A case of EGFR mutation in squamous cell carcinoma

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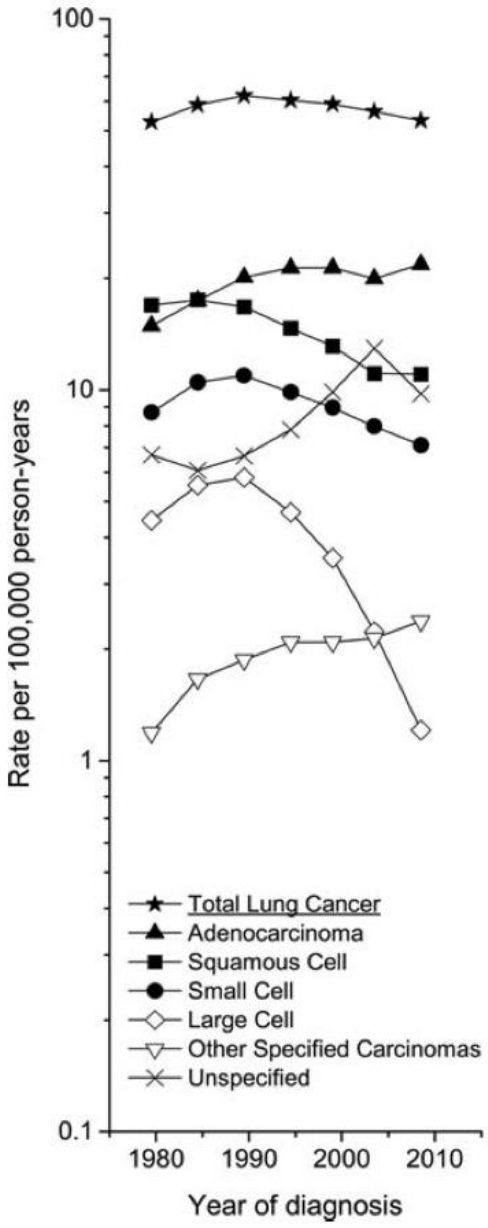
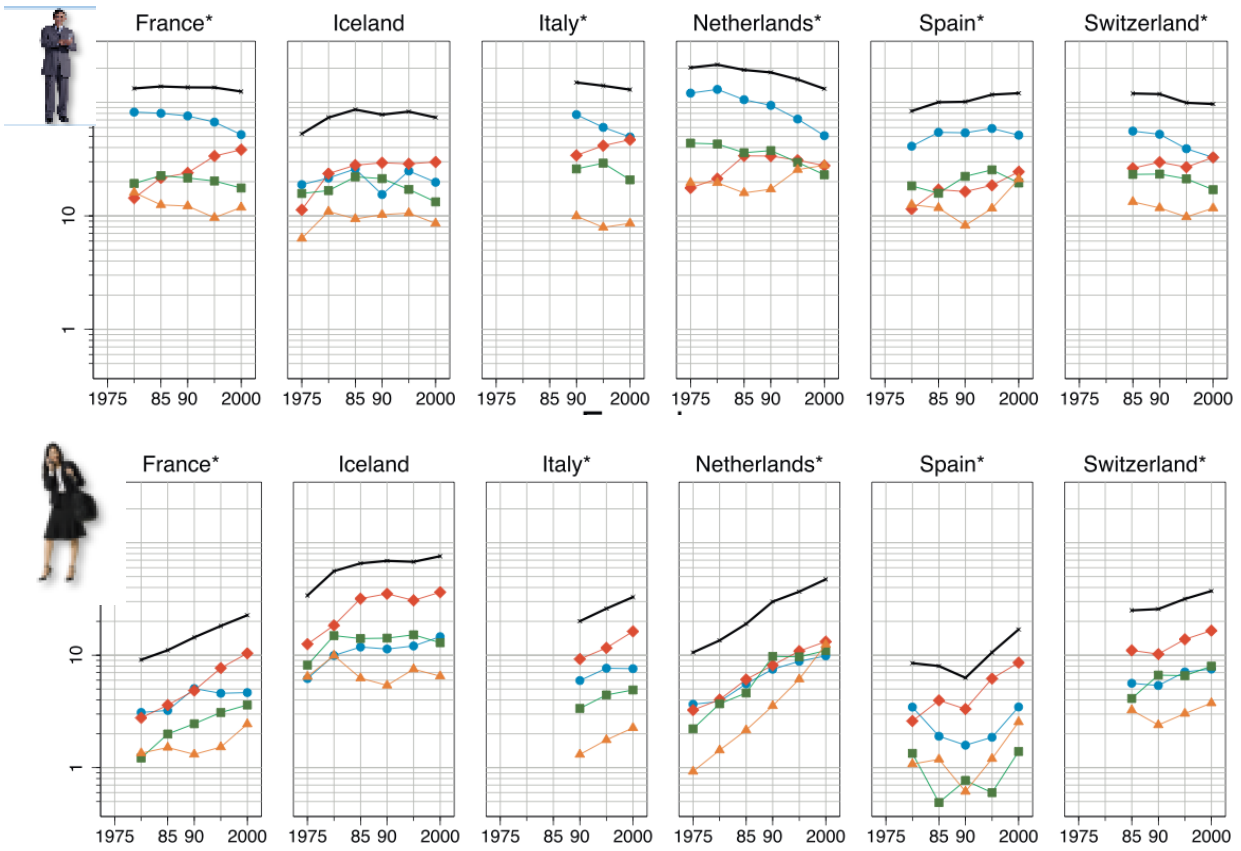
Disclosures

Speakers honoraria from: Astra Zeneca, Boehringer Ingelheim, MSD, Eli Lilly.

US



Europe



Riedel Lewis D et al, Cancer 2014;
J. Lortet-Tieulent Lung Cancer 2014

Clinical history

A.M.C, female, 59 years old
Never smoker (<100 cigarettes at a young age)

Medical history:
2000: breast cancer (surgery + adjuvant chemo-radiotherapy)

No allergy

Oncological history:
January 2013: the patient reported from 2 months a progressive cough

She started an antibiotic treatment (amoxicillin) and stheroid (prednison) without any benefit and appearance of a cervical pain

A chest X-Ray showed a right upper lobe lesion

Oncological visit

In February 2013 the patient reffered to an oncologist:

✓Good clinical conditions (ECOG PS: 0)

✓SatHbO2: 98%

✓No relevant clinical evidence

✓Cervical pain treated with tramadol 100 mg td

Basing on these data a total body CT scan, a CT-PET scan and a FBS were requested

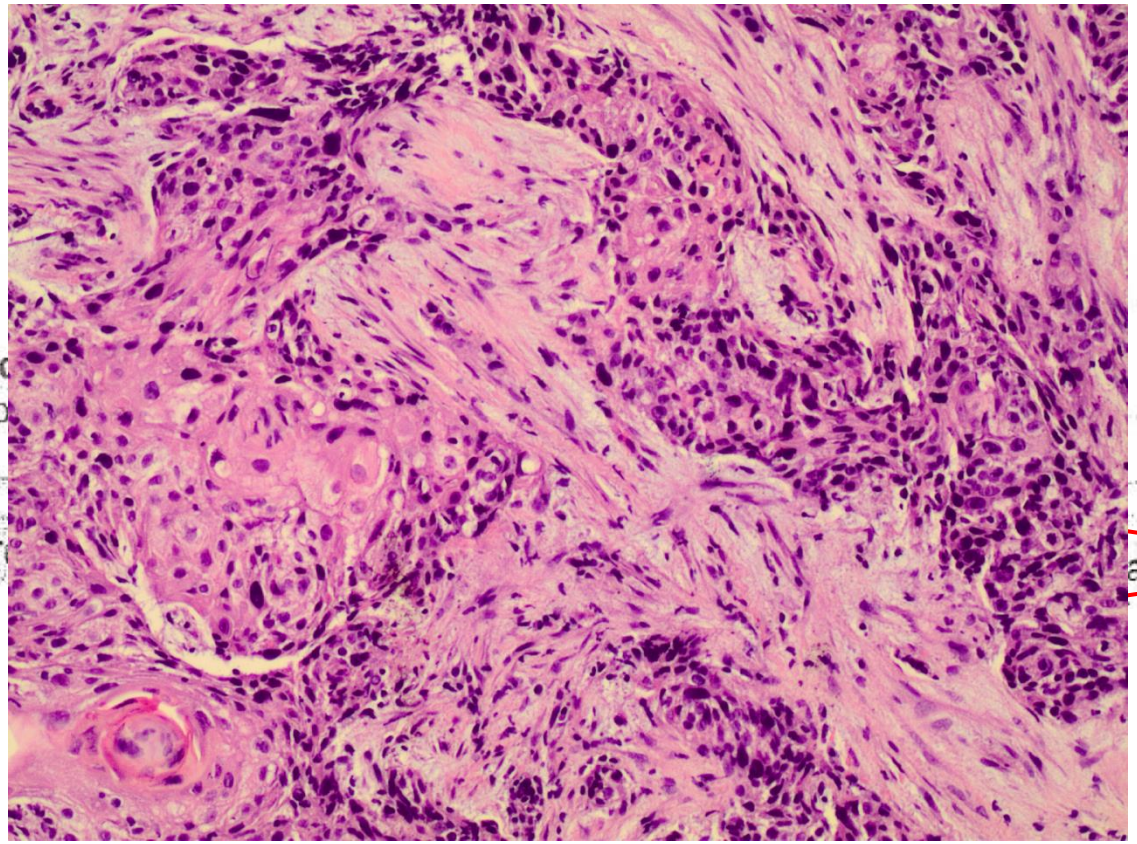
✓**CT-scan**: evidence of a right upper lobe lesion (25 mm) with hilar and mediastinal lymph-nodes involvment. No evidence of abdominal and cerebral metastases

✓**CT-PET scan**: high metabolic activity of the lung and lymph-nodal lesions. Evidence of bone metastases (C1 and pelvis)

✓**FBS**: presence of a right upper lobe bronchial lesion

The citologic and histologic exams were positive for:

SQUAMOUS CELL LUNG CANCER



DIAGNOSI

Descrizione mac
Frammenti multip

Diagnosi:
Frammenti di par

ante, infiltrante.

First line treatment

According to the diagnostic data the patient started:

- ✓ Cisplatin 75 mg/mq d1
  Navelbine 30 mg/mq d1,8

- ✓ Zoledronic acid 4 mg

- ✓ Palliative cervical and lombar radiotherapy

At the end of the six cycle of chemotherapy the CT scan showed a partial response and from July the patient continued with her 3 months CT follow-up

Follow-up

On March 2014 the CT scan showed a progressive disease due to an increase of mediastinal lymph-nodes, appearance of a liver and multiple cerebral metastases.

The patient performed a whole brain radiotherapy (30 Gy)

Several considerations were done at that point: possibility to include the patient in a phase II/III trial with a PD1 inhibitor, evaluation of EGFR and Alk status on the basis of the non smoking exposure.

