Prevalence and clinical outcomes for patients with ALK positive adenocarcinoma in Europe: preliminary results from the European Thoracic Oncology Platform Lungscape Project


*equal contribution
F.H. Blackhall / ETOP declare no conflict of interest

F. H. Blackhall has received honoraria from Pfizer for speaker and advisory board roles
Lungscape project responsibilities

Project design and guidance:
• Lungscape steering committee

Project execution:
• ETOP office
• Frontier Science Foundation – Hellas (FSF-H)

Lungscape financial support:
• Consortium approach

Contributions for this specific project:
• Pfizer
• ALK gene fusion (ALK+) is a validated therapeutic target for non-small cell lung cancer (NSCLC)\textsuperscript{1}

• The prevalence of ALK+ NSCLC is low, varies by population examined and detection method used \textsuperscript{2} – predominantly found in adenocarcinoma & more frequent in never-smokers

• Prevalence in early stage NSCLC is not well understood

• The ETOP Lungscape iBiobank is a decentralised resource of 2400 NSCLC cases from 11 countries \textsuperscript{3} – and created as a platform to evaluate prevalence & clinical significance of NSCLC biomarkers including ALK in a European population

\textsuperscript{1} Kwak et al NEJM 2010
\textsuperscript{2} Scagliotti et al. EJC 2012
\textsuperscript{3} Peters et al. #1179O ESMO 2012
Immunohistochemistry (IHC) and Fluorescent In Situ Hybridisation (FISH) for detection of ALK gene fusion.
Aims

• Determine prevalence of ALK positivity in resected stage I-III lung adenocarcinomas in Europe using immunohistochemistry (IHC) then fluorescent in situ hybridisation (FISH) for confirmation

• Explore correlation of ALK positivity with outcome

• Compare IHC to FISH for ALK + detection
Methods: case selection and immunohistochemistry

- Ibiobank central electronic database built for annotated comprehensive clinical data collection

- Sites: selection of eligible patients for Lungscape (tissue tracking, clinical data review and capture) (Peters et al: abstract 11790)

- Immunohistochemistry (IHC): clone 5A4 antibody (Novocastra), establishment of protocol, internal and external quality assessment followed by local IHC on whole sections (Thunnissen et al: abstract 193P)
Methods: case matching and FISH confirmation

• IHC ALK+ cases matched 1:2 with IHC ALK negative cases using a predefined algorithm with the following priority: stage, gender, smoking status, center, year of surgery, age at surgery - IHC 2+ & 3+ cases matched first

• Fluorescent in situ hybridisation (FISH) performed on the IHC ALK+ cases and the matched IHC ALK negative controls

• FISH: Vysis break apart FISH probe (Abbott, per manual) internal and external quality assessment followed by local FISH on whole sections.
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 + \[ N=69 \]
- ALK IHC - \[ N=138 \]

ALK IHC +

- 9 FISH ND
- 38 FISH -

ALK IHC -

- 22 FISH +
- 1 FISH +
- 23 FISH +
- 46 FISH -
- 137 FISH -

ALK FISH 1:2 Matched sub-cohort
\[ N=69 \]
Statistical analysis

- **Full data set**: Fisher’s exact test or Chi-square test to explore differences in patient & tumor characteristics by ALK status. Log-rank test and Cox proportional hazards models (univariate & multivariate) to explore differences in hazard for ALK positive vs negative patients (OS, RFS)

- **Case-matched data sets**: Stratified Cox proportional hazards regression & Conditional logistic regression (OS, RFS)

**DEFINITIONS:**

- Overall survival (OS): date of surgery to death from any cause
- Relapse free survival (RFS): date of surgery to first relapse or death from any cause
# Results: patient characteristics

