



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

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## 2 | Disclosures

F.H. Blackhall / ETOP declare no conflict of interest

**F. H. Blackhall has received honoraria from Pfizer for speaker and advisory board roles**

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## 4 | 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

## 5 | Background and rationale

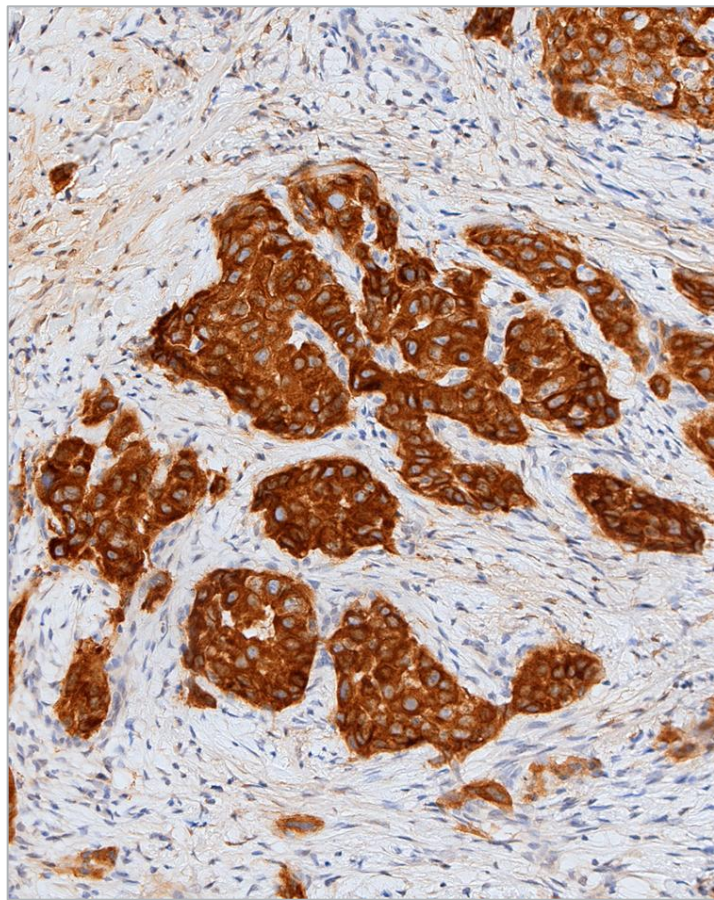
- ALK gene fusion (ALK+) is a validated therapeutic target for non-small cell lung cancer (NSCLC)<sup>1</sup>
- The prevalence of ALK+ NSCLC is low, varies by population examined and detection method used <sup>2</sup> – predominantly found in adenocarcinoma & more frequent in never-smokers
- Prevalence in early stage NSCLC is not well understood
- The ETOP Lungscape iBiobank is a decentralised resource of 2400 NSCLC cases from 11 countries <sup>3</sup> – and created as a platform to evaluate prevalence & clinical significance of NSCLC biomarkers including ALK in a European population

<sup>1</sup> Kwak et al NEJM 2010

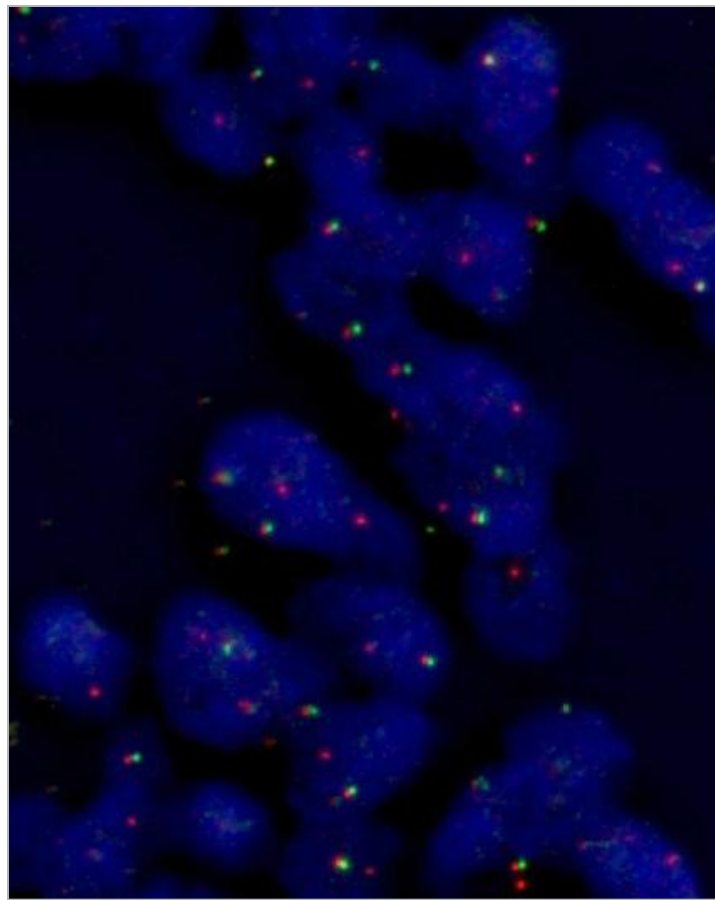
<sup>2</sup> Scagliotti et al. EJC 2012

<sup>3</sup> Peters et al. #11790 ESMO 2012

6 | Immunohistochemistry (IHC) and Fluorescent In Situ Hybridisation (FISH) for detection of ALK gene fusion



ALK IHC 3+



ALK FISH+  
(single red signals)

## 7 | 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

## 8 | Methods: case selection and immunohistochemistry

- Ibiobank central electronic database built for annotated comprehensive clinical data collection
- Sites: selection of eligible patients for Lungscope (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)

