# Molecular testing in lung cancer.

P.Pauwels (UZA/UZG)

M. Kockx ( ZNA/ HistoGeneX)

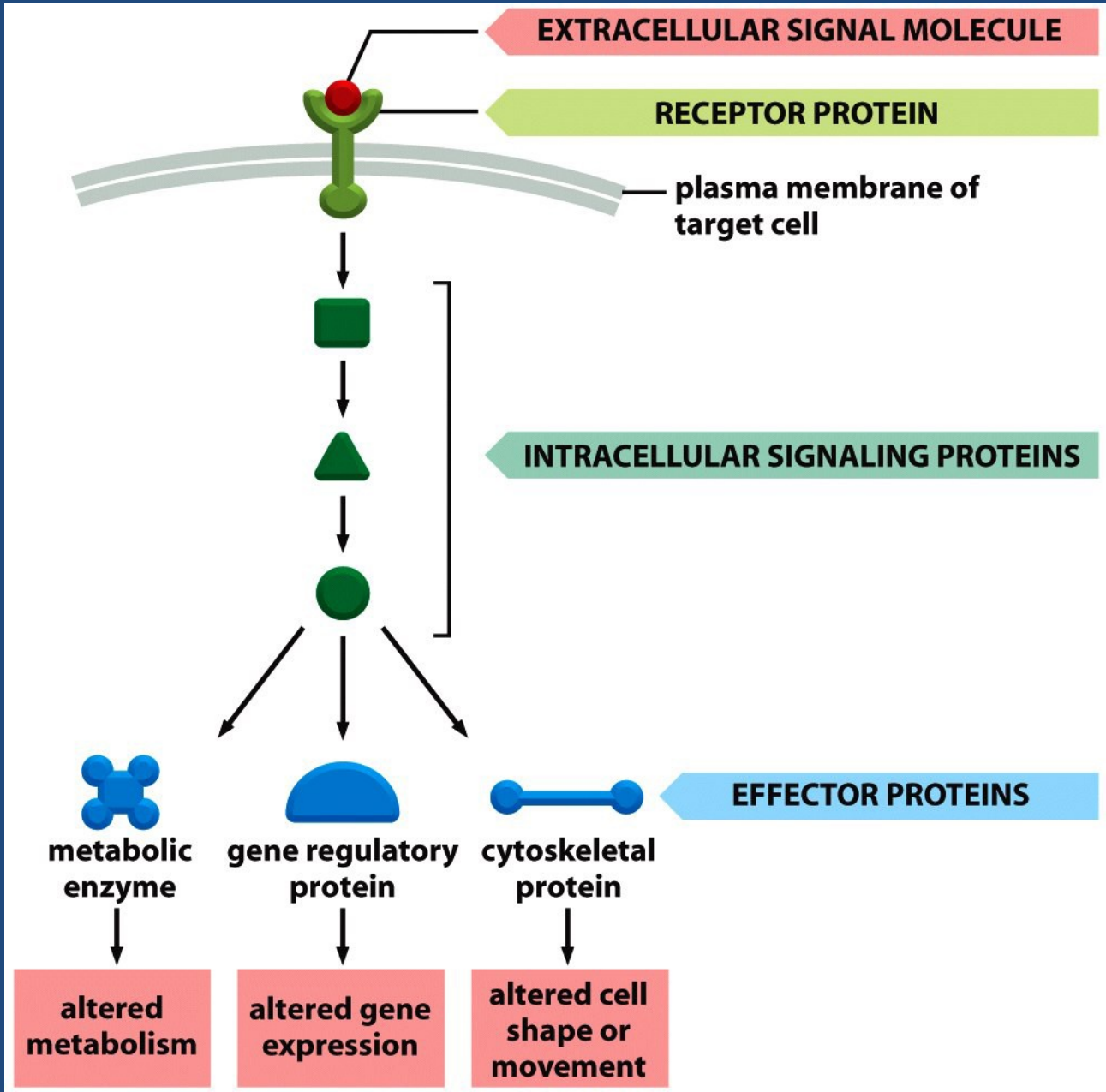
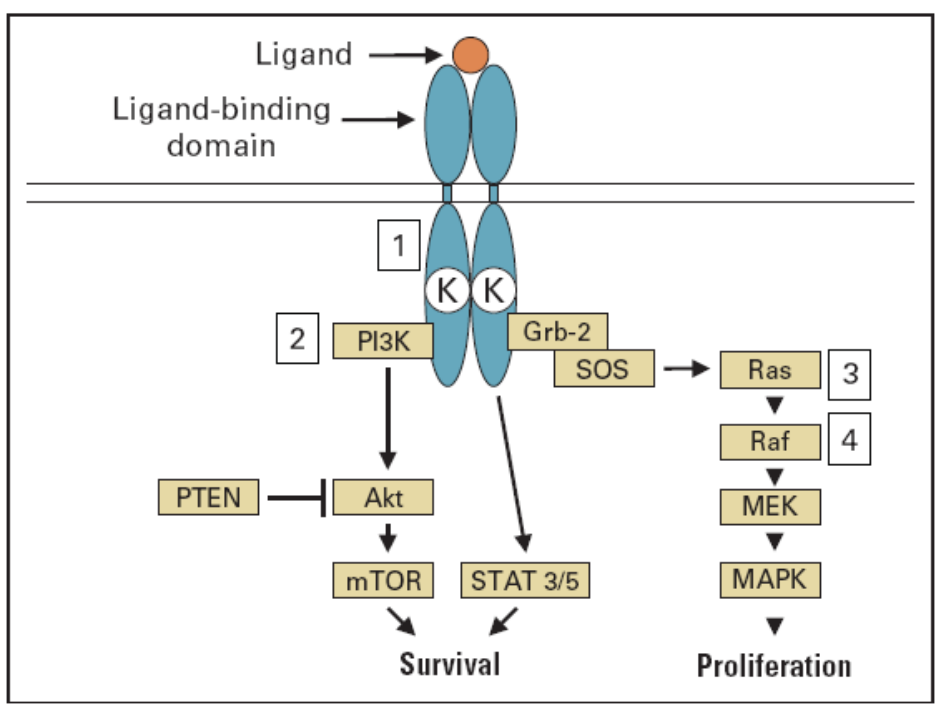



Figure 15-1 Molecular Biology of the Cell 5/e (© Garland Science 2008)

