Molecular testing in lung cancer.

P. Pauwels (UZA/UZG)
M. Kockx (ZNA/ HistoGeneX)
EXTRACELLULAR SIGNAL MOLECULE

RECEPTOR PROTEIN

plasma membrane of target cell

INTRACELLULAR SIGNALING PROTEINS

metabolic enzyme

gene regulatory protein

cytoskeletal protein

EFFECTOR PROTEINS

altered metabolism

altered gene expression

altered cell shape or movement

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

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

Pao & Miller JCO 2005;23:2556-2568
<table>
<thead>
<tr>
<th>Anti-EGFR agent/reference</th>
<th>EGFR expression</th>
<th>Response rate % (no./total no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cetuximab</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cunningham et al. [4] (BOND study)</td>
<td>EGFR-expressing cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≤10%</td>
<td>7 (4/56)</td>
</tr>
<tr>
<td></td>
<td>&lt;10 to ≤20%</td>
<td>31 (5/16)</td>
</tr>
<tr>
<td></td>
<td>&gt;20 to ≤35%</td>
<td>0 (0/7)</td>
</tr>
<tr>
<td></td>
<td>&gt;35%</td>
<td>9 (3/32)</td>
</tr>
<tr>
<td></td>
<td>EGFR staining intensity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Faint</td>
<td>5 (1/21)</td>
</tr>
<tr>
<td></td>
<td>Weak or moderate</td>
<td>13 (7/55)</td>
</tr>
<tr>
<td></td>
<td>Strong</td>
<td>12 (4/34)</td>
</tr>
<tr>
<td><strong>Saltz et al. [20]</strong></td>
<td>EGFR status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1+</td>
<td>6 (1/17)</td>
</tr>
<tr>
<td></td>
<td>2+</td>
<td>13 (4/30)</td>
</tr>
<tr>
<td></td>
<td>3+</td>
<td>0 (0/10)</td>
</tr>
<tr>
<td><strong>Panitumumab</strong></td>
<td>EGFR-expressing cells</td>
<td></td>
</tr>
<tr>
<td>Hecht et al. [22]</td>
<td>≤1%</td>
<td>6 (2/35)</td>
</tr>
<tr>
<td></td>
<td>1–9%</td>
<td>8 (4/51)</td>
</tr>
<tr>
<td></td>
<td>≤9%</td>
<td>7 (6/89)</td>
</tr>
<tr>
<td>Berlin et al. [21]</td>
<td>≥10%</td>
<td>8 (3/39)</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th></th>
<th>21-g Needle Aspiration</th>
<th>19-g Needle Aspiration</th>
<th>Transbronchial Biopsy</th>
<th>CT-Guided Needle Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of cells per biopsy/aspiration</td>
<td>≥100</td>
<td>≥150</td>
<td>≥300</td>
<td>≥500</td>
</tr>
<tr>
<td>No. of biopsies</td>
<td>4</td>
<td>4</td>
<td>4–5</td>
<td>2–3</td>
</tr>
</tbody>
</table>
Image 18: 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).
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>EGFR</th>
<th>P</th>
<th>K-ras</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n = 219)</td>
<td>9 (4.1)</td>
<td>&lt;.0001</td>
<td>67 (30.6)</td>
<td>.001</td>
</tr>
<tr>
<td>Female (n = 199)</td>
<td>42 (21.1)</td>
<td></td>
<td>34 (17.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking habit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current (n = 268)</td>
<td>4 (1.5)</td>
<td>&lt;.0001</td>
<td>89 (33.2)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Never (n = 118)</td>
<td>42 (35.6)</td>
<td></td>
<td>3 (2.5)</td>
<td></td>
</tr>
<tr>