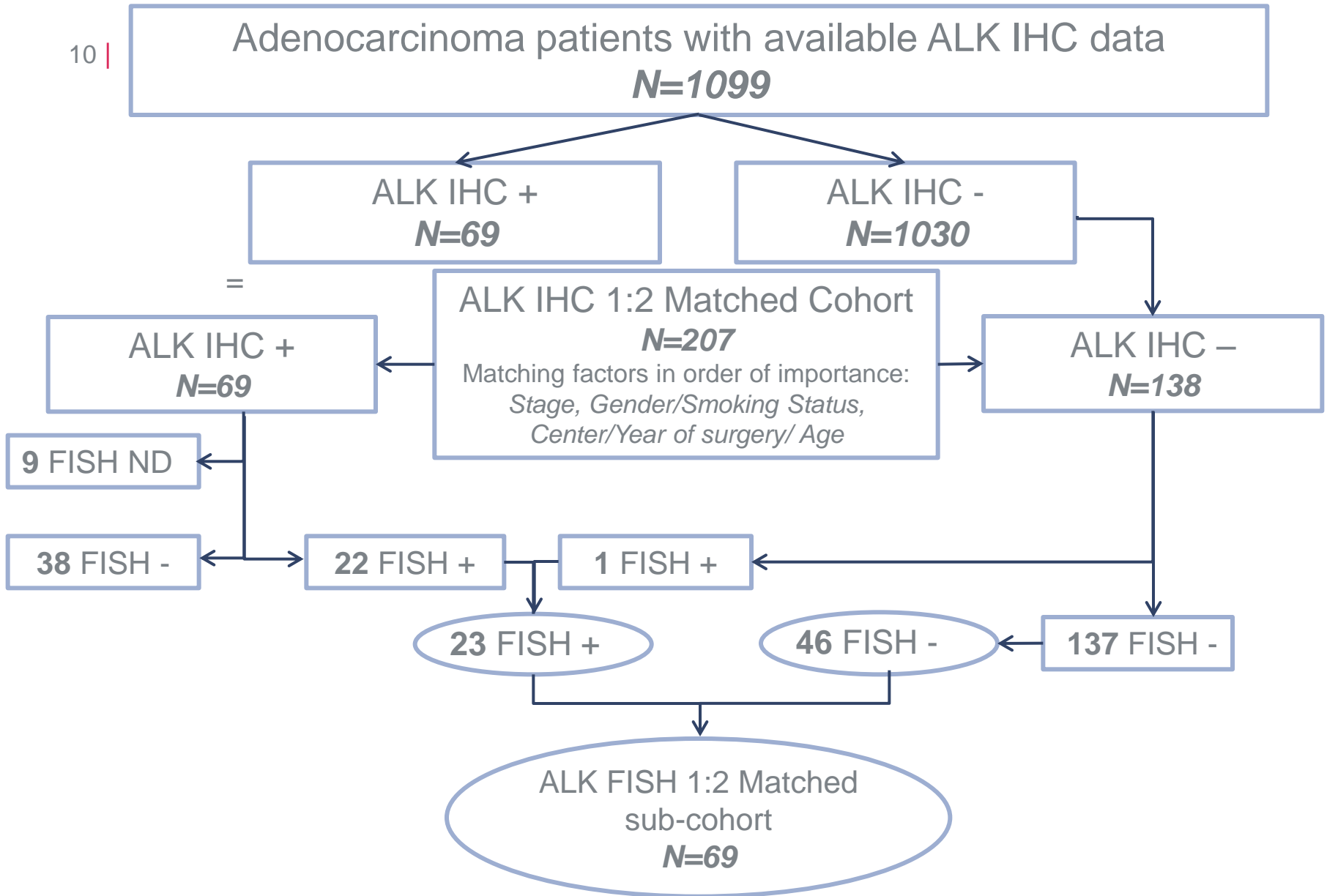


## 9 | Methods: case matching and FISH confirmation

- 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

10 |



## 11 | 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

## 12 | Results: patient characteristics

CHARACTERISTIC (%)	Total N=1099
Male : Female	55.5 : 44.5
Caucasian	99.2
Current smoker	32.9
Former smoker	48.6
<b>Never smoker</b>	<b>14.6</b>
Smoking unknown	3.9
<b>Stage Ia / Ib</b>	<b>27.5 / 26.4</b>
Stage IIa / IIb	14.1 / 9.5
Stage IIIa / IIIb	20.9 / 1.6
Adjuvant CT	21.3

## 13 | 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%)

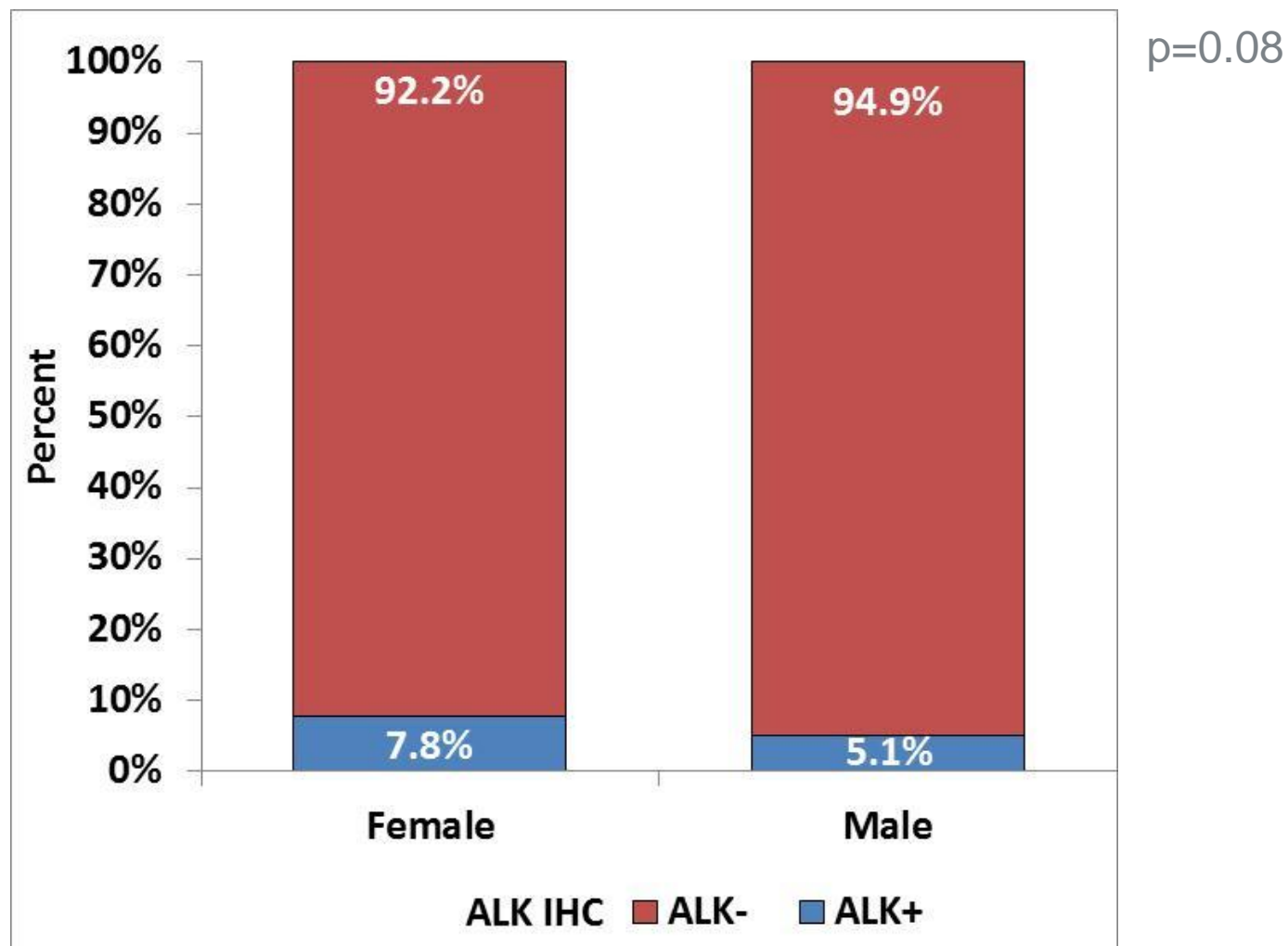
Mean H-score <sup>1</sup>:

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

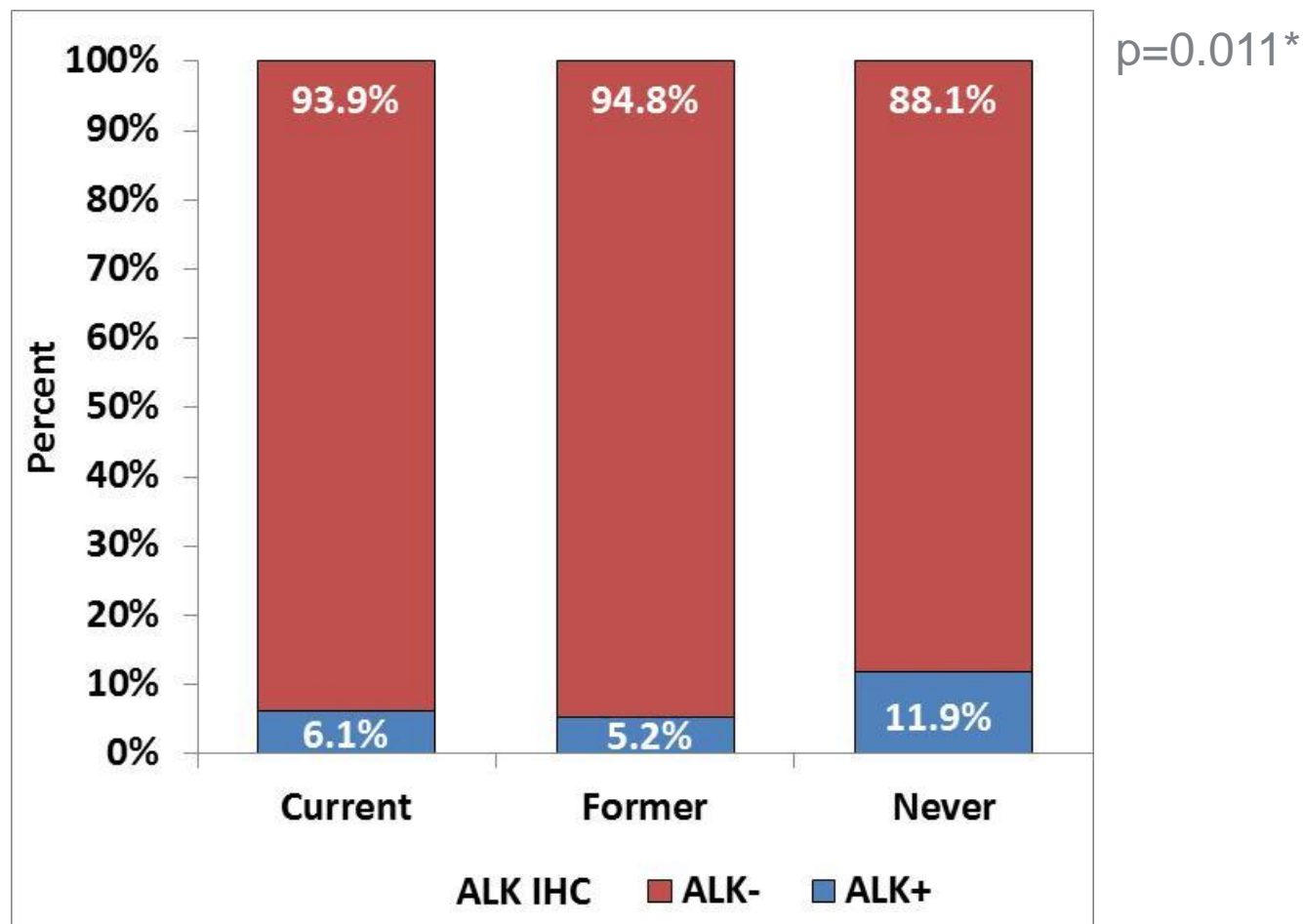
➤ Homogeneous staining (100% cells stained) : 23% cases