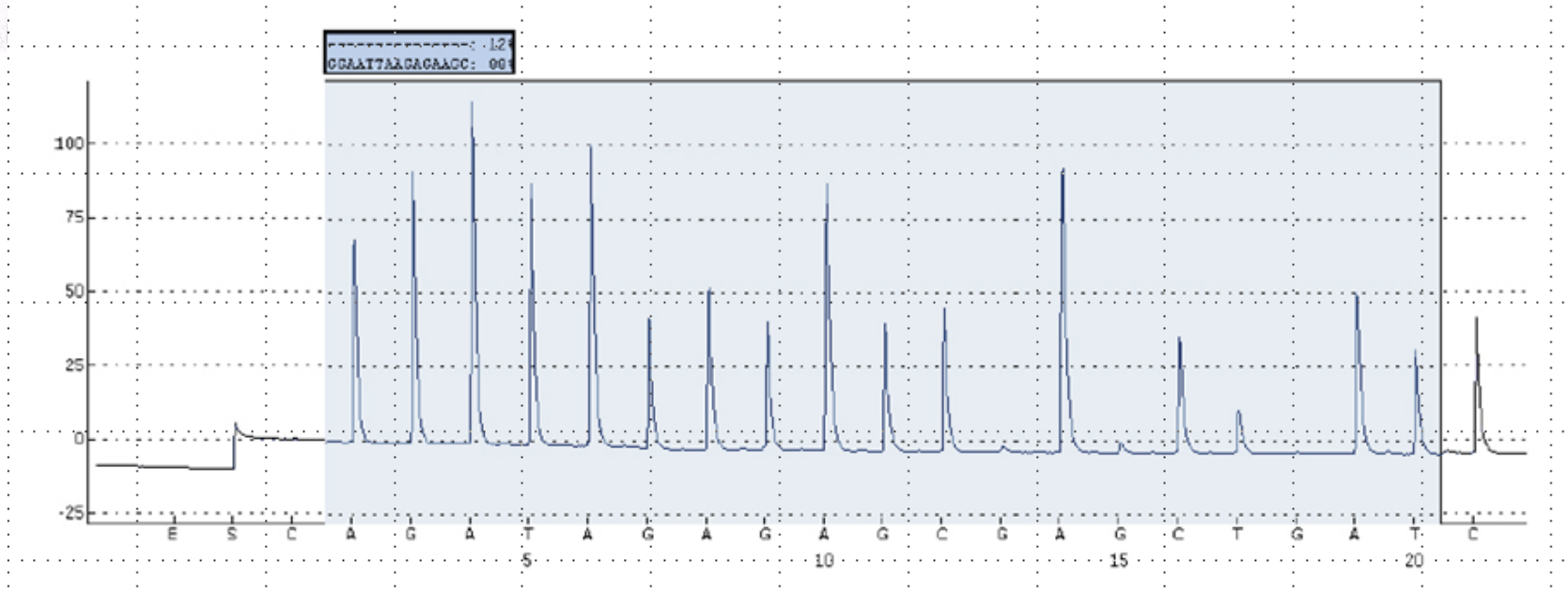
EGFR and ALK status

EGFR mutation: POSITIVE

METODO:
Real Time Polymerase Chain Reaction su DNA estratto da materiale in paraffina per ricerca mutazioni EGF-R e Pirosequenziamento (con Kit EGFR TKI – Diatech Pfarmacogenetics).

DIAGNOSI:
E' stata eseguita la determinazione delle principali mutazioni del gene **EGF-R** mediante PCR e pirosequenziamento.
Area tumorale inferiore al 50%.

RISULTATI:
esone 18: **non mutato**
esone 19: **deleto (88% WT; 12% deleto)**
esone 20: **non mutato**
esone 21: **non mutato**



ALK traslocation: NEGATIVE

MICROSCOPIA:
Indagine FISH con SONDA: Spec **ALK dual color Dual Color break apart probe** FISH, Split Signal ZytoLight

REGIONI ANALIZZATE:
TARGET ORANGE (572 nm): "CMYC major breakpoint distal region" 2p.23
TARGET GREEN (528 nm): "CMYC major breakpoint proximal region toc myc gene" 2p.23
CONTRASTO NUCLEARE : DAPI.

Numero di cellule neoplastiche in interfase esaminate: 118
Cellule con co-localizzazione (appaiamento del segnale): 114 (97,4%)
Cellule con traslocazione (split del segnale):4 (2,6%)

COMMENTO: il segnale non risulta traslocato

COMMENTO: Il segnale non risulta traslocato.

Second opinion

On April 2014 the patient referred to our center for a second opinion:

- ✓ Good clinical conditions (ECOG PS: 1)
- ✓ SatHbO2: 98%
- ✓ Neurologically asymptomatic

We asked for a revision by our Pathologist.

Histology revision and EGFR status

Histology revision

EGFR mutation: NEGATIVE

Reperto microscopico:

Metodiche eseguite:

-materiale isolato per microdissezione dopo colorazione idoneo per analisi mutazionale (cellule tumorali >50 % e n° tra 100 e 1000)

-DNA genomico estratto con colonnine a membrana di silice(Qiagen/Macherey-Nagel) Lettura spettrofotometrica.(58.6 ng/microl)

1)***Amplificazione in real-time (tecnologia ARMS/Scorpion)*** *con Therascreen EGFR RGQ PCR KIT IVD-CE(ditta Qiagen) per le mutazioni:*

- **esone18: p.Gly719Cys/Ser/Asp/Ala senza identificarle;**
- **esone19: principali delezioni senza identificarle**
- **esone21: p.Leu861Gln e p.Leu858Arg.**

2)**Amplificazione real-time end-point e sequenziamento** *con pirosequenziatore PyroMark MA96(sec. Righi et al. BMC Cancer 2013, 13:114)*

- **esone 18: p.Gly719Cys; p.Gly719Ser; p.Gly719Asp; p.Gly719Ala;**
- **esone 19: delezioni tra i codoni 746 e 753;**
- **esone 21: p.Leu861Gln; p.Leu858Arg.**

test è eseguito secondo le "Raccomandazioni per l'analisi mutazionale del gene EGFR nel carcinoma polmonare" Linee guida AIOM-SIAPEC-IAP- Pathologica2010;102:123-126

Il laboratorio partecipa al programma di controllo di qualità nazionale per EGFR carcinoma polmonare dell'AIOM-SIAPC-IAP

RISULTATO

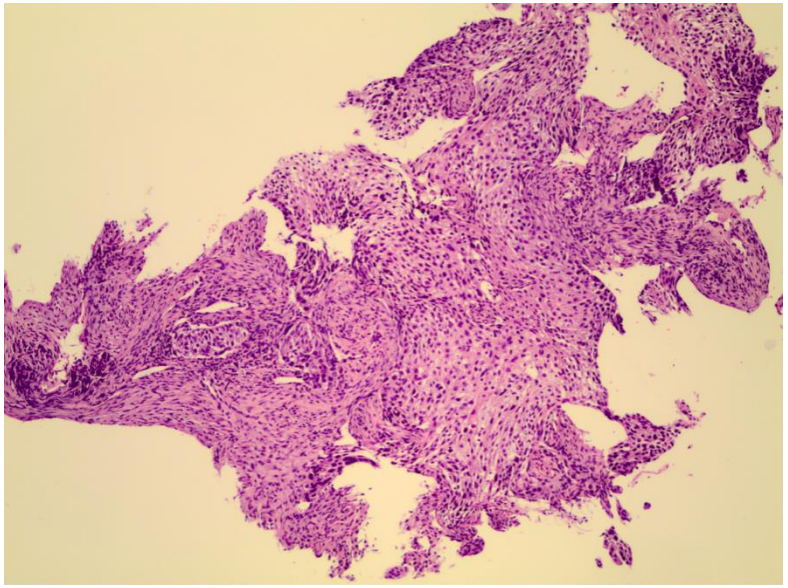
ESONE 18: non sono state evidenziate le mutazioni testate

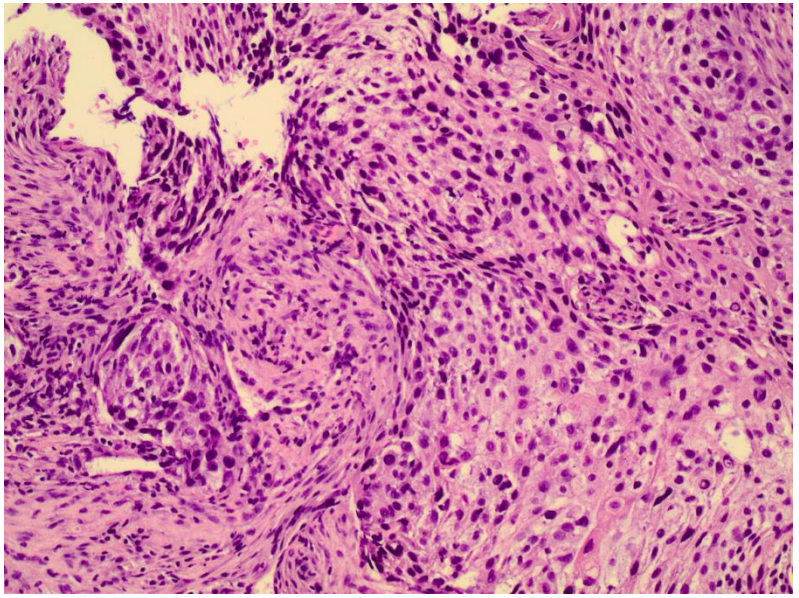
ESONE 19: non sono state evidenziate le mutazioni testate

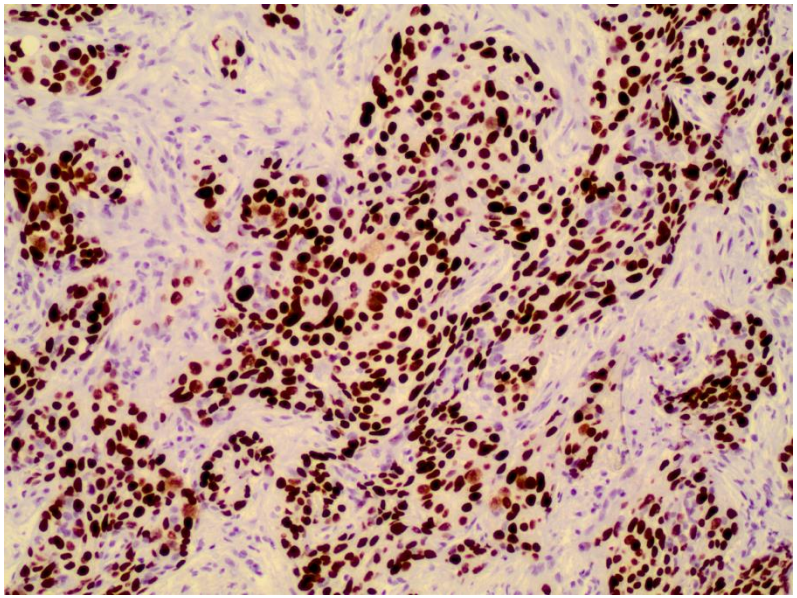
ESONE 21: non sono state evidenziate le mutazioni testate

