor. 

=> 368 specimen sequenced. 

<table>
<thead>
<tr>
<th>Exon</th>
<th>Cases</th>
<th>Frequency Exon 18</th>
<th>Frequency Exon 19</th>
<th>Frequency Exon 20</th>
<th>Frequency Exon 21</th>
</tr>
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<tbody>
<tr>
<td>Exon 19</td>
<td>32</td>
<td>8.7%</td>
<td>57.1%</td>
<td>45.0%</td>
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<td>Exon 21</td>
<td>14</td>
<td>3.8%</td>
<td>25.0%</td>
<td>40-45</td>
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<tr>
<td>Exon 18</td>
<td>6</td>
<td>1.6%</td>
<td>10.7%</td>
<td>5.0%</td>
<td></td>
</tr>
<tr>
<td>Exon 20</td>
<td>4</td>
<td>1.1%</td>
<td>7.2%</td>
<td>&lt;1.0%</td>
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</table>

Proven EGFR-Mutations 

Based on the experience of >3000 cases it is strongly recommended to test all 4 exons.

*the study was supported by Astra Zeneca

The results correspond with those from the other institutes of the German panel institutes of the German Soc. of Path.
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(US, EU filed)
RR 60%, PFS 8 months, OS 14 - 16

**EGFR-TKIs in EGFR-mut NSCLC**
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RR 60 – 80%, PFS 10 – 13 months,
OS 19 – 30 months

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Oncogenic $EML4$–$ALK$ gene product results from a genomic translocation

21 variants

constitutive activation

cell growth

Modified according to Soda et al. nature 448:561 (2007).
**Tumour Responses to Crizotinib by Patient**

**Study A8081001**  
*N=116¹*

**PROFILE 1005**  
*N=240²*

*Decrease or increase from baseline (%)*

- PD  
- SD  
- PR  
- CR

*Mature population, excluding those with early death, indeterminate response and non-measurable disease*

Rapid Responses Seen In Some Patients

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• Who to test

• When to test

• Future development
Acquisition of Tissue Samples

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

For biopsy material this means in general:

- The bigger the better
- The specimen in total should be 0.3 x 0.3 x 0.3 cm at a minimum
- Cytology, pleural effusion cell pellets or EBUS-FNA are sufficient for all tests, but the number of tumor cells have to be adequate

EBUS-FNA; endobronchial ultrasound fine-needle aspirate; EGFR, epidermal growth factor receptor; ERCC1, excision repair cross-complementation group 1; EVG, Elastic Van Gieson; H&E, hematoxylin and eosin; PAS, periodic acid shift; syn, synaptophysin; TTF1, thyroid transcription factor

Thunnissen E, et al. Virchows Arch 2012;
1. Histology/cytology

2. Immunohistochemistry
   PEC: p63, CK5
   AdCA: TTF1, CK7, NapsinA
   SCLC: CD56, panCKAE1/3
   LCC
   other e.g. lymphoma metastases

3. Molecular diagnostic

...and, and, and

Slide given by R. Büttner, Köln
Pellet & thrombin

- FFPE protocols for FISH and IHC
- Preservation of DNA and proteins

- DNA quality and morphology ↓
- Often not available (tumor amount)
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## Methods assessing *EGFR* mutations

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Sensitivity</th>
<th>Mutations identified</th>
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</thead>
<tbody>
<tr>
<td><strong>Low sensitivity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct sequencing</td>
<td>20</td>
<td>Known and new</td>
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<tr>
<td>TaqMan PCR</td>
<td>10</td>
<td>known only</td>
</tr>
<tr>
<td>Loop-hybrid mobility shift assay</td>
<td>10</td>
<td>Known only</td>
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<tr>
<td><strong>Medium sensitivity</strong></td>
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<tr>
<td>Pyro-sequencing</td>
<td>5</td>
<td>Known and new</td>
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<tr>
<td>PCR-SSCP</td>
<td>5</td>
<td>Known and new</td>
</tr>
<tr>
<td>dHPLC</td>
<td>5</td>
<td>Known and new</td>
</tr>
<tr>
<td>Cycleave PCR</td>
<td>5</td>
<td>Known and new</td>
</tr>
<tr>
<td>PCR-RFLP and length analysis</td>
<td>5</td>
<td>Known only</td>
</tr>
<tr>
<td>MALDI-TOF MS-based genotyping</td>
<td>5</td>
<td>Known only</td>
</tr>
<tr>
<td>Scorpions ARMS - Thera screen</td>
<td>1</td>
<td>Known only</td>
</tr>
<tr>
<td>PNA-LNA PCR clamp</td>
<td>1</td>
<td>Known only</td>
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<tr>
<td><strong>High sensitivity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Next generation sequencing</td>
<td>0.1</td>
<td>Known (many)</td>
</tr>
</tbody>
</table>