<sup>1</sup> modified from Rüschoff J, et al. Virchows Arch. 2010; 457: 299-307

14 | ALK IHC according to gender, N=1099

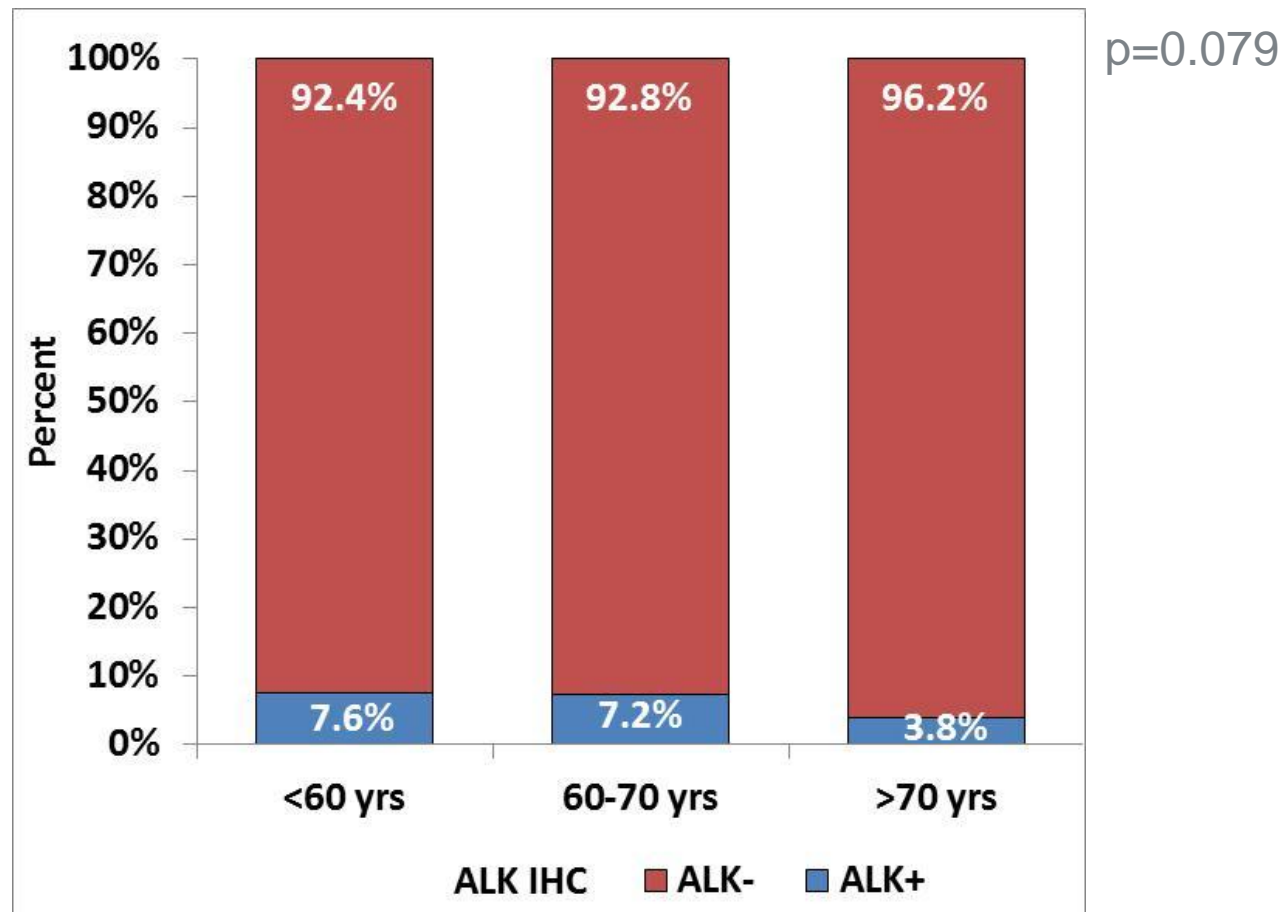


15 | ALK IHC according to smoking status, N=1099



*\*Excluding category "Unknown"*

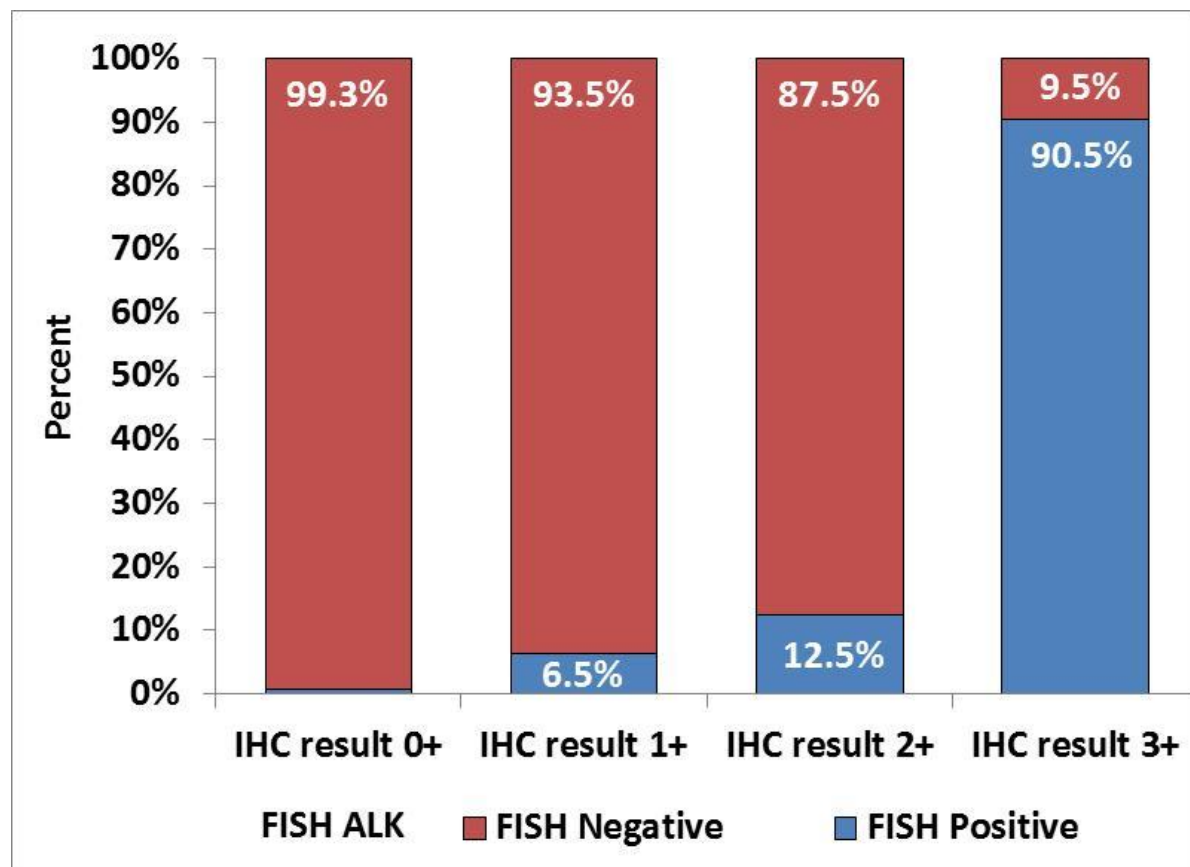
## 16 | ALK IHC according to age groups, N=1099



Median age lower for ALK + (p=0.042)