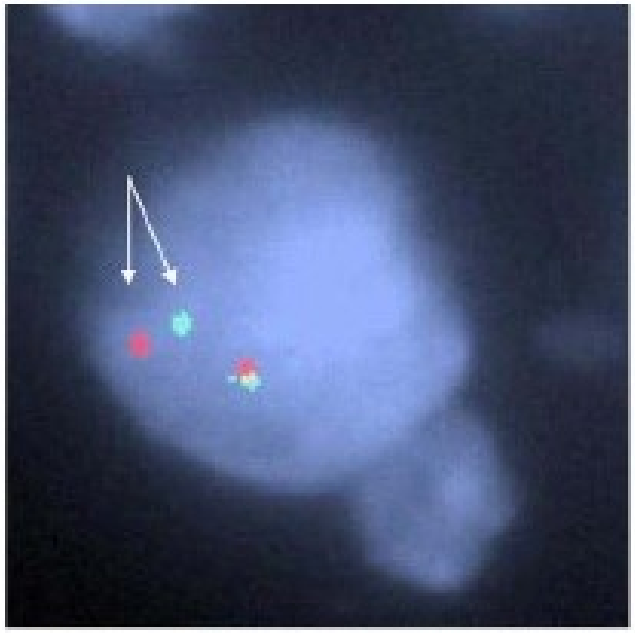
# EGFR



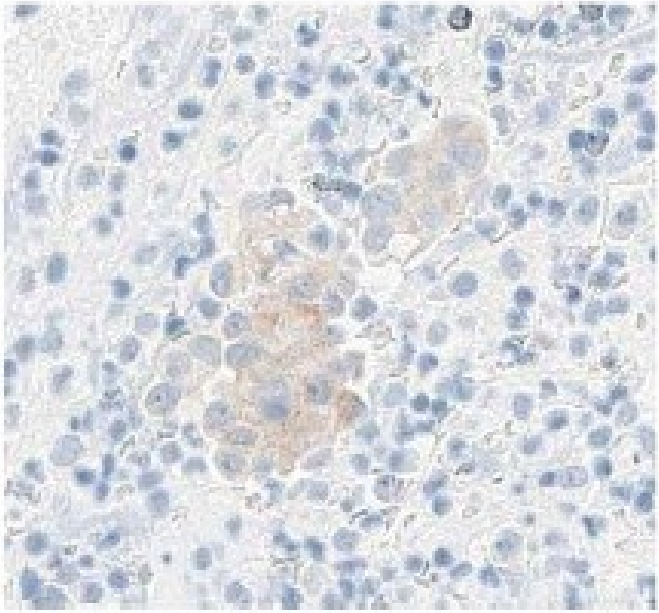

Pao & Miller JCO 2005;23:2556-2568

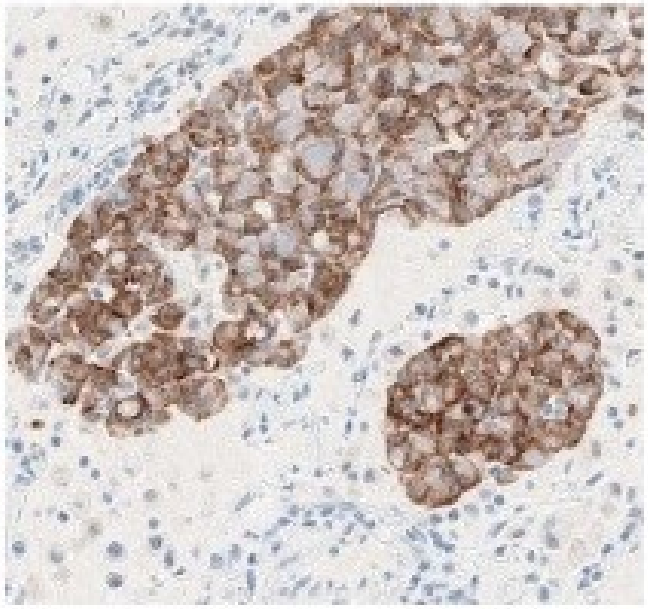
- Exist as monomers
- Binds ligand, changes shape and homo/hetero-dimerize
- Autophosphorylation of tyrosine residues
- Recruitment of adaptor/signaling molecules
- Downstream signaling

A



B





Influence of EGFR expression on rate of response to cetuximab or panitumumab as monotherapy in colorectal cancer

| Anti-EGFR agent/reference | EGFR expression | Response rate % (no./total no.) |
|---|---|---|
| Cetuximab | | |
| Cunningham et al. [4] (BOND study) | EGFR-expressing cells | |
| | ≤10% | 7 (4/56) |
| | <10 to ≤20% | 31 (5/16) |
| | >20 to ≤35% | 0 (0/7) |
| | >35% | 9 (3/32) |
| | EGFR staining intensity | |
| | Faint | 5 (1/21) |
| | Weak or moderate | 13 (7/55) |
| | Strong | 12 (4/34) |
| Saltz et al. [20] | EGFR status | |
| | 1+ | 6 (1/17) |
| | 2+ | 13 (4/30) |
| | 3+ | 0 (0/10) |
| Panitumumab | | |
| Hecht et al. [22] | EGFR-expressing cells | |
| | <1% | 6 (2/35) |
| | 1–9% | 8 (4/51) |
| | ≤9% | 7 (6/89) |
| Berlin et al. [21] | ≥10% | 8 (3/39) |

EGFR expression was measured using immunohistochemical analysis with standardized kit.