Molecular Methods used at Institute of Pathology, Charité Berlin

Sanger sequencing

Pyro sequencing

I an own test series we found differences between Sanger- and pyro-sequencing only in 1 or 2 cases out of 200.

An explanation could be that tumor selection was done quite rigorously.

p.G12D

p.G12D
Integrating Next Generation Sequencing in Diagnostic Pathology

Genomic DNA

Multiplex PCR
Ion AmpliSeq Primer Pool

Partially digested primer sequences

Adapters
A  P1

Barcode adapters
X  P1

Ligate adapters

Nonbarcoded library

Barcoded library

Ion AmpliSeq™ Cancer Panel targets 46 critical genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
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<tr>
<td>ABL1</td>
<td>ERBB4</td>
<td>KDR</td>
<td>PTPN11</td>
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<tr>
<td>AKT1</td>
<td>FBXW7</td>
<td>KIT</td>
<td>RB1</td>
</tr>
<tr>
<td>ALK</td>
<td>FGFR1</td>
<td>KRAS</td>
<td>RET</td>
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<tr>
<td>APC</td>
<td>FGFR2</td>
<td>MET</td>
<td>SMAD4</td>
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<tr>
<td>ATM</td>
<td>FGFR3</td>
<td>MLH1</td>
<td>SMARC1</td>
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<tr>
<td>BRAF</td>
<td>FLT3</td>
<td>MPL</td>
<td>SM0</td>
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<tr>
<td>CDH1</td>
<td>GNAS</td>
<td>NOTCH1</td>
<td>SRC</td>
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<tr>
<td>CDKN2A</td>
<td>HRAS</td>
<td>NPM1</td>
<td>STK11</td>
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<tr>
<td>CSF1R</td>
<td>HRAS</td>
<td>NRAS</td>
<td>TP53</td>
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<tr>
<td>CTNNB1</td>
<td>IDH1</td>
<td>PDGFRα</td>
<td>VHL</td>
</tr>
<tr>
<td>EGFR</td>
<td>JAK2</td>
<td>PIK3CA</td>
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<tr>
<td>ERBB2</td>
<td>JAK3</td>
<td>PTEN</td>
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</tbody>
</table>
Two CE-marked ALK testing kits are currently available and recommended by ESMO/CAP

- Vysis ALK break-apart FISH probe
- VENTANA anti-ALK (D5F3) primary antibody

Wild type ALK rearranged

FISH, fluorescence, in-situ hybridisation

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How should we test for ALK?: What the guidelines say

Level of evidence/grade of recommendation

- Definitive assessment of ALK rearrangement is determined by FISH
- IHC methods may be employed for screening and may become validated for therapy
- Methodologies should be validated by an external quality assurance programme

“Laboratories should use an ALK FISH assay using dual-labelled break-apart probes for selecting patients for ALK TKI therapy; ALK IHC, if carefully validated, may be considered as a screening methodology.”

Consensus opinion: “A pathologist should be involved in the selection [and interpretation] of sections for ALK FISH…”


TKI, tyrosine kinase inhibitor
ALK immunocytochemistry on tissue – (D5F3)
High concordance between IHC and FISH tests

100% concordance between FISH and IHC across most centres
Cleveland Clinic Foundation, USA¹
  • 318 samples from 296 patients, July 2010 to August 2012
  • Ventana ALK D5F3 primary antibody coupled with OptiView detection

Relative to FISH, IHC had 94% sensitivity and 100% specificity
National Cancer Center Hospital, Tokyo, Japan²
  • 80 samples
  • Ventana ALK D5F3 primary antibody

98.8% concordance between IHC and FISH
Charité Berlin, Inst. of Pathology³
  • 800 samples
  • Ventana ALK D5F3 primary antibody coupled with OptiView detection
  • Web-based training for IHC interpretation (not yet published)

3. Dietel et al. in preparation
Parallel FISH and Immunohistochemical Studies of ALK Status in 3244 Non–Small-Cell Lung Cancers Reveal Major Discordances

Results: ... Thus, a single FISH or IHC analysis alone would have failed to detect approx. one-fourth of ALK-positive cases with similar findings in 2 centers.