## 17 | Association of ALK IHC and FISH, N=198



36.7% of IHC+ are FISH+

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

FISH Sensitivity=36.7%  
22 FISH + / 60 IHC +

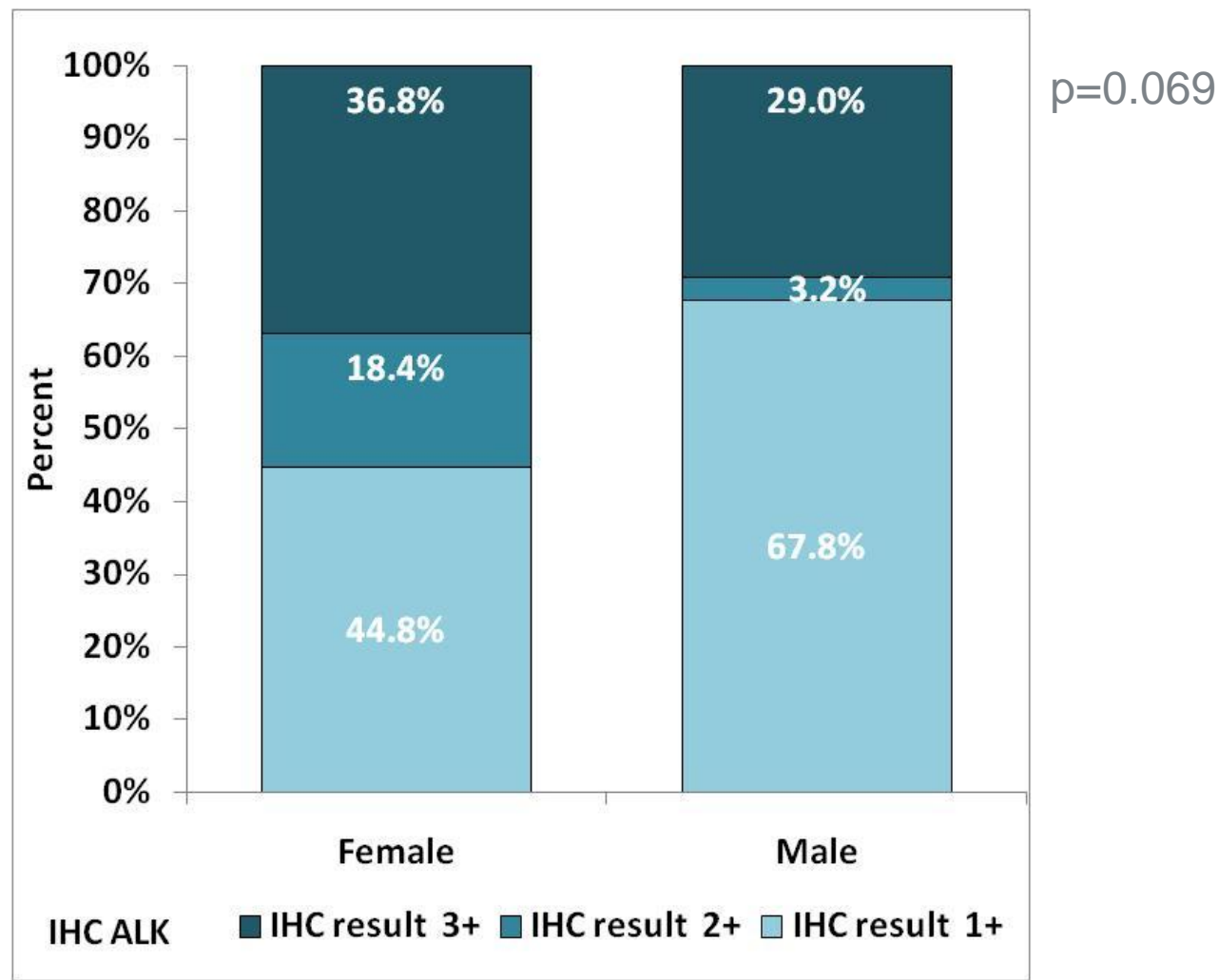
FISH Specificity=99.3%  
137 FISH - / 138 IHC –  
*p<0.001*

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

FISH Sensitivity = 90.5%  
19 FISH + / 21 IHC 3 +

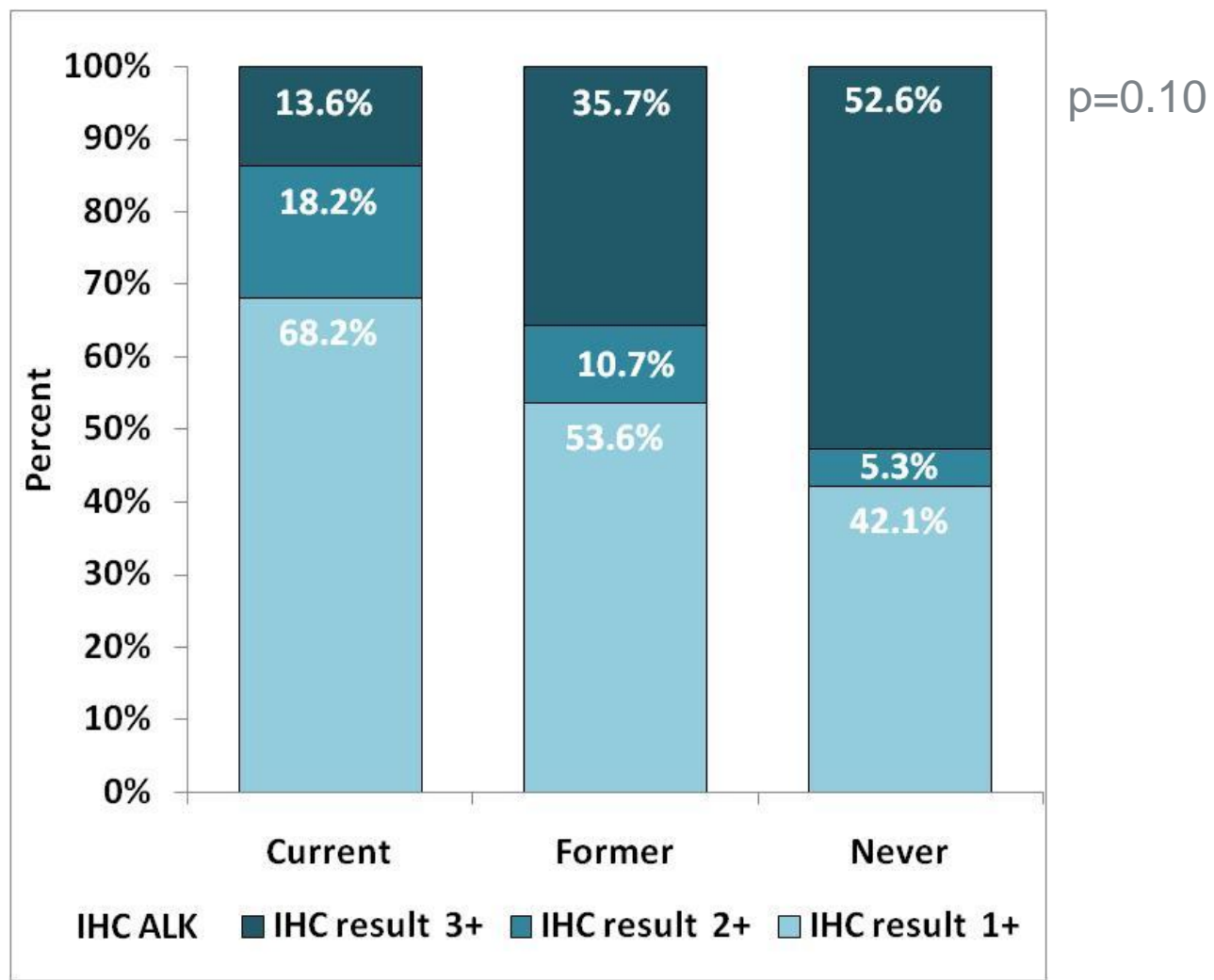
FISH Specificity = 97.7%  
173 FISH - / 177 IHC 0+/1+/2+  
*p<0.001*

