IAP/ESP Joint Conference, Cologne Sept 25-29, 2016
Predictive Pathology, Guiding and Monitoring Therapy

Short Course SC-01 Lung Pathology:
Recent advances in molecular assessment of lung cancer

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www.ngm-cancer.com
2015 data from the German Cancer Research Center (DKFZ)

Lung cancer is the current most urgent medical need in oncology.....
Molecular Targets

Treatment Beyond resistance

Molecular Diagnostics

Liquid Diagnostics

Treatment with Immune Checkpoint Inhibitors

~5,000 Lung Cancer Genomes connected to clinical data (NGM-L)
Lung Cancer serves as a Paradigm

The differences between chemotherapy regimens are negligible

Wahrscheinlichkeit Überleben

months

E1504

cisplatin/paclitaxel
cisplatin/gemcitabine
cisplatin/docetaxel
carboplatin/paclitaxel
In **unselected** patients targeted drugs will add only marginal benefits (if at all)

EGFR-TKI mono

anti-VEGF mab + chemotherapy

Do we **hit the right target** in a specific patient ???

Taregeted therapies vs Personalised Therapies
Targeted Therapies: Match the right patient to the right drug
Special thanks to:

Patients
Sabine Merkelbach and team
Jürgen Wolf and team
Roman Thomas and team
Options for Personalised Therapies for NSCLC (2015)

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<thead>
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<th>Gene</th>
<th>Alteration</th>
<th>Frequency</th>
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<tr>
<td>EGFR</td>
<td>Mutation</td>
<td>10-15%</td>
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<tr>
<td>ALK</td>
<td>Rearrangement</td>
<td>4-5%</td>
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<tr>
<td>RET</td>
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<td>1%</td>
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<tr>
<td>MEK1</td>
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<td>FGFR1</td>
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<td>KRAS, NF1, p53</td>
<td>Mutation</td>
<td>30-35%</td>
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<tr>
<td>NRAS</td>
<td>Mutation</td>
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<tr>
<td>PIK3CA</td>
<td>Mutation</td>
<td>1-3%</td>
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<tr>
<td>PTEN</td>
<td>Deletion</td>
<td>4%</td>
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- **Standard 1st line**
  - CINC280, Crizotinib
  - off label Dabrafenib, Rez.
  - off label Lapatinib, Rez.?
  - off label Dasatinib, Rez.?
  - off label Cabozantinib o.
    Vandetanib, Rez.

- **Clinical Studies**
  + NTRK1, 2, 3 NRG1
  + 3 additional therapies applying immune checkpoint antibodies
  CTLR-4, PD1, PD-L1
  DLL3-Conjugate
  RAS-Inhibitors

**Legend**
- Green: drugs approved in NSCLC
- Yellow: drugs approved in NSCLC, but for other molecular subtype
- Blue: drugs approved in other cancer
- White: drugs in clinical development
Improved survival of $\text{EGFR}^{\text{mut}}$ und $\text{ALK}^{\text{transl}}$ patients with personalised therapy as compared to chemotherapy

Subkkohorten des Network Genomic Medicine (NGM)

The Clinical Lung Cancer Genome Project and Network Genomic Medicine, Sci Transl Med 2013
Overcoming resistance by structure-based compound design

Zhou et al., Nature 2009
Clinical efficacy of next-gen EGFR inhibitors

**CO-1686**

Centrally confirmed T790M+ patients within therapeutic dose range (N=40)

-100
-80
-60
-40
-20
0
20
40
60
80
100

ORR to date: 58%

**AZD9291**

Best percentage change from baseline in target lesion:
T790M+ evaluable patients, expansion cohorts only (N=107)

- ORR* = 64% (69/107; 95% CI 55%; 73%) in patients with EGFR T790M+ NSCLC
- Overall disease control rate (CR+PR+SD) = 54% (101/197; 95% CI 86%; 98%)

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<th>Dose (mg)</th>
<th>N (107)</th>
<th>ORR</th>
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<td>20</td>
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<td>50%</td>
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<td>40</td>
<td>29</td>
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<td>80</td>
<td>34</td>
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<td>160</td>
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<td>240</td>
<td>6</td>
<td>83%</td>
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**OS of patients treated in clinical trials**

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<th>No. of pts</th>
<th>mOS</th>
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<td>1 = clin trial</td>
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<tr>
<td>2 = no trial</td>
<td>824</td>
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<td>934</td>
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P < 0.0001 (log-rank test)

<table>
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<tr>
<th>No. of pts</th>
<th>mOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = clin trial</td>
<td>25</td>
</tr>
<tr>
<td>2 = no trial</td>
<td>83</td>
</tr>
<tr>
<td>Total</td>
<td>108</td>
</tr>
</tbody>
</table>