Conclusions: .... Many pre-analytic factors may account for the apparent discrepancies ....
... The significant level of discrepancies supports the need of combined testing to optimize the detection of ALK-inhibitor-eligible patients .....
Following an expert meeting at Ventana-Roche together with Pfizer Oncology the

Harmonization-Study was initiated to test **reproducibility** of immunohistochemistry (IHC-D5F3/Roche-VENTANA detection system) in ALK-rearranged NSCLC in 16 labs around Europe.

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Manfred Dietel, Maximilian von Laffert, Michael Hummel
Institute of Pathology, Charité, Berlin, Germany

A cooperation between Inst. of Pathology (Charité, Berlin), Pfizer Oncology and Ventana/Roche Diagnostic.

Framework Conditions

• Prior to the TMA-based case testing, each participating instrument was qualified using the VENTANA ALK 2 in 1 Control Slides.
• Qualification was done by Ventana staff.
• All participants used the same AB, dilution, detection system etc.
• A webinar-based training was given to all observers.
• This training included an overview of the ALK Interpretation Guide, a guided review of 50 patient cases using digital whole slide images, and a proficiency exam certifying each observer.
ALK-negative cases (n=7): binary evaluation ROCHE-VENTANA (pos. vs. neg.)

<table>
<thead>
<tr>
<th>observer</th>
<th>case 2</th>
<th>case 4</th>
<th>case 13</th>
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ALK-positive cases (n=8): binary evaluation ROCHE-VENTANA (poAs. vs. neg.)

<table>
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<tr>
<th>observer</th>
<th>case 1</th>
<th>case 3</th>
<th>case 8</th>
<th>case 11</th>
<th>case 14</th>
<th>Case 15</th>
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Consequence

When the technical details, the AB, the criteria for evaluation etc. are clearly defined and the pathologists are trained properly IHC is a reliable and reproducible approach for predictive diagnoses.

Von Laffert et al. J Thorac Oncol 2014 published online
NSCLC molecular testing algorithm at University Hospital Charité

NSCLC (adenocarcinoma, large cell, NOS)

- EGFR
  - Following board decision

- ALK-IHC
  - Reflex testing

- KRAS
  - Following board decision

  - ALK-IHC
    - Currently, for all IHC+ cases

  - ALK-FISH

  - Combined pathology report
What is the irreplaceable role of anatomic pathology in the procedure of molecular biomarker analysis?

One key point is *tissue selection!*
Teilnahmezertifikat

4. Ringversuch EGFR-Mutationsbestimmung beim NSCLC.

2013

Prof. Dr. med. Manfred Dietel
Charité - Universitätsmedizin Berlin
Institut für Pathologie
Charitéplatz 1
10117 Berlin

hat am Ringversuch ‘EGFR-Mutationstestung beim NSCLC’ mit Erfolg teilgenommen.

Leitung des Ringversuches:
Prof. Dr. med. P. Schirmacher, Prof. Dr. med. M. Dietel, Dr. R. Penzel, Dr. Chr. Schewe

Prof. Dr. med. P. Schirmacher
Deutsche Gesellschaft für Pathologie e. V.

Prof. Dr. med. W. Schlasko
Bundesverband Deutscher Pathologen e. V.

Institut für Pathologie – Charité Berlin

Combined Report on Anatomic and Molecular Pathology

Material:
External colon biopsy, FFPE - block no. ##### -10

Clinical Data:
Met. ### cancer ###

Histopath.:
Malignant epithelial …………

Combined patho-report: Metastasized adenocarcinoma of the ##### with ### mutation as indicated.
Final recommendations based on > 5000 cases processed in routine diagnostic

- Primarily (2006) 15% of participants failed → QC is absolutely essential
- Today the failure rate is < 5%.
- Clinicians should cooperate only with institutes, which passed the quality control (although in Germany there is no respective law)
- Clinical studies should involve only certified institutes,
- Currently mutation analyses are mandatory for routine diagnostic only of exon 19 und 21, but 18 and 20 are also recommended,
- otherwise 5 - 10% of all mutated cases will not be detected.
- The certified institutes are published on the home pages of the DGP

* Quality in Pathology, an initiative of the German Soc. of Pathology (DGP) and the German Association of Pathologists (BDP)
Structure of the talk on NSCLC Testing

• General introduction on molecular pathology
• What to test: sampling / tissue / cytology
• How to test
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    • IHC
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• Who to test
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• Future development
Main Issue: The report should be readily understandable for clinicians. It should contain a clear statement to select appropriate therapy.