Giudizio diagnostico:

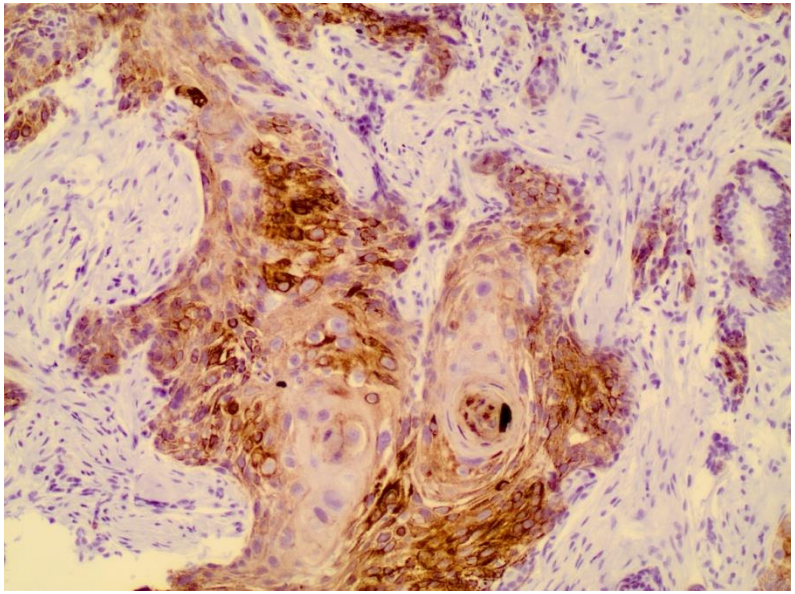
Campione WILD TYPE per le mutazioni testate del gene EGFR esone 18 - 19 - 21







p40: positive



CK5: positive

Third advice

Considering the discrepancies between the data a third pathological center was involved:

Histological revision:

DIAGNOSI

Le sezioni mostrano un carcinoma squamocellulare infiltrante moderatamente differenziato con focali aree di cheratinizzazione (TTF-1 negativo).

EGR mutation: NEGATIVE

L'analisi molecolare, eseguita mediante PCR e MassARRAY (Sequenom), ha evidenziato quanto segue:

EGFR
esone 18 (codone 719): NON MUTATO
esone 19 (codoni 746-754): NON MUTATO
esone 20 (codoni 768, 770-771, 790): NON MUTATO
esone 21 (codoni 858-861): NON MUTATO

ALK traslocation: NEGATIVE

La determinazione dell'espressione della proteina ALK è risultata NEGATIVA (score 0 sec. Park HS et al. LUNG CANCER 2012).

2nd line treatment

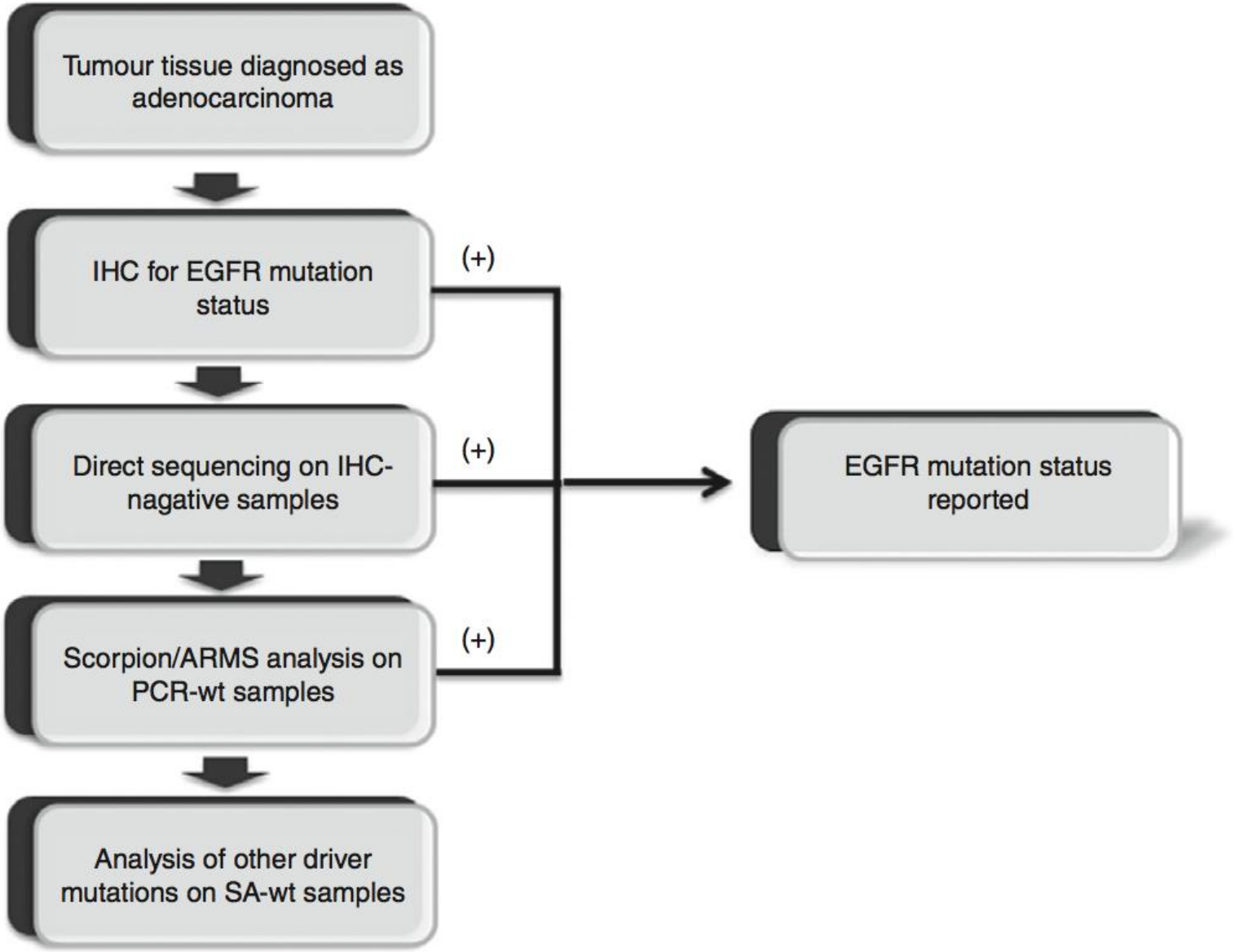
✓ Before receveing the results of the third opinion the patient started a second line treatment with a reversible Tki , without any benefit and a rapid clinical progression.

✓ The patient died in May 2014 (*diagnosis March 2013*)

- some series have argued that responses to EGFR-targeted therapies in SCC are due to pathological **misclassification**

- increasingly being recognized that different mutation testing systems have **differing sensitivities for detection** of EGFR mutations

Different Tecniques, Different Results…..
do we need an alghoritm?



Ho HL et al, Respirology (2013) 18, 1261–1270

Clinical history

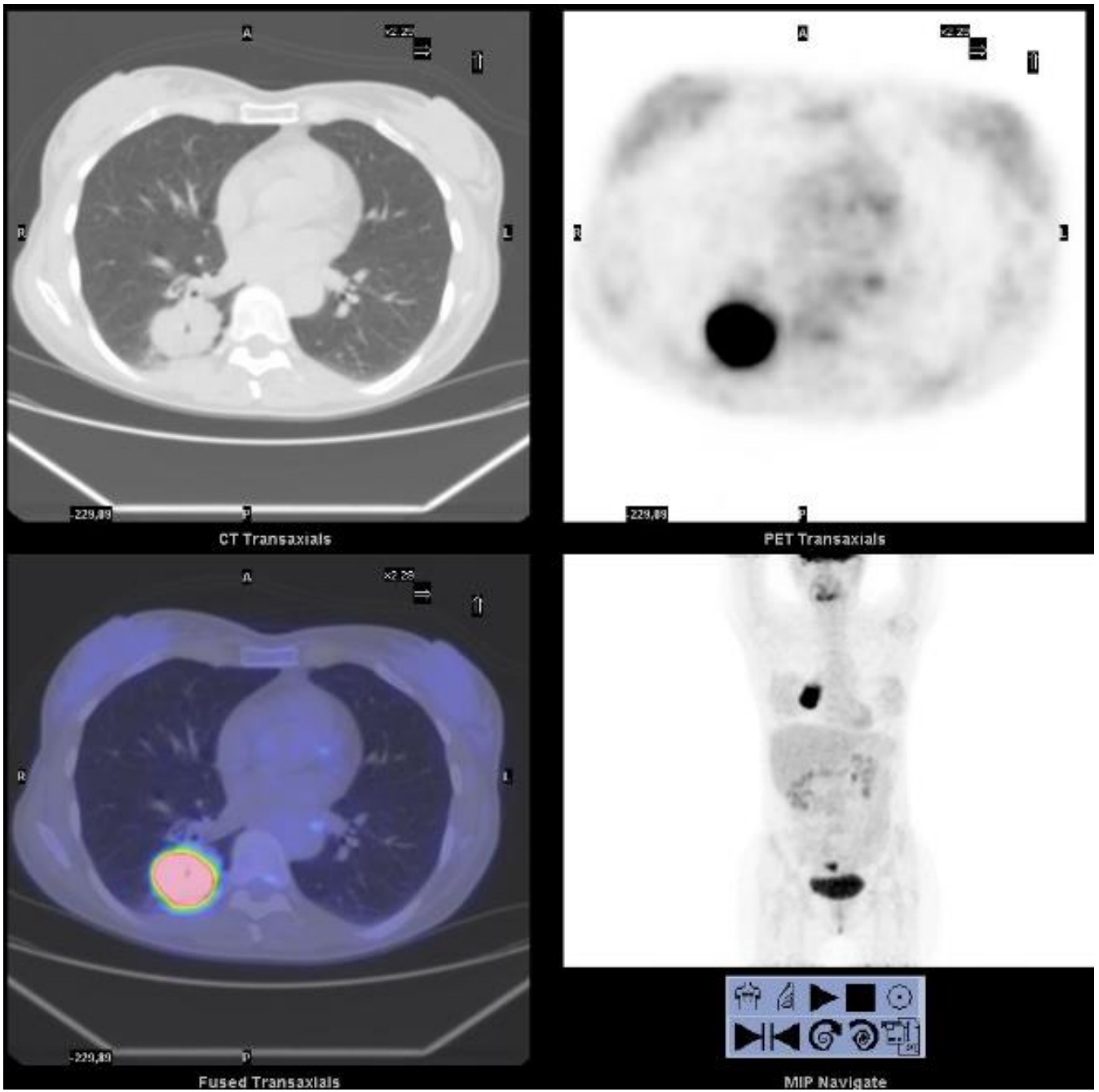
E.B., female, 57 years old
Never smoker

Medical history:
2001: conization for a high grade dysplasia

No allergy

Oncological history:

