ESMO ADVANCED COURSE ON BIOMARKERS FOR PRECISION MEDICINE:

NGS for tissue biopsies

Reinhard Buettner, Institute for Pathology and Center for Integrated Oncology (CIO) Cologne / Germany

Zürich, 28-29 November 2019

Reinhard.Buettner@uk-koeln.de
DISCLOSURE OF INTEREST

- Lectures and Advisory Boards for
  AbbVie, Amgen, AstraZeneca, Bayer, BMS, Boehringer-Ingelheim, Illumina, Lilly, Merck-Serono, MSD, Novartis, Qiagen, Pfizer, Roche, Targos MP Inc

- Co-Founder and Scientific Advisor for Targos Mol. Pathology Inc

- Testifying Advisor for MSD in GBA-Assessment for Pembrolizumab
The Center for Integrated Oncology CIO

Lung Cancer:
Targeted Therapies
Immune Therapies
Changing Standard Care
Our Mission:
Identify Options for Targeted Therapies

- Target Oncogenic Driver Mutations
  - EGFR-TKIs, KRAS-Inhibitors
- Exploit Intrinsic DNA Repair Deficiencies
  - BRCA, MMR, PoLE/D-deficiency
- Direct Therapeutics to Tumour Cell Surface Antigens
  - HER2 mAbs, Tumour Vaccination, CAR-Tcells
- Select Tumours for Immune Therapies
  - Immune Checkpoints, Tumour Mutational Burden
Systemic therapy of NSCLC is increasingly molecularly guided

**NSCLC stage IIIB, IV**

<table>
<thead>
<tr>
<th>EGFR (ca. 12%)</th>
<th>ALK (ca. 4%)</th>
<th>ROS1 (ca. 2%)</th>
<th>BRAF V600 (ca. 1.5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erlotinib</td>
<td>Alectinib</td>
<td>Crizotinib</td>
<td>Dabrafenib + Trametinib</td>
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<tr>
<td>Gefitinib</td>
<td></td>
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<td>Afatinib</td>
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</table>

**Non-squamous cell carcinoma**

**Squamous cell carcinoma**

**PD-L1 (tumor cells)**

- PDL1 > 50%
  - Pembrolizumab
- PDL1 – / < 50%
  - Pembrolizumab

**Resistance mutations**

- T790M
- Osimeritinib

**Targeted therapy**

- MET, RET, HER2, NTRK1,2,3, KRASG12C, FGFR1,2,3
- > targeted therapy

**Chemotherapy**

- Nivolumab: PD-L1 independent
- Pembrolizumab: only PD-L1
- Atezolizumab: PD-L1 independent
Systemic therapy of NSCLC is increasingly molecularly guided

**NSCLC stage IIIB, IV**

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<td>ca. 2 %</td>
<td>ca. 1.5 %</td>
</tr>
</tbody>
</table>

**Non-squamous cell carcinoma**

- **EGFR**
  - Erlotinib
  - Gefitinib
  - Afatinib

- **ALK**
  - Crizotinib

- **ROS1**
  - Crizotinib

- **BRAF**
  - Dabrafenib + Trametinib

**Resistance mutations**

- T790M

**PDL1 > 50%**

- Pembrolizumab

**MET, RET, HER2, NTRK1,2,3, KRASG12C, FGFR1,2,3**

- Targeted therapy

- Chemotherapy

**PDL1 – / < 50%**

- Pembro/Chemotherapy

**ALK/FGFR/EGFR/MET**

- TKI

**Squamous cell carcinoma**

- **ALK/FGFR/EGFR/MET**

**PDL1 < 1%**

- Chemo-free IO
  - Ipi/Nivo

**TMB ?**

**Systemic therapy of NSCLC**

- **BRAF V600**
  - ca. 1.5 %

- **Dabrafenib + Trametinib**

- **Pembrolizumab**
  - PD-L1 (tumor cells)

- **Pembrolizumab**
  - PD-L1 independent
  - Pembrolizumab: only PD-L1 +
  - Atezolizumab: PD-L1 independent

