etop

Information | Research

Lungscape

A project by ETOP

ETOP Lungscape iBiobank
ELCC 2016, Geneva
Prof. Rolf Stahel

3rd ETOP meeting November 2010

LUNGSCAPE project: General objective

- **Studying the molecular epidemiology of lung cancer in Europe**
  - Coordinate and harmonize procedures among lung cancer specialists across Europe.
  - Facilitate analysis of larger series of cases.
- **Goals**
  - Expedite knowledge of the prevalence and context of current and emerging molecular biomarkers with clinical significance.
  - Provide a platform for future marker-driven ETOP studies.

Stepwise evolution

Step 1:

Retrospective analysis of ~2500 completely resected NSCLC from a limited number of sites: Immunohistochemistry, selected FISH and mutation testing on formalin-fixed paraffin-embedded tumor tissue

Step 2:

Prospective studies including biopsies and advanced disease and increasing to the number of participating sites which evolved into collaboration with EORTC:

SPECTAlung – a prospective multiplex analysis of thoracic malignacies (chair Benjamin Besse) and masterprotocol (chairs Rafal Dziuadziuszko and Solange Peters)

Lungscape: Organization – as it has evolved

Steering Committee: Rolf Stahel (chair), Solange Peters (iBiobank), Keith Kerr C (pathology), Lukas Bubendorf (pathology), Erik Thunnissen (pathology and quality control), Stephen Finn (pathology), Rafael Rosell, Miguel A. Molina (molecular testing), Urania Dafni (statistics), Walter Weder (surgery), Rosita Kammler (ETOP translational research coordinator)

Study Leads:

ALK IHC and FISH: Fiona Blackhall: commicated and published

ALK RT- PCR: Igor Letovanec: communicated

ALK NGS: Stephen Finn

MET IHC and SISH: Lukas Bubendorf.: communicated

RANK/ RANKL-L: Erik Thunnissen

PTEN: Alex Soltermann

Multiplex Mutation Testing: Keith Kerr, Miguel Angel Molino: communicated

PD-L1: Keith Kerr, Erik Thunnissen

Lungscape

A project by ETOP

- **Belgium**
- Leuven:
  J. Vansteenkiste, E. Verbeken, C. Dooms, L.Vliegen

- **Denmark**
- Aarhus:
  P. Meldgaard, L.B. Madsen

- **Greece**
- ETOP Statistical Center, Frontier Science Hellas:
  U. Dafni, Z. Tsourti, X. Pedeli, P. Zygoura

- **Germany**
- Heidelberg:
  H.Dienemann, A. Warth, T. Muley

- **Ireland**
- Dublin:
  S. Finn, S. Gray, K. Gately

- **Italy**
- Chieti:
- A. Marchetti, F. Buttitta, A. Di Lorito, G. de Luca

- **Poland**
- Gdansk:
  R. Dziadziuszko, W. Biernat, A. Sejda,

- **Spain**
- Barcelona:
  E. Felip, J. Hernandez-Losa, I.Sansano, M. T. Salcedo, M. Canela,
- Badalona:
  R. Rosell, M.A. Molina
- Valencia:
  C. Camps, M. Martorell, M.C. Calabuig, A. Navarro, E. Jantus-Lewintre

- **Beyond Europe:**
- **China**
- Shanghai Chest Hospital:
  S. Lu, Z. Jie, Q. Tan
- **USA**
- Roswell Park Cancer Institute: A. Adjei, R. Cheney, M. Reid

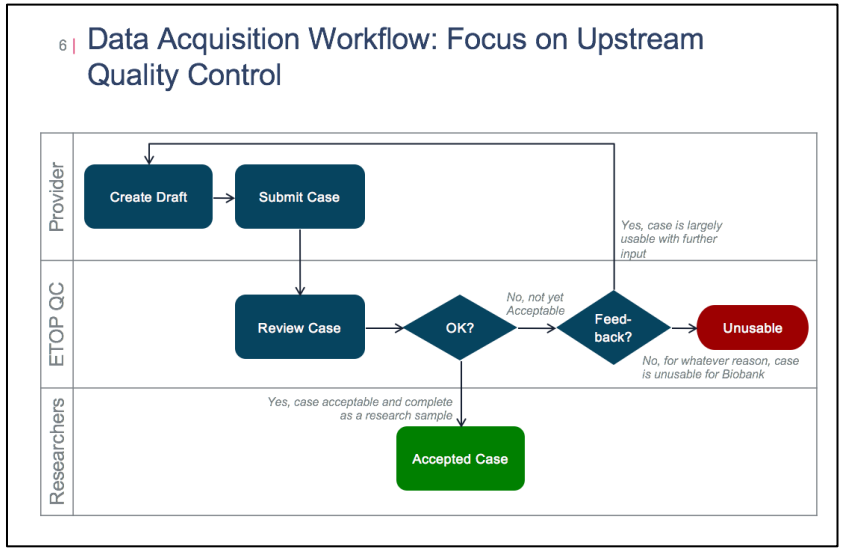
- **Switzerland**
- ETOP Coordinating Office:
  A. Hiltbrunner, S. Peters, R. Kammler, T. Geiger, B. Ruepp, M.Marbot, R. King, R. Maibach, R. Stahel
- Basel:
  L. Bubendorf, S. Savic
- Zurich:
  W. Weder, A. Soltermann, V. Tischler

- **The Netherlands**
- Amsterdam VUMC:
- E. Thunnissen, E. Smit
- Amsterdam NKI:
  P. Baas, K. Monkhorst, B. van de Weil
- Maastricht:
  A.-M. Dingemans, E-J.M. Speel

- **United Kingdom**
- Aberdeen:
  K.M. Kerr, N. Price, M. Nicolson
- Manchester:
  F. Blackhall, D. Nonaka, R. Peck, L. Franklin, A.M. Quinn

Methodology: Case inclusion criteria

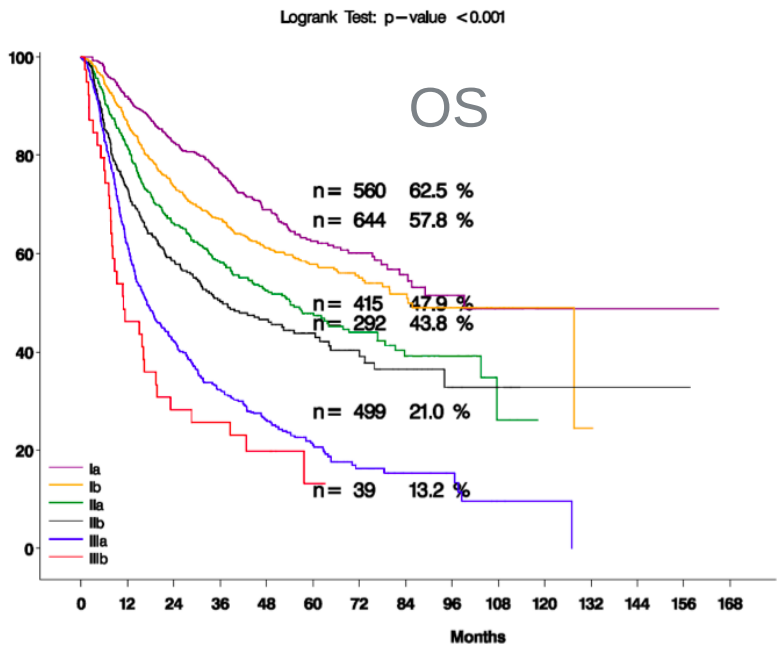
- Documented ethical approval for tissue sample and associated clinical data
- Histological diagnosis of NSCLC
- Radically resected non-pretreated stage IA-IIIB NSCLC
- Well characterized, clinically annotated formalin-fixed paraffin embedded tissue in sufficient quantity and quality
- Diagnosis after January 2003
- At least 3 years of follow-up
- Mandatory comprehensive clinical data available