- April 2009: during pre-operative exams for inguinal hernioplastic a chest X-ray showed a 4 cm lesion on the right lung
- CT scan showed two pulmonary lesions in the right upper and lower lobe
- CT-PET confirmed only the lesion in the lower lobe


CT Transaxials
PET Transaxials
Fused Transaxials
MIP Navigate

Surgical visit

In May 2009 the patient reffered to a thoracic surgeon:

✓Good clinical conditions (ECOG PS: 0)

✓SatHbO2: 100%

Basing on these data a bronchoscopy and a spirometry were requested

✓FBS: no endobronchial lesion

✓Spirometry: no contraindication for surgery

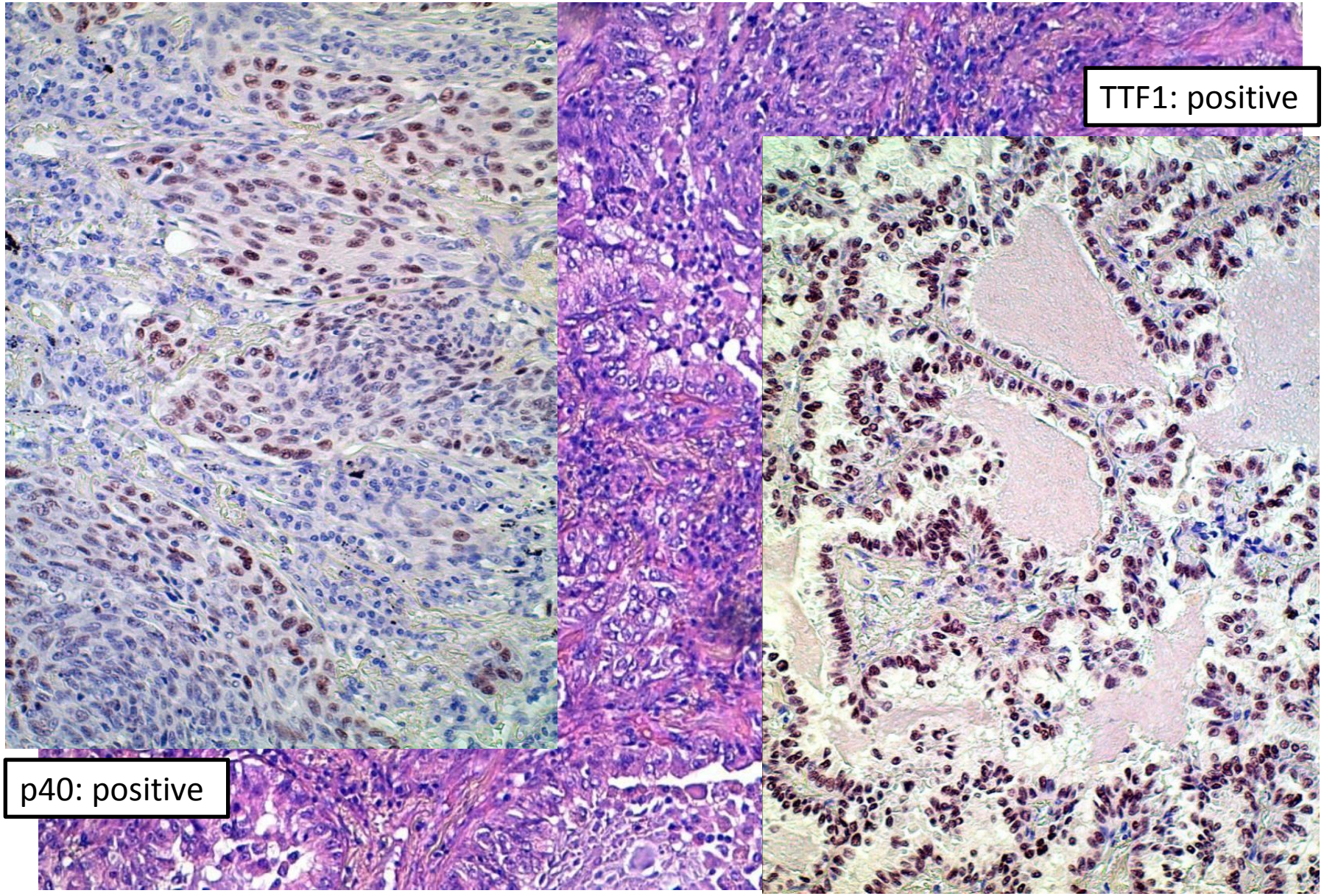
Surgical resection (21.05.2009)

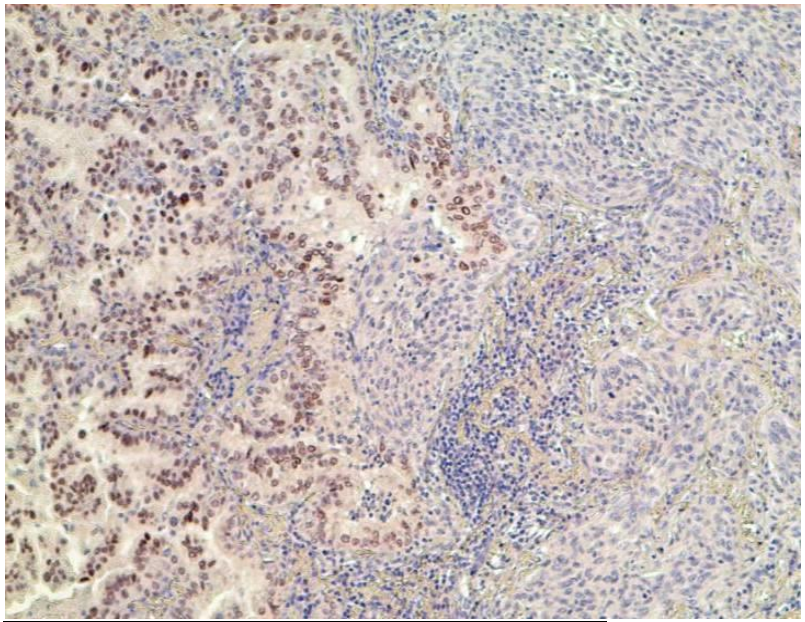
- ✓ A right lower lobe lobectomy plus atypical resection at the upper lobe lesion were performed

Giudizio diagnostico:
A: FRAMMENTO DI PARENCHIMA POLMONARE CON NODO DI CARCINOMA BRONCHIOLO-ALVEOLARE, NON MUCINOSO.
STADIO sec.UICC 2002: pT1 Nx
Margine di resezione parenchimale indenne

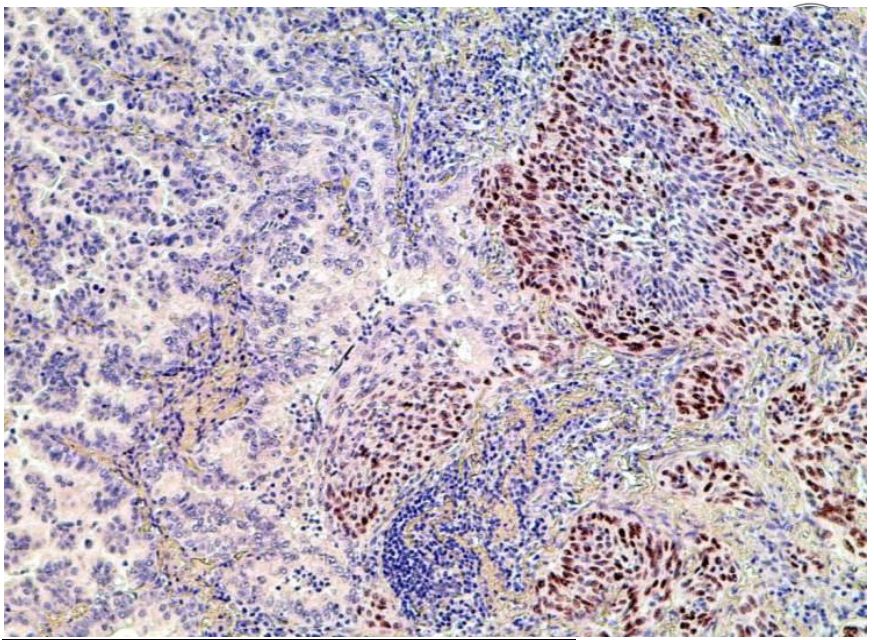
B: LOBO POLMONARE CON CARCINOMA ADENOSQUAMOSO
Grado: scarsamente differenziato (G3)
Dimensioni: diametro massimo cm. 4,5
Necrosi intratumorale: presente estesa in componente squamosa
Invasione vascolare: presente
Pleura viscerale: indenne
Margini di resezione vascolare e bronchiale: indenni da infiltrazione neoplastica (vedi campione K)
Stazioni linfonodali con iperplasia reattiva: da B a J
Stazioni linfonodali con metastasi di carcinoma: nessuna
Reperti associati: //
Parenchima peritumorale: assenti alterazioni di rilievo
C-D-E-F-G-H-I-J: FRAMMENTI LINFONODALI CON ANTRACOSI ED ISTIOCITOSI DEI SENI (2+5+4+3+2+6+4+2 frammenti esaminati)
K: FRAMMENTO DI PARETE BRONCHIALE INDENNE.
STADIO sec.UICC 2002: pT2 pN0

