Biomarkers in lung cancer

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Disclosure slide

• no Conflicts of Interest to declare
Prevalence and clinical outcomes for patients with ALK positive adenocarcinoma in Europe: preliminary results from the European Thoracic Oncology Platform Lungscape Project


*equal contribution
In 2012: adenocarcinoma has been split in multiple molecular subtypes

Adenocarcinoma

Others

Squamous cell carcinoma

EGFR

Unknown

KRAS

ERBB2

PIK3CA

ROS1 rearrangement

ALK rearrangement

NRAS

BRAF

MET amplification

**EML4–ALK rearrangement**

- **Chr 2p**
- **Inversion**
- **ALK fusion protein**

**Cell survival**
- PI3K–AKT–MTOR–S6K
- BAD

**Proliferation**
- RAS–MEK–ERK

- **PLCγ–PIP2–IP3**

Targeting EML4-ALK

Crystal S; Shaw A et al, Clin Cancer Res 2012
ETOP Lungscape Project

- European Thoracic Oncology Platform (ETOP) has shown the feasibility of a non centralized review of IHC for ALK translocation in NSCLC from a large number of centers in Europe, building a comprehensive database

  - Large cohort of non metastatic patients
  - Assessing Prognostic value
  - Patients’ characteristics

Peters S, J Thorac Oncol 2011;6:S994
Characteristics of “EML4-ALK” patients

ETOP Study

- 3-5% of NSCLC patients: YES
- Male = Female: YES
- Young: YES
- Non-smokers: never smokers, former light smokers: YES
- In most cases mutually exclusive with EGFR and Kras mutations: Don’t know
- Refractory or poorly sensitive to EGFR TKI: Don’t know
- Same profile of response to chemotherapy (when compared to EGFR mutated patients)
Blackhall et et al,
Prognostic value of ALK

• The prognostic value of ALK remains controversial
RFS Multivariate Cox Model: 
N=1099; RFS events=591 
HR IHC 3+ vs IHC 0+/1+/2+ = 0.41 
95% CI (0.19, 0.86), p=0.0189 
Adjusted for Stage, Gender & PS

OS Multivariate Cox Model: 
N=1099; Deaths=513 
HR IHC 3+ vs IHC 0+/1+/2+ = 0.32 
95% CI (0.13, 0.79), p=0.0127 
Adjusted for Stage, Gender, PS & Age
The prognostic significance of ALK rearrangement in NSCLC has not been settled

  - did not demonstrate any significant differences in overall survival (OS) for patients with NSCLC by EML4-ALK status in the era before crizotinib.
- Zhang et al.: AACR 2012
  - no survival difference according to ALK status after adjusting for disease stage, histology, and EGFR/KRAS mutant status.
- Lee et al.: Cancer 2012
  - patients with ALK-rearranged NSCLC had the shortest overall survival compared to wild-type patients, but the difference was not significant.
- Kim et al. Cancer 2012
  - able to demonstrate that patients with ALK-rearranged NSCLC had a significant worse OS outcomes after factoring in age, sex, histology, stage, and performance status.
  - EML4-ALK status is a poor prognostic factor for relapse-free survival after factoring in stage, sex, age, and treatment.
Worse Disease-Free Survival in Never Smokers with ALK Lung Adenocarcinoma

PFS/RFS survival curves for FISH/IHC3 (positive) and FISH-negative/ IHC0/1

300 never-smokers with lung adenocarcinoma from the observational Mayo Clinic Cohort

ALK positivity was 12.2% by IHC and confirmed at 8.2% of tumors by FISH, with complete concordance between IHC 3+/0 and FISH+/-

Overall survival of lung adenocarcinoma patients with *EML4-ALK*

Median, 14.7 versus 10.3 months; \( p \ 0.009, \)
The prognostic significance of $ALK$ rearrangement in NSCLC has not been settled

- Conversely, Wu et al. *J Thorac Oncol. 2012* found that patients with $EML4$-$ALK$ NSCLC identified from pleural effusion cytology had a significantly improved survival outcome compared with patients without $EML4$-$ALK$ NSCLC.