Charité’s pathology report* includes

- standard patient identifier
- histological diagnosis, including all relevant morphological characteristics
- technical information on the assay performed, assay sensitivity
- percentage of tumor cells
- number of cell analyzed
- each exon sequenced
- each mutation detected
- clinically significant mutations, terminology adapted to local agreement
- clear statement on the likelihood of the tumor to respond.

Who to test for ALK? ESMO/CAP/IASLC/AMP 2013 guidelines

Major recommendations

ALK-testing should be performed in

• all patients with advanced-stage adenocarcinoma,
• regardless of sex, race, smoking history, or other clinical risk factors,
• selected squamous tumours (from patients with minimal or remote smoking history) should be strongly considered for testing

ESMO, Europ. Soc. For Medical Oncology
AMP, Association for Molecular Pathology; CAP, College of American Pathologists; EGFR, epidermal growth factor receptor; IASLC, International Association for the Study of Lung Cancer

Recommendations from the CAP/IASLC/AMP guidelines: How quickly should results be available? Who should be involved

“EGFR and ALK results should be available within 2 weeks (10 working days) of receiving the specimen in the laboratory”

Due to the clinician’s demand and patient’s wish in Germany we try to provide the results within 5 days

Consensus opinion: “A pathologist should (must be – by German law) be involved in the selection and interpretation of sections for ALK FISH…….”

Structure of the talk on NSCLC Testing

- General introduction on molecular pathology
- How to test
  - Methods / quality control
- What to test: sampling / tissue / cytology
  - FISH
  - IHC
  - Sanger-, pyro- or NG Sequencing
- How to report
- Who to test
- When to test
- Future development
AZD9291 Clinical Development Programme in Advanced and Metastatic EGFR T790M+ NSCLC

**AURA1**
Ongoing, recruitment completed

- Single-arm, Phase I dose escalation (N = 31) and Phase II extension (N=222) 2nd line (prior EGFR TKI only) and ≥3rd line
- Phase I: Safety, tolerability, PK and antitumour activity
d- Phase II: Efficacy and tolerability 80 mg QD in T790M NSCLC

**AURA2**
Ongoing, recruitment completed

- Single-arm, Phase II (N=210) 2nd line (prior EGFR TKI only) and ≥3rd line
- Efficacy and tolerability
- 80 mg QD in T790M NSCLC

**AURA3**
Recruitment completed

- Randomised comparative Phase III (N~410)
- Efficacy and safety of AZD9291 80 mg QD vs platinum-based doublet CT in patients with T790M, advanced/metastatic NSCLC following prior EGFR TKI

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AZD9291 is not approved in Germany. The content of these slides are not for the purposes of therapeutic recommendation.
AZD9291 – 66% ORR in T790M positive patients*

*as assessed by central tumor tissue testing

Best percentage change from baseline in target lesion

DCR (CR+PR+SD) in patients with centrally tested T790M positive tumours was 90% (141 / 157; 95% CI 84, 94)

<table>
<thead>
<tr>
<th></th>
<th>20 mg</th>
<th>40 mg</th>
<th>80 mg</th>
<th>160 mg</th>
<th>240 mg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (157)</td>
<td>10</td>
<td>32</td>
<td>61</td>
<td>41</td>
<td>13</td>
<td>157</td>
</tr>
<tr>
<td>ORR (95% CI)</td>
<td>50% (19, 81)</td>
<td>59% (41, 76)</td>
<td>66% (52, 77)</td>
<td>51% (35, 67)</td>
<td>54% (25, 81)</td>
<td>59% (51, 68)</td>
</tr>
</tbody>
</table>