Adenosquamous carcinoma

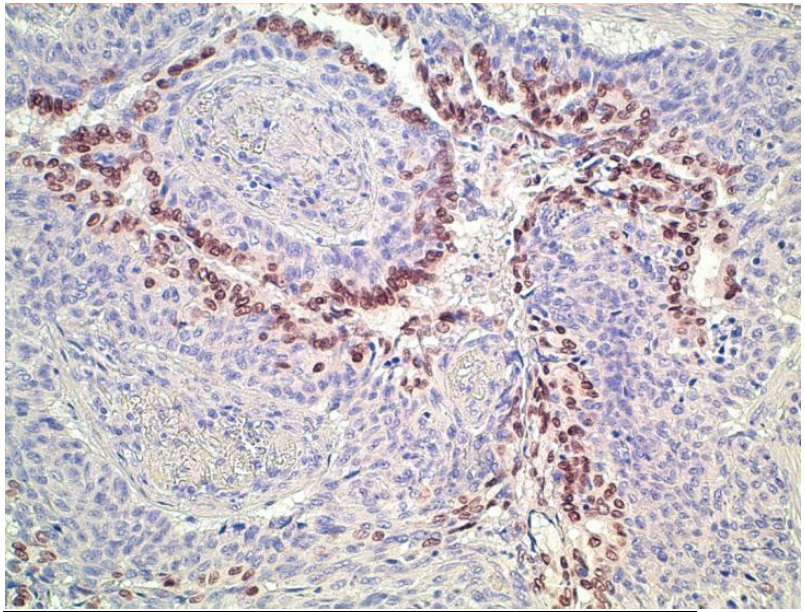




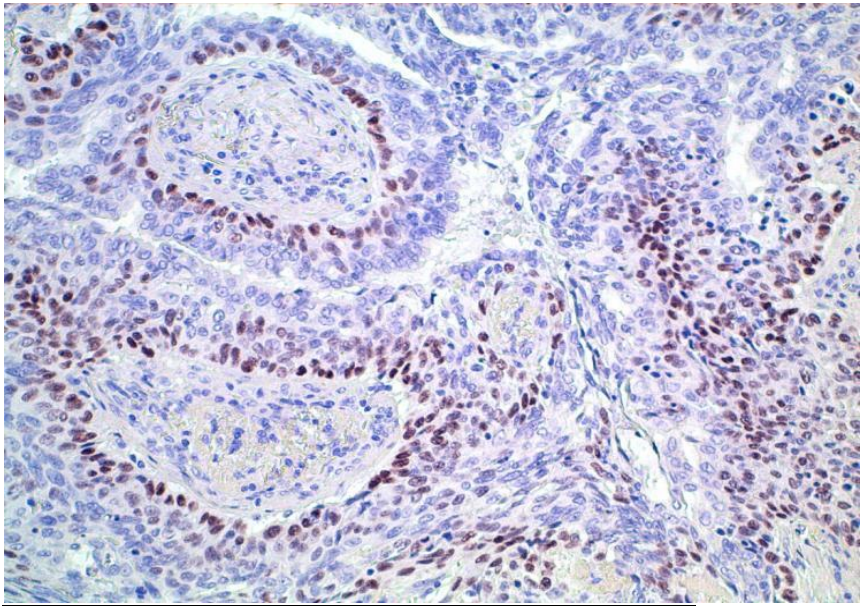
TTF1 adenosquamous collision



p40 adenosquamous collision

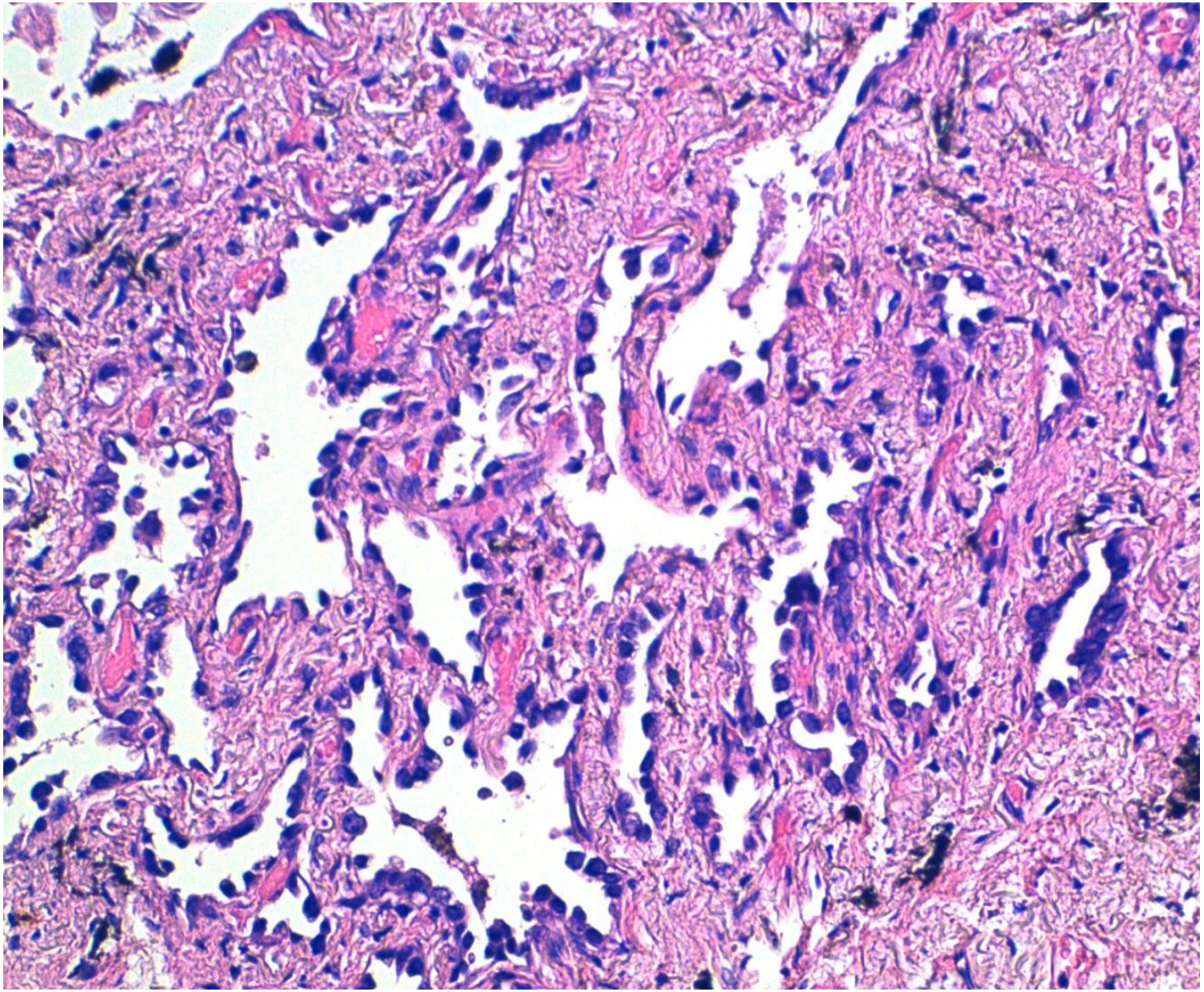


TTF1 adenosquamous intermingled



p40 adenosquamous intermingled

Bronchoalveolar carcinoma



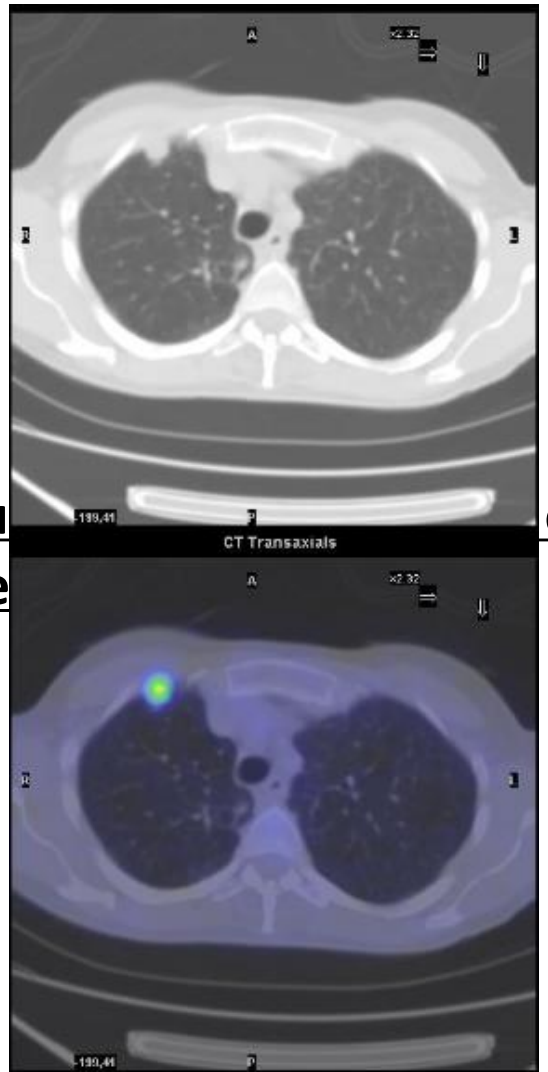
Oncological evaluation and follow-up

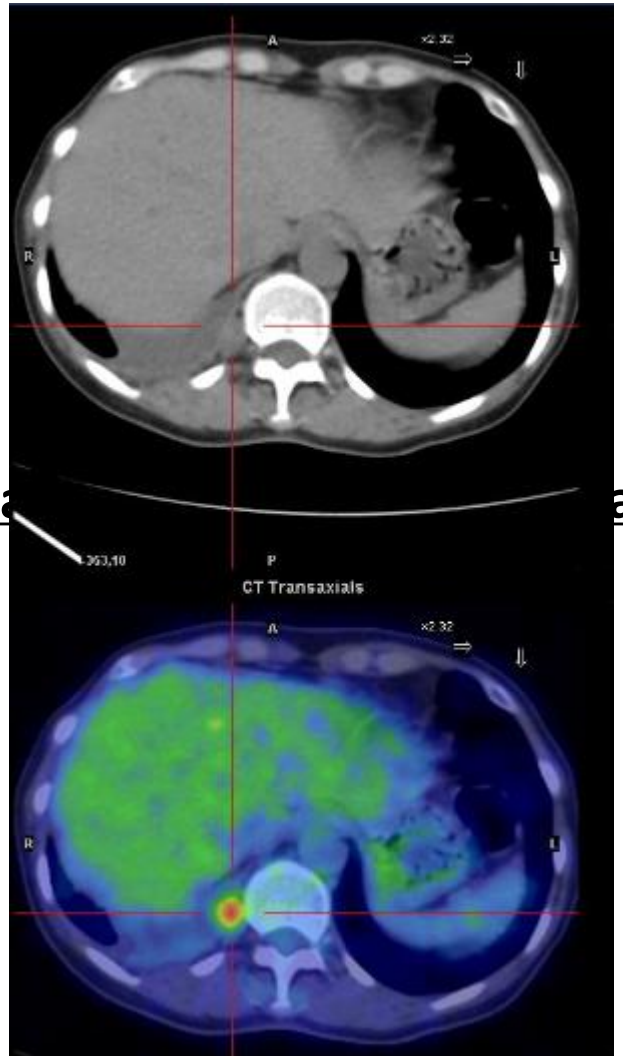
- ✓ No indication to an adjuvant treatment but only follow-up