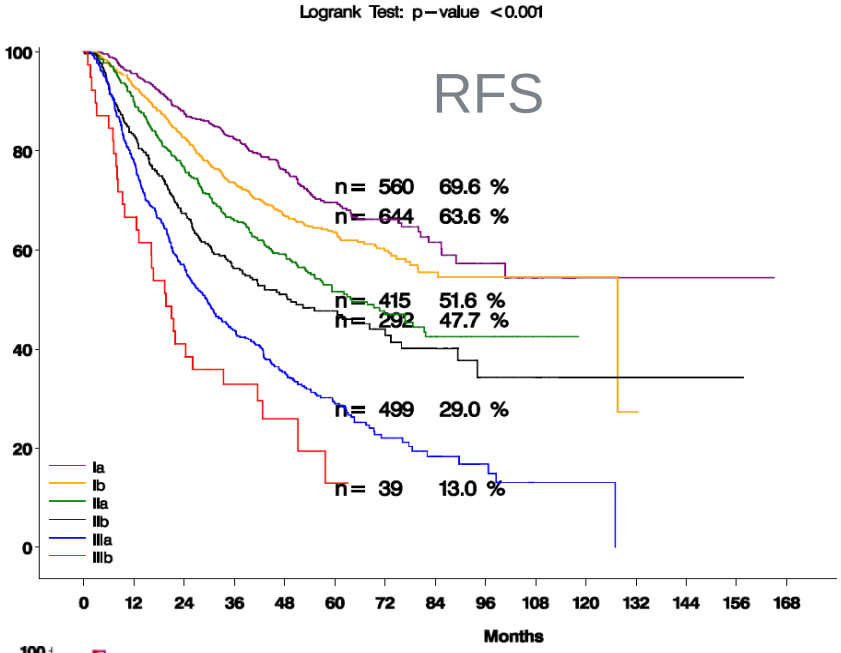


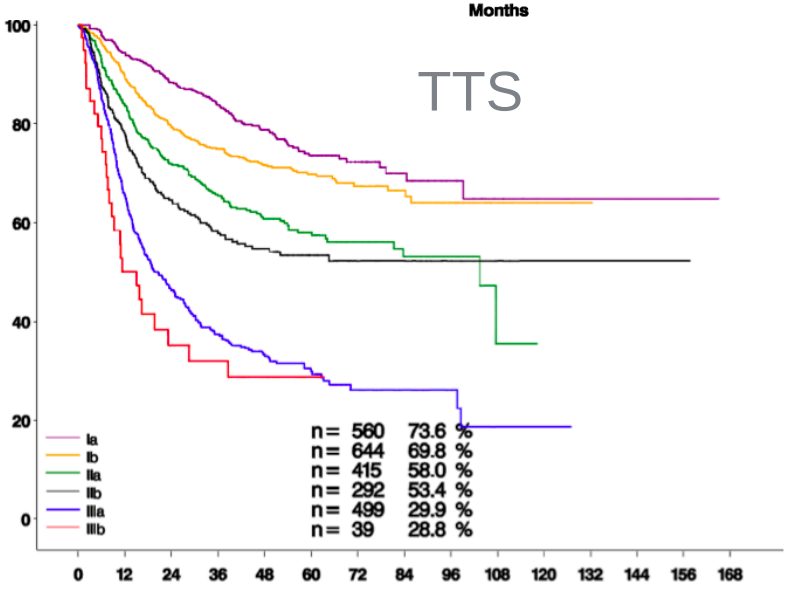
Lungscape cohort

Center	Adeno.	Squam.	Other	Total
University Hospital **Leuven**, Belgium	90	100	10	**200**
University Hospital **Basel**, Switzerland	60	90	16	**166**
University Hospital **Zurich**, Switzerland	166	124	19	**309**
Shanghai Lung Cancer Center, China	111	7	19	**137**
Universitätsklinikum **Heidelberg**, Germany	50	49	3	**102**
Aarhus University Hospital, Denmark	184	124	28	**336**
University Hospital **Valencia**, Spain	22	23	1	**46**
Vall d'Hebron University Hospital **Barcelona**, Spain	80	57	33	**170**
Royal Infirmary **Aberdeen**, UK	75	65	17	**157**
Lung Cancer Group **Manchester**, UK	53	24	2	**79**
St James' Hospital **Dublin**, Ireland	119	144	18	**281**
Ospedale Clinicizzatto **Chieti**, Italy	107	59	1	**167**
The Netherlands Cancer Institute **Amsterdam**, NL	38	26	12	**76**
Free University Medical Center **Amsterdam**, NL	41	48	13	**102**
University Medical Centre **Maastricht**, NL	44	43	6	**93**
Medical University **Gdansk**, Poland	86	109	5	**200**
Roswell Park Cancer Institute **Buffalo**, USA	51	25	12	**88**
Total	**1377**	**1117**	**215**	**2709**

Outcomes according to stage







Peters et al.
Journal of Thoracic Oncology (2014) 9(11):1675-84.

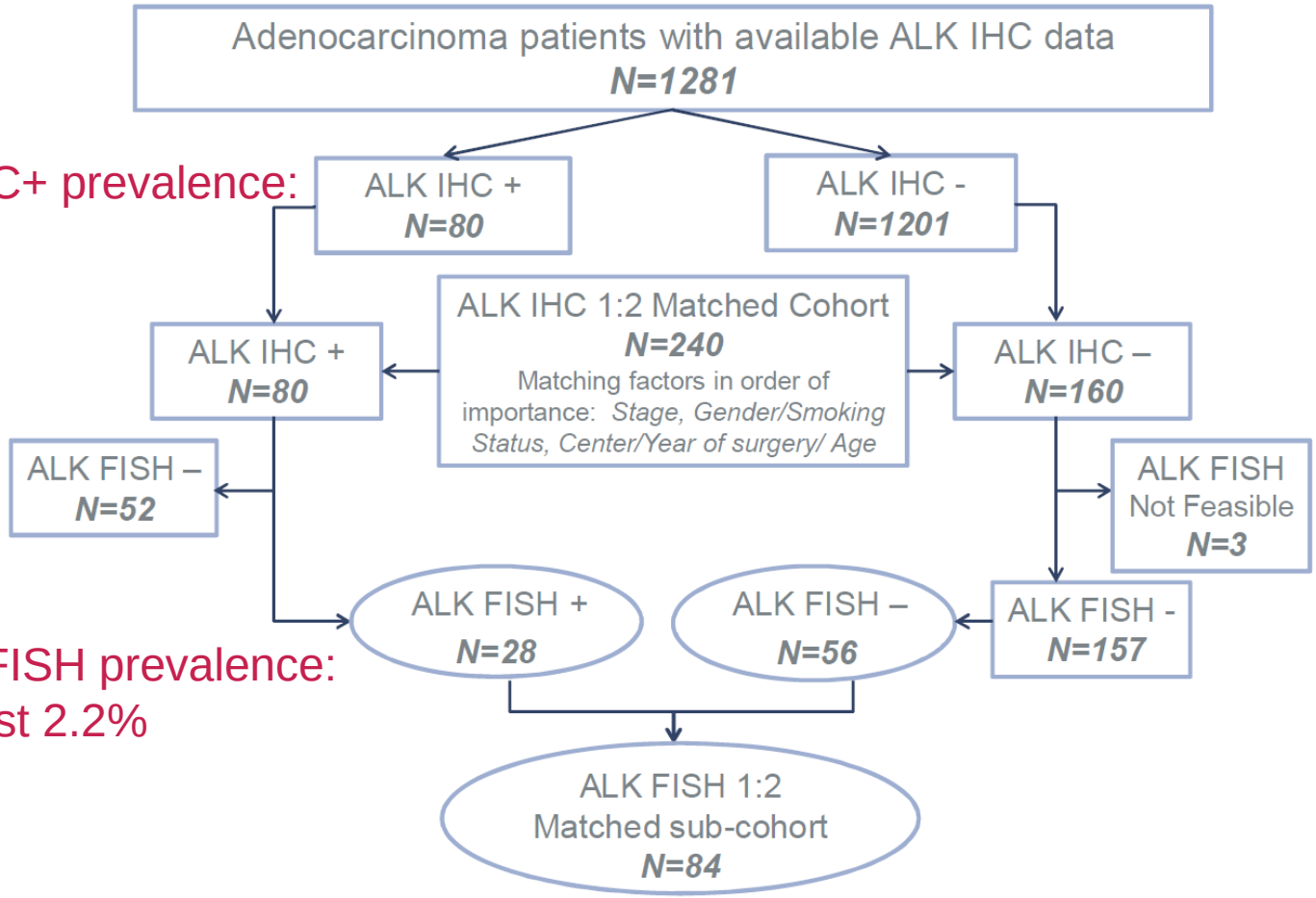
Selected conclusions from the Lungscape collection

- Application of the 7th TNM classification has been successful in distinguishing prognostic categories in our dataset with OS similar to IASLC dataset
- Multivariate survival analysis of OS identified age, gender, PS and previous history of cancer as independent prognostic characteristics in addition to TNM stage
- Histology and PS were independent prognostic factors for TTR in addition to TNM stage by multivariate analysis
- Among the outcomes measurements in Lungscape, TTR might be best suited to determine the prognostic impact of molecularly defined subgroups