- **Nivolumab**
  - PD-L1 independent
## Targetable mutations in NSCLC

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alteration</th>
<th>frequency (NSCLC)</th>
<th>drugs</th>
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<tbody>
<tr>
<td>EGFR</td>
<td>Ex 19 del., L858R</td>
<td>10 %</td>
<td>erlotinib, gefitinib, afatinib, osimertinib</td>
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<tr>
<td>ALK</td>
<td>fusions</td>
<td>3%</td>
<td>crizotinib, alectinib, ceritinib, ..........</td>
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<tr>
<td>ROS 1</td>
<td>fusions</td>
<td>1%</td>
<td>crizotinib, (cabozantinib, ponatinib....)</td>
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<tr>
<td>BRAFV600</td>
<td>mutation</td>
<td>2%</td>
<td>dabrafenib + trametinib</td>
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<tr>
<td>MET</td>
<td>amplification (GCN&lt;10)</td>
<td>1%</td>
<td>crizotinib, capmatinib ......</td>
</tr>
<tr>
<td>MET</td>
<td>exon 14 skipping</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td>fusions</td>
<td>&lt; 1%</td>
<td></td>
</tr>
<tr>
<td>RET</td>
<td>fusions</td>
<td>&lt; 1%</td>
<td>cabozantinib, vandetanib, alectinib, LOXO-292</td>
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<td>NRG1</td>
<td>fusions</td>
<td>&lt; 1%</td>
<td>afatinib</td>
</tr>
<tr>
<td>HER2</td>
<td>mutation</td>
<td>1-2%</td>
<td>(trastuzumab, pertuzumab). TAK 788</td>
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<tr>
<td>NTRK 1-3</td>
<td>fusions</td>
<td>&lt; 1% (?)</td>
<td>larotrectinib, entrectinib</td>
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<tr>
<td>EGFR</td>
<td>exon 20 insertion</td>
<td>&lt; 1%</td>
<td>Poziotinib, TAK 788</td>
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<tr>
<td>FGFR 1-3</td>
<td>fusions, mutations</td>
<td>1% (each)</td>
<td>erdafinib, BGJ398.........</td>
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<tr>
<td>KRAS</td>
<td>mutation</td>
<td>20%</td>
<td>AMG510</td>
</tr>
<tr>
<td>NRAS</td>
<td>mutation</td>
<td>1%</td>
<td>?</td>
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</table>
Cancer Gene Panel – „academic“ panel

- based on a hybrid-capture panel developed in Cologne
- driver mutations for different tumor entities included, as well as translocations and CNVs
- technology: DNA-based with Agilent reagents, use of molecular barcodes

### Sequencing Strategies:

1. Multiplex PCR Panels (+IHC, FisH)
2. Hybrid Capture Panels, (DNA and RNA)
3. Sequencing ctDNA „Liquid biopsy“
4. Biomarker for IO, PD-L1, TMB, Immune infiltrate

<table>
<thead>
<tr>
<th>Gene</th>
<th>Target</th>
<th>Gene</th>
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<td>IDH1</td>
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<td>RASHG2</td>
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</tbody>
</table>

MS Marker

- MSI Marker
- KRAS_BMI1
- MMK1A_NAFG
- POLE3_CNR
- NR_22_2223B
- DAT32, IMSH2
- ACVR2A, CNR
- MSH6_CNR
- MSH6_CNR
- MSH6_CNR
- MSH6_CNR
What materials are being tested?

- FFPE from slides or blocks
- Cytology specimens
- Peripheral blood
3rd gen. EGFR-TKI as 1st line therapy are superior to 1st gen. inhibitors

Initial biopsy

EGFR del 19 L858R

Erlotinib, Gefitinib (8-10m)

PD: rebiopsy

T790M

Osimertinib (8-10m)

Osimertinib (18.9m)

- FLAURA
- Phase III
- Osimertinib vs. Standard EGFR-TKI

Soria et al, NEJM 2018

Osimertinib

Standard EGFR-TKI

Median PFS, months (95%KI)

18.9 (15.2; 21.4)

10.2 (9.6; 11.1)

HR 0.46

(95%KI 0.37; 0.57)

p<0.0001
Overcoming resistance by structure-based compound design
Second-generation EGFR inhibitors override EGFR\textsuperscript{G724S}-induced osimertinib resistance in lung adenocarcinoma patients, Nat. Comm. 2018

- Osimertinib first-line suppresses emerging T790M, but
- G724S and C797S are the most frequent resistant mutations after Osimertinib.
Genomic profiling identifies outcome-relevant mechanisms of innate and acquired resistance to third-generation EGFR TKI therapy in lung cancer

Michels, Heydt et al, JCO Prec. Oncology 2019
Jana Fassunke, Nat. Comm., Nov 2018
Sequential therapy in EGFRmut NSCLC: increasingly molecularly guided

1st gen. EGFR-TKI
PFS: 10 m

2nd gen. EGFR-TKI
PFS: 14.7 m (dacom.)

alternatively: 1st gen. EGFR TKI + bevacizumab:
T790M+: PFS 16 m / T790M-: PFS 10 m