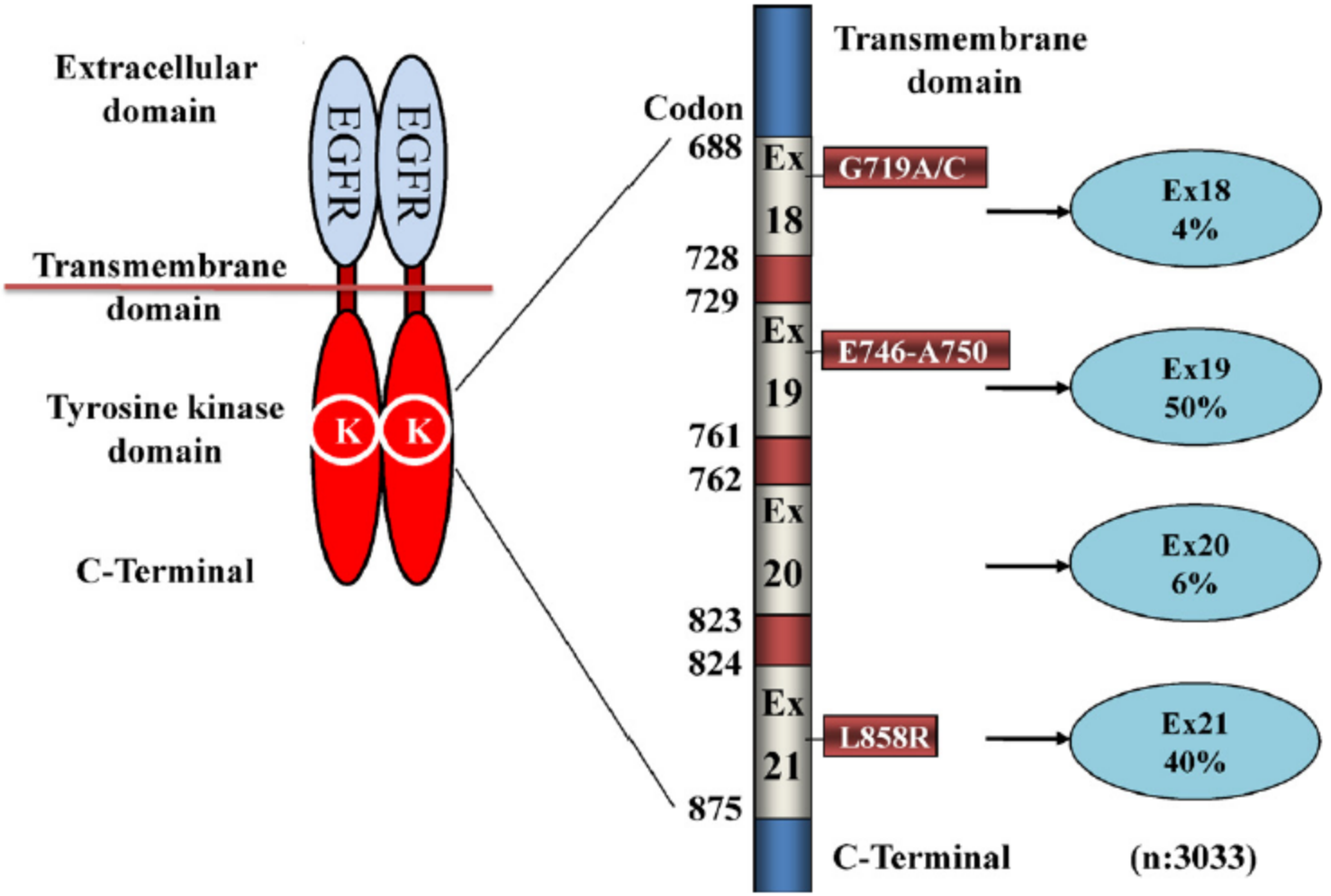



**Fig. 3.** Frequency of *EGFR* mutation in NSCLC ($n = 3033$) [54].

# Consensus for *EGFR* Mutation Testing in Non-small Cell Lung Cancer

## *Results from a European Workshop*

*Robert Pirker, MD,* Felix J. F. Herth, MD, PhD, FCCP,† Keith M. Kerr, MD, FRCPath,‡ Martin Filipits, PhD,* Miquel Taron, PhD,§‖ David Gandara, MD,¶ Fred R. Hirsch, MD,# Dominique Grunenwald, MD,** Helmut Popper, MD,†† Egbert Smit, MD, PhD,‡‡ Manfred Dietel, MD,§§ Antonio Marchetti, MD, PhD,‖‖ Christian Manegold, MD,¶¶ Peter Schirmacher, MD,## Michael Thomas, MD, PhD,† Rafael Rosell, MD, PhD,§‖ Federico Cappuzzo, MD,*** and Rolf Stahel, MD†††; on Behalf of the European EGFR Workshop Group*

**TABLE 1.** Biopsy Techniques

| | 21-g Needle Aspiration | 19-g Needle Aspiration | Transbronchial Biopsy | CT-Guided Needle Biopsy |
|---|---|---|---|---|
| Total no. of cells per biopsy/ aspiration | ≥100 | ≥150 | ≥300 | ≥500 |
| No. of biopsies | 4 | 4 | 4–5 | 2–3 |

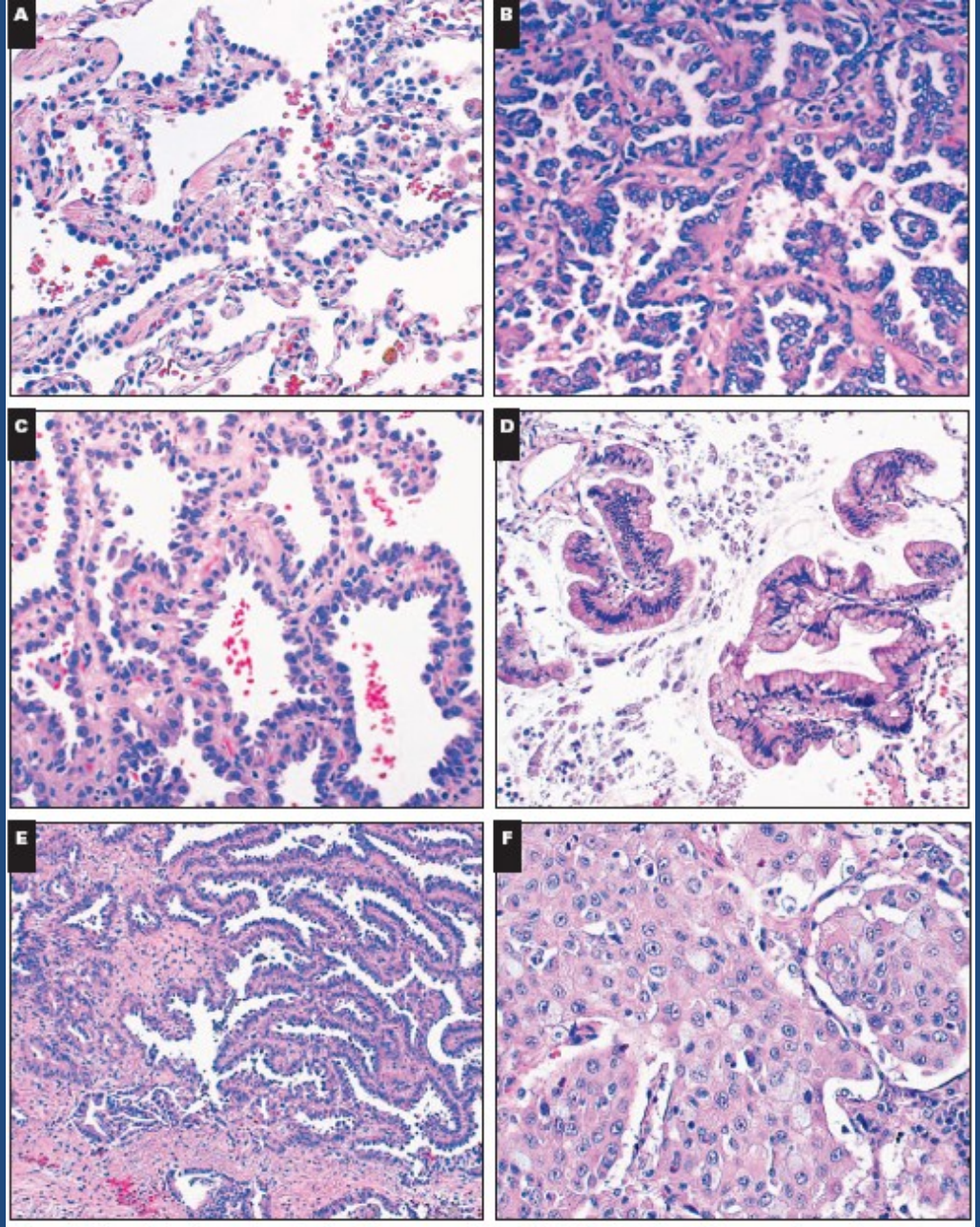



**Image 1** Histopathologic examples of atypical adenomatous hyperplasia (**A**, H&E, ×230), papillary-type adenocarcinoma (**B**, H&E, ×200), nonmucinous-type bronchioloalveolar carcinoma (BAC; **C**, H&E, ×200), mucinous-type BAC (**D**, H&E, ×200), mixed acinar/conventional adenocarcinoma with nonmucinous-type BAC (**E**, H&E, ×100), and adenocarcinoma, solid with mucus type (**F**, H&E, ×200).