Peters et al, ASCO, 2013

Lungscape 001 - ALK

ALK IHC+ prevalence: 6.2%

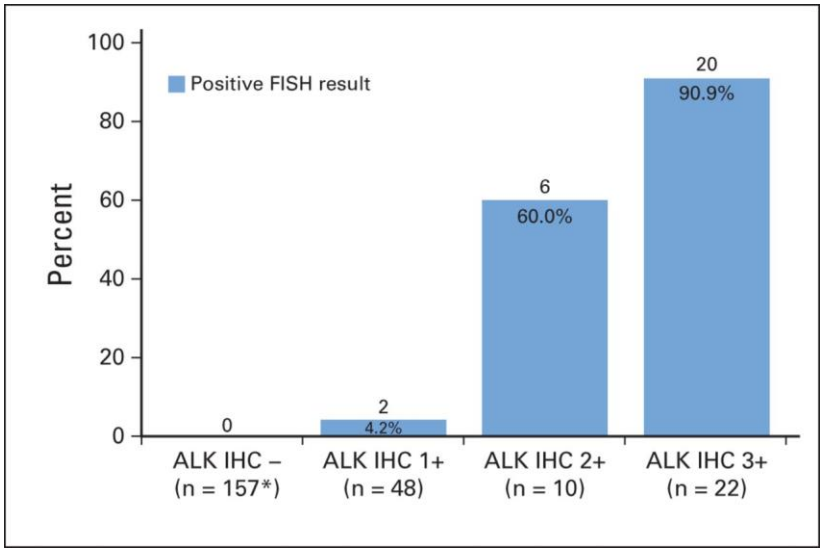


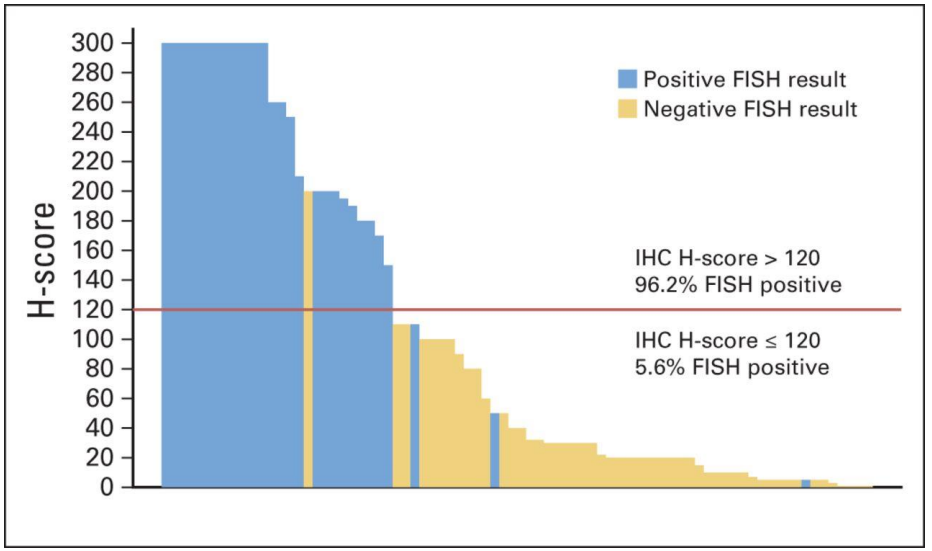
ALK FISH prevalence: at least 2.2%

5A4 antibody (Novocastra; Leica Biosystems, Buffalo Grove, IL)

Blackhall et al, JCO 2014

Lungscape 001 - ALK

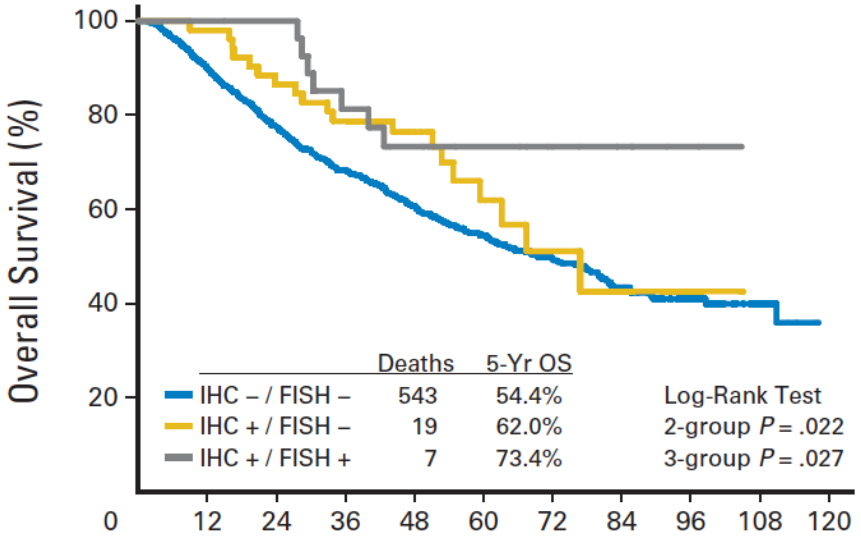




A screening strategy based on IHC or H-score could be envisaged

Blackhall et al, JCO 2014

Lungscape 001 - ALK



Multivariable models, adjusted for patient, tumor, and treatment characteristics, and matched cohort analysis confirmed that ALK FISH positivity is a predictor for better overall survival (OS)

Blackhall et al, JCO 2014

ALK RT-PCR Results

ALK RT-PCR result according to ALK FISH status and H-score levels (N=71)

RT-PCR Status	ALK FISH				All patients
	Negative		Positive		
	H-score<120	H-score≥120	H-score<120	H-score≥120	
Negative	52	0	1(*)	2(**)	55
Positive	0	1(***)	1(****)	14	16
Total	52	1	2	16	71

Non-concordant cases:

- Overall, there are 66 cases out of 71 (93%) where all three methods were in agreement.
- There are 2 cases where a negative RT-PCR contradicts a positive result supported by both FISH and H-score
- There was one negative RT-PCR which agrees with an H-score less then120 but contradicts a positive FISH result
- There were 2 more positive RT-PCR cases in disagreement either negative FISH or H-score results

Lelovanec et al, WCLC 2015

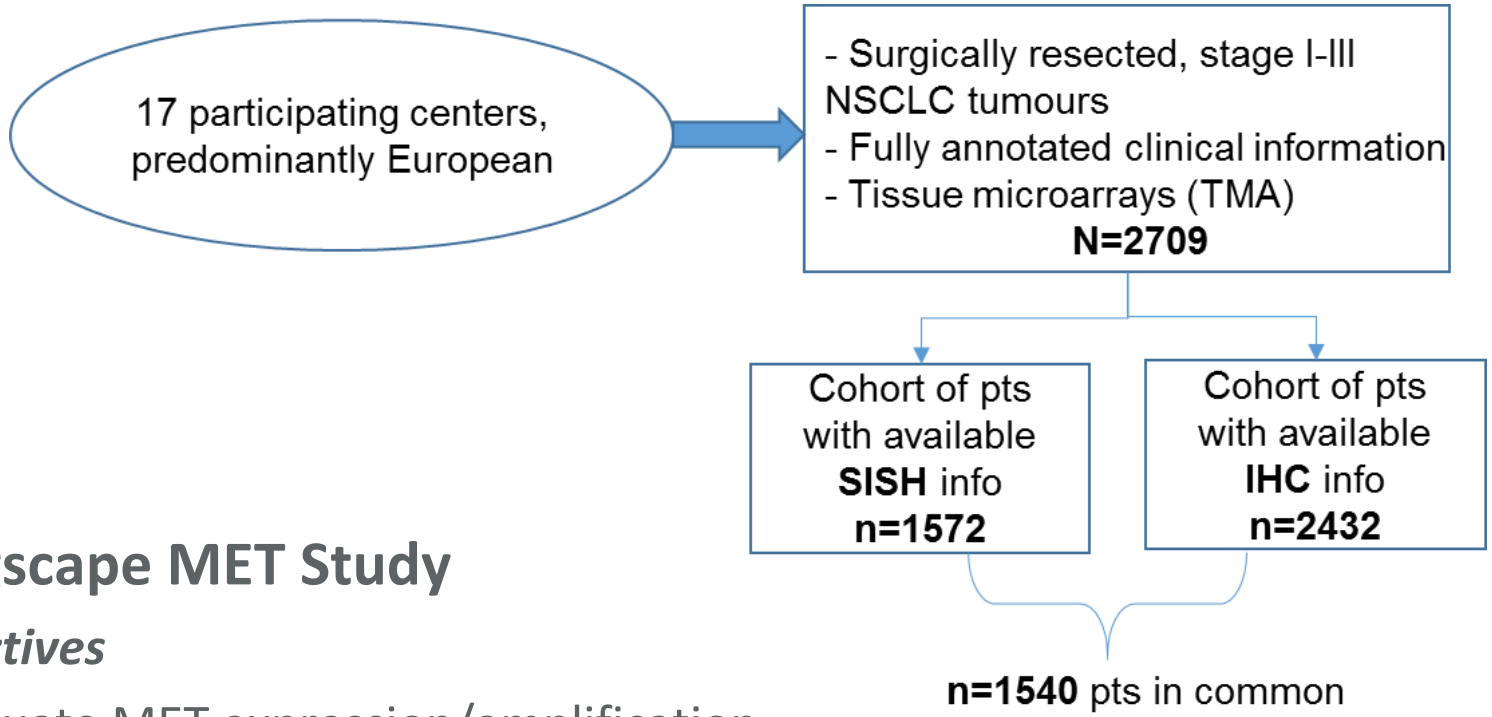
ALK RT-PCR Discussion and Conclusion

- RT-PCR is a very good tool for sorting discordant IHC/FISH cases
- However, we do not recommend using this technique as single method due to :
  - lower sensitivity of RT-PCR, as not all variants are covered
  - limitations of RNA preservation, especially for older archive material or material from centres with unstandardized fixation protocols

- NGS Project:
  95 samples re-analyzed by RT-PCR, 95 by NGS

Letovanec et al, WCLC 2015

Lungscape 002 - MET



Lungscape MET Study

Objectives

- Evaluate MET expression/amplification
- Explore long-term outcome
- Compare IHC with SISH
- Correlate MET with other oncogenic driver alterations.