<table>
<thead>
<tr>
<th>CHARACTERISTIC (%)</th>
<th>Total N=1099</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male : Female</td>
<td>55.5 : 44.5</td>
</tr>
<tr>
<td>Caucasian</td>
<td>99.2</td>
</tr>
<tr>
<td>Current smoker</td>
<td>32.9</td>
</tr>
<tr>
<td>Former smoker</td>
<td>48.6</td>
</tr>
<tr>
<td><strong>Never smoker</strong></td>
<td>14.6</td>
</tr>
<tr>
<td>Smoking unknown</td>
<td>3.9</td>
</tr>
<tr>
<td>Stage Ia / Ib</td>
<td>27.5 / 26.4</td>
</tr>
<tr>
<td>Stage IIA / IIB</td>
<td>14.1 / 9.5</td>
</tr>
<tr>
<td>Stage IIIA / IIIB</td>
<td>20.9 / 1.6</td>
</tr>
<tr>
<td>Adjuvant CT</td>
<td>21.3</td>
</tr>
</tbody>
</table>
Results: ALK IHC

69 were positive among 1099 cases (6.3%; 95% CI [4.9%, 7.9%])

- IHC 1+ = 38 (55.1%)
- IHC 2+ = 8 (11.6%)
- IHC 3+ = 23 (33.3%)

Mean H-score:

- IHC 1+ : 30.8
- IHC 2+ : 86.1
- IHC 3+ : 239.5

- Homogeneous staining (100% cells stained) : 23% cases

ALK IHC according to gender, N=1099

- Female: 92.2% ALK+ (7.8% ALK-)
- Male: 94.9% ALK+ (5.1% ALK-)

p=0.08
ALK IHC according to smoking status, N=1099

- Current: 93.9%
- Former: 94.8%
- Never: 88.1%

*Excluding category “Unknown”

p=0.011*
ALK IHC according to age groups, N=1099

Median age lower for ALK + (p=0.042)
Association of ALK IHC and FISH, N=198

For ALK IHC + vs – (IHC 1+/2+/3+ vs IHC 0+)

- FISH Sensitivity = 36.7%
  - 22 FISH + / 60 IHC +
- FISH Specificity = 99.3%
  - 137 FISH - / 138 IHC –
  - \( p < 0.001 \)

For ALK IHC 3+ vs 0/1+/2+

- FISH Sensitivity = 90.5%
  - 19 FISH + / 21 IHC 3 +
- FISH Specificity = 97.7%
  - 173 FISH - / 177 IHC 0+/1+/2+
  - \( p < 0.001 \)

36.7% of IHC+ are FISH+
ALK IHC+ cases according to gender, N=69

"IHC 1+ & 2+" vs "IHC 3+", p=NS

p=0.069
ALK IHC+ cases according to smoking status, N=69

"IHC 1+ & 2+" vs "IHC 3+", p=0.026
ALK IHC+ cases according to age groups, N=69

"IHC 1+ & 2+" vs "IHC 3+", p=0.08

p=0.058
RFS and OS by ALK IHC status, N=1099

Log-rank test: p=0.005

Log-rank test: p=0.006

Note: Number of patients and 5-year RFS / OS, depicted in the pictures

RFS Multivariate Cox Model:
N=1099; RFS events= 591
HR $\text{ALK}^+ \text{ vs } \text{ALK}^-$ = 0.52,
95% CI (0.35, 0.78), p=0.0014
Adjusted for Stage, Gender & PS

OS Multivariate Cox Model:
N=1099; Deaths=513
HR $\text{ALK}^+ \text{ vs } \text{ALK}^-$ = 0.51,
95% CI (0.33, 0.79), p=0.0025
Adjusted for Stage, Gender, PS & Age
RFS and OS for IHC 3+ vs IHC 0/1+/2+, N=1099

Log-rank test: p=0.0185

**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

Log-rank test: p=0.0091

**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

Note: Number of patients and 5-year RFS / OS, depicted in the pictures
RFS and OS by ALK IHC status, matched cohort, N=207

Matching Factors: Stage, Gender/Smoking Status, Center/Year of surgery/ Age

Stratified log-rank test: p=0.091

Stratified log-rank test: p=0.012

Note: Number of patients and 5-year RFS / OS, depicted in the pictures

Conditional Logistic Regression – RFS event at 3 years
N=207; RFS events at 3 years=96
OR Yes vs No=0.52, 95% CI (0.26, 1.01), p=0.06