DOI: 10.1309/AJCPH0TRMPXVZW2F

**Table 2**

**Statistical Correlations Between Baseline Characteristics of the Entire Series (n = 418) of Lung Tumors and of *EGFR* and K-*ras* Mutations***

| Characteristic | *EGFR* | *P* | K-*ras* | *P* |
|---|---|---|---|---|
| Sex | | <.0001 | | .001 |
| Male (n = 219) | 9 (4.1) | | 67 (30.6) | |
| Female (n = 199) | 42 (21.1) | | 34 (17.1) | |
| Smoking habit | | <.0001 | | <.0001 |
| Current (n = 268) | 4 (1.5) | | 89 (33.2) | |
| Never (n = 118) | 42 (35.6) | | 3 (2.5) | |
| Former (n = 32) | 5 (16) | | 10 (31) | |
| Histotype 1 | | | | |
| Adenocarcinoma (n = 181)† | 33 (18.2) | | 50 (27.6) | |
| nmBAC (n = 10) | 2 (20) | | 2 (20) | |
| mBAC (n = 13) | 0 (0) | | 10 (77) | |
| Mixed adenocarcinoma/BAC (n = 37) | 12 (32) | | 8 (212) | |
| Colloid (n = 9) | 0 (0) | | 4 (44) | |
| Signet-ring cell (n = 9) | 0 (0) | | 4 (44) | |
| Papillary (n = 14) | 2 (14) | | 6 (43) | |
| Solid with mucus (n = 9) | 0 (0) | | 3 (33) | |
| Fetal type (n = 2) | 0 (0) | | 0 (0) | |
| Adenosquamous (n = 4) | 3 (75) | | 0 (0) | |
| Squamous cell (n = 31) | 0 (0) | | 1 (3) | |
| Large cell (n = 6)‡ | 0 (0) | | 3 (50) | |
| Small cell (n = 6) | 0 (0) | | 1 (17) | |
| LCNEC (n = 20) | 0 (0) | | 3 (15) | |
| Typical carcinoid (n = 20) | 0 (0) | | 0 (0) | |
| Atypical carcinoid (n = 5) | 0 (0) | | 0 (0) | |
| Sarcomatoid (n = 13)§ | 0 (0) | | 7 (37) | |
| Cystic blastoma (n = 1) | 0 (0) | | 0 (0) | |
| Mucoepidermoid (n = 5) | 0 (0) | | 0 (0) | |
| Adenoid cystic (n = 3) | 0 (0) | | 0 (0) | |
| Sclerosing hemangioma (n = 14) | 0 (0) | | 0 (0) | |

## Can Cytology Samples Be Used?

Cytology samples may be suitable for analysis but further research is needed to fully understand the clinical reliability of mutational data obtained from these samples. Until then, clinicians should be encouraged to provide tissue biopsy samples whenever possible.

**TABLE 3.** Recommendations for *EGFR* Mutation Testing in NSCLC

| | |
|---|---|
| Which patient? | NSCLC patients[a] |
| Time point | At diagnosis |
| | When possible at disease progression |
| Sample source | Most easily accessible |
| | Biopsy preferred over cytology |
| Fixation | 10% neutral-buffered formalin |
| | Bouin's fluid should not be used |
| Tumor cell content | ≥50% Tumor cells for DNA sequencing |
| | Lower % acceptable with higher sensitivity techniques |
| *EGFR* mutation analysis method | No gold standard yet |
| Report to include | Detail of biopsy sample and tissue extracted |
| | Type of mutation analysis |
| | Mutation present/absent |
| | Interpretation |

[a] Local policy may determine which patients are tested. In European studies, the prevalence of *EGFR* mutation in definitively diagnosed squamous cell carcinoma, neuroendocrine carcinomas, and mucinous bronchioloalveolar-pattern adenocarcinomas is effectively zero.[45] A pragmatic approach could be to exclude from testing those patients with a confident diagnosis of the above tumor types, but to test all those with other NSCLC subtypes, and all "never smokers," regardless of tumor type. In cases in which subtype is unclear, testing is indicated.

NSCLC, non-small cell lung cancer.

Human Pathology (2010) 41, 94–102

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Original contribution

# A comparison of *EGFR* and *KRAS* status in primary lung carcinoma and matched metastases

**Sara E. Monaco MD*, Marina N. Nikiforova MD, Kathleen Cieply BS, Lisa A. Teot MD, Walid E. Khalbuss MD, PhD, Sanja Dacic MD, PhD**

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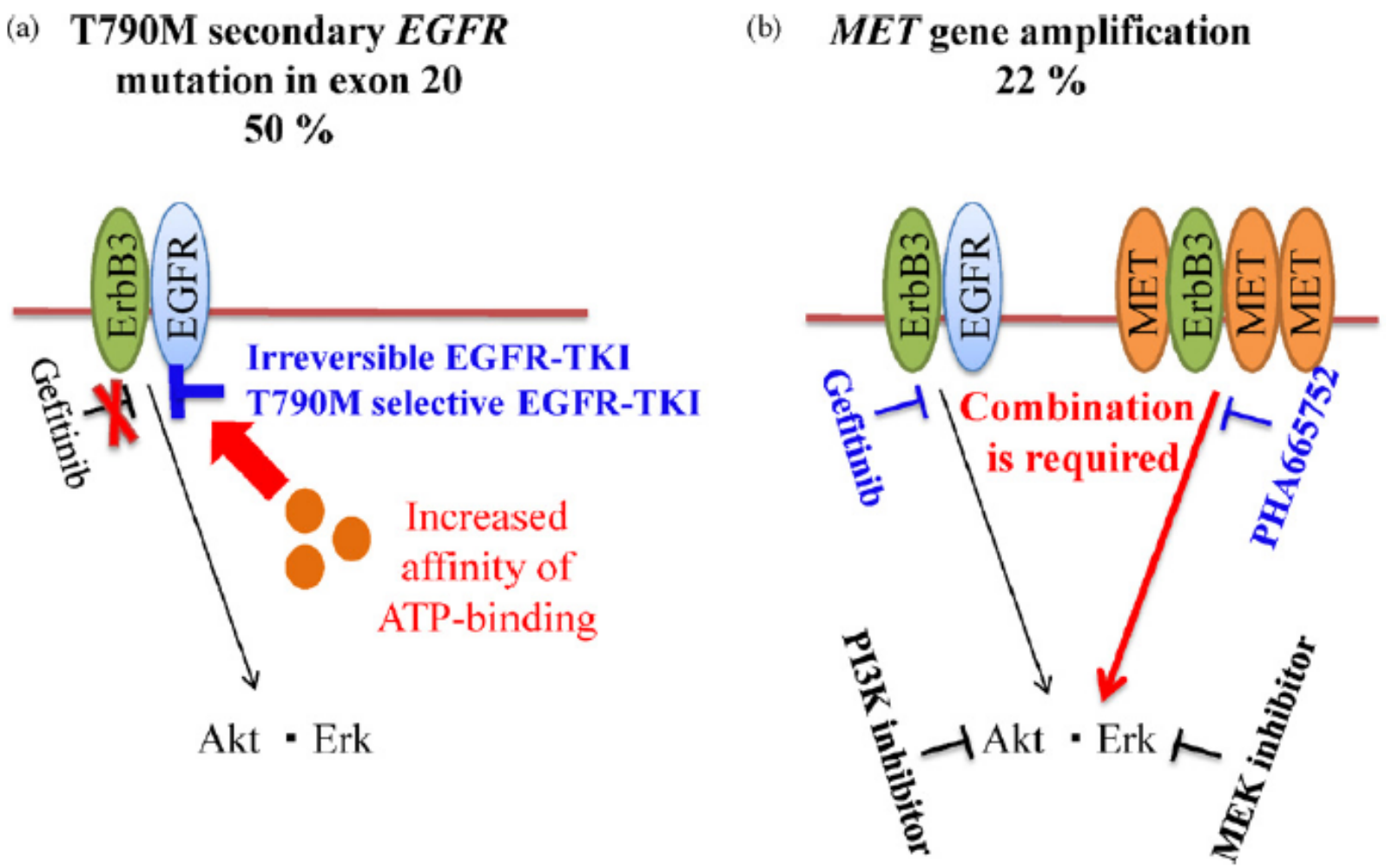
Received 13 May 2009; revised 29 June 2009; accepted 30 June 2009

# The law is the law…..

- What do you want, first line TKI?
- You will be disappointed…..
- First line = gefitinib = mutation analysis.
- The patient progressed under chemo, can I give TKI?? Erlotinib = IHC!