- Takeuchi et al. *Nat Med 2012* receptor kinase fusion-positive NSCLC is an independent favorable prognostic factor after taking into consideration age, sex, stage, and smoking status.

- All of these studies are limited by the small number of $ALK$-positive patients, different comparison group of patients, the heterogeneous treatment patients received, and differences in the balance of prognostic factors (e.g., smoking status, surgical treatment, age) compared with $ALK$-rearranged patients.

- Blackhall’s results are based on a large series of non metastatic patients (Stade I to III), not treated with crizotinib.
Several Methods of detection

FISH ALK (Break apart probe)

No translocation

EML4-ALK Fusion

False positive risk: 5 %

Vysis (Abbott)
Methods of detection

• Immunohistochemistry
  – Several antibodies
    • ALK1 clone, sensitivity of 90% & specificity 97.8%
    • 5A4 clone detecting EML4-ALK v1, v2, v3, v6 et v7 & KiF5B-ALK variant
    • D5F3 clone

• Multiplex RT-PCR
Correlation of ALK IHC and FISH

<table>
<thead>
<tr>
<th>ALK IHC</th>
<th>ALK FISH</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>234</td>
</tr>
<tr>
<td>1+</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>2+</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>3+</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Total (%)</td>
<td>25 (9.5%)</td>
<td>237 (91.5%)</td>
</tr>
</tbody>
</table>

262 patients who were either EGFR wild-type or non-responders to previous EGFR tyrosine kinase inhibitors (TKI).
Flow chart

Adenocarcinoma patients with available ALK IHC data

N=1099

ALK IHC +
N=69

ALK IHC -
N=1030

ALK IHC 1:2 Matched Cohort
N=207
Matching factors in order of importance:
Stage, Gender/Smoking Status,
Center/Year of surgery/ Age

ALK IHC +
N=69

9 FISH ND

38 FISH -

22 FISH +

1 FISH +

23 FISH +

46 FISH -

137 FISH -

ALK FISH 1:2 Matched sub-cohort
N=69

www.esmo2012.org
Different translocation variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Protein Kinase</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>E13;A20</td>
<td>WD</td>
<td>33% - 40%</td>
</tr>
<tr>
<td>E06;A20</td>
<td>WD</td>
<td>29% - 37.5%</td>
</tr>
<tr>
<td>E20;A20</td>
<td>WD</td>
<td>9% - 17.5%</td>
</tr>
<tr>
<td>E14;A20</td>
<td>WD</td>
<td>3% - 2.5%</td>
</tr>
<tr>
<td>E18;A20</td>
<td>WD</td>
<td>2%</td>
</tr>
<tr>
<td>E15;A20</td>
<td>WD</td>
<td>2%</td>
</tr>
<tr>
<td>E02;A20</td>
<td>WD</td>
<td>2% - 2.5%</td>
</tr>
<tr>
<td>E17;A20</td>
<td>WD</td>
<td>1%</td>
</tr>
</tbody>
</table>

- FISH with Break Apart Rearrangement Probe confirms the presence of an ALK rearrangement but provides no information about the specific type of ALK fusion itself.
- RT-PCR can also identify the EML4-ALK variant

References:
- Crystal S; Shaw A et al, Cl Cancer Res 2012
How to screen ALK

Biological samples sent for molecular diagnosis EGFR, Ras, ALK, etc

All adenocarcinomas?

IHC ALK

FISH in case of

IHC+

Some prespecified populations: non smokers...
ADC TTF1 + (P63 +)?
Some histological pattern?

If discrepancies IH/FISH
If less than 60 TC for FISH analysis

FISH (other probes)?
RT-Q-PCR?