Bubendorf et al, WCLC 2015

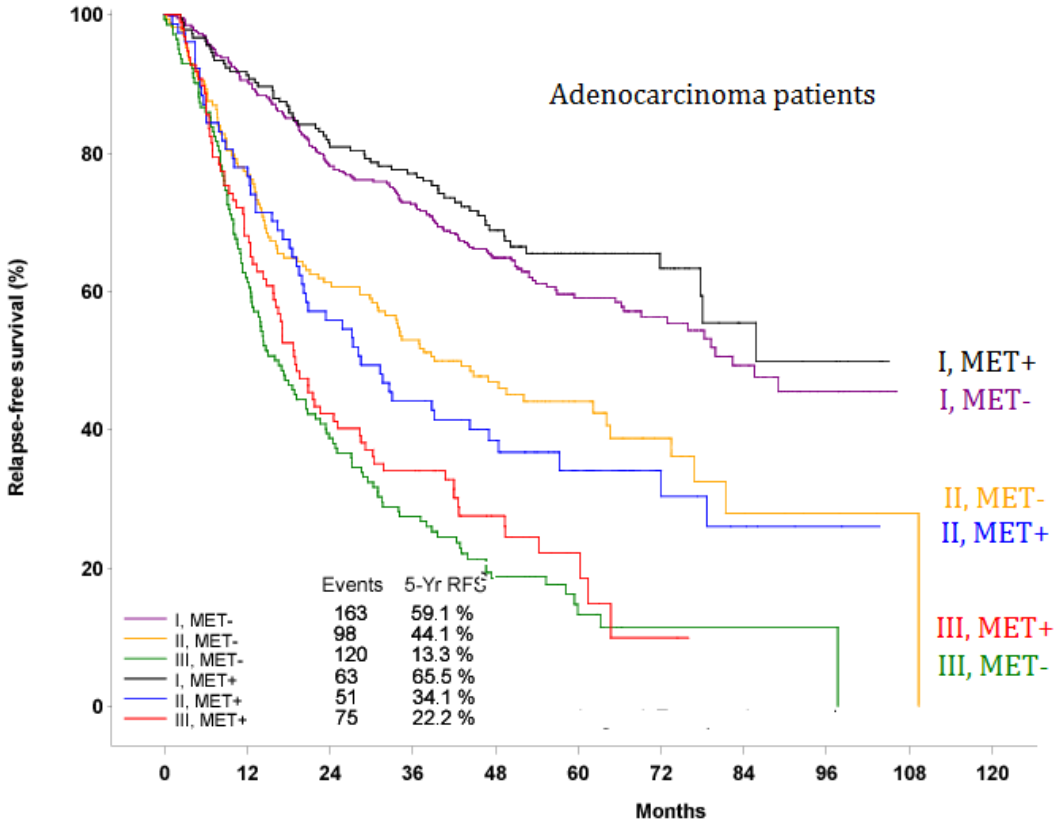
Lungscape 002 – MET: Expression by IHC

Prevalence

IHC MET	n (%)	95% CI
(+)	579 (**23.8**)	(22.1, 25.5)
(-)	1853 (76.2)	(74.5, 77.9)
All	2432	

MET expression is not prognostic for any outcome measurement

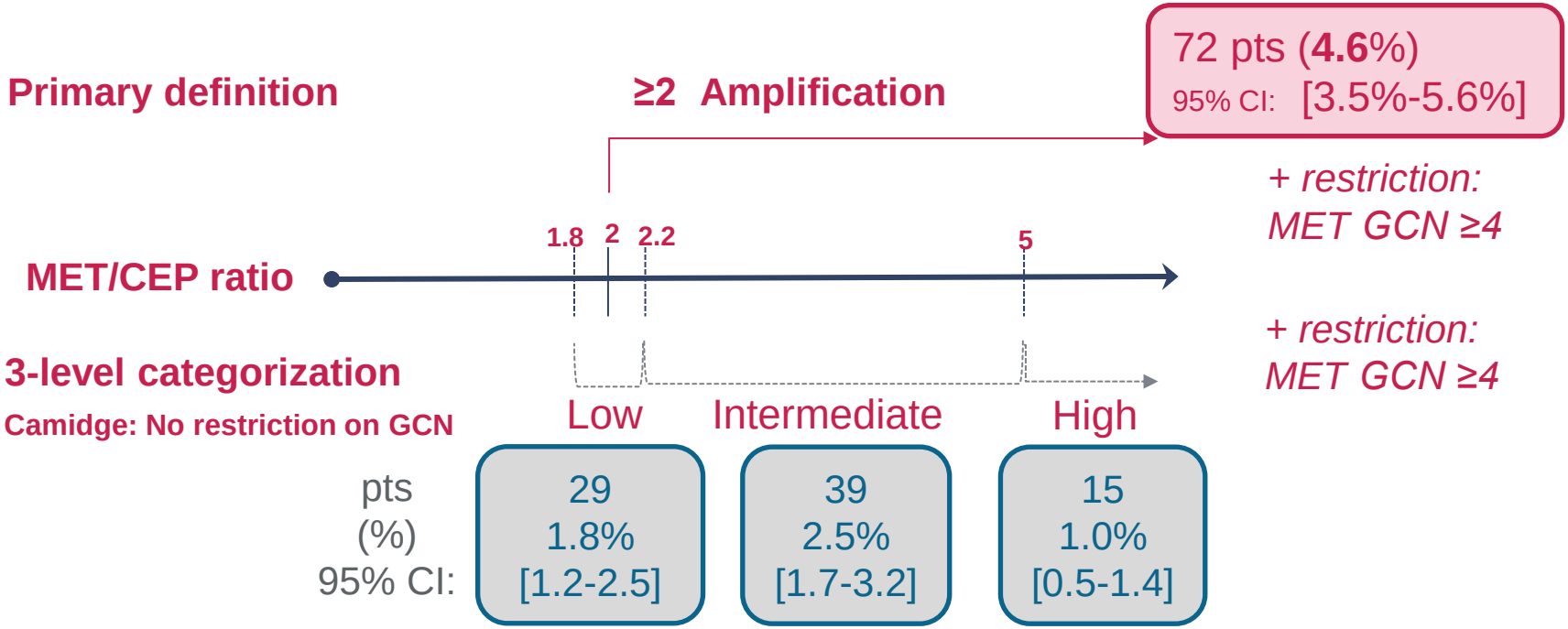
Relapse-free Suvival by Stage and MET status:



No association of MET status with outcome (RFS, TTR, OS; all histological subtypes)

Bubendorf et al, WCLC 2015

Lungscape 002 – MET: Amplification: Definitions and prevalence in 1572 pts samples examined with SISH



High MET GCN

- threshold of the value 5
- threshold of median (2.28)

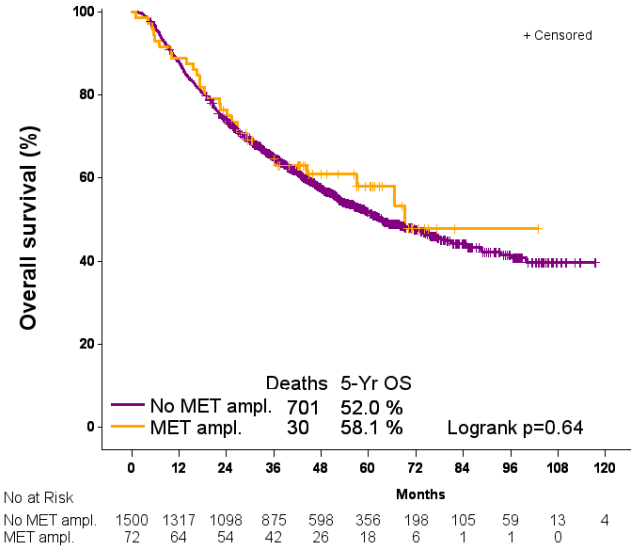
65 pts - **4.1%**, 95% CI: [3.2%-5.1%]

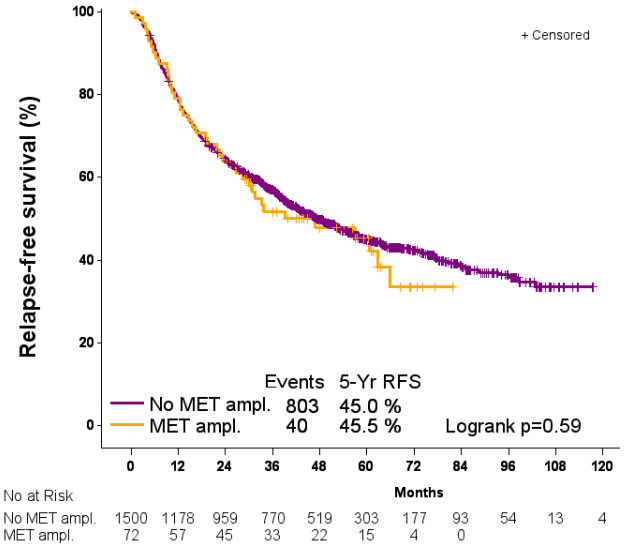
50% by definition

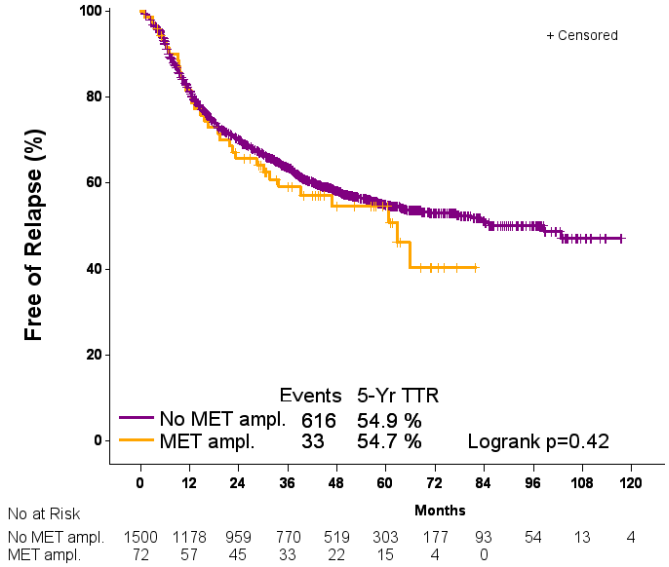
Dziadziuszko, JTO, 2012.; Cappuzzo JCO, 2009;
Camidge ASCO JCO 2014; Schildhaus CCR 2015