(a) T790M secondary *EGFR* mutation in exon 20 50 %
ErbB3
EGFR
Gefitinib
Irreversible EGFR-TKI
T790M selective EGFR-TKI
Increased affinity of ATP-binding
Akt ▪ Erk
(b) *MET* gene amplification 22 %
ErbB3
EGFR
MET
ErbB3
MET
MET
Gefitinib
Combination is required
PHA665752
PI3K inhibitor
Akt ▪ Erk
MEK inhibitor

Cancer Cell
Article

# Preexistence and Clonal Selection of *MET* Amplification in *EGFR* Mutant NSCLC

Alexa B. Turke,[1,2,10] Kreshnik Zejnullahu,[3,4,10] Yi-Long Wu,[5] Youngchul Song,[1] Dora Dias-Santagata,[1] Eugene Lifshits,[1] Luca Toschi,[3,4] Andrew Rogers,[3,4] Tony Mok,[6] Lecia Sequist,[1] Neal I. Lindeman,[7] Carly Murphy,[7] Sara Akhavanfard,[1] Beow Y. Yeap,[1,2] Yun Xiao,[4,7] Marzia Capelletti,[3,4] A. John Iafrate,[1] Charles Lee,[7] James G. Christensen,[8] Jeffrey A. Engelman,[1,2,11,*] and Pasi A. Jänne[2,3,4,9,11,*]

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JOURNAL OF CLINICAL ONCOLOGY

EDITORIAL

# *EML4-ALK*: Honing In on a New Target in Non–Small-Cell Lung Cancer

Leora Horn and William Pao, *Vanderbilt-Ingram Cancer Center, Nashville, TN*

# Clinical Activity Observed in a Phase 1 Dose-Escalation Trial of an Oral MET and ALK Inhibitor, PF-02341066

EL Kwak[1], DR Camidge[2], J Clark[1], GI Shapiro[3], RG Maki[4], MJ Ratain[5], B Solomon[6], Y-J Bang[7], S-H Ou[8], R Salgia[5]

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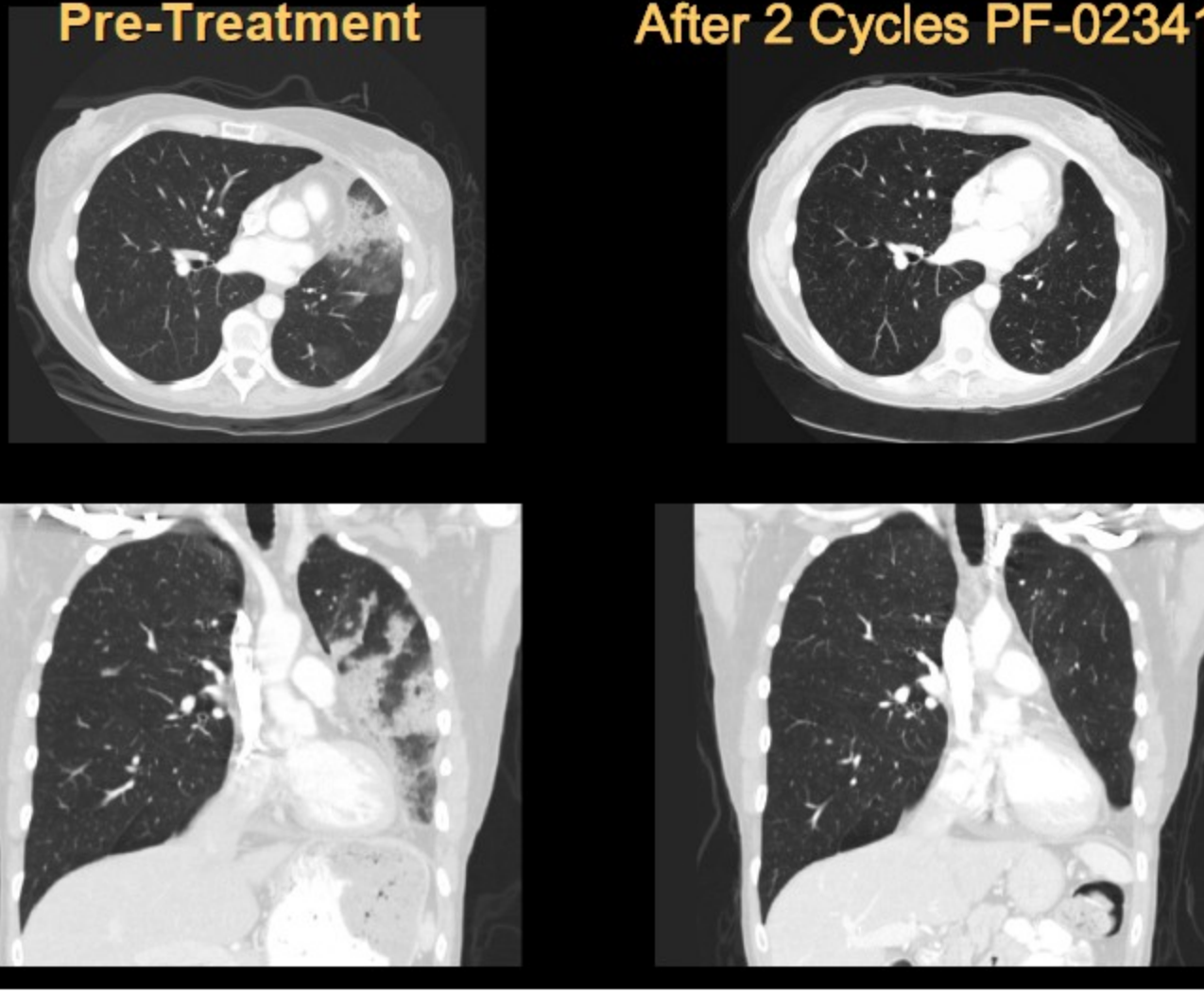
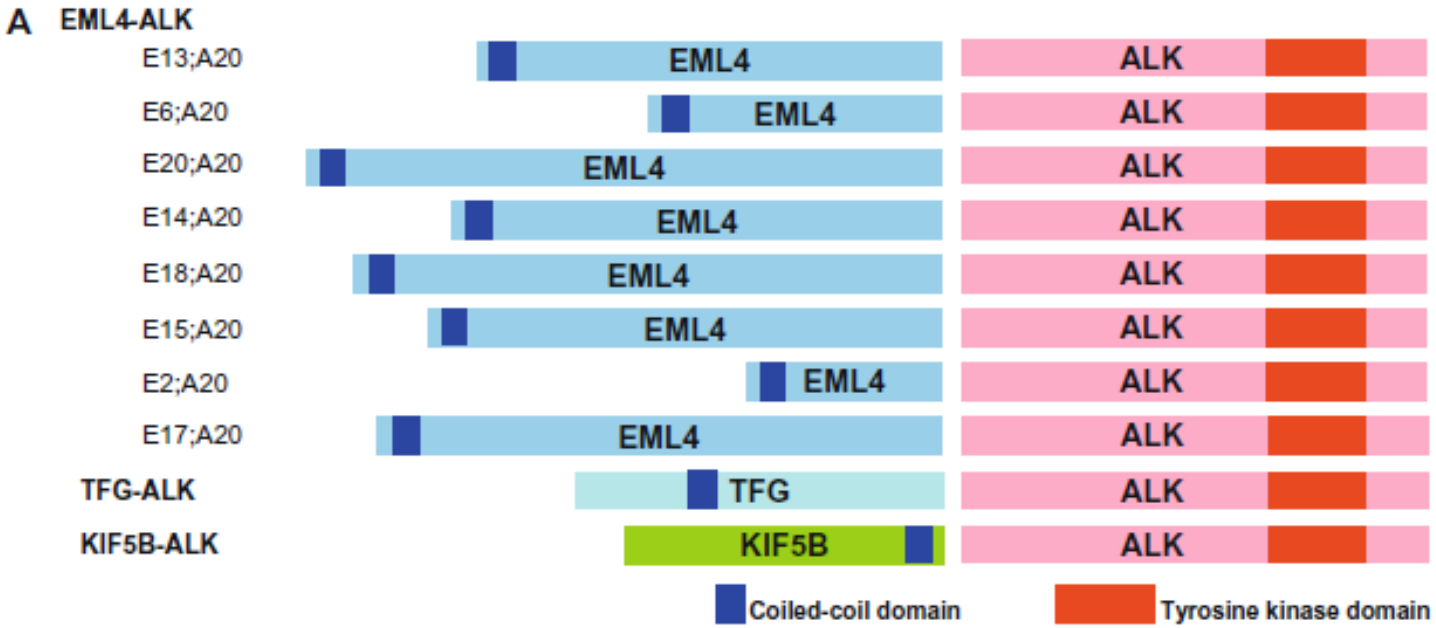
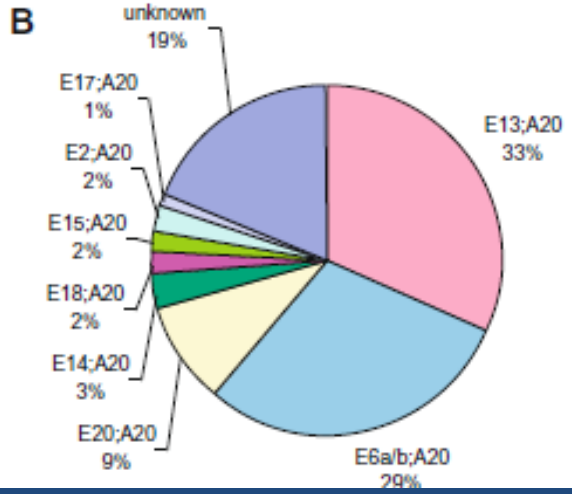
3. Dana-Farber Cancer Institute
4. Memorial Sloan-Kettering Cancer Center
5. University of Chicago Cancer Center
6. Peter MacCallum Cancer Centre
7. Seoul National University
8. University of California at Irvine