- ✓ The patient started her regular follow-up every 3 months

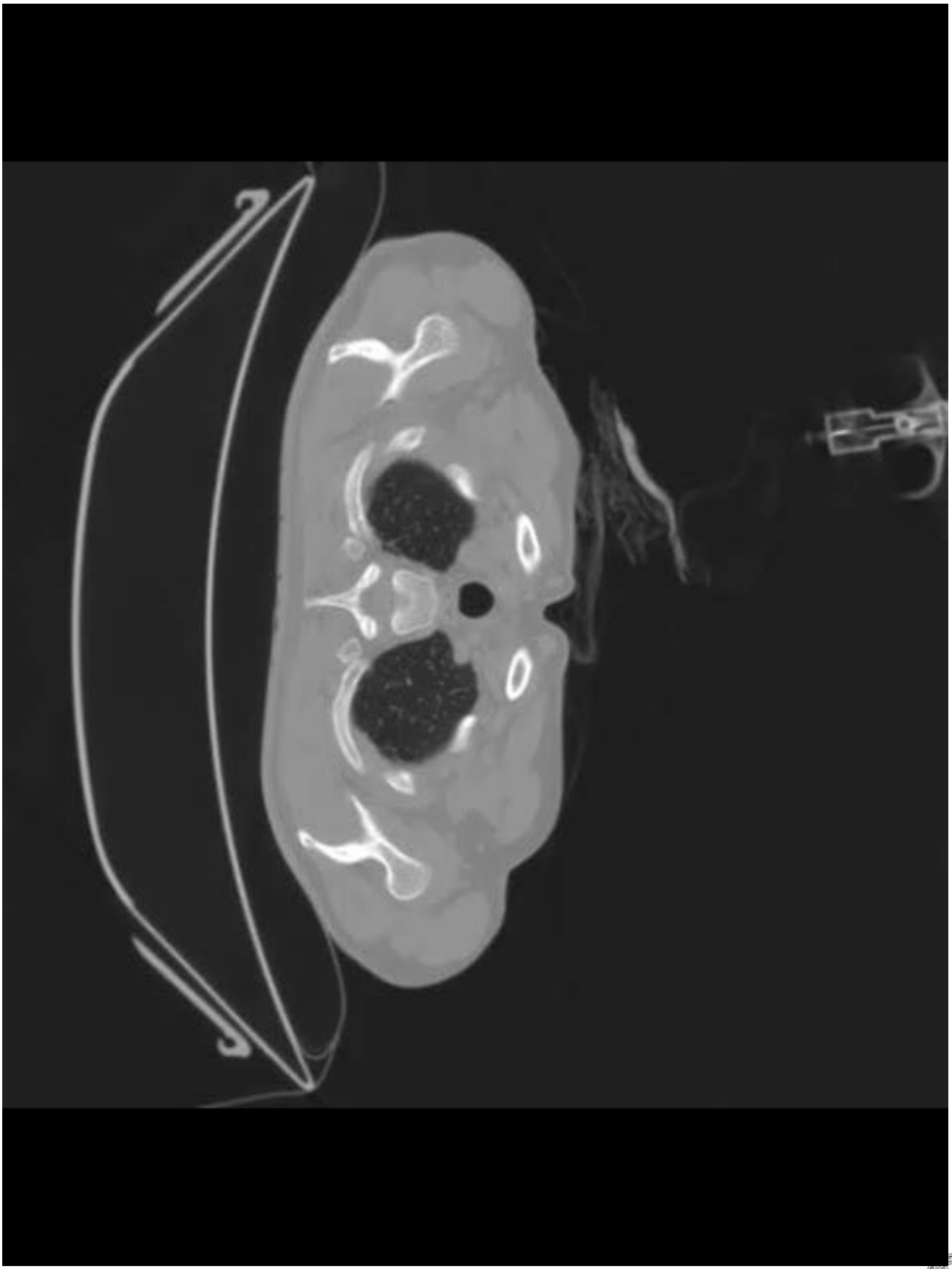
October 2009

- ✓ The CT scan showed a right upper lobe lesion and evidence of a pleural thickening near to T11; the CT-PET confirmed the lesion in the right upper lobe (SUV: 8.7) and in the parietal pleura (SUV: 4.7)



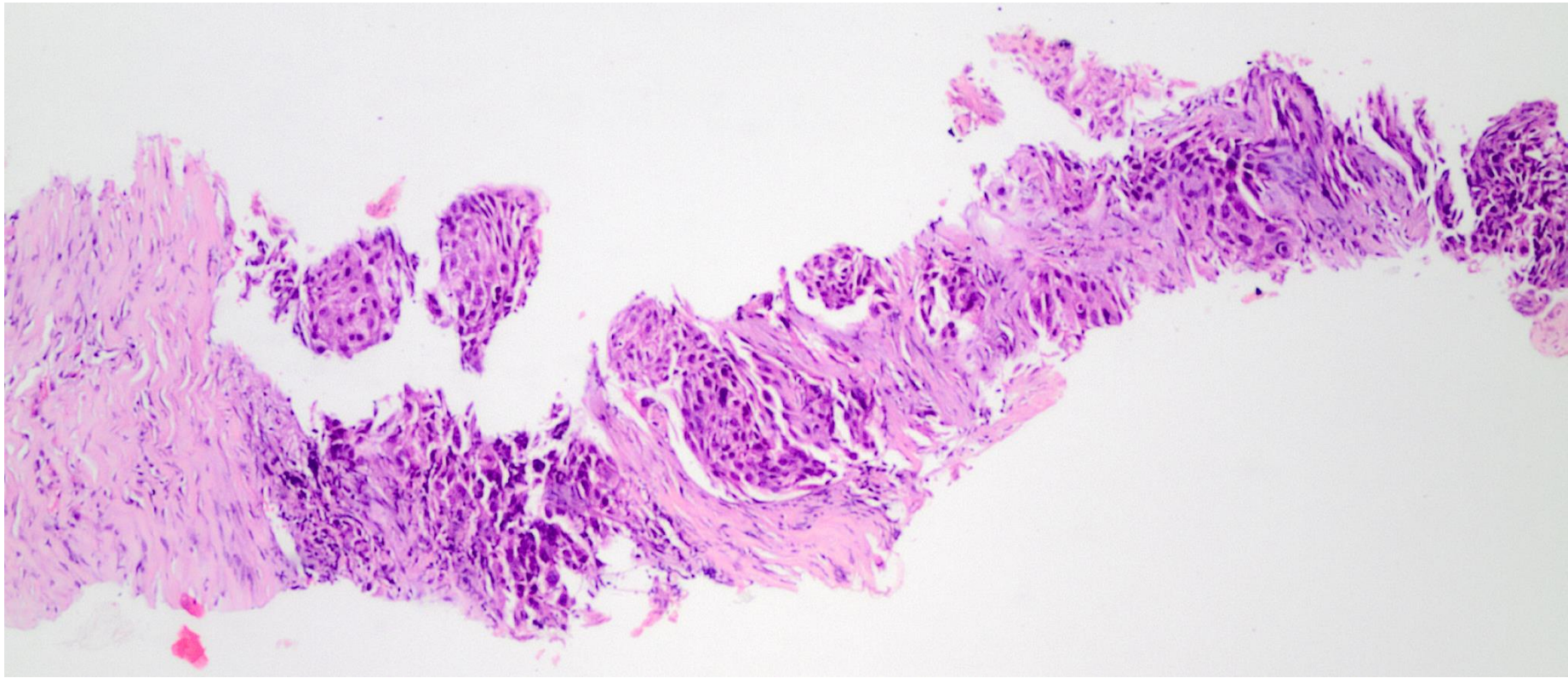


- ✓ **Su of the disea as pe**



Sede del prelievo: *Polmone lobo superiore destro*
Numero di nodi: *1*
Dimensioni: *1 cm.*
Caratteristiche della lesione : *solida, a contatto con la pleura.*
Tipo ago: *TRU-CUT*
Numero di prelievi : *1* ***Accesso :*** *anteriore*

Giudizio diagnostico:
QUADRO CITOLOGICO COMPATIBILE CON PRESENZA DI CELLULE DI CARCINOMA (possibile CARCINOMA SQUAMOSO).



Surgical resection (04.01.2010)

- ✓ The thoracic surgeon proposed an atypical resection of the lesion

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A Pleura parietale adiacente a neoplasia (esame estemporaneo al congelatore) B Pleura viscerale in sede costo-frenica (esame estemporaneo al congelatore) C Polmone destro: resezione atipica D Pleura parietale adiacente a neoplasia E Pleura viscerale

Giudizio diagnostico:

A) FRAMMENTI DI TESSUTO CONNETTIVO FIBROADIPOSO E MUSCOLARE SCHELETRICO DELLA PARETE TORACICA, INDENNI DA NEOPLASIA.

B) FRAMMENTO PLEURICO INDENNE DA NEOPLASIA.

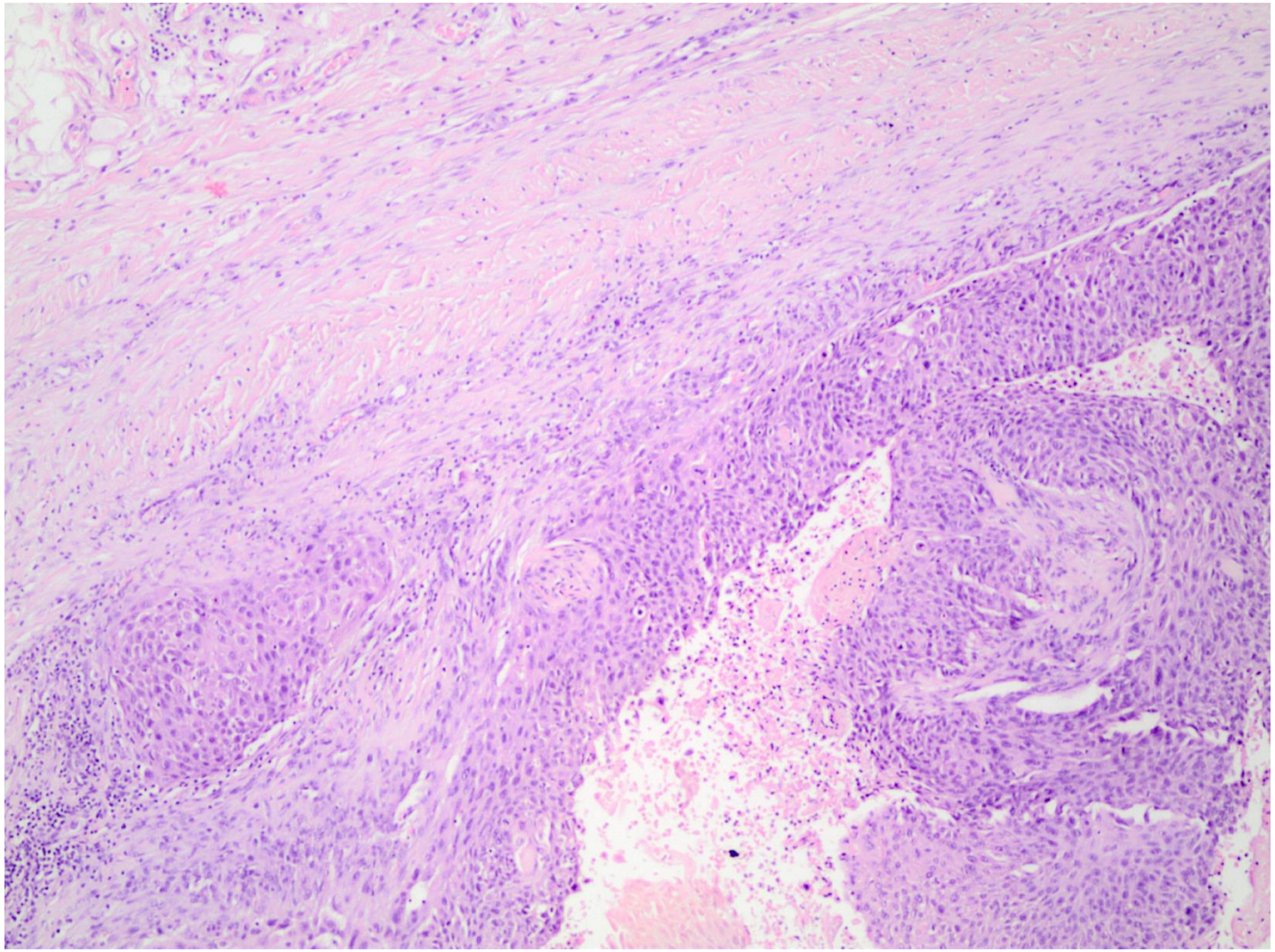
C) FRAMMENTO DI PARENCHIMA POLMONARE CON NODO DI CARCINOMA SQUAMOSO SCARSAMENTE DIFFERENZIATO (G3) INFILTRANTE LA PLEURA VISCERALE.