## 18 | ALK IHC+ cases according to gender, N=69



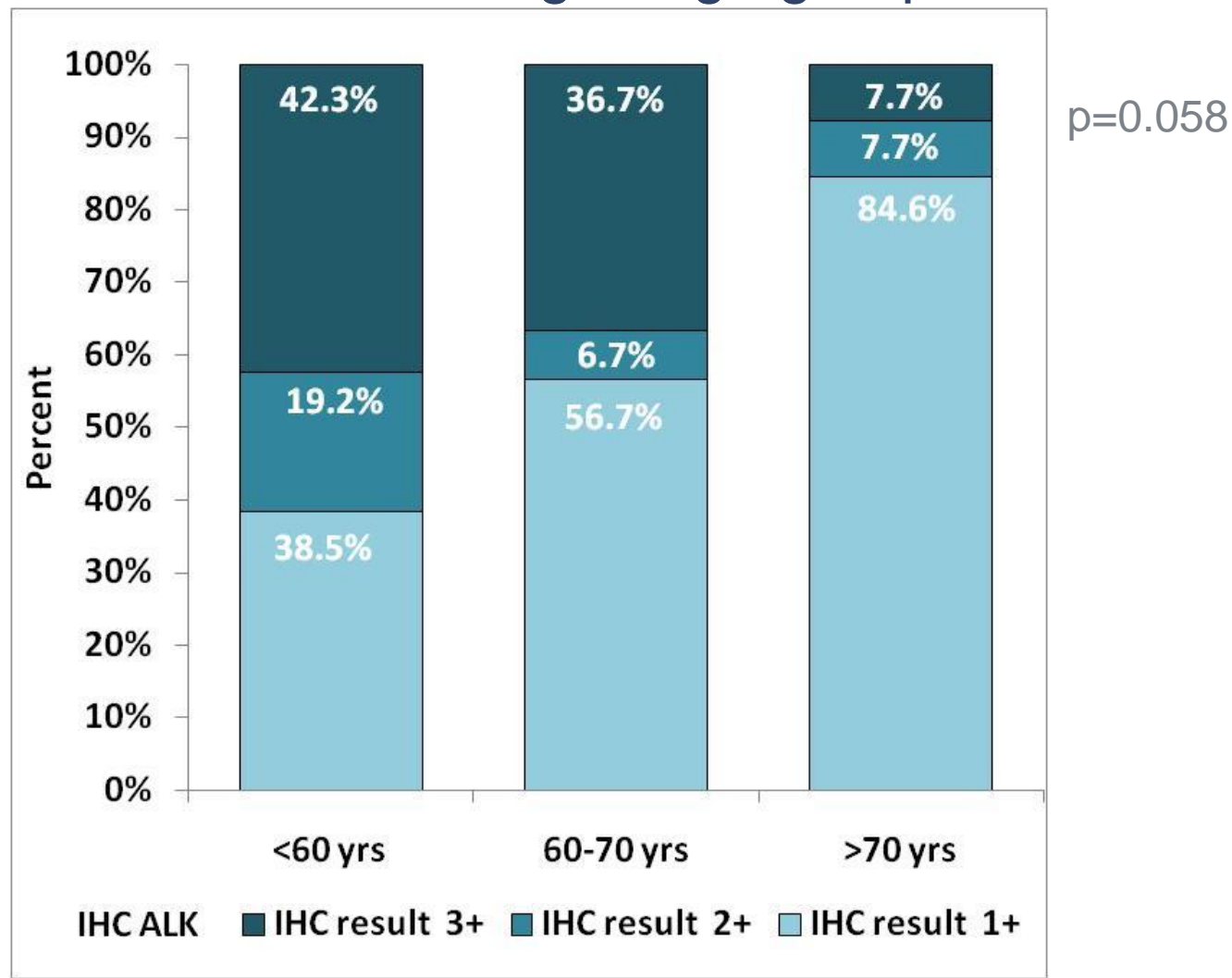
“IHC 1+ & 2+” vs “IHC 3+”, p=NS

19 | ALK IHC+ cases according to smoking status, N=69



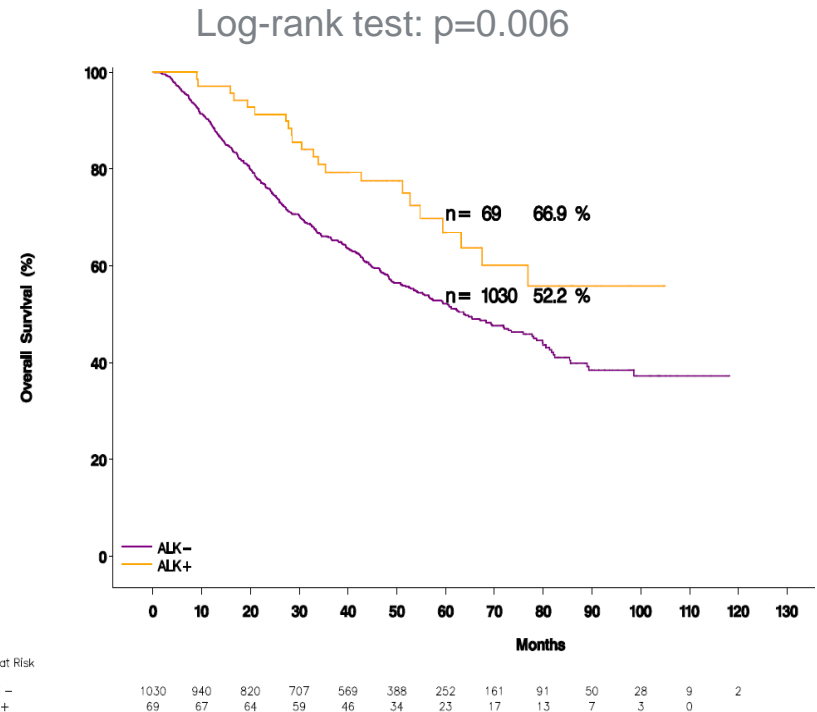
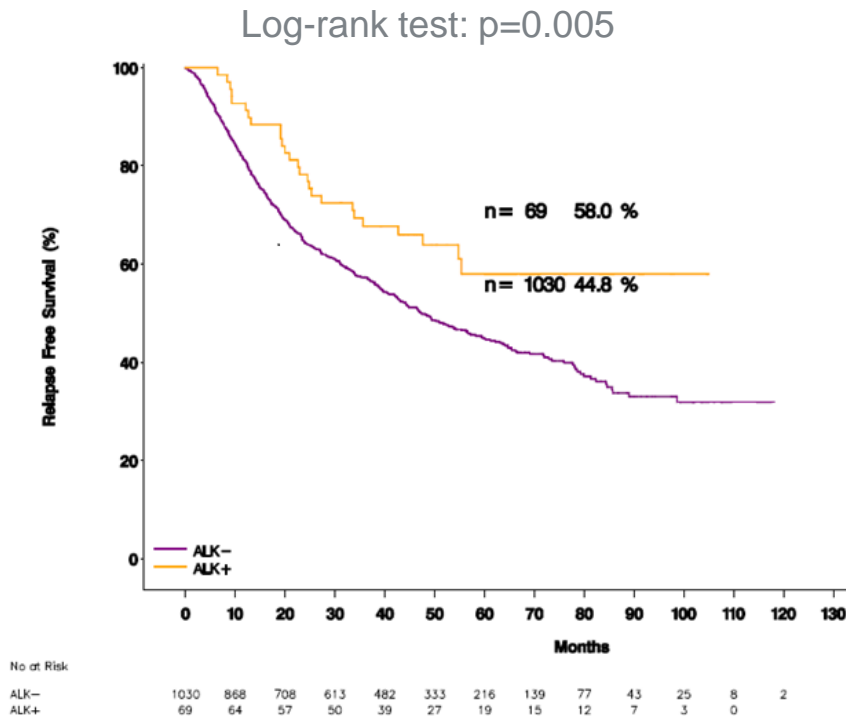
“IHC 1+ & 2+” vs “IHC 3+”, p=0.026

## 20 | ALK IHC+ cases according to age groups, N=69



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

## 21 | RFS and OS by ALK IHC status, N=1099

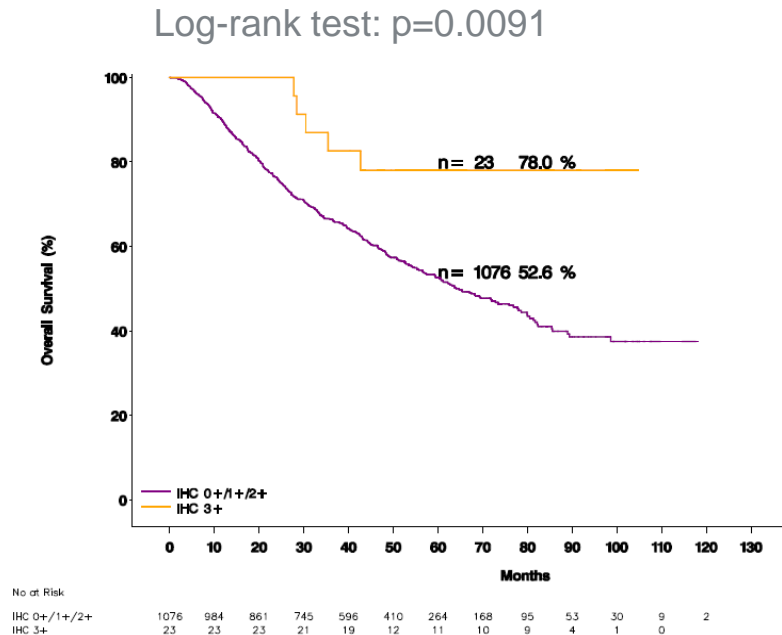
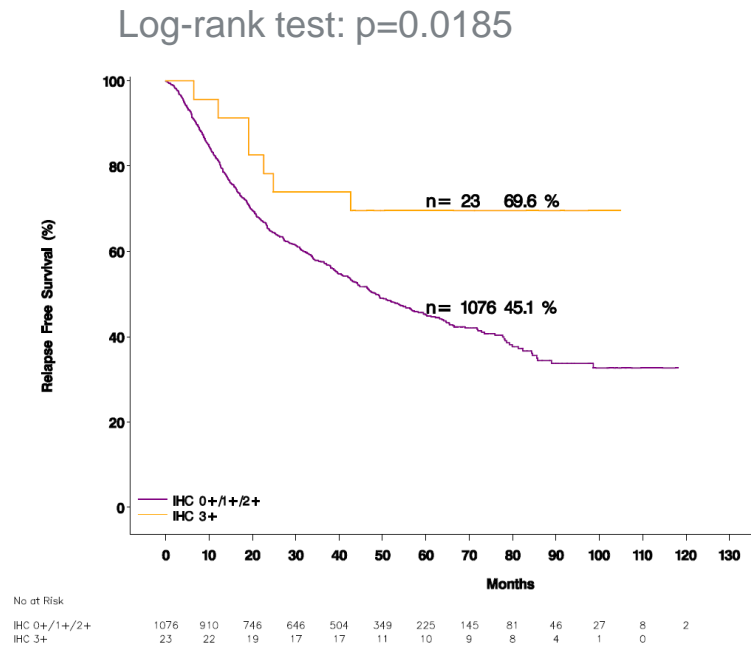