Multi-pathway Inhibition as Strategy to treat TKI-resistant NSCLC

Institut für Pathologie – Charité Berlin
2nd Generation ALK-Inhibitors

Other ALK-inhibitors in development include:
- ceritinib and alectinib

Irreversibly binding, pan-HER inhibitors in clinical development include:
- dacomitinib (Phase 3) and HM781-36B (Phase 1; solid tumours)

Dacomitinib (PF-00299804; Pfizer Inc.) is an investigational compound not currently licensed for use in any market

Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer

Edward B. Garon, M.D., Naiyer A. Rizvi, M.D., Rina Hui, M.B., B.S., Natasha Leighl, M.D., Ani S. Balmanoukian, M.D., Joseph Paul Eder, M.D., Amita Patnaik, M.D., Charu Aggarwal, M.D., Matthew Gubens, M.D., Leora Horn, M.D., Enric Carcereny, M.D., Myung-Ju Ahn, M.D., Enriqueta Felip, M.D., Jong-Seok Lee, M.D., Matthew D. Hellmann, M.D., Omid Hamid, M.D., Jonathan W. Goldman, M.D., Jean-Charles Soria, M.D., Marisa Dolled-Filhart, Ph.D., Ruth Z. Rutledge, M.B.A., Jin Zhang, Ph.D., Jared K. Lunceford, Ph.D., Reshma Rangwala, M.D., Gregory M. Lubiniecki, M.D., Charlotte Roach, B.S., Kenneth Emancipator, M.D., and Leena Gandhi, M.D., for the KEYNOTE-001 Investigators*
Immunotherapy of Cancer

Stimulation of the immunosystem by blocking immun-suppressive receptor protein interactions => PD-1/PD-L1

The Role of Anti-PD-L1 Immunotherapy in Cancer – OncLive - published online
Check-point Inhibitors: IHC of PD-L1

<table>
<thead>
<tr>
<th>Case 11</th>
<th>Pathologists</th>
<th>Modus</th>
<th>Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor, E1L3N</td>
<td>P1 2 3 4 4 5</td>
<td>4</td>
<td>56%</td>
</tr>
<tr>
<td>Tumor, SP142</td>
<td>P5 4 5 4 4 4</td>
<td>5; 4</td>
<td>56%</td>
</tr>
<tr>
<td>ImmuneCells, E1L3N</td>
<td>P1 0 0 1 1 1</td>
<td>0</td>
<td>56%</td>
</tr>
<tr>
<td>ImmuneCells, SP142</td>
<td>P1 1 1 1 1 1</td>
<td>1</td>
<td>89%</td>
</tr>
</tbody>
</table>
OS by PD-L1 Expression, Evaluable Patients by Prior Treatment

Previously Treated

<table>
<thead>
<tr>
<th>PS</th>
<th>Median (95% CI), mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥50%</td>
<td>NR (9.3-NR)</td>
</tr>
<tr>
<td>1-49%</td>
<td>7.3 (5.8-12.1)</td>
</tr>
<tr>
<td>&lt;1%</td>
<td>8.6 (5.5-12.0)</td>
</tr>
</tbody>
</table>

Treatment Naive

<table>
<thead>
<tr>
<th>PS</th>
<th>Median (95% CI), mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥50%</td>
<td>NR (NR-NR)</td>
</tr>
<tr>
<td>1-49%</td>
<td>16.2 (8.6-16.2)</td>
</tr>
<tr>
<td>&lt;1%</td>
<td>10.4 (3.4-NR)</td>
</tr>
</tbody>
</table>

OS was assessed in all patients whose samples were stained within 6 months of cutting.
Analysis cut-off date: August 29, 2014.
Personalized medicine is based on a “combined morphological-molecular pathology report” including classical morphology (HE/IHC/FISH) and diverse molecular analyses – to do this in a fast and reliable manner will be the future challenge of pathology.
Conclusions

- Durable antitumor activity in a large, advanced NSCLC population
  - Median duration of response exceeds 1 year

- Manageable toxicity profile
  - Low incidence of possibly immune-mediated AEs

- PS ≥50% identifies those patients with the greatest likelihood of benefit
  - Represents 23.2% of the screened NSCLC population
  - ORR (validation set): 45.2%
    - Previously treated: 43.9%, treatment naive: 50.0%
  - Median PFS: 6.3 months
  - Median OS: not reached

- Benefit in the previously treated PS ≥50% subgroup substantially exceeds that expected from cytotoxic chemotherapy