# 48 yo Female Non-Smoker with NSCLC ALK Fusion





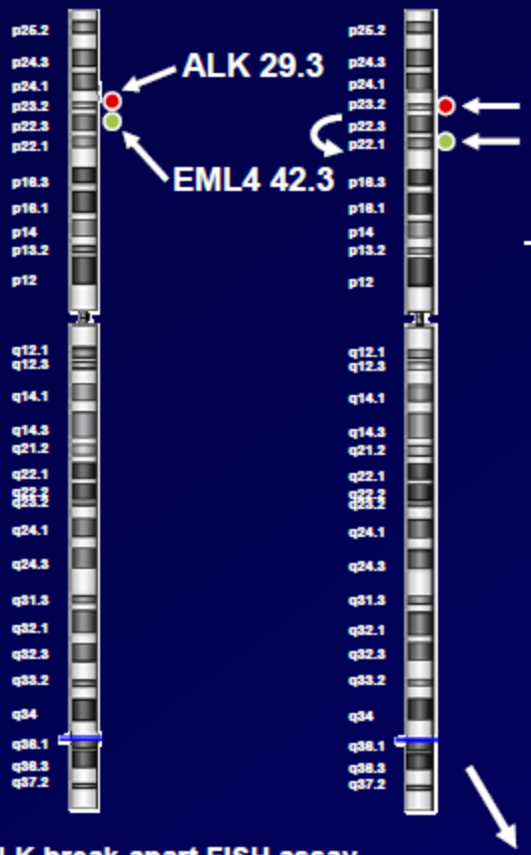

**EML4-ALK Variants**

| | |
|---|---|
| E13;A20 | E13;A20 (variant 1), E13;ins69 A20 |
| E6;A20 | E6a/b;A20 (variant 3a/b) |
| E20;A20 | E20;A20 (variant 2), E20;ins18A20 |
| E14;A20 | E14;ins11del49A20(variant 4'), E14;del12A20 (variant 7) |
| E18;A20 | E18;A20 (variant 5') |
| E15;A20 | E15 del19;del20A20 (variant 4) |
| E2;A20 | E2;A20 &E2;ins117A20 (variant 5a/b) |
| E17;A20 | E17;ins68A20 |

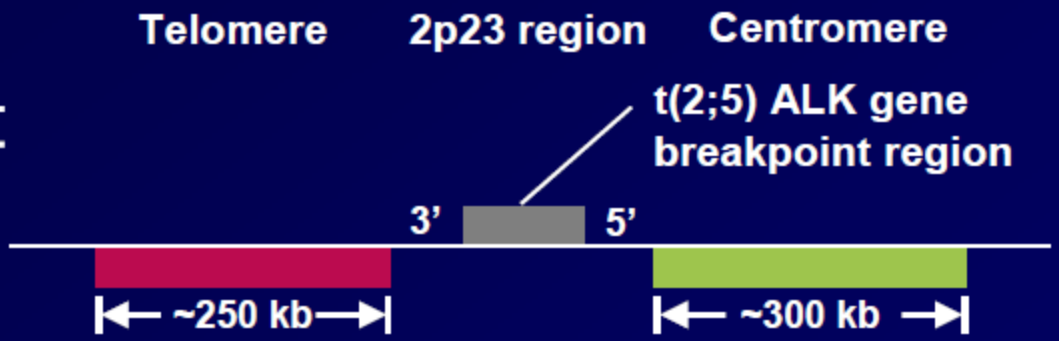
**NSCLC Cell lines**

H3122 and DFCI032 contain E13;A20. H2228 contain E6;A20

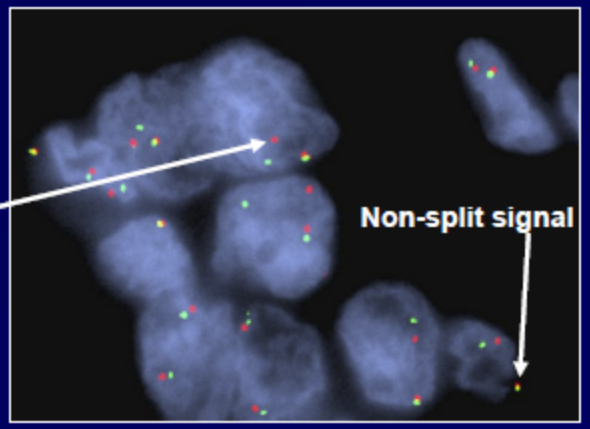
# FISH Assay for *ALK* Rearrangement*




ALK break-apart FISH assay
[Courtesy John Iafrate, Massachusetts General Hospital]




Break-apart FISH assay for *ALK*-fusion genes[1]




*Assay is positive if rearrangements can be detected in ≥15% of cells
FISH = fluorescence in situ hybridization

[1]Shaw AT et al. J Clin Oncol 2009;27:4247–4253

# Summary

- Treatment with crizotinib resulted in impressive clinical activity in patients with *ALK*-positive advanced NSCLC
  - ORR: 57%
  - DCR at 8 weeks: 87%
  - PFS probability at 6 months: 72%
- Crizotinib was well tolerated
  - The most frequent adverse events were mild and moderate gastrointestinal events and mild visual disturbances

# ALK translocation.

- How to test?
- How long does it take?
- Where?
- Where is crizotinib?