*Note: Number of patients and 5-year RFS / OS, depicted in the pictures*

RFS Multivariate Cox Model:  
 N=1099; RFS events= 591  
 HR<sub>ALK+ vs -</sub> = 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<sub>ALK+ vs -</sub> = 0.51,  
 95% CI (0.33, 0.79),  $p=0.0025$   
 Adjusted for Stage, Gender, PS & Age

## 22 | RFS and OS for IHC 3+ vs IHC 0/1+/2+, N=1099



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

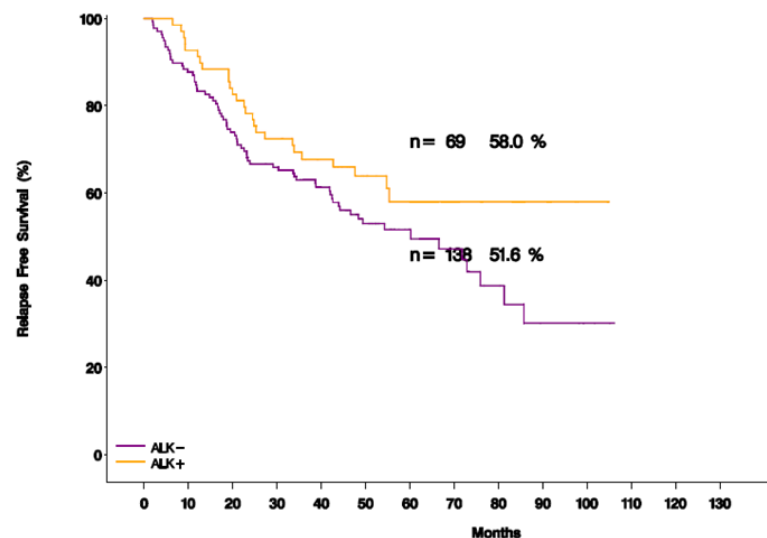
RFS Multivariate Cox Model:  
 N=1099; RFS events=591  
 HR IHC 3+ vs IHC 0+/1+/2+ =0.41  
 95% CI (0.19, 0.86),  $p=0.0189$   
 Adjusted for Stage, Gender & PS

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

## 23 | RFS and OS by ALK IHC status, matched cohort, N=207

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

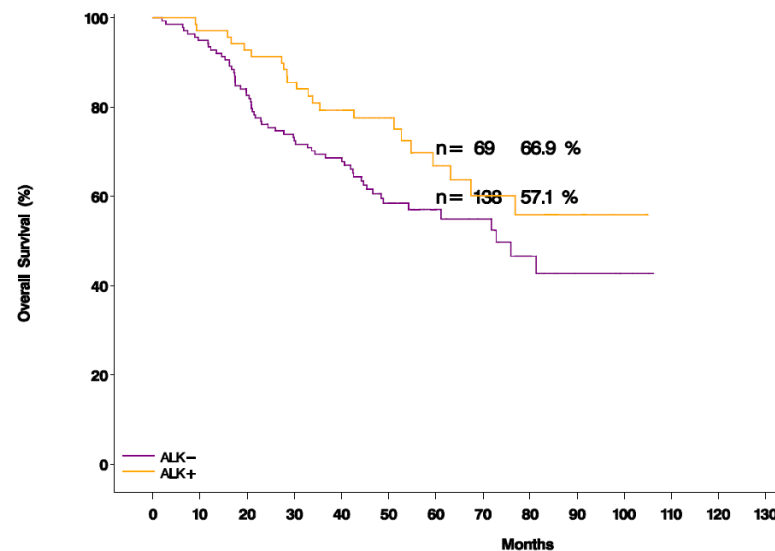
Stratified log-rank test:  $p=0.091$



No at Risk

ALK- 138 121 102 89 73 49 25 20 11 6 3 0  
ALK+ 69 64 57 50 39 27 19 15 12 7 3 0

Stratified log-rank test:  $p=0.012$



No at Risk

ALK- 138 131 114 98 81 52 28 23 14 7 3 0  
ALK+ 69 67 64 59 46 34 23 17 13 7 3 0

*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<sub>Yes vs No</sub>=0.52, 95% CI (0.26, 1.01),  $p=0.06$

Conditional Logistic Regression – OS event at 3 years

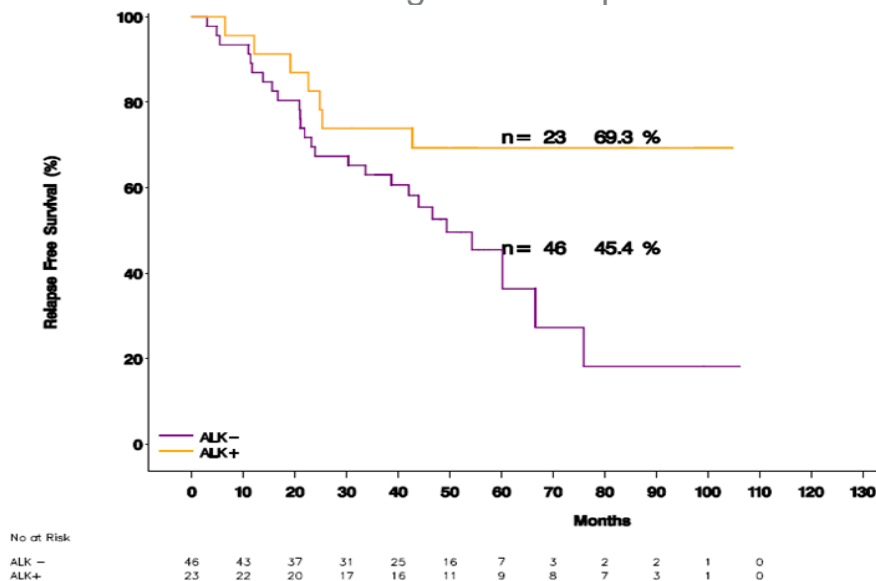
N=207; Deaths at 3 years=82

OR<sub>Yes vs No</sub>=0.53, 95% CI (0.26, 1.07),  $p=0.077$

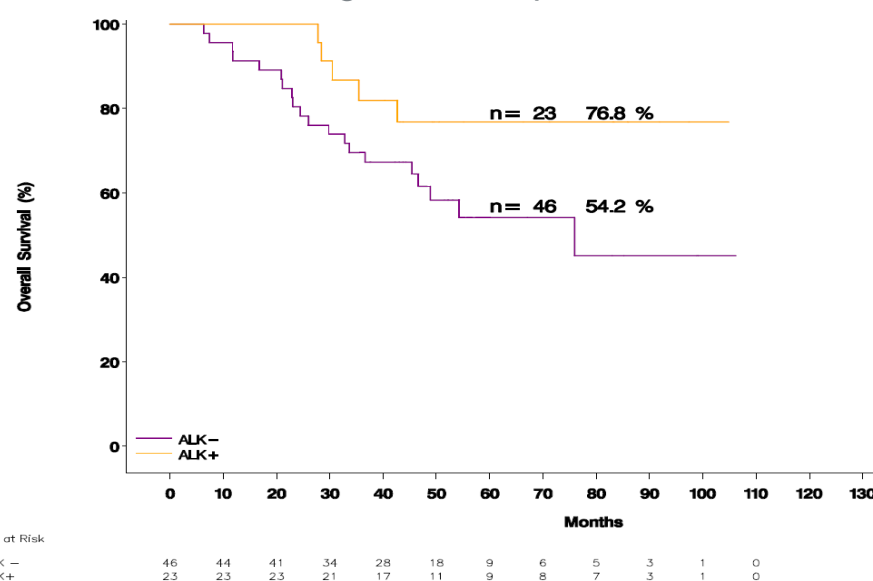
## 24 | RFS and OS by ALK FISH status, matched cohort, N=69