P = 0.002 (log-rank test)

Kostenko A, submitted
3rd generation ALK

1: 21 pts - mOS 8
2: 45 pts – mOS 23
3: 19 pts – mOS 35

Total mOS = 22

P < 0.0001
Crizotinib-resistant ALK\textit{fus} NSCLC: Rapid clinical development of 2\textsuperscript{nd} generation ALK-inhibitors

Soda, Nature 2007

Bang, ASCO 2010, Kwak NEJM 2010
FDA-approval: 2011
EMEA-approval: 2012

Tumor responses to LDK378

Shaw, ASCO 2013, NEJM 2014
2014: FDA accelerated approval track
Molecular mechanisms of acquired resistance in ALK fus+ NSCLC are increasingly understood.

# Next Gen - ALK inhibitors

<table>
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<tr>
<th>ALK TKI</th>
<th>ROS1 activity</th>
<th>Status</th>
<th>Ongoing Studies</th>
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<tbody>
<tr>
<td>Ceritinib</td>
<td>Yes</td>
<td>FDA approved (4-29-14) EMA approved (5-8-15)</td>
<td>Phase 3 (vs chemo)</td>
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<tr>
<td>Alectinib</td>
<td>No</td>
<td>Approved in Japan (7-4-2014) FDA Breakthrough Therapy Designation</td>
<td>Phase 3 (vs crizotinib)</td>
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<tr>
<td>Brigatinib</td>
<td>Yes</td>
<td>Investigational FDA Breakthrough Therapy Designation</td>
<td>Phase 2</td>
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<tr>
<td>X-396</td>
<td>Yes</td>
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<td>Phase 1</td>
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<tr>
<td>TSR-011</td>
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<td>Investigational</td>
<td>Phase 1/2a</td>
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<tr>
<td>Entrectinib</td>
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<td>Phase 1/2a</td>
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<tr>
<td>CEP-37440</td>
<td>Unk</td>
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<td>Phase 1</td>
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<tr>
<td>Lorlatinib</td>
<td>Yes</td>
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<td>Phase 1/2</td>
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</table>
Aquired resistance to Next Gen ALK-inhibitors

Baseline

WT EML4-ALK

After 8 weeks of crizotinib

Alk S1206Y
(sensitive to ceritinib)

After 34 months of crizotinib

After 12 weeks of ceritinib

After 15 months of ceritinib

ALK G1202R
(resistant to ceritinib)

Friboulet et al., Cancer Discov 4(6): 662-73, 2014
How to perform

Comprehensive Molecular Diagnostics?
EBUS-TBNA, EBB, TBB, Cytoblock

Diagnostics

1. Histology /Cytology

2. Immunohistochemistry

PEC: p63, CK5
AdCA: TTF1, CK7, NapsinA
SCLC: CD56, panCKAE1/3
LCC
exclude lymphomas, metastases

3. Single parameter molecular diagnostics (companion diagnostics
 ...... etc, etc....

4. Whole Genome Sequ vs

Multiplexing
Informative Gene Sets
80% of DNA Extracts have the minimal required amount of material

Multiplex PCR
- 10 to 50ng of gDNA
- DDR2 Panel
- Lung Panel

Library Preparation
- Adapter ligation including BC
- Enrichment (10 cycles)

MiSeq (Illumina)
- 48 Patients are loaded (DDR2 Panel)
- 24 Patients loaded (Lung Panel)
- Minimal coverage 500x

Quantification (Quibit)
Normalization
Pooling

Lung Panel
for all NSCLC
189 Amplicons
- NRAS Exon2-3
- DDR2 Exon1-18
- PTEN Exon7
- FGFR2 Exon5-17
- HRAS Exon2-3
- KRAS Exon2-3
- AKT1 Exon4
- MAP2K1 Exon2
- ERBB2 Exon19-20
- STK11 Exon1-9
- KEAP1 Exon1-6
- ALK Exon19-28
- NFE2L2 Exon1-5
- PIK3CA Exon1-2,9,20
- EGFR Exon18-21
- MET Exon16-19
- BRAF Exon11,15
- JAK2 Exon12,14

DDR2 Panel
for squamous
35 Amplicons
- BRAF Exon11, 15
- DDR2 Exon1-18

Timeline
Day 1: DNA → Multiplex PCR
Day 2: Library Prep → MiSeq loading
Day 3: MiSeq ready → Fastq files
Day 4: Alignment, BAM → Data