STADIO sec. AJCC 2009: rpT3(PL3) - NX

D) FRAMMENTO DI PLEURA PARIETALE CON ESTESA INFILTRAZIONE DA PARTE DI CARCINOMA SQUAMOSO.

E) FRAMMENTI DI TESSUTO CONNETTIVO FIBROADIPOSO E MUSCOLARE SCHELETRICO, INDENNI DA NEOPLASIA.

Squamous carcinoma



May 2010

- ✓ At the first CT scan after surgery an evidence of hepatic and renal relapse of the disease was documented

- ✓ The patient started a 1st line treatment with:
  - Cisplatin 75 mg/mq day 1
  - Docetaxel 70 mg/mq day 1

  Every 21 days

- ✓ Toxicity profile: CTCAE grade 3-4 gastro-intestinal toxicity

- ✓ The CT scan after 3 cycles of therapy showed a stable disease

Considering the treatment response, the toxicity profile, and the smoking habit the evaluation of the EGFR mutation was requested

EGFR status

Metodiche eseguite:

-materiale isolato da sezioni in bianco deparaffinate idoneo per analisi mutazionale con una percentuale di cellule tumorali > 50 % ottenuto per microdissezione

-DNA genomico estratto con "QIAamp DNA Mini Kit" Quiagen e valutato per lettura spettrofotometrica.(95,1 ng/microl)

-Amplificazione end-point e sequenziamento con pirosequenziatore PyroMark "ID" utilizzando il kit "EGFRTKI response(sensitivity) con certificazione IVD CE della ditta Diatech che anlizza le mutazioni possibili nell'esone 18 codone 719 (sensibilità allele mutato 7,5%), esone 19 codone 746-750del (sensibilità allele mutato 2 %), esone 21 codone 861 e 858 sensibilità allele mutato 7,5%).

DNA genomico utilizzato per test: 100 ng

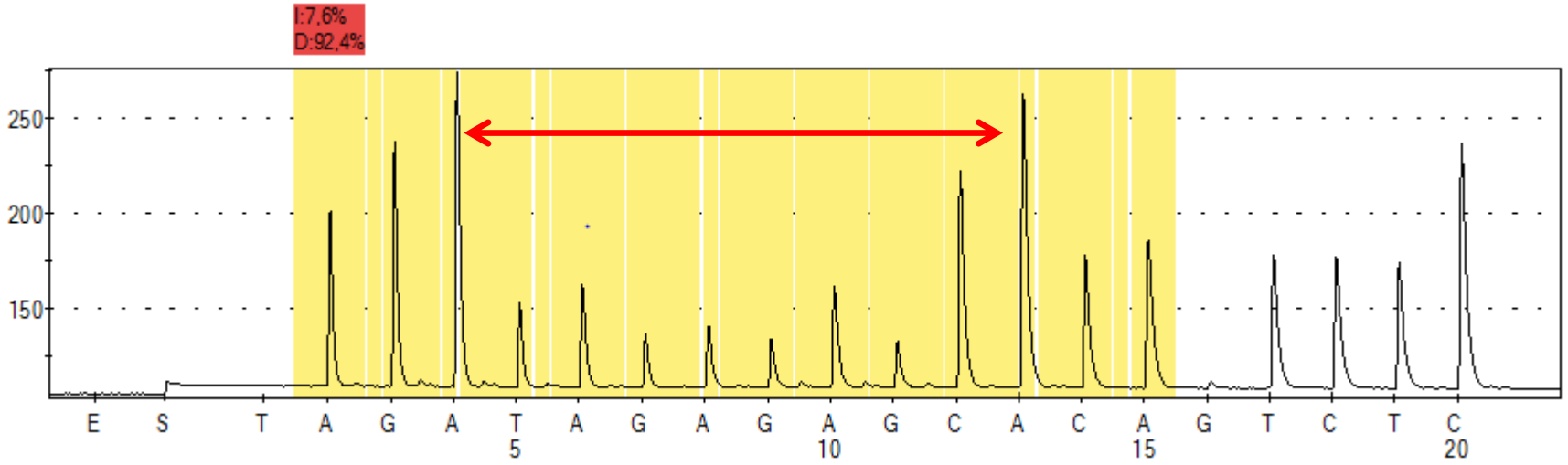
RISULTATO

ESONE 18 = non sono state evidenziate mutazioni nel codone 719

ESONE 19 = *e' stata evidenziata una delezione compresa nei codoni 746-750* (regione codificante 2234-2258)

ESONE 21 = non sono state evidenziate mutazioni nel codone 861 e 858

CAMPIONE MUTATO NELL'ESONE 19 del GENE EGFR.



Second line treatment

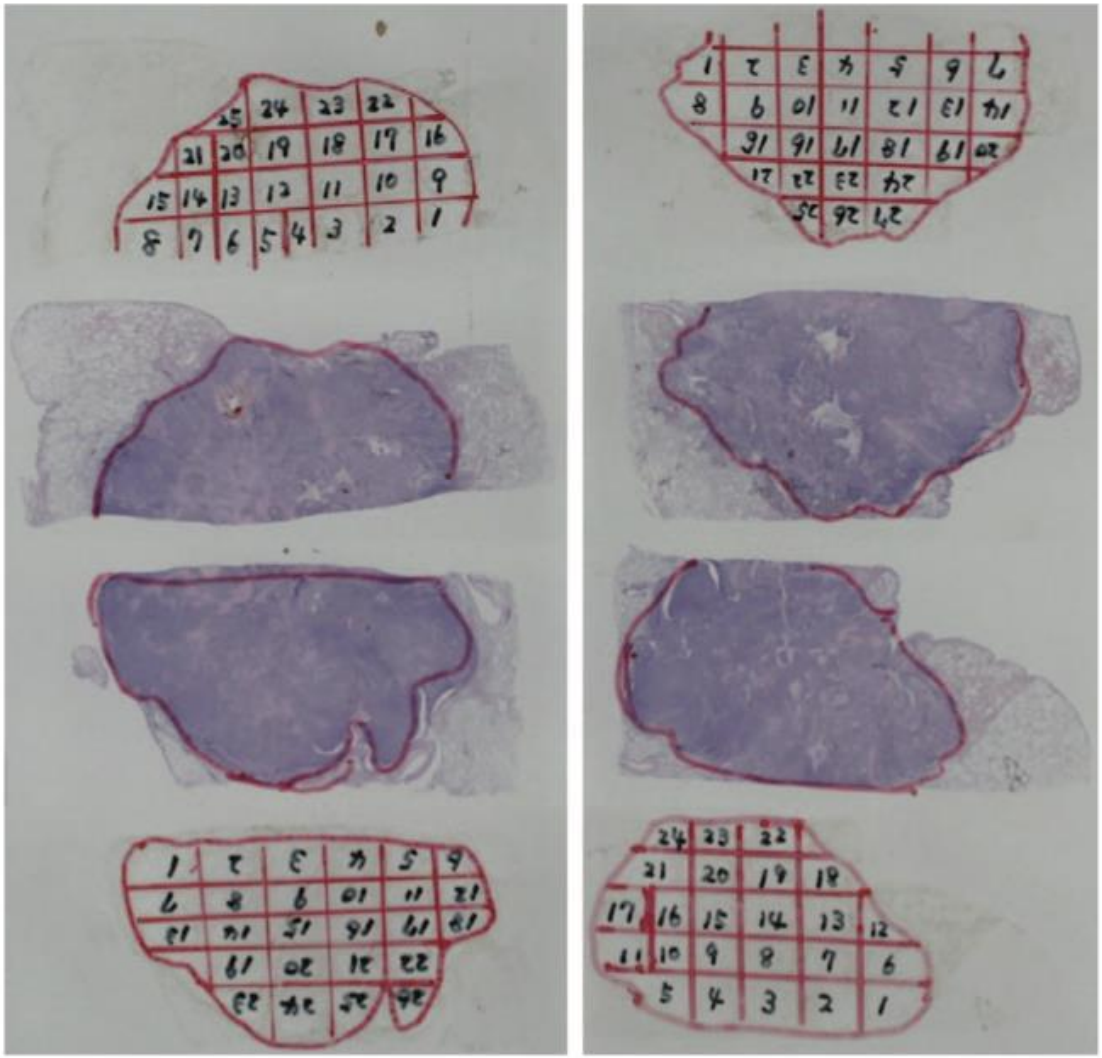
- ✓ Considering the EGFR status and the toxicity profile during chemotherapy the patient started a treatment with:
  - Gefitinb 250 mg/die
- ✓ The patient was treated from August 2010 to March 2011
- ✓ The CT scan of November 2010 showed a stable disease
- ✓ The treatment was well tollerated without any significant toxicity

Progressive disease

- ✓ On March 2011 evidence of disease progression due to the appearance of a bone (vertebral) lesion
- ✓ A palliative radiotherapy treatment was performed (20 Gy), interrupted due to appearance of neurological toxicity
- ✓ A 3rd line with Afatinib 40 mg was proposed (EAP)
- ✓ The patient died on 1st June 2011 for progression disease (*relapse 6 mo after surgery, on Oct 2010*)

- tumor **heterogeneity**, particularly in biopsy samples, has been identified as a barrier to detection of mutation-positive disease (even if *"large pathological series have demonstrated that tumor heterogeneity for EGFR mutations is in fact quite infrequent")*