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<sub>Yes vs No</sub>=0.19, 95% CI (0.04, 0.91),  $p=0.037$

Conditional Logistic Regression – OS event at 3 years

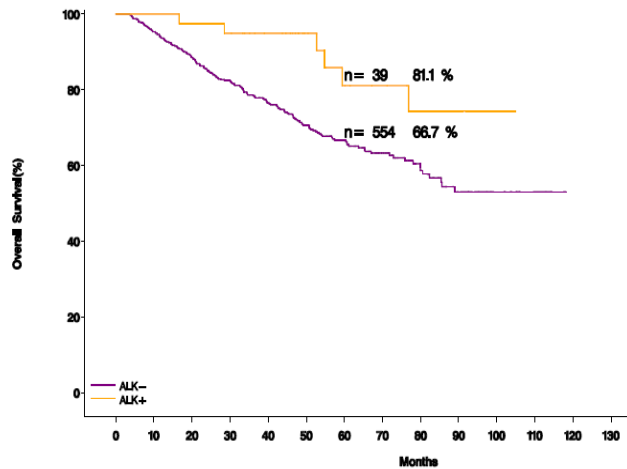
N=69; Deaths at 3 years=25

OR<sub>Yes vs No</sub>=0.22, 95% CI (0.05, 1.10),  $p=0.057$



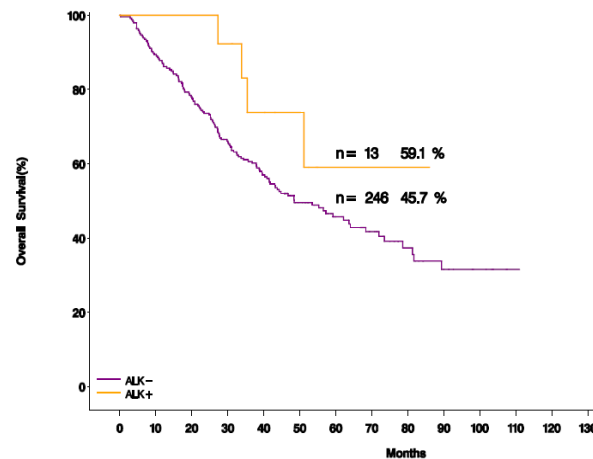
## 25 | OS by ALK IHC status & stage, N=1099

Stage Ia & Ib



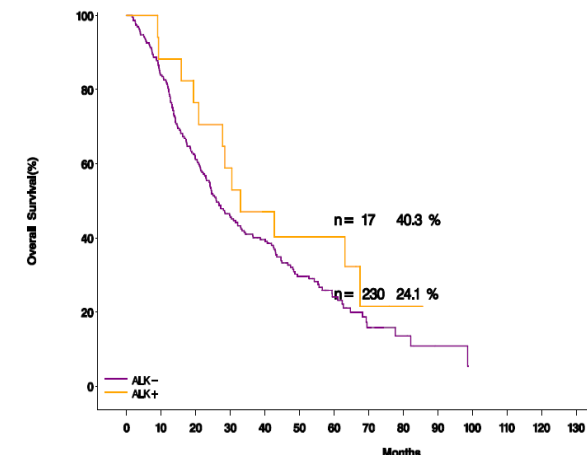
log rank p=0.014

Stage IIa & IIb



log-rank p=0.159

Stage IIIa & IIIb



log-rank p=0.086

**interaction p=NS**

*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*

## 26 | 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.

## 27 | Conclusions (2)

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

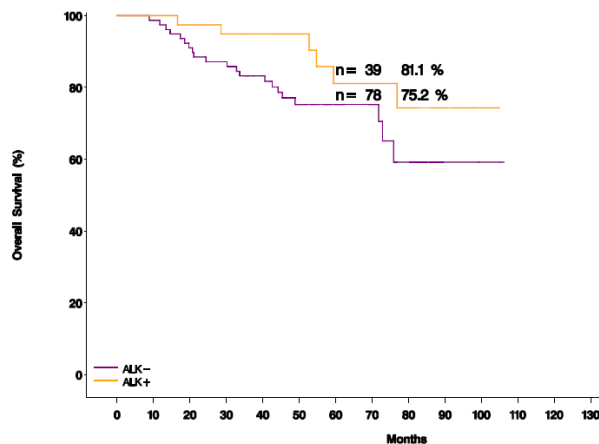
## 28 | Acknowledgements

- The ETOP steering committee, ETOP staff and all participating ETOP members
- S Peters - Lungscape database coordination and quality assessment (Peters et al. #11790 ESMO 2012)
- E Thunnissen, K Kerr, L Bubendorf, D Nonaka for pathology protocol, internal and external quality assessment (Thunnissen et al: abstract 193P)
- Urania Dafni and Frontier Science Foundation – Hellas for statistical design and analysis
- Pfizer for support of this study
- Abbott Molecular for supporting ongoing Lungscape research

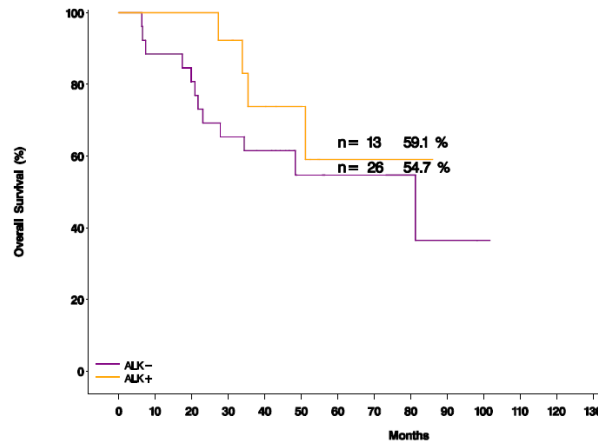
## ADDITIONAL SLIDES

# 30 | OS by ALK IHC status & stage, matched cohort, N=207

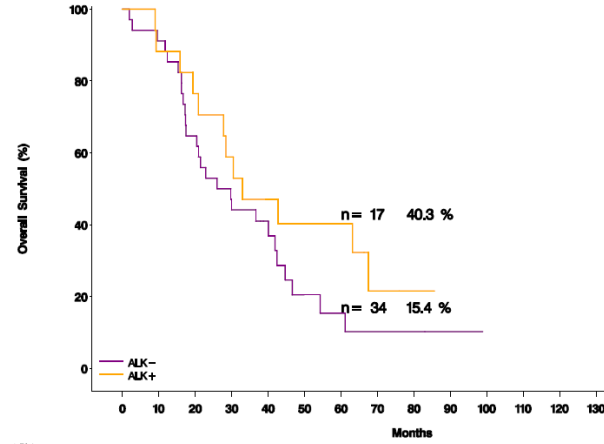
## Stage Ia & Ib



## Stage IIa & IIb



## Stage IIIa & IIIb

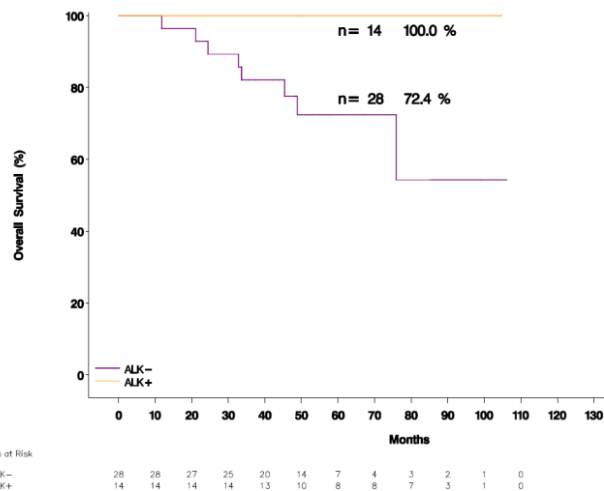


*Overall survival (OS) by ALK IHC status for Stage “Ia & Ib”, “IIa & IIb” and “IIIa & IIIb”*