Bubendorf et al, WCLC 2015

MET amplification: Non-significant association with outcome (OS, RFS, TTR)







Also, no significant association with outcome, based to any of the alternative MET amplification or high MET GCN definition

Bubendorf et al, WCLC 2015

Lungscape 002 – MET: Conclusiones

MET **overexpression** (by IHC):

- in 23.8% of NSCLC (all histologies)

MET **amplification** (by SISH):

- in 4.6% of NSCLC (all histologies)

MET amplification and GCN≥5

- are associated with strong MET expression
- have no association with patient's or tumor's baseline characteristics
- have no influence on prognosis

MET **mutation** (exon 14)

- analysis ongoing

Bubendorf et al, WCLC 2015

Lungscape 002 – PTEN: Selection of antibody and external quality assessment

A) Compare 3 different PTEN antibodies

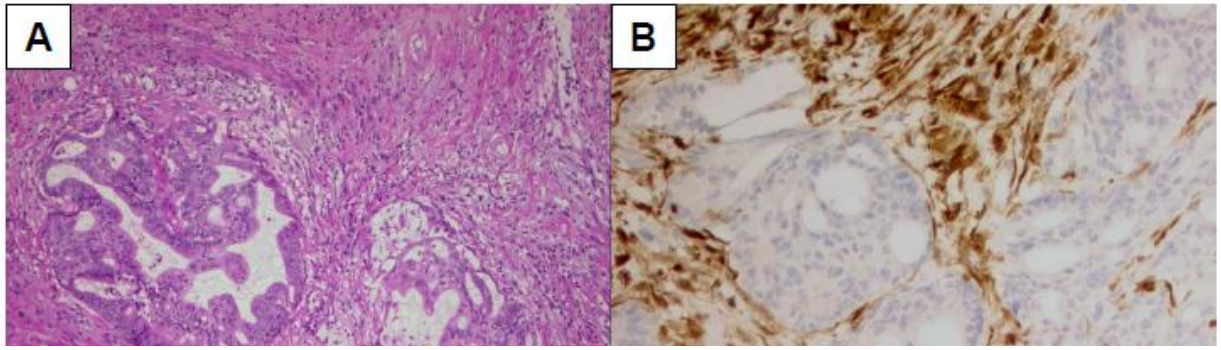
- MmAb 6H2.1 DAKO Cytomation, 1:100, Leica Bond
- RmAb 138G6 Cell Signaling, 1:200, Ventana Benchmark
- RmAb 218 Spring Bioscience, 1:100 Ventana Benchmark

B) **Compare pathologist scores vs. image analysis computer scores**

Soltermann et al,
Schweiz. Gesellschaft für Pathologie 2015

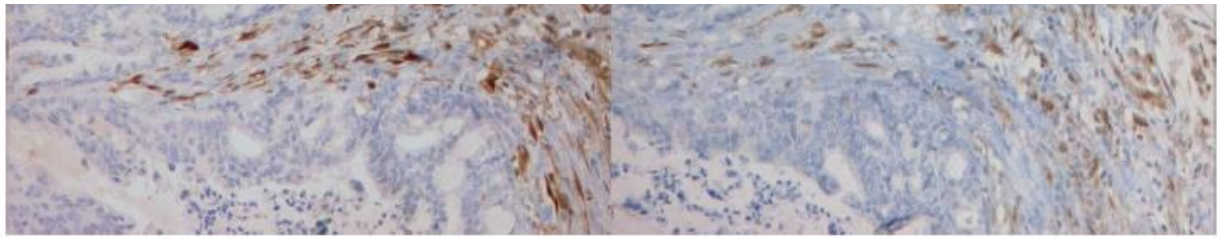
PTEN antibody comparison

Mucinous Endometrium Carcinoma



SP 218 anti-PTEN antibody
(SpringBioscience 1:100 on Ventana BenchMark)

- yields a **clean IHC** and **separates 6 positive from 6 negative PTEN cases**, based on mean averages
- used for part B and the Lungscape cohort

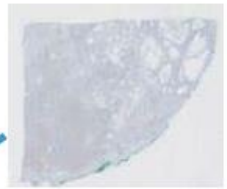


A) H&E B) DAKO 6H2.1 C) CST 138G6 D) SP 218

Soltermann et al, Schweiz. Gesellschaft für Pathologie 2015

Pathologist vs Computer scoring comparison

6 positive and 6 negative PTEN cases selected in Zurich



TMA was prepared

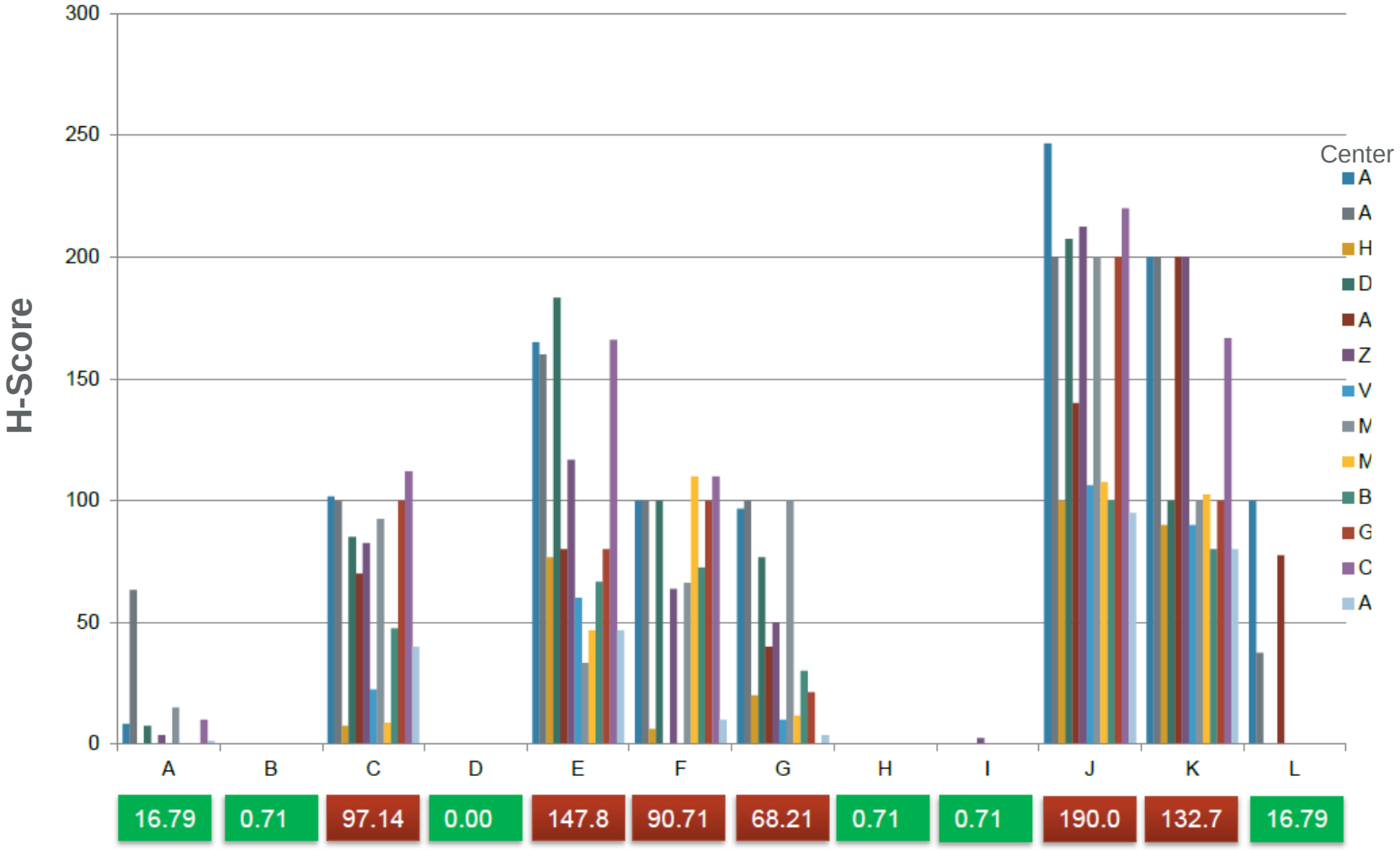
16 ETOP centres stained TMA and gave H scores