Modified from S Lantuejoul
Association between Tumor EGFR and KRAS Mutation Status and Clinical Outcomes in NSCLC Patients Randomized to Sorafenib plus Best Supportive Care (BSC) or BSC Alone: Subanalysis of the Phase III MISSION Trial

Tony S. Mok,1 Luis Paz-Ares,2 Yi-Long Wu,3 Silvia Novello,4 Erzsebet Juhasz,5 Osvaldo Aren,6 Yan Sun,7 Vera Hirsh,8 Egbert F. Smit,9 Chetan Lathia,10 Teng Jin Ong,10 and Carol Peña10

1Chinese University of Hong Kong, Hong Kong, China; 2Instituto de Investigaciones Biomédicas de Sevilla, Hospital Universitario Virgen del Rocío/Universidad de Sevilla/CSIC, Seville, Spain; 3Guangdong General Hospital and Guangdong Academy of Medical Sciences, Guangzhou, China; 4University of Turin, Turin, Italy; 5Koranyi National Institute of TB and Pulmonology I and XIV, Budapest, Hungary; 6Instituto Nacional de Cancer, Santiago, Chile; 7Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China; 8McGill University Health Centre, Montreal, Que., Canada; 9Department of Pulmonary Diseases, VU University Medical Center, Amsterdam, The Netherlands; 10Bayer HealthCare Pharmaceuticals, Montville, NJ, USA
Meta-analysis showing no significant difference between the chemotherapy plus multitargeted antiangiogenic TKI and chemotherapy alone groups for overall survival in patients with advanced NSCLC (HR 0.93, 95% CI 0.83–1.03).

- G. Herbst 2010: 0.91 (0.79, 1.07) 44.13
- De Boer 2011: 0.88 (0.65, 1.13) 16.11
- V. Heymach 2008: 1.15 (0.75, 1.77) 3.57
- V. Heymach 2007: 0.91 (0.55, 1.52) 3.94
- G. Scaglotti 2010: 1.15 (0.94, 1.41) 16.80
- D. Goss 2010: 0.73 (0.57, 1.06) 15.40
- Overall (I-squared = 15.0%, p = 0.313): 0.93 (0.83, 1.03) 100.00
Overall survival based on EGFR mutation status

**Pts with EGFR mut (in tumor or plasma)**
- Sorafenib N=44; Placebo N=45
- HR=0.48 (95% CI 0.3, 0.76)
- P-value=0.002
- Sorafenib median OS= 13.9 mo (423d)
- Placebo median OS= 6.5 mo (197d)

**Pts with EGFR wt**
- Sorafenib N=122; Placebo N=136
- HR=0.92 (95% CI 0.7, 1.21)
- P-value=0.559
- Sorafenib median OS= 8.3 mo (253d)
- Placebo median OS= 8.4 mo (256d)

Biomarker*treatment interaction analysis: p-value=0.023
Results: biomarker analysis

<table>
<thead>
<tr>
<th></th>
<th>EGFR mutation</th>
<th>KRAS mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor positive</td>
<td>12 / 90 (13%)</td>
<td>20 / 71 (28%)</td>
</tr>
<tr>
<td>Plasma positive</td>
<td>85 / 346 (25%)</td>
<td>62 / 346 (18%)</td>
</tr>
<tr>
<td>Either tumor or plasma</td>
<td>89 / 347 (26%)</td>
<td>68 / 347 (20%)</td>
</tr>
</tbody>
</table>
High rate of EGFR mutations in MISSION subgroup analysis

- EGFR and KRAS mutations are found in 5% to 15% and 15% to 20% of unselected whites with lung adenocarcinomas, respectively, to be mostly mutually exclusive.
- A rate of 26% of REGF mutation could be overestimated.
Detection of EGFR mutations in plasma cfDNA

• Various methods have been reported
  – Plasma DNA analyzed by mass spectrometry-based genotyping (Sequenom) and mutant-enriched PCR (Brevet et al, Lung Cancer 2011)
  – Scorpions ARMS, could be more sensitive (L Qin, Chin Med J 2011)
Epidermal Growth Factor Receptor Mutations in Plasma DNA Samples Predict Tumor Response in Chinese Patients With Stages IIIB to IV Non–Small-Cell Lung Cancer