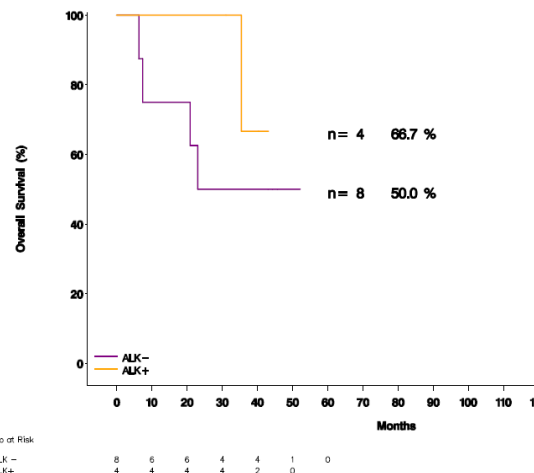
*Note: Number of patients and 5-year OS, depicted in the pictures*

# 31 | OS by ALK FISH status & stage, matched cohort, N=69

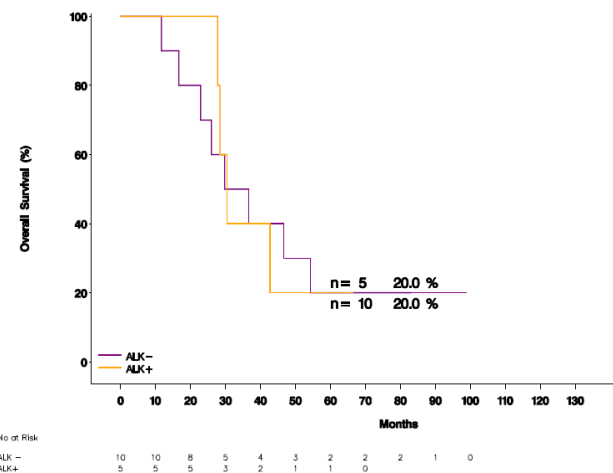
Stage Ia & Ib



Stage IIa & IIb



Stage IIIa & IIIb

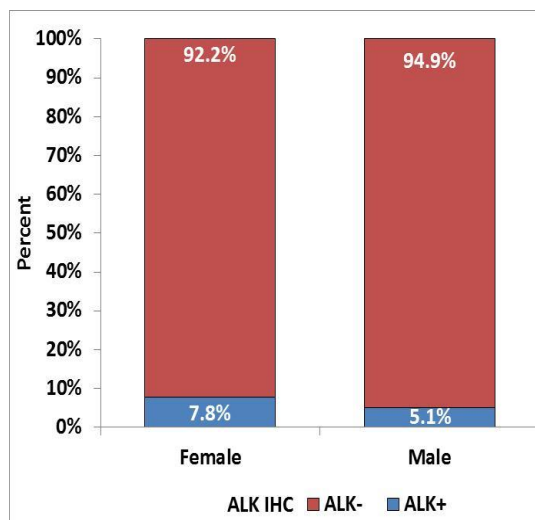


*Overall survival (OS) by ALK FISH status for Stage “Ia & Ib”, “IIa & IIb” and “IIIa & IIIb”*

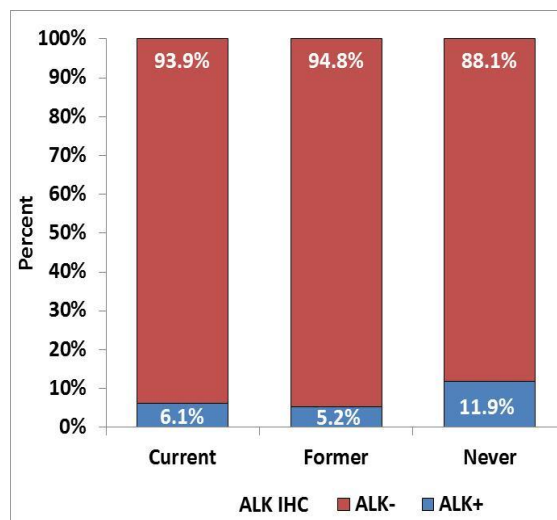
*Note: Number of patients and 5-year OS, depicted in the pictures*

## 32 | ALK IHC according to gender, smoking status & age groups; N=1099

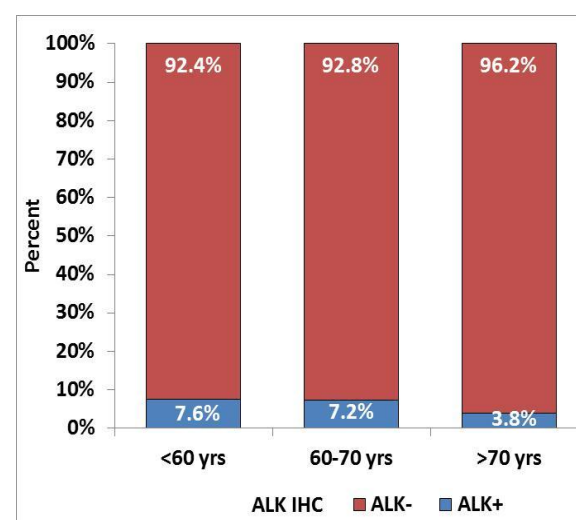
p=0.08



p=0.011\*



p=0.079



\*Excluding category “Unknown”

Median age lower  
for ALK IHC + : p=0.042

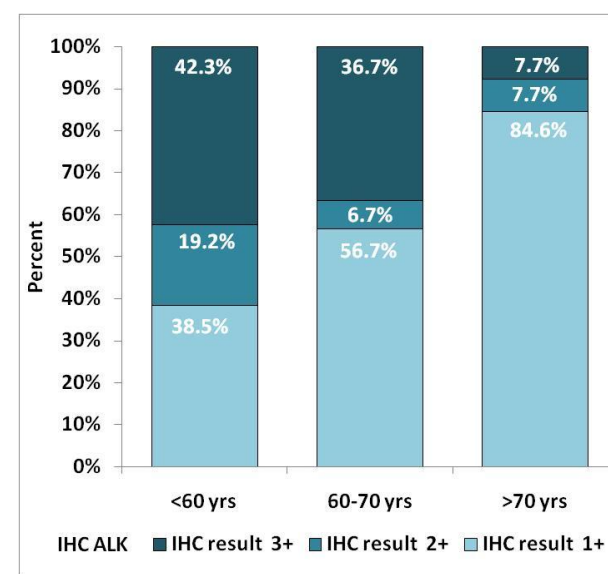
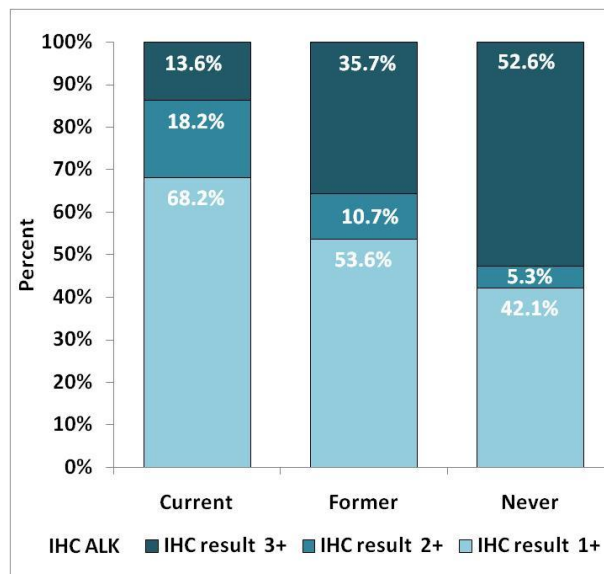
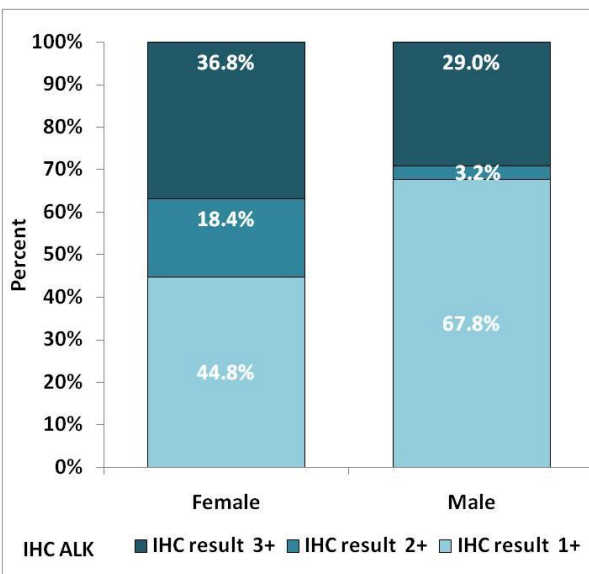


# 33 | ALK IHC 1+, 2+ & 3+ according to gender, smoking status & age groups; N=69 IHC ALK positive cases

p=0.068

p=0.10

p=0.059



“IHC 1+ & 2+” vs “IHC 3+”, p=NS

“IHC 1+ & 2+” vs “IHC 3+”, p=0.026

“IHC 1+ & 2+” vs “IHC 3+”, p=0.08