**Overview /Results of EGFR mutation testing for Hospitals at HistoGeneX / HistoGeneX 2010 /**

- ZNA Middelheim
- CMP Pathology
- UZ GENT
- AZ Sint Jan Brugge – Oostende
- Institut Jules Bordet
- Universitair Ziekenhuis Antwerpen
- Sint-Elisabethziekenhuis
- AZ Sint Lucas
- AZ St-Jozef
- AZ Jan Palfijn-Gallifort
- AZ Sint-Augustinus
- AZ Sint-Maarten
- AZ Sint Jan Brugge
- Monica Deurne
- AZ KLINA
- AZ Jan PORTAELS
- AZ Gezondheidszorg Oostkust

Average turn around time EGFR testing: 6 days

BELAC ISO15189:2007

HistoGeneX
8 11970 17 233

ISO15189:2007
Reg. Nr: 257-MED

# Histological Gene Expression systems
## DNA EGFR RAPPORT

P341 - EGFR Mut

### Gegevens Patient en Aanvrager

00000292

| | |
|---|---|
| Naam Patient | |
| Geboortedatum | |
| Mutualiteits Gegevens | |
| Geslacht | |

| | |
|---|---|
| Aanvrager Naam | |
| Hospitaal | |
| Rizivnummer | |
| Straat | nr. |
| PostCode | |
| Plaats | |
| Fax# | |

| | |
|---|---|
| Staalnummer | |
| Bloknummer | |
| Weefsel | |
| Specimen Type | ☐ Paraffineblok ☐ Blanco coupes |
| Additioneel Materiaal | ☐ HE coupe ☐ IHC coupe |
| Datum Staalname | |
| Fixatief | |
| Fixatie Tijd | |

### TUMOR INFO

| | |
|---|---|
| Tumor Type | Adenocarcinoma |
| Biopsie Type | Resection (>5mm) |
| Biopsie Kwaliteit | Suitable for Analysis |

### EGFR ARMS MUTATIE-ANALYSE

| | | |
|---|---|---|
| Reden ontbreken EGFR-analyse | | |
| Exon 19 deletie | Positive | * |
| L858R | Negative | EGFR c.2573T>G |
| L861Q | Negative | EGFR c.2582T>A |
| G719X | Negative | G719S or EGFR c.2156G>A; G719A or EGFR c.2156G>C; G719C or EGFR c.2156G>T |
| S768I | Negative | EGFR c.2303G>T |
| Exon 20 insertie | Negative | EGFR c.2307_2308ins9, c.2307_2308ins9, c.2310_2311insGGT |
| Globaal EGFR Mutatie Resultaat | **Positive** | |
| Commentaar | | |

Datum: _____ Naam Patholoog: _____ Report Version: 0

* EGFR c.2235_2249del15, c.2236_2252>AAT (complex), c.2236_2253del18, c.2237_2251del15, c.2237_2254del18, c.2237_2255>T (complex), c.2236_2259del15, c.2238_2255del18, EGFR c.2238_2248>GC (complex), c.2238_2252>GCA (complex), c.2239_2247del9, c.2239_2253del15, c.2239_2256del18, c.2239_2248TTAAGAGAAG>C (complex), c.2239_2256>CA (complex), c.2240_2251del12, c.2240_2257del18, c.2240_2254del15, c.2239_2251>C (complex)

*Gebruikte test: EG-03 and EG-04: DxS EGFR Mutation Test Kit for the detection of 29 mutations in the Epidermal Growth Factor Receptor (EGFR) gene.*

*De test is in staat de 28 meest voorkomende EGFR mutaties in exon 18 en exon 21 te detecteren. De aanwezigheid van de mutaties wordt gedetecteerd met een analytische specificiteit van 95% met een detectielimiet van 1% in een achtergrond van wild-type genomisch DNA. Een negatief test-resultaat kan de afwezigheid van een EGFR mutatie of ziekte niet met zekerheid uitsluiten. Wanneer de hoeveelheid amplificeerbaar DNA beperkt is, kan een beperkte aanwezigheid van bepaalde mutaties ongedetecteerd blijven.*

HistoGeneX N.V.
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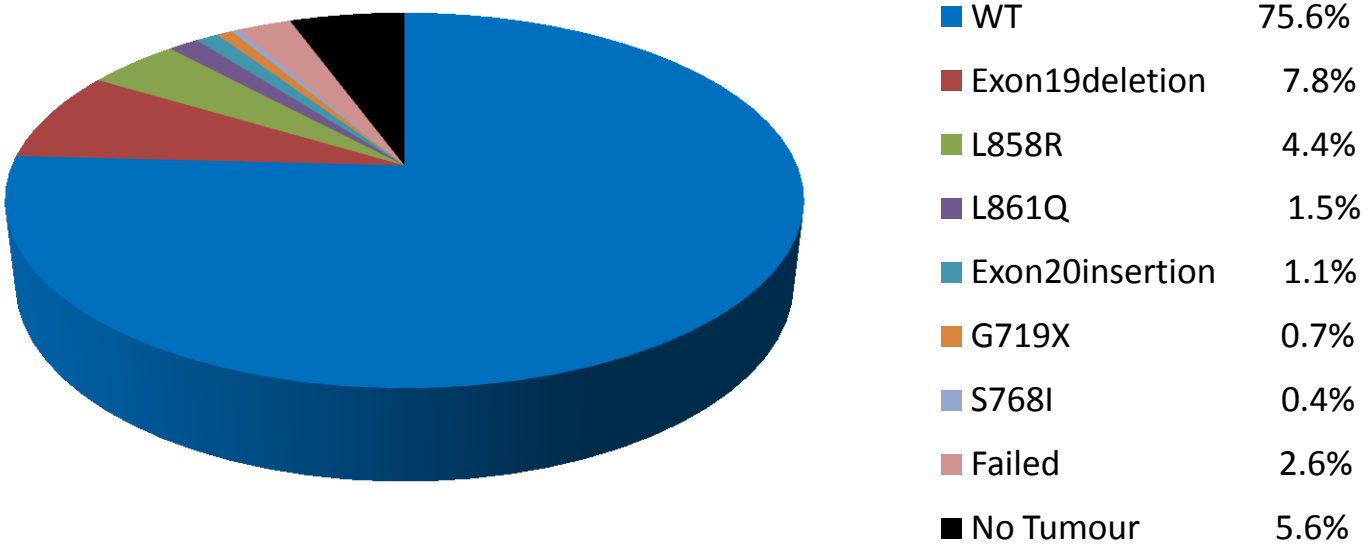
B-2600 Berchem