Enrichment
Amplify targets using Ion AmpliSeq™ Primer Pool
Partially digest primer sequences
Ligate adapters
Barcoded library

Plus FISH
Plus PD-L1

König K,
JTO 2015
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<tr>
<td>C: 2 (0%, 0+, 2-)</td>
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<td>G: 10456 (100%, 52.55+, 52.01-)</td>
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<tr>
<td>T: 3 (0%, 0+, 3-)</td>
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<tr>
<td>N: 0</td>
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**DEL: 206**

**G**

---

**EGFR**

c.2235_2249del   p.E746_A750del
Hybrid Selection instead of Multiplex PCR:
- Fragmentation of DNA (Covaris)
- Ligation of Adapter and Barcodes

Advantage:
- fusions can be integrated

Disadvantage:
- more sample input (10x)
- more data output
Composition of **aCIO** (= all cancers in one) panel **LTCGv3.0**

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<tr>
<th>Gene</th>
<th>target</th>
<th>Gene</th>
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83 genes and gene regions

**Mutation analysis**

**Rearrangement analysis**

Size of **aCIO**

(= all cancers in one) panel:

- estimated total target region: 2.26 Mb
- suggested mean coverage: 200-400 x

S Merkelbach Bruse, M Odenthal S Dümcke, R Büttner
Liquid Diagnostics

In practice
1. Optimised Handling of Blood Specimens

Use EDTA-vials
Prepare plasma within 2 hours

Use cell-free DNA vials
Prepare plasma within 4 days
⇒ can be shipped at room temperature
⇒ adaptor for puncture necessary
**Liquid Biopsy:**

**Verwendung von humanem Vollblut für die Testung auf eine T790M Mutation**

**Blutentnahme und Versand**

### Blutentnahme

- Bitte entnehmen Sie 10 ml Blut
- Bitte verwenden Sie zur Blutentnahme Cell-Free DNA BCT Röhrchen, z. B. von der Firma Streck
- Blutentnahme nach CLSI H3-A66 mit einer Venofix-Safety-Kanüle G21
- Patientenarm muss in einer abwärts gerichteten Position gehalten werden, damit kein Rückfluss entstehen kann
- Röhrchen möglichst aufrecht halten
- Bitte befüllen Sie die Röhrchen vollständig
- Röhrchen vom Adapter entfernen und unverzüglich 10 mal invertieren

### Achtung

1. Zellfreie Streck-Röhrchen sind aus Glas hergestellt.
2. Zellfreie Streck-Röhrchen enthalten chemische Zusatzstoffe, ein Rückfluss aus den Röhrchen muss daher unbedingt vermieden werden.

### Versand

- Röhrchen nicht einfrieren – die Röhrchen können bei Raumtemperatur gelagert und verschickt werden
- Röhrchen gut gesichert verschicken, z. B. in einem Schutzgefäss
- Vollblut sollte spätestens an Tag 4 zu Plasma verarbeitet werden (bitte bei der Versendung der Proben einbeziehen)
- Versand erfolgt in einem mit UN3373 gekennzeichneten Paket

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**Verpackungsanweisung P650**

**Infos und SOP:** Jana.Fassunke@uk-koeln.de
The molecular mechanisms of resistance are increasingly understood.

MET-inhibitors (like crizotinib) can overcome acquired EGFR-TKI resistance caused by MET amplification.

Scheffler et al., JTO 2015
Reimbursement: Integrated Care Contract (ICC)

First flatrate model for NGS genotyping in Germany

NGM
AOK RH

Public health insurances

First Integrated Care Contract (ICC) (§140a SGB V) in 2014

Further health insurances joined in 2015: Barmer GEK; TK; BKK VBU; BKK NOVITAS and other private and public → over 42,4 Mio. insurants

NGS-based genotyping for 35% of all German annually newly diagnosed inoperable lung cancer pts is covered by the ICC

Increase in survival of EGFRmut lung cancer:
2005 9 months
2015 55 months
Summary

There is a medical need for comprehensive molecular profiling beyond single „companion“ biomarkers

Prediction of therapies,
Treatment beyond resistance
Classification of entities,
Classification of multiple tumors vs metastasis,
Prognosis

There is a need for robust quality controls

There is a need for interdisciplinary diagnostic centers connected to clinical networks (nNGM)

Integrated Care is a model for reimbursement and steering Personalised Therapies
Thank you for your attention

Need more information?

See our website (English or German language)
www.ngm-cancer.com

Want regular updates and info?

Send an email to: juliane.sueptitz@uk-koeln.de

Ask for registering your email and get regular newsletters:
LCGC Newsletter 2016/03 and more....