Chemo
PFS: 5m

T790M+
Osimertinib
PFS: 10 m

T790M-
Osimertinib
PFS: 10 m

T790M-
Chemo
PFS: 5m

PD: rebiopsy

EGFR C797S
EGFR G724S
hl MET amp.
HER2 amp.
KRAS Mut

Predictability in Evolution
SFB 1310
Combined Therapies Suppress Resistance
2nd NGM Evaluation 2018: OS benefit with sequential therapies

Michels et al., JCO Precision Med 2018

C. Alidousty, J Pathol, June 2018: TP53 mutant ALK+ Lung Cancers are CIN high

**TP53 mut**

**TP53 wt**

mut, mutation; wt, wild type.
Michels et al. JCO Prec Onc 2018
Lin and Shaw, JTO 2017
Immunotherapy, biomarkers

Histology-based

Tumor Mutational Burden; Neoantigens

Checkpoints (PD-L1/2, ...)

Microenvironment; Associated immune cells

‘Tissue agnostic’

TMB & GEP: Groups

TMB vs. GPE: 4 groups

- **A** Reduced: Lack of immunogenicity
- **B** Moderate: Immune evasion
- **C** Moderate: Stromal/endothelial TME
- **D** Strong: Intense cytolytic activity

**Distribution in TCGA data (n=6384)**

- Colorectal, MSI-h
- Lung SCC
- Skin cutaneous melanoma
- Lung adenocarcinoma
- Stomach adenocarcinoma
- Head and neck SCC
- Bladder urothelial carcinoma
- Cervical SCC and endocervical
- Uterine corpus endometrial
- Colorectal, MSS
- Breast, HER2+
- Liver hepatocellular
- Pancreatic adenocarcinoma
- Breast, invasive
- Breast, TNBC
- Kidney renal clear cell
- Prostate adenocarcinoma
- Ovarian serous
- Thyroid carcinoma
- Glioblastoma multiforme

Results of the first German Harmonization-Study PD-L1 IHC

09/2016 | Institute of Pathology, University Cologne, www.pdl1.de


SFB „Lung Cancer“

ICPs: PD-L1, LAG3, VISTA

Lymphocytes: CD3, CD4, CD8, CD20, FoxP3, CD25

Myelo-Monocytic: CD68, GrB, S100, CD163

CAFs: aSMA, NG2, FAP, TenC

Vessels: desmin, VEGFR1/2, TIE1
CD3 (0-2000/mm²): PD1
PFS: Nivolumab + Chemotherapy and Nivolumab + Ipilimumab
By TMB, Checkmate 227, ASCO 2018

TMB ≥10 mut/Mb and <1% Tumor PD-L1 Expression

<table>
<thead>
<tr>
<th></th>
<th>Nivo + chemo (n = 43)</th>
<th>Nivo + ipi (n = 38)</th>
<th>Chemo (n = 48)</th>
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</thead>
<tbody>
<tr>
<td>Median PFS,a mo</td>
<td>6.2</td>
<td>7.7</td>
<td>5.3</td>
</tr>
<tr>
<td>HR (vs chemo)</td>
<td>0.56</td>
<td>0.48</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.35, 0.91)</td>
<td>(0.27, 0.85)</td>
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TMB <10 mut/Mb and <1% Tumor PD-L1 Expression

<table>
<thead>
<tr>
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<th>Nivo + chemo (n = 54)</th>
<th>Nivo + ipi (n = 52)</th>
<th>Chemo (n = 59)</th>
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<tbody>
<tr>
<td>Median PFS,b mo</td>
<td>4.7</td>
<td>3.1</td>
<td>4.7</td>
</tr>
<tr>
<td>HR (vs chemo)</td>
<td>0.87</td>
<td>1.17</td>
<td>(95% CI)</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.57, 1.33)</td>
<td>(0.76, 1.81)</td>
<td></td>
</tr>
</tbody>
</table>

Exploratory analysis

TMB ≥10 mut/Mb and <1% Tumor PD-L1 Expression:

- Nivolumab + chemotherapy: 1-y PFS = 45%
- Nivolumab + ipilimumab: 1-y PFS = 27%
- Chemotherapy: 1-y PFS = 8%

TMB <10 mut/Mb and <1% Tumor PD-L1 Expression:

- Nivolumab + chemotherapy: 1-y PFS = 18%
- Nivolumab + ipilimumab: 1-y PFS = 16%
- Chemotherapy: 1-y PFS = 16%
OS and PFS With NIVO + IPI vs NIVO vs Chemo in Patients With Tumor PD-L1 Expression ≥ 1%

Dosages were NIVO (3 mg/kg Q2W) + IPI (1 mg/kg Q6W), and NIVO (240 mg Q2W). Subsequent systemic therapy was received by 35% of patients in the NIVO + IPI arm, 44% of patients in the NIVO arm, and 54% of patients in the chemo arm; subsequent immunotherapy was received by 6%, 8%, and 43%, respectively.