Table 2. Correlation of EGFR Mutations Between Plasma DNA and Primary Tumor DNA

<table>
<thead>
<tr>
<th>Correlate</th>
<th>Tumor</th>
<th>Case Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGFR-Positive</td>
<td>EGFR-Negative</td>
</tr>
<tr>
<td>Plasma</td>
<td>63</td>
<td>16</td>
</tr>
<tr>
<td>EGFR-positive</td>
<td>14</td>
<td>137</td>
</tr>
<tr>
<td>EGFR-negative</td>
<td>77</td>
<td>153</td>
</tr>
<tr>
<td>Case number</td>
<td>77</td>
<td>153</td>
</tr>
</tbody>
</table>

Abbreviation: EGFR, epidermal growth factor receptor.
*Correlation index = 0.74.
Detection of specific mutation in circulating DNA

Tumor

DNA

Direct sequencing

Mutation
for example, APC 1338 C→T

Plasma

DNA

Real-time PCR

Total DNA concentration
for example, 11,500 DNA fragments per sample

BEAMing

Percentage mutant DNA
for example, 0.27%

Mutant DNA concentration
for example, 31 mutant DNA fragments per sample

Diehl F Nature Medicine 2008
## Results: biomarker analysis

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</tbody>
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### EGFR

<table>
<thead>
<tr>
<th></th>
<th>Tumor</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Wild-type</td>
<td>Mutant</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>Wild-type</td>
<td>75</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Mutant</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Total number</td>
<td>89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concordance (%)</td>
<td>93.3*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### KRAS

<table>
<thead>
<tr>
<th></th>
<th>Tumor</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild-type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>Wild-type</td>
<td>45</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Mut</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Total number</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concordance (%)</td>
<td>84.3*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
 EGFR mutational status discrepancies in non small cell lung cancer (primary tumor versus distant metastasis).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Year</th>
<th>N</th>
<th>Main site</th>
<th>EGFR all mutations</th>
<th>EGFR activating mutation$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$\Delta$ (%)</td>
<td>$+/-$ (%)</td>
</tr>
<tr>
<td>Matsumoto</td>
<td>2006</td>
<td>8</td>
<td>Brain 100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cortot</td>
<td>2010</td>
<td>21</td>
<td>Brain 62%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kalikaki</td>
<td>2008</td>
<td>25</td>
<td>Lung 36%</td>
<td>24</td>
<td>16</td>
</tr>
<tr>
<td>Park$^a$</td>
<td>2008</td>
<td>101</td>
<td>Node 100%</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Gow$^a$</td>
<td>2009</td>
<td>67</td>
<td>Brain 38%</td>
<td>38</td>
<td>13</td>
</tr>
<tr>
<td>Schmid</td>
<td>2009</td>
<td>48</td>
<td>Node 100%</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Sun</td>
<td>2011</td>
<td>80</td>
<td>Node 100%</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Han</td>
<td>2011</td>
<td>37</td>
<td>Pleura 57%</td>
<td>16</td>
<td>11</td>
</tr>
</tbody>
</table>

$\Delta$: discrepancies; $+/-$: samples being positive in the primary tumor and negative in the metastatic setting; $-/+$: samples being negative in primary tumor and positive in the metastatic setting.

$^a$ By direct sequencing.

$^b$ Activating EGFR mutation on exons 19 and 21.

Vignot S et al  Critical Reviews in Oncology/Hematology (2012)
Sorafenib mechanisms of action

Cell proliferation, differentiation, Migration, invasion, metastasis

Vascular permeability

Cell survival
Antitumor Activity of Sorafenib in Human Cancer Cell Lines with Acquired Resistance to EGFR and VEGFR Tyrosine Kinase Inhibitors

Floriana Morgillo*, Erika Martinelli, Teresa Troiani, Michele Orditura, Ferdinando De Vita, Fortunato Ciardiello
Division of Medical Oncology, Department of Clinical and Experimental Medicine and Surgery "F. Magrassi e A. Lanzara" Second University of Naples, Naples, Italy

• Sorafenib reduced the activation of MEK and MAPK and caused an inhibition of cell proliferation, invasion, migration, anchorage-independent growth in vitro and of tumor growth in vivo of all TKI-resistant CALU-3 and HCT116 cell lines.