Full sections were stained and scanned in Zurich

Link of scanned slides was send to 16 ETOP centres and H score was given

Automated analysis

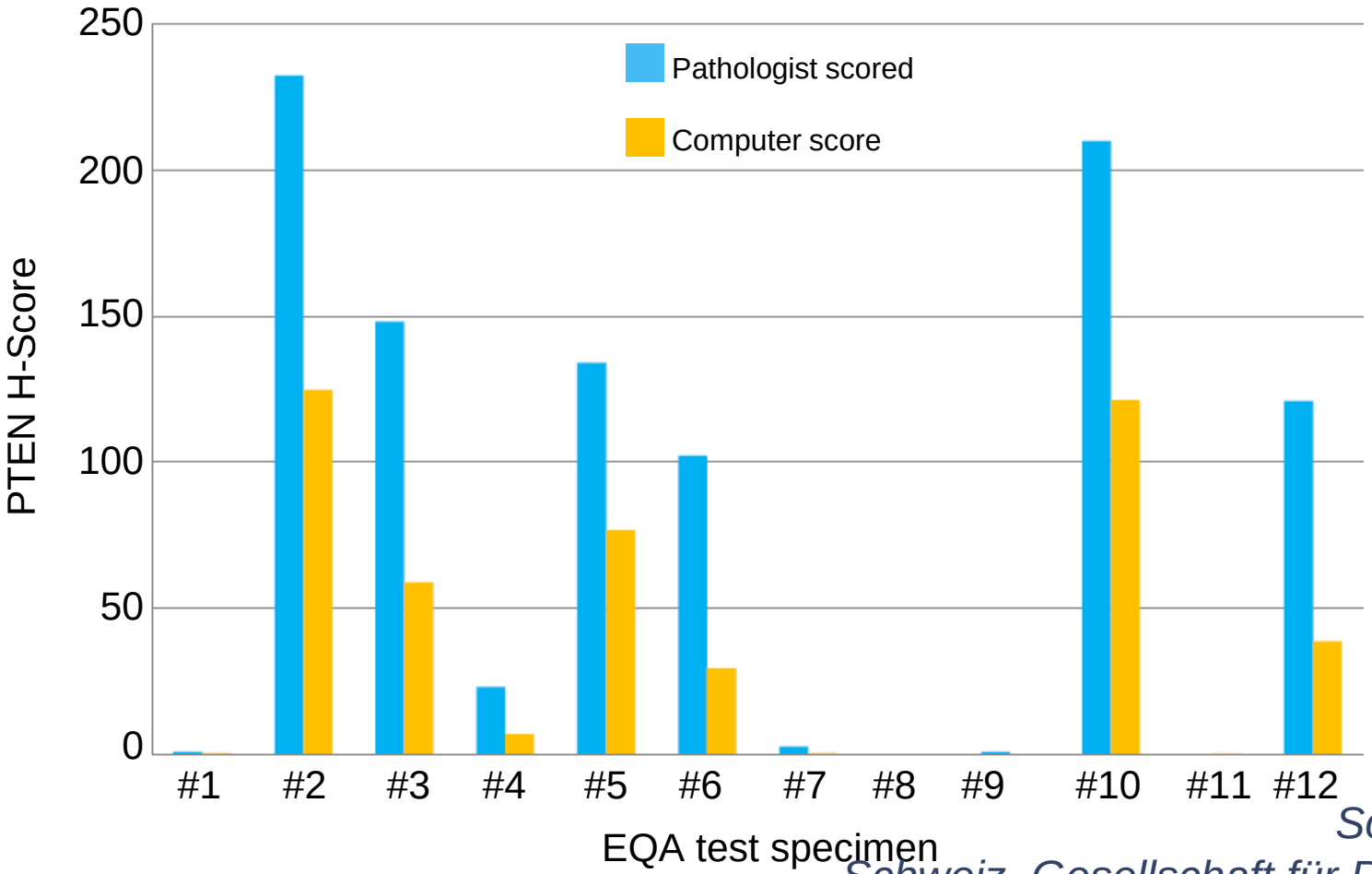
Lungscape pathologists' H-scores of EQA TMA



Lungscape – 002: PTEN IHC

EQA:

- Pathologist read vs. computerized image-analysis.



Soltermann et al, Schweiz. Gesellschaft für Pathologie 2015

Lungscape 002 PTEN IHC - Conclusions

EQA

- SP 218 anti-PTEN antibody yields a clean IHC and separates 6 positive from 6 negative PTEN cases, based on mean averages.
- Computer-based measurement of PTEN immunoreactivity is in alignment with pathologist's scores (but overall lower).

Ongoing

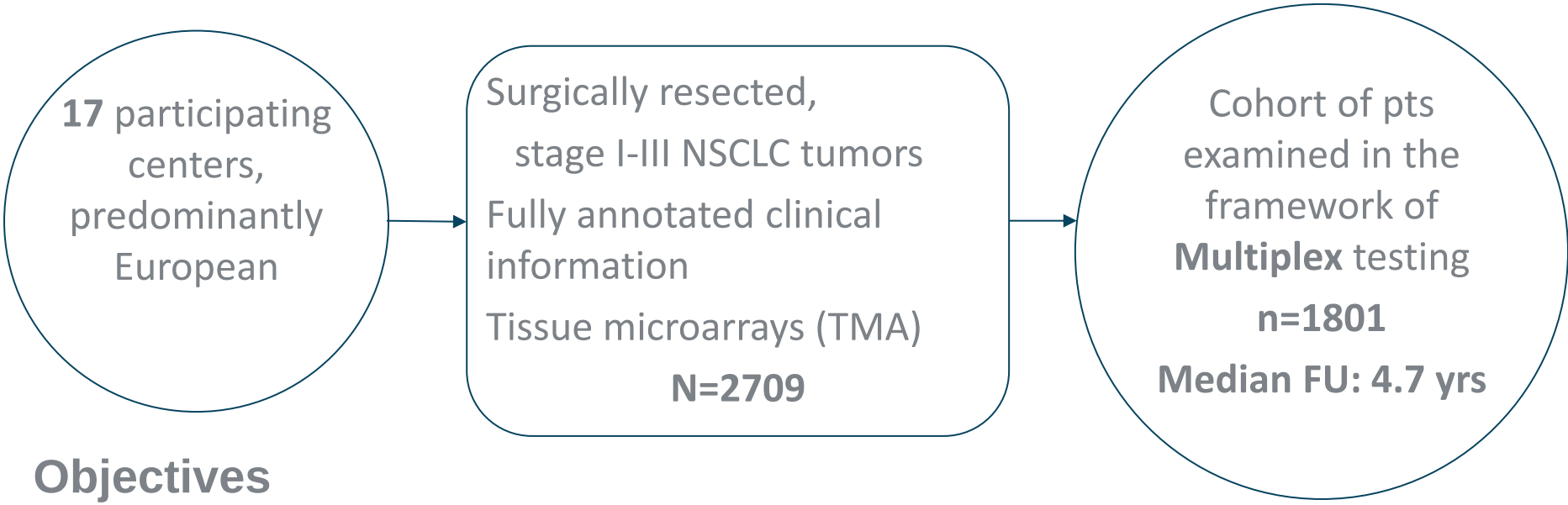
- Statistical analysis of PTEN IHC in 2240 samples from the Lungscape cohort (computer and pathologists' scores).

Soltermann et al,
Schweiz. Gesellschaft für Pathologie 2015

Lungscape - 003 Multiplex Mutation Analysis

- Tumor sections selected for maximum tumor content
- DNA extracted at participating centers from FFPE sections
- Local quality assurance verified in a central collaborating laboratory - samples standardized for genomic analysis
- Gene mutation testing by Fluidigm technology:
  - A microfluidics-based PCR platform,
  - Allele-specific multiplex test covering **13 genes** (**~150 mutations**),
  - Mutant allele detection sensitivity is >1%