Hetereogeity or not Heterogeneity, that is the question



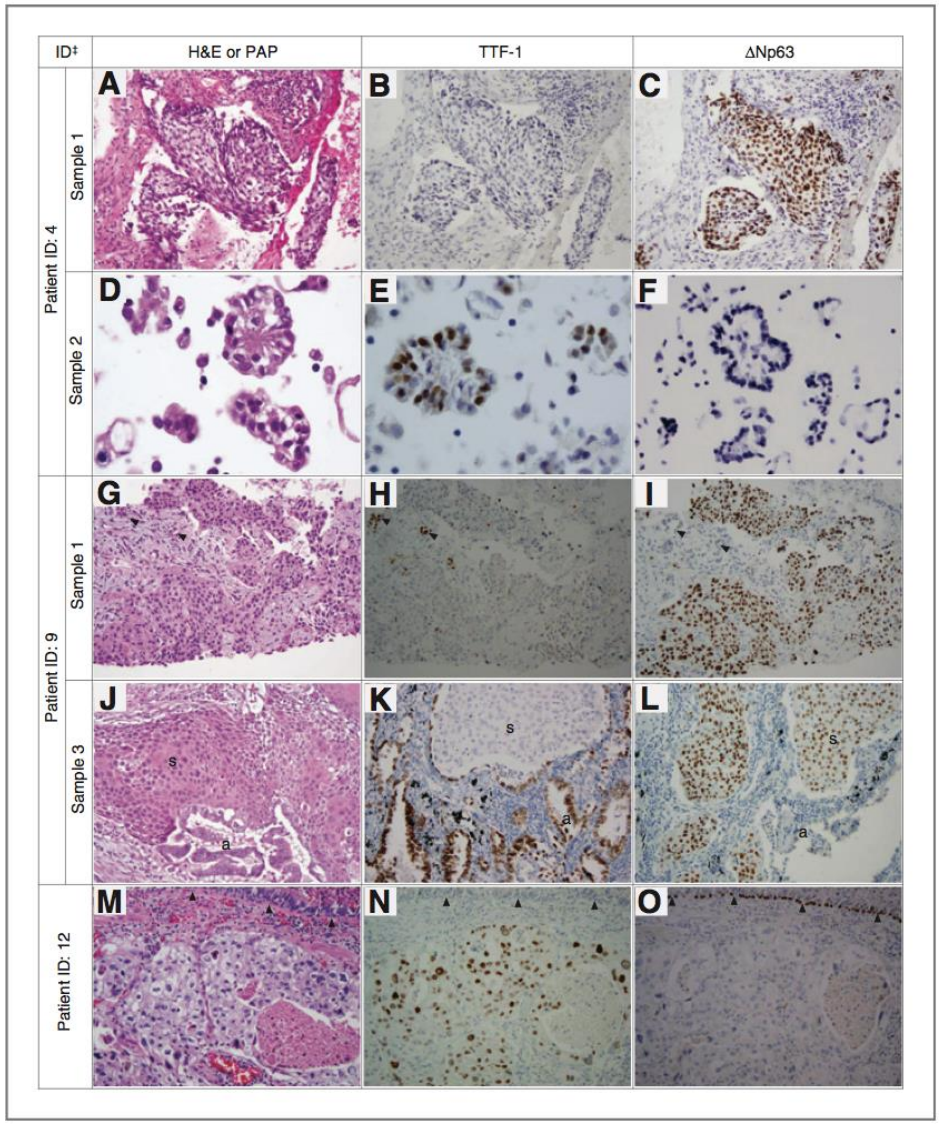
Five ADCs with the *EGFR* mutation were dissected into more than 100 pieces

Identical *EGFR* mutation among the pieces

Yatabe Y et al, J Clin Oncol 2011 29:2972-2977

- subsequent biomarker analyses in the major randomized studies (in unselected population) have demonstrated levels of EGFR mutations at **2%** in the squamous population, 13% in the non-adenocarcinoma population and 11% in the total population (including adenoca)

Do we really deal with PURE Squamous carcinomas?



- The screen of 95 biomarker-verified SQCCs revealed no EGFR [0%; 95% confidence interval (CI), 0%–3.8%] mutations.

Rekhtman N et al, Clin Cancer Res; 18(4) 2012

- There were no EGFR mutations in 454 squamous carcinomas using a dual technical approach: direct sequencing of polymerase chain reaction (PCR) and PCR single-strand conformation polymorphism (SSCP) analysis.

Marchetti A et al, J Clin Oncol 865; 2005

26/381
6.8%

EGFR mutations in SqCC

Smoking habit	Number	%
Never Smoker	16	61.6
Light Smoker	1	3.8
Former Smoker (>15 years)	4	15.4
Former Smoker (<15 years)	3	11.5
Smoker	2	7.7

Normanno N et al, AIOM 2011

- **recommending EGFR mutation testing in SQC we assume that sensitizing-EGFR mutations as a predictive biomarker for EGFR-TKi treatment in SQC are as good as they are in ADC…. however, this fact has never been carefully examined**

***EGFR* mutation rates and the response to EGFR-tKI treatment in SQC**

	n	Female (%)	Nonsmoker (%)	ORR (%)	*EGFR* mutation (%)
Hata et al. [16]	41	12 (29.3)	6 (14.6)	9.7	2/34 (5.9)
Lee et al. [17]	71	15 (21.1)	13 (18.3)	8.7[a]	1/37 (2.7)
Tseng et al. [18]	92	17 (18.5)	18 (19.6)	17.4	2/27 (7.4)
Chiang et al. [19]	37	9 (24.3)	12 (32.4)	16.2	–
Pooled	242	53 (21.9)	49 (20.2)	13.3	5/98 (5.1)

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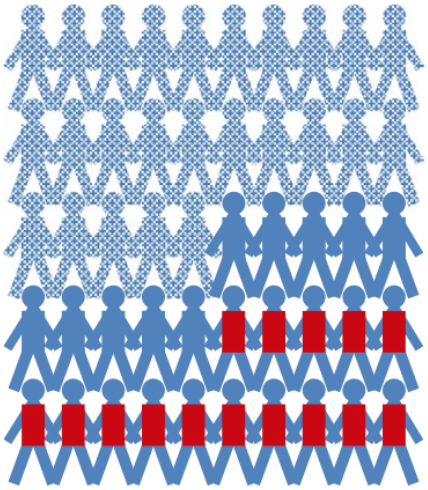
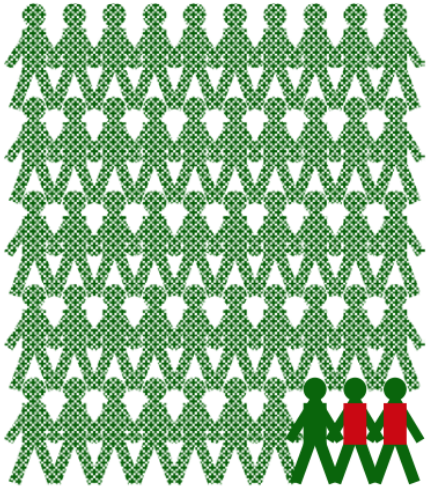
Should EGFR mutations be tested in advanced lung squamous cell carcinomas to guide frontline treatment ?



EGFR WT EGFR mutant EGFR-TKI responder

Expected EGFR-TKI response rate in East Asian LADC
= 1 x EGFR mutation rate x $RR_{EGFR\ M+}$
= 1 x 50% x 60%
= 30%



Expected EGFR-TKI response rate in East Asian LSQC
= 1 x EGFR mutation rate x $RR_{EGFR\ M+}$
= 1 x 5% x 60%
= 3%

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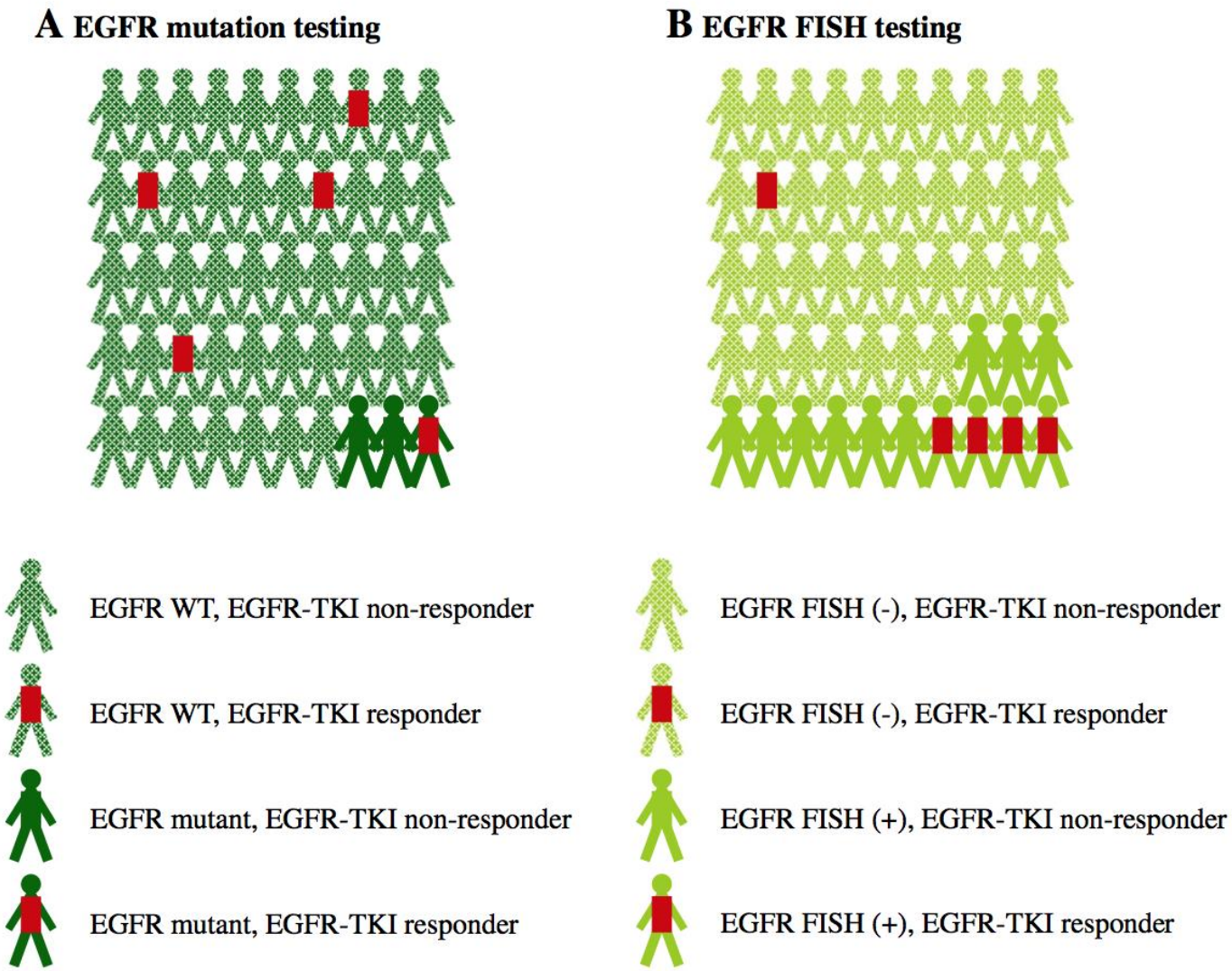
Is EGFRm status a "valid biomarker" also in SQC as in ADC?



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Conclusion

- In squamous cell carcinoma smoking habit guide the decision to perform/not perform EGFR analysis
- Be sure that you're dealig with a "pure" squamous
- Take into consideration that differences in results are possible for different tecniques
- Take into consideration that we assume that sensitizing-EGFR mutations as a predictive biomarker for EGFR-TKi treatment in SQC are as good as they are in ADC, but this is not clearly demonstrated