• These data suggest that resistance to EGFR inhibitors is predominantly driven by the RAS/RAF/MAPK pathway and can be overcame by treatment with sorafenib

www.esmo2012.org
EGFR pathway and angiogenesis

• The BATTLE trial:
  – Sorafenib had a higher disease control rate in EGFR-wild-type patients (P < 0.001), but a worse disease control rate in patients with EGFR mutation (P = 0.01) or high EGFR polysomy (P = 0.05) compared with other agents. Kim ES. Cancer Discov 2011;1:44-53

• Randomized, Phase II trial sorafenib + erlotinib versus erlotinib + placebo.
  – 67 patients with tumors bearing wild-type EGFR, sorafenib/erlotinib group showed a superior median PFS (3.38 months in sorafenib/erlotinib group versus 1.77 months, P = 0.018) and a superior mean overall survival (8 months for sorafenib/erlotinib versus 4.5 months, P = 0.019). Spigel DR J Clin Oncol 2011;29(18):2582-9
EGFR pathway and angiogenesis

• Despite the importance of both the EGFR pathway and angiogenesis in lung cancer, inhibition of both these pathways using the combination of sorafenib plus erlotinib, does not appear to improve the therapeutic ratio.

PM Ellis Critical Reviews in Oncology/Hematology 84 (2012) 47–58
## Treatment summary

| EGFR mutation |  | EGFR wild type |  |
|---------------|  |  |  |  |
| Sorafenib n=44 (%) | Placebo n=45 (%) | Sorafenib n=122 (%) | Placebo n=136 (%) |
| On-study |  |  |  |  |
| Duration of therapy |  |  |  |  |
| Mean (weeks) | 16.6 | 6.1 | 19.6 | 12.4 |
| Dose interruption, n (%) | 13 (30) | 6 (13) | 60 (49) | 27 (20) |
| Dose reduction, n (%) | 8 (18) | 1 (2) | 47 (39) | 8 (6) |
| Post-progression Therapy |  |  |  |  |
| Any | 26 (59) | 25 (56) | 54 (44) | 84 (62) |
| 2+ | 16 (36) | 9 (20) | 22 (18) | 33 (24) |
| Anti-EGFR | 19 (43) | 8 (18) | 18 (15) | 37 (27) |

P=0.01
Progression-free survival based on EGFR mutation status

Pts with EGFR mut (in tumor or plasma)
- Sorafenib N=44; Placebo N=45
- HR=0.27 (95% CI 0.16,0.46)
- P-value<0.001
- Sorafenib median PFS= 2.7 mo (83d)
- Placebo median PFS= 1.4 mo (42d)

As the PFS was prolonged in patients receiving sorafenib, in patients in 3rd to 4th line of treatment, did it give time to have access to anti EGFR targeted treatment

Biomarker*treatment interaction analysis: p-value=0.015
Sorafenib benefit in EGFR mutated subgroup

- OS outcome may be biased by the unbalanced use of post-study EGFR TKI (sorafenib arm 43%; placebo arm 18%)
- The patient subgroup with available samples for biomarker analysis is not representative of the overall population
- Limited sample size (47% of overall population)
- Biomarker analyses in MISSION were retrospective
Perspectives

• Liquid biopsy with blood sample reflecting metastatic or micrometastatic disease
  – Could be repeated during treatment
  – Emergence of treatment resistance
• Circulating cell free DNA for KRAS and EGFR
• Circulating tumor cell for ALK translocation
  – (Ilie et al Ann Oncol2012)
The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers

Luis A. Diaz Jr\textsuperscript{1,2}, Richard T. Williams\textsuperscript{3}, Jian Wu\textsuperscript{1,4}, Isaac Kinde\textsuperscript{1}, J. Randolph Hecht\textsuperscript{5}, Jordan Berlin\textsuperscript{6}, Benjamin Allen\textsuperscript{7}, Ivana Bozic\textsuperscript{7}, Johannes G. Reiter\textsuperscript{7,8}, Martin A. Nowak\textsuperscript{7}, Kenneth W. Kinzler\textsuperscript{1}, Kelly S. Oliner\textsuperscript{2} & Bert Vogelstein\textsuperscript{1}
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• Dr Anne Vincent-Salomon
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