Lungscape - 003 Multiplex Mutation Analysis

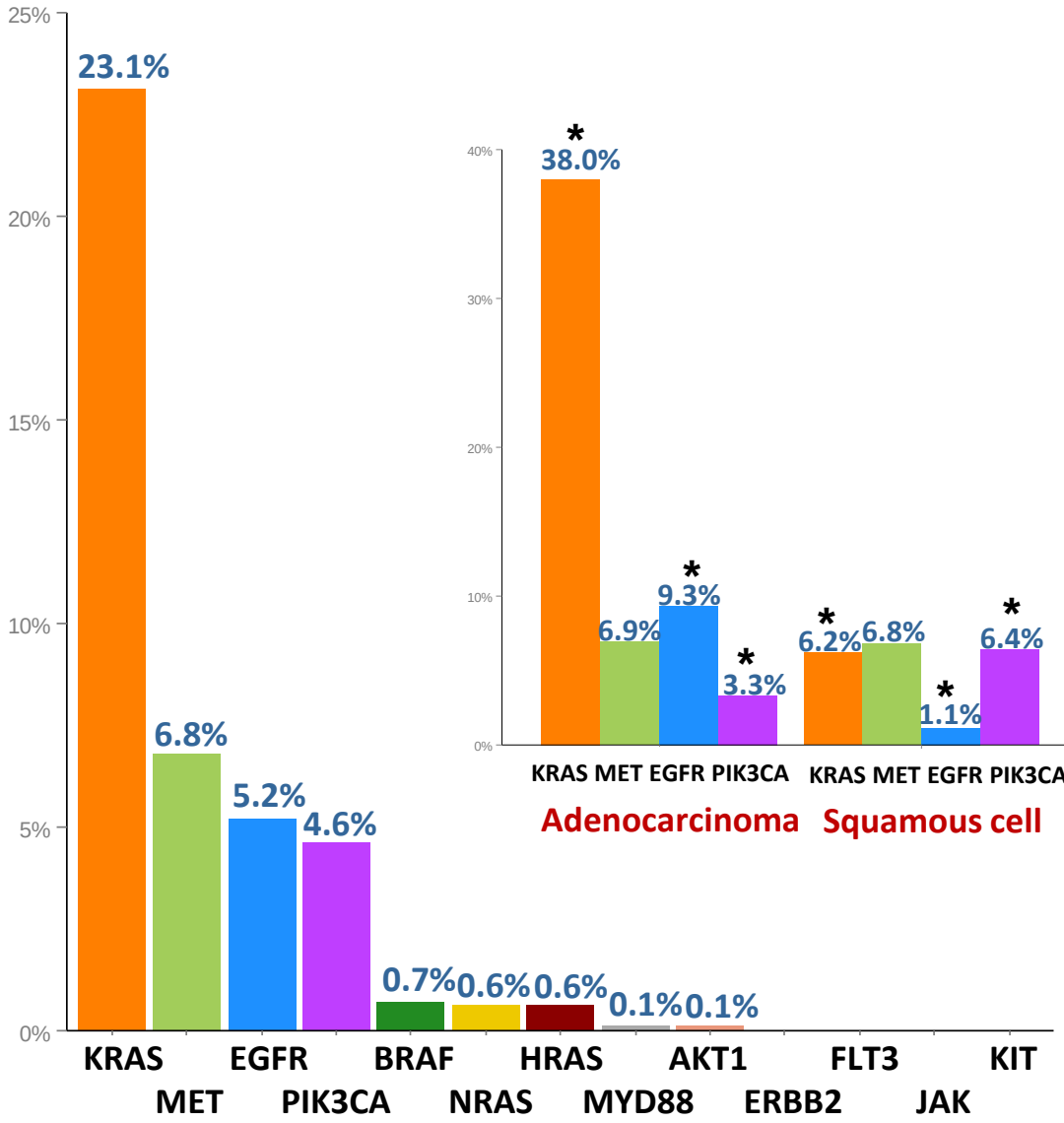


Objectives

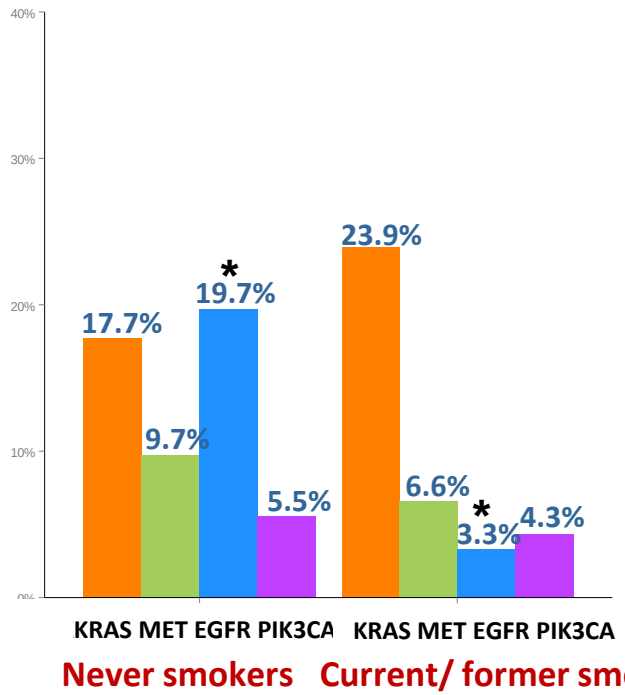
- Determine the prevalence of selected cancer related mutations
- Analyze their interrelationship
- Correlate:
  - utation pattern with other molecular alterations, e.g. ALK, MET
  - presence with clinico-pathological characteristics and outcome RFS-primary endpoint, TTR, OS

Kerr et al, ECCO-ESMO 2015

Prevalence of cancer related gene mutations



* Significant at 0.05



Kerr et al, ECCO-ESMO 2015

Baseline characteristics and gene mutation status

- **EGFR** mutation prevalence:

  Gender: 9.5% in **females** vs. 2.9% in males; p<0.001

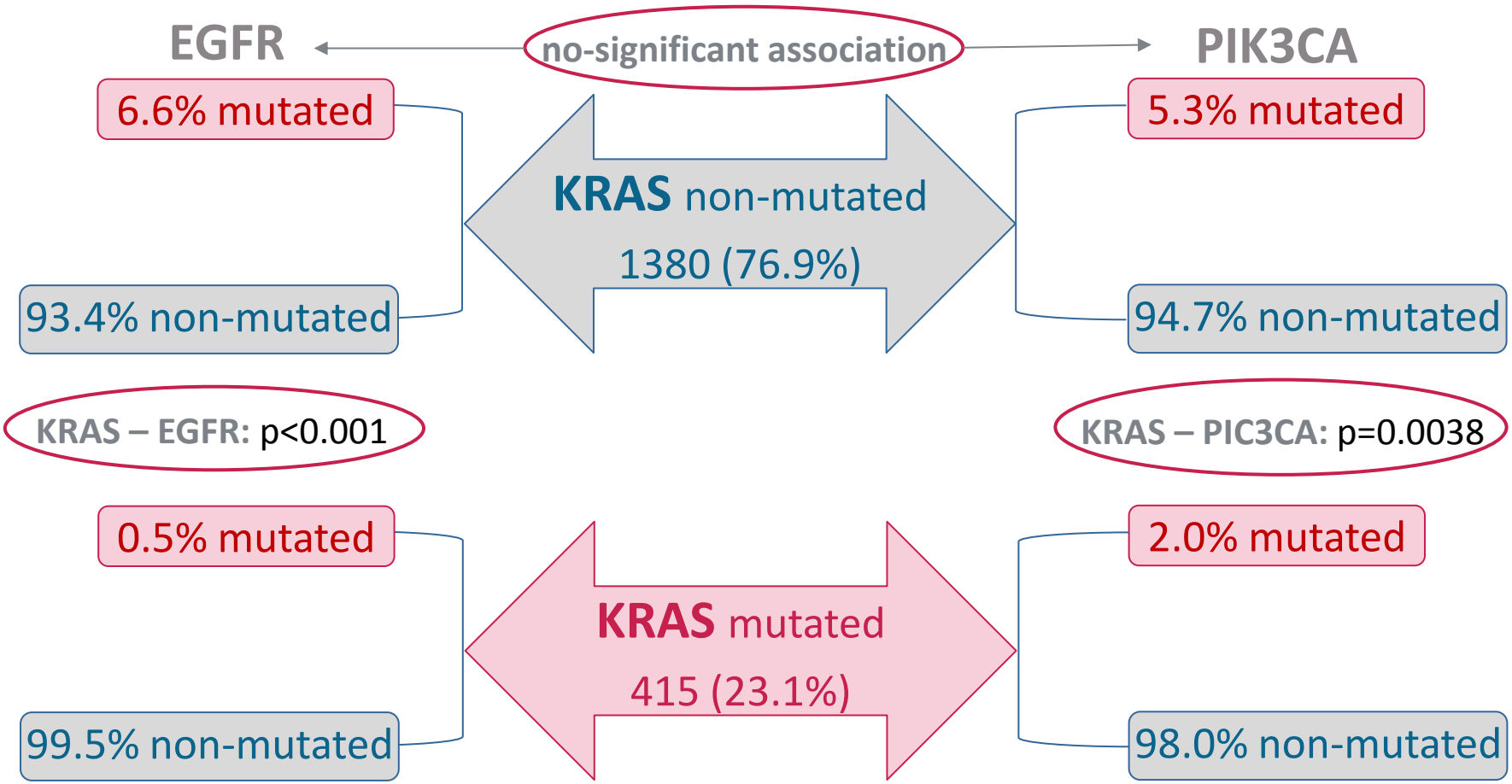
  Smoking status: 19.7% in **non-smokers** vs. 3.3% in smokers; p<0.001

  Histology: 9.3% in **adenocarcinoma**, 1.1% in squamous cell, 2.4% in undifferentiated, 1.8% in other; p<0.001