<td>Former (n = 32)</td>
<td>5 (16)</td>
<td></td>
<td>10 (31)</td>
<td></td>
</tr>
<tr>
<td><strong>Histotype 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma (n = 181)†</td>
<td>33 (18.2)</td>
<td></td>
<td>50 (27.6)</td>
<td></td>
</tr>
<tr>
<td>nmBAC (n = 10)</td>
<td>2 (20)</td>
<td></td>
<td>2 (20)</td>
<td></td>
</tr>
<tr>
<td>mBAC (n = 13)</td>
<td>0 (0)</td>
<td></td>
<td>10 (77)</td>
<td></td>
</tr>
<tr>
<td>Mixed adenocarcinoma/BAC (n = 37)</td>
<td>12 (32)</td>
<td></td>
<td>8 (212)</td>
<td></td>
</tr>
<tr>
<td>Colloid (n = 9)</td>
<td>0 (0)</td>
<td></td>
<td>4 (44)</td>
<td></td>
</tr>
<tr>
<td>Signet-ring cell (n = 9)</td>
<td>0 (0)</td>
<td></td>
<td>4 (44)</td>
<td></td>
</tr>
<tr>
<td>Papillary (n = 14)</td>
<td>2 (14)</td>
<td></td>
<td>6 (43)</td>
<td></td>
</tr>
<tr>
<td>Solid with mucus (n = 9)</td>
<td>0 (0)</td>
<td></td>
<td>3 (33)</td>
<td></td>
</tr>
<tr>
<td>Fetal type (n = 2)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Adenosquamous (n = 4)</td>
<td>3 (75)</td>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Squamous cell (n = 31)</td>
<td>0 (0)</td>
<td></td>
<td>1 (3)</td>
<td></td>
</tr>
<tr>
<td>Large cell (n = 6)†</td>
<td>0 (0)</td>
<td></td>
<td>3 (50)</td>
<td></td>
</tr>
<tr>
<td>Small cell (n = 6)</td>
<td>0 (0)</td>
<td></td>
<td>1 (17)</td>
<td></td>
</tr>
<tr>
<td>LCNEC (n = 20)</td>
<td>0 (0)</td>
<td></td>
<td>3 (15)</td>
<td></td>
</tr>
<tr>
<td>Typical carcinoid (n = 20)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Atypical carcinoid (n = 5)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Sarcomatoid (n = 13)†</td>
<td>0 (0)</td>
<td></td>
<td>7 (37)</td>
<td></td>
</tr>
<tr>
<td>Cystic blastoma (n = 1)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Mucoepidermoid (n = 5)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Adenoid cystic (n = 3)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Sclerosing hemangioma (n = 14)</td>
<td>0 (0)</td>
<td></td>
<td>0 (0)</td>
<td></td>
</tr>
</tbody>
</table>
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>
<thead>
<tr>
<th>Table 3. Recommendations for <em>EGFR</em> Mutation Testing in NSCLC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Which patient?</strong></td>
</tr>
<tr>
<td><strong>Time point</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Sample source</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Fixation</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Tumor cell content</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>EGFR mutation analysis method</strong></td>
</tr>
<tr>
<td><strong>Report to include</strong></td>
</tr>
<tr>
<td></td>
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<td></td>
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<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> 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. 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.
A comparison of \textit{EGFR} and \textit{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

Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA 15232, USA

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%

(b) MET gene amplification
22%

Irreversible EGFR-TKI
T790M selective EGFR-TKI

Increased affinity of ATP-binding

Akt • Erk

Combination is required

Akt • Erk

MEK inhibitor

PI3K inhibitor

PHA665752

Gefitinib
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,3,7 Marzia Capelletti,3,4 A. John Iafrate,1 Charles Lee,7 James G. Christensen,8 Jeffrey A. Engelman,1,2,11,* and Pasi A. Jänne5,7,11,*

1Massachusetts General Hospital Cancer Center, Boston, MA 02129, USA
2Department of Medicine, Harvard Medical School, Boston, MA 02115, USA
3Lowe Center for Thoracic Oncology, Boston, MA 02115, USA
4Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA 02115, USA
5Guangdong Lung Cancer Institute and Cancer Center, Guangdong General Hospital, Guangzhou, China
6The Chinese University of Hong Kong, Hong Kong, China
7Department of Pathology, Brigham and Women’s Hospital, Boston, MA 02115, USA
8Pfizer Global Research and Development, Department of Research Pharmacology, La Jolla Laboratories, La Jolla, CA 92121, USA
9Department of Medicine, Brigham and Women’s Hospital, Boston, MA 02115, USA
10These authors contributed equally to this work
11These laboratories contributed equally to this work
*Correspondence: jengelman@partners.org (J.A.E.), pjanne@partners.org (P.A.J.)
DOI 10.1016/j.ccr.2009.11.022
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 Kwak¹, DR Camidge², J Clark¹, GI Shapiro³, RG Maki⁴, MJ Ratain⁵, B Solomon⁶, Y-J Bang⁷, S-H Ou⁸, R Salgia⁵

1. Massachusetts General Hospital
2. University of Colorado Cancer Center
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
FISH Assay for ALK Rearrangement*

*Assay is positive if rearrangements can be detected in ≥15% of cells

FISH = fluorescence in situ hybridization

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
**Histological Gene Expression systems**
**DNA EGFR RAPPORT**

**P341 - EGFR Mut**

**Gegevens Patient en Aanvrager**

- Naam Patient: 
- Geboortedatum: 
- Mutualiteit: 
- Gegevens: 
- Gestacht: 

**Staatsnummer**

- Weerstand:
- Specimen Type:
  - Paraaffineblok
  - Blanco coupes

**Aanvrager Naam**

- Hospital:
- Rizinummer:
- Straat:
- Post Code:
- Floors:
- Fax:

**TUMOR INFO**

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

**EGFR ARMS MUTATIE-ANALYSE**

- Reden ontbreken EGFR-analyse:

**Exon 19 delen**

- Positive

**LS5R**

- Negative

**L858R**

- Negative

**G719X**

- Negative

**S768I**

- Negative

**Exon 20 Insertie**

- Negative

**Global EGFR Mutatie Resultaat**

- Positive

**Commentaar**

**Datum**

**Naam Patholoog:**

**Report Visible: 0**

---

**Gebuurtstest:** EG-69 en EG-94: DNA EGFR Mutation Test Kit voor de detekstie van 28 mutaties in de Epidemal 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 deze mutaties wordt geassocieerd met een prognostische vermindering van 25% en een doelstelling van 15% in een achtergrond van niet-

**Genealogie test-resultaten:** kan de aanwezigheid van een EGFR mutatie of ziekte niet met zekerheid uitsluiten. Wanneer de hoeveelheid amfibiel DNA behoren is, kan de bepaalde aanwezigheid van bepaalde mutaties ongedeeld blijven.
Overview /Results of EGFR mutation testing at HistoGeneX
HistoGeneX 2010

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

- WT: 75.6%
- Exon19deletion: 7.8%
- L858R: 4.4%
- L861Q: 1.5%
- Exon20insertion: 1.1%
- G719X: 0.7%
- S768I: 0.4%
- Failed: 2.6%
- No Tumour: 5.6%
Overview /Results of EGFR mutation testing at HistoGeneX
HistoGeneX 2010

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>72.7%</td>
</tr>
<tr>
<td>Exon19 deletion</td>
<td>14.1%</td>
</tr>
<tr>
<td>L858R</td>
<td>7.1%</td>
</tr>
<tr>
<td>L861Q</td>
<td>2.0%</td>
</tr>
<tr>
<td>Exon20 insertion</td>
<td>2.0%</td>
</tr>
<tr>
<td>G719X</td>
<td>0%</td>
</tr>
<tr>
<td>S768I</td>
<td>0%</td>
</tr>
<tr>
<td>Failed</td>
<td>2.0%</td>
</tr>
</tbody>
</table>

| 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%