Conditional Logistic Regression – OS event at 3 years
N=207; Deaths at 3 years=82
OR Yes vs No=0.53, 95% CI (0.26, 1.07), p=0.077
RFS and OS by ALK FISH status, matched cohort, N=69

Matching Factors: Stage, Gender/Smoking Status, Center/Year of surgery/ Age

Stratified log-rank test: p=0.15

Stratified log-rank test: p=0.058

Note: Number of patients and 5-year RFS / OS, depicted in the pictures

Conditional Logistic Regression – RFS event at 3 years
N=69; RFS events at 3 years=33
OR Yes vs No=0.19, 95% CI (0.04, 0.91), p=0.037

Conditional Logistic Regression – OS event at 3 years
N=69; Deaths at 3 years=25
OR Yes vs No=0.22, 95% CI (0.05, 1.10), p=0.057
Overall survival (OS) by ALK IHC status for Stage “Ia & Ib”, “IIa & IIb” and “IIIa & IIIb”

Note: Number of patients and 5-year OS, depicted in the pictures
Conclusions

• We report on the first large European dataset evaluating prevalence and outcome of ALK positive stage I-III resected lung adenocarcinoma patients, using IHC and FISH confirmation.

• Tumour tissue and annotated clinical data were available for 1099 patients treated in 15 different institutions.

• Case matching according to main prognostic clinical parameters was performed for ALK IHC and FISH positive cases in a 1:2 ratio with IHC negative and FISH negative cases respectively.
In early stage completely resected adenocarcinoma:

- Prevalence of ALK IHC is 6.3%
- Prevalence of ALK FISH is at least 2.1%
- ALK IHC+ is an independent prognostic factor for OS & RFS
- ALK FISH+ is associated with a trend to better survival (p=0.058)
- High concordance between ALK IHC (0 and 3+) with FISH (-ve and +ve, respectively) is demonstrated
Acknowledgements

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• S Peters - Lungscape database coordination and quality assessment (Peters et al. #1179O ESMO 2012)

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• Abbott Molecular for supporting ongoing Lungscape research
ADDITIONAL SLIDES
Overall survival (OS) by ALK IHC status for Stage “Ia & Ib”, “IIa & IIb” and “IIIa & IIIb”

Note: Number of patients and 5-year OS, depicted in the pictures
Overall survival (OS) by ALK FISH status for Stage “Ia & Ib”, “IIa & IIb” and “IIIA & IIIB”

Note: Number of patients and 5-year OS, depicted in the pictures
ALK IHC according to gender, smoking status & age groups; N=1099

- Female: 92.2% ALK IHC+, 7.8% ALK IHC-
- Male: 94.9% ALK IHC+, 5.1% ALK IHC-
  p=0.08

- Current: 93.9% ALK IHC+, 6.1% ALK IHC-
- Former: 94.8% ALK IHC+, 5.2% ALK IHC-
- Never: 88.1% ALK IHC+, 11.9% ALK IHC-
  p=0.011*

- <60 yrs: 92.4% ALK IHC+, 7.6% ALK IHC-
- 60-70 yrs: 92.8% ALK IHC+, 7.2% ALK IHC-
- >70 yrs: 96.2% ALK IHC+, 3.8% ALK IHC-
  p=0.079

*Excluding category “Unknown”

Median age lower for ALK IHC+: p=0.042
ALK IHC 1+, 2+ & 3+ according to gender, smoking status & age groups; N=69 IHC ALK positive cases

p=0.068

p=0.10

p=0.059

“ALK IHC 1+ & 2+” vs “ALK IHC 3+”, p=NS

“ALK IHC 1+ & 2+” vs “ALK IHC 3+”, p=0.026

“ALK IHC 1+ & 2+” vs “ALK IHC 3+”, p=0.08