  Tumor size: 6.3% in tumors **≤4 cm** vs. 3.4% in tumors >4 cm; p=0.0047

Kerr et al, ECCO-ESMO 2015

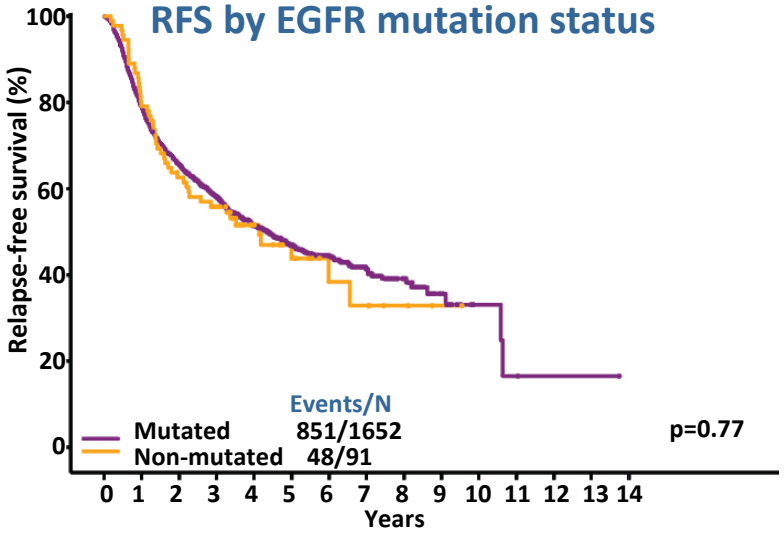
Associations between gene mutations

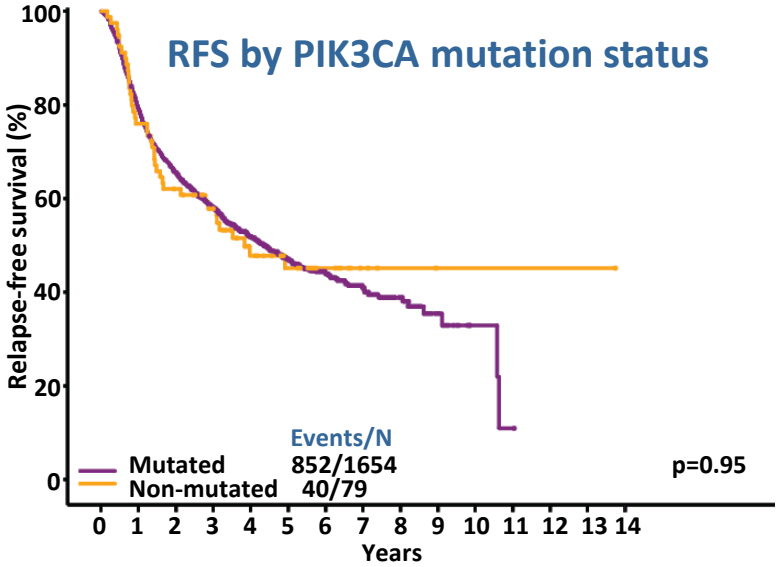


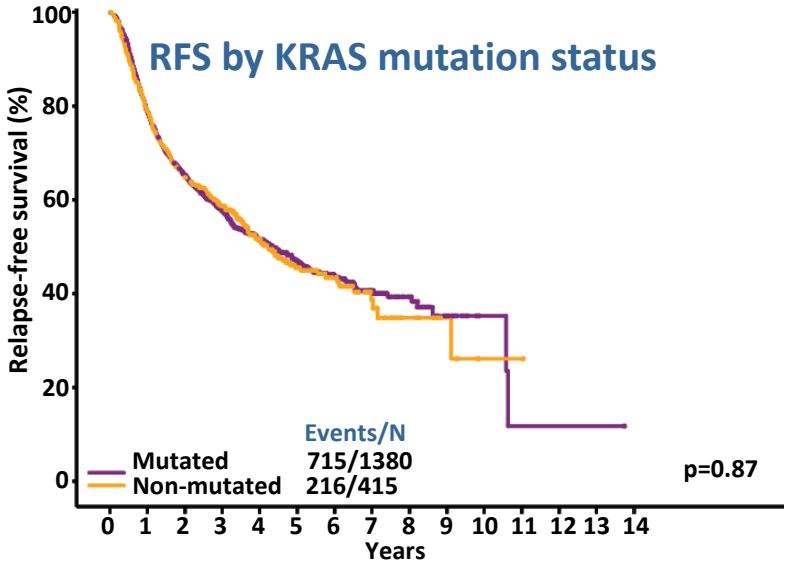
Kerr et al, ECCO-ESMO 2015

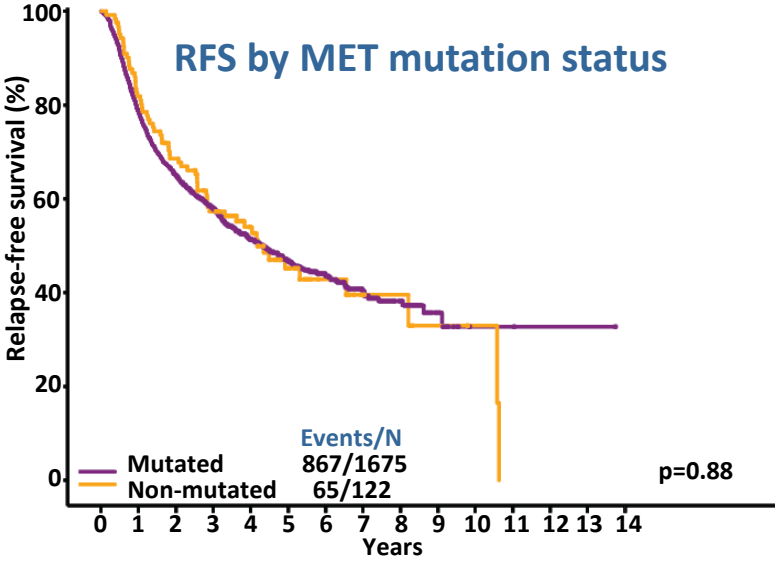
RFS and gene mutation status: Non-significant association

Median RFS: 4.3 years (95% CI: 3.9, 4.9)









Kerr et al, ECCO-ESMO 2015

Conclusions: Lungscape 003 Multiplex Mutation Analysis

In this predominantly European, clinically annotated cohort:

- **KRAS**, **EGFR** and **PIK3CA** are the most prevalent gene mutations of those sought.
- KRAS is negatively associated with PIK3CA and EGFR.
- MET IHC showed positive association with KRAS and EGFR mutations (no significant association of ALK IHC).
- Non-significant differences detected between mutated and non-mutated cases with respect to RFS, TTR and OS for the four genes of interest.

Kerr et al, ECCO-ESMO 2015

Lungscape - 004 RANK/L

6 | SPLENDOUR: A randomised, open-label
evaluating the addition of denosumab to s
line anticancer treatment in advanced NS

Screening, eligibility and enrolment **Trial treatment**

First-line Stage IV NSCLC

Stratify:
- Bone mets
- Region
- ECOG PS
- Histology

RANDOMISE

A: 4-6 cycles chemotherapy every 3 weeks + BSC

B: 4-6 cycles chemotherapy every 3 weeks → Denosumab every 3-4 weeks

Translational research:

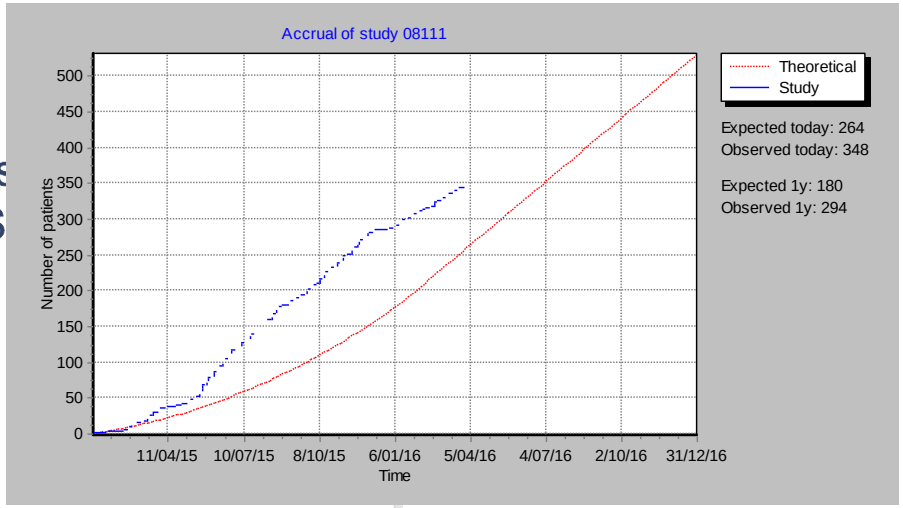
FFPE

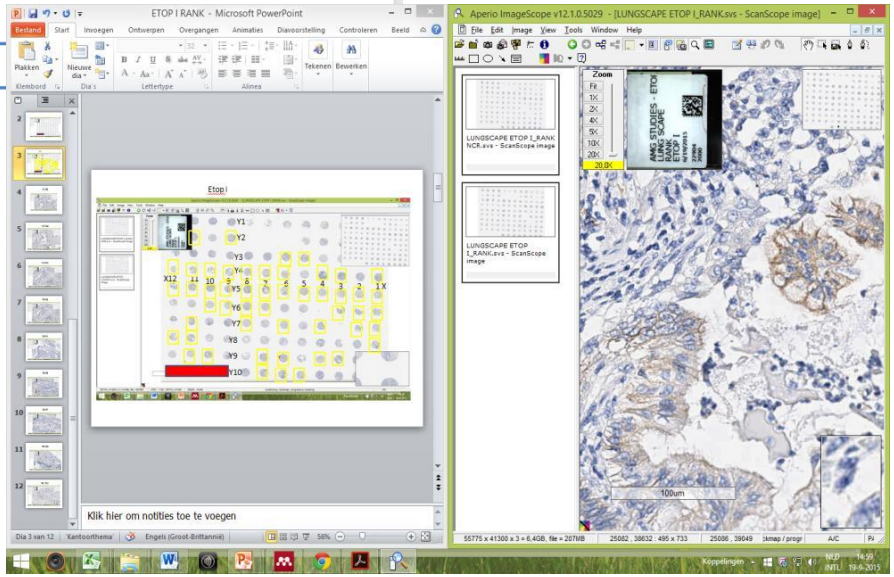
Blood

Blood

Sample size: 1000

Primary endpoint: Overall survival





Lungscape 005 PD-L1

- DAKO PD-L1 training and exam held in Amsterdam

Thank you for listening!

Lungscape
A project by ETOP

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Information | Research

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