Tel.: +32-(0)3-2181080/82
Fax: +32-(0)3-2804861
www.histogenex.com

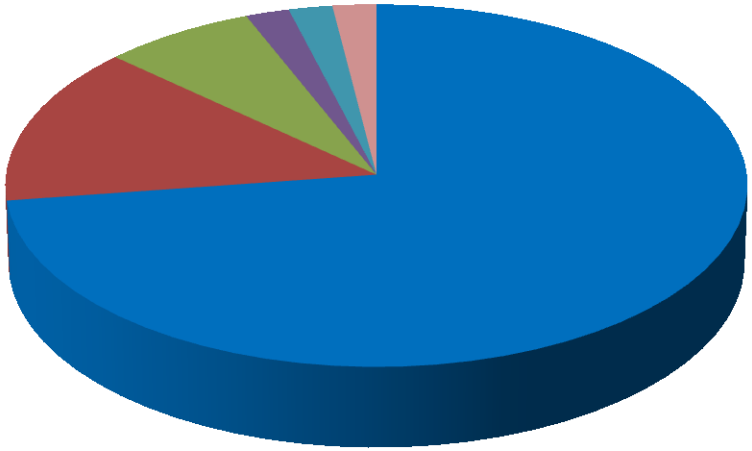
Bank J Van Breda & Co
IBAN BE58 0451 0771 3270
BTW nr: BE 0474654355

# Overview /Results of EGFR mutation testing at HistoGeneX
# HistoGeneX 2010

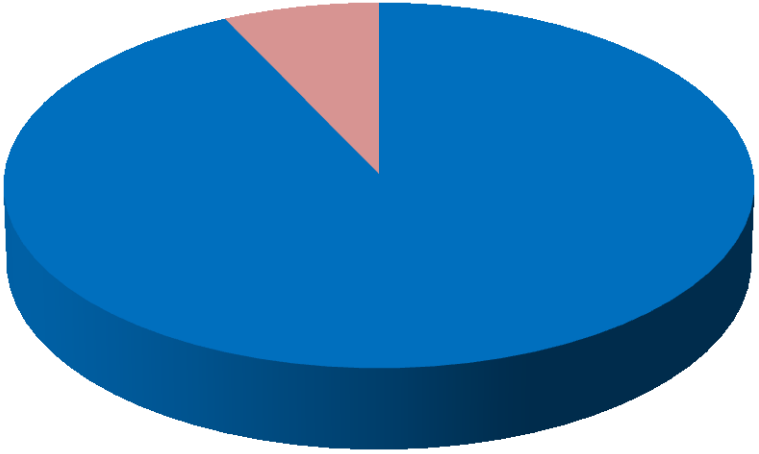
Total amount of samples received
from 12 May 2009 until now:
n=270



# Overview /Results of EGFR mutation testing at HistoGeneX
# HistoGeneX 2010



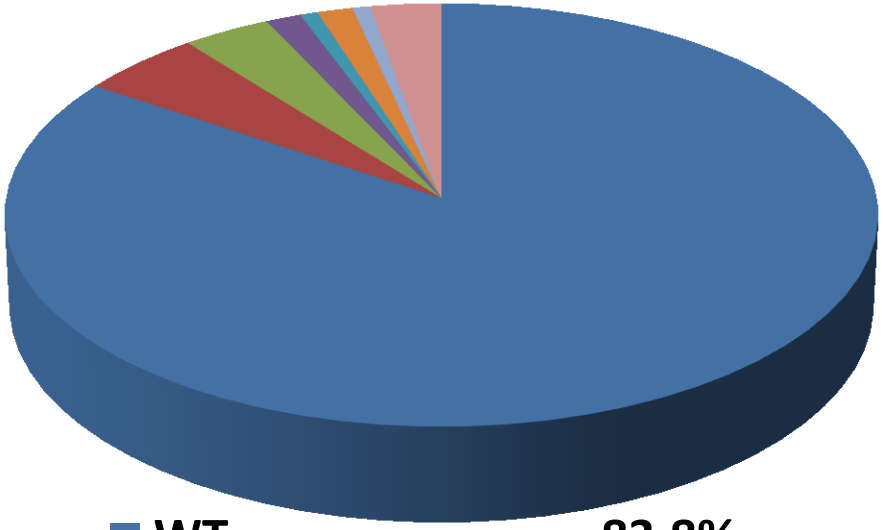
| | |
|---|---|
| WT | 72.7% |
| Exon19deletion | 14.1% |
| L858R | 7.1% |
| L861Q | 2.0% |
| Exon20insertion | 2.0% |
| G719X | 0% |
| S768I | 0% |
| Failed | 2.0% |



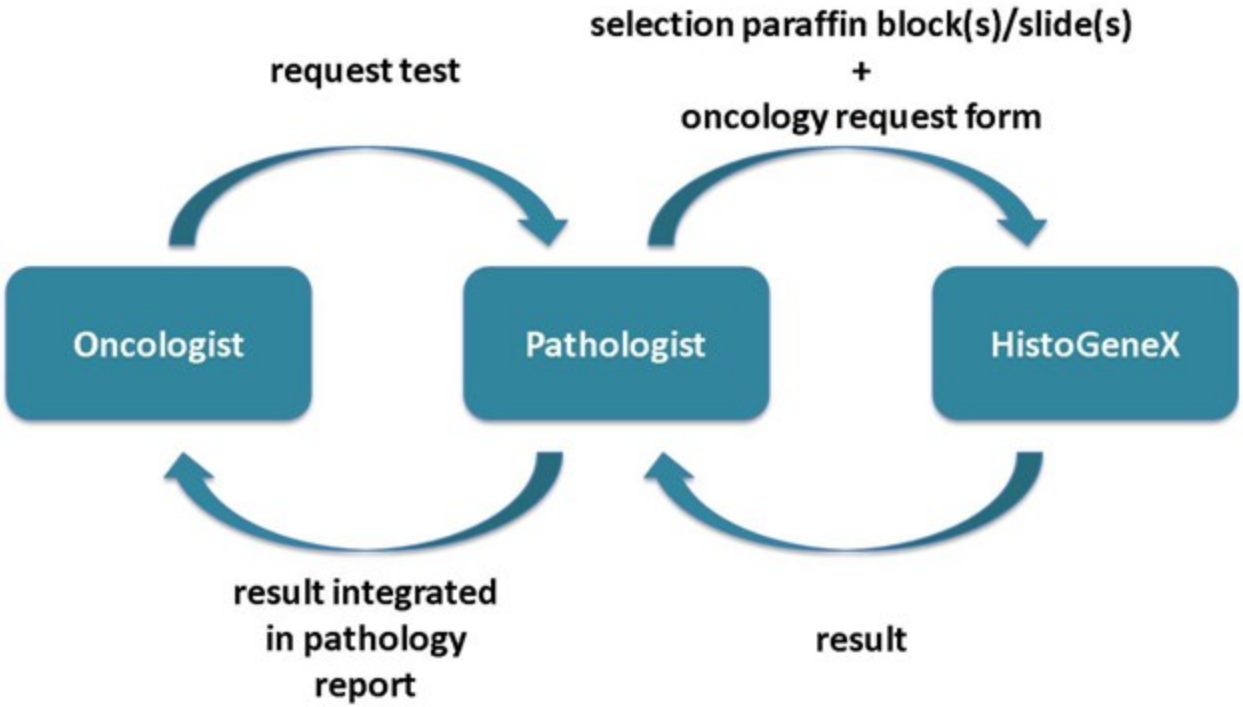
| | |
|---|---|
| WT | 92.9% |
| Failed | 7.1% |

**Overview /Results of EGFR mutation testing at HistoGeneX**
**HistoGeneX 2010**

NSCLC / Not specified
n=142 (53%)



| | |
|---|---|
| **WT** | **83.8%** |
| **Exon19deletion** | **4.9%** |
| **L858R** | **3.5%** |
| **L861Q** | **1.4%** |
| **Exon20insertion** | **0.7%** |
| **G719X** | **1.4%** |
| **S768I** | **0.7%** |
| **Failed** | **2.8%** |

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