OS (n = 396) NIVO (n = 396) Chemo (n = 397)

Median OS, mo 17.1 15.7 14.9

HR (vs chemo)ᵃ 0.79 0.88
CI 0.65–0.96ᵇ 0.75–1.04ᶜ

PFS by BICR (n = 396)

NIVO + IPI NIVO Chemo

Median PFS, mo 5.1 4.2 5.6

HR (vs chemo)ᵈ 0.82 0.99
95% CI 0.69–0.97 0.84–1.17

No. at risk

NIVO + IPI 396 341 295 264 244 212 190 165 153 145 129 91 41 9 1 0
NIVO 396 330 299 265 220 201 176 153 139 129 115 70 36 10 2 0
Chemo 397 358 306 250 218 190 166 141 126 112 93 57 22 6 1 0

⁴HR (95% CI) for NIVO + IPI vs NIVO, 0.90 (0.76–1.07); ⁹7.72% CI; ⁹5% CI; ⁴HR (95% CI) for NIVO + IPI vs NIVO, 0.83 (0.71–0.97).
Supplementary Appendix: CM227

**OS With NIVO + IPI vs Chemo in Patients With Tumor PD-L1 Expression < 1%**

<table>
<thead>
<tr>
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<th>NIVO + IPI (n = 187)</th>
<th>Chemo (n = 186)</th>
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<tr>
<td>Median OS, mo</td>
<td>17.2</td>
<td>12.2</td>
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<td>HR (vs chemo)</td>
<td>0.62</td>
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<tr>
<td>CI</td>
<td>0.48–0.78^a</td>
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</table>

Dosages were NIVO (3 mg/kg Q2W) + IPI (1 mg/kg Q6W), and NIVO (360 mg Q3W) plus chemo. Subsequent systemic therapy was received by 44% of patients in the NIVO + IPI arm, 41% of patients in the NIVO + chemo arm, and 53% of patients in the chemo arm; subsequent immunotherapy was received by 4%, 4%, and 36%, respectively.

^a95% CI.
## Cancer Gene Panel: Overview

<table>
<thead>
<tr>
<th>Feature</th>
<th>academic</th>
<th>Illumina</th>
<th>Thermo</th>
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§ data from Campesato et al., Oncotarget 2016

**Mutect2-Pipeline**

1. **Alignment (bwa)**
2. **Umi-read grouping/deduplication (fg-bio)**
3. **Realignment of all readgroups (bwa)**
4. **Variant calling (GATK Mutect2)**
5. **Annotation (snpEFF; COSMIC)**
6. **Filtering (Annotation, coverage, AF tumor, quality, ratio AF tumor vs. AF pon)**
7. **Nonsynonymous calls / Covered regions**

- **Blacklist**
  - Panel of normals (pon)
  - ExAC_non_TCGA pop.-freq. ≥ 0.01%

- **ExAC_non_TCGA pop.-freq. ≥ 0.01%**
Illumina TMB
IlluminaNonSyn. TMB
Thermo TML
NEO
Qiagen biomedical workflow
Qiagen – Mutect2
Academic panel - Mutect2
Current status of nNGM

nNGM-centers and network partners

2018: molecular diagnostics of ca. 10,000 pts. with advanced NSCLC
= ca. 1/3 of the target population

> Personalized cancer medicine becomes reality
Task Force 1a/b
Mol. Diagnostics
QC

Task Force 2
Documentation
Evaluation

Task Force 3
Medical Reports
Counselling

Task Force 4
Clinical Trials

Task Force 5
Reimbursement
HC Providers

Task Force 6
Research

nNGM
National Network
Genomic Medicine
Lung Cancer

Speakers: J Wolf, R Büttner, C von Kalle
Integration of Diagnostics and Specialised Cancer Therapies

Assertion of Quality under Financial Constraints

Implementing Personalised Medicine

Early Implementation of Innovation

Assertion of Knowledge in a Public Health Care System

Independence of Therapies from Diagnostic Interests

Gain of ~ 75,000 patient-years/year
Molecular Diagnostic Centers in nNGM
High-end diagnostics – Clinical trials – New approaches

Thanks to EFRE/NRW, German Cancer Aid
Thanks to our Patients