**EGFR and lung cancer**

Epidermal growth factor (EGF) pathway inhibition is now established as an option for the first-, second- and third-line treatments of NSCLC.

TKI have received global approval for treatment in the second- and third-line settings.

Only a subset of patients with advanced NSCLC treated with TKI experience dramatic benefits.

**Biomarkers predicting response to TKI inhibitors**

- Life-long never smoker
- Asian people
- Women
- Adenocarcinoma histology
- Activating mutations in the EGFR TK domain

Paez JG et al Science 2004, 304:1497

- Histology
- EGFR-IHC
- EGFR-FISH
- EGFR mutational status
- K-Ras mutational status

**Incidence trend of lung cancers by histotypes. Ticino, 1996-2009, both genders**

**Clinicopathological predictors of EGFR/KRAS mutational status in primary lung adenocarcinomas**
Modern Pathology 2010, 23:159-168

- History of never-smoking
- Mild lymphocytic response
- Female gender
- Absence of solid growth pattern
- Older age
- History of smoking
- Mucinous differentiation
EGFR Mutation specific antibodies
Clin Cancer Res 2009;15(9) May 1, 2009

Conclusions: This simple assay for detection of EGFR mutations in diagnostic human tissues provides a rapid, sensitive, specific, and cost-effective method to identify lung cancer patients responsive to EGFR-based therapies.

SATURN study

<table>
<thead>
<tr>
<th>% of evaluable samples</th>
<th>HR (95% CI)</th>
<th>Interaction</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR IHC+</td>
<td>84</td>
<td>0.69 (0.58– 0.82)</td>
<td>0.63</td>
</tr>
<tr>
<td>EGFR IHC–</td>
<td>16</td>
<td>0.77 (0.51–1.14)</td>
<td></td>
</tr>
<tr>
<td>EGFR mutation+</td>
<td>11</td>
<td>0.10 (0.04– 0.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>EGFR wild-type</td>
<td>89</td>
<td>0.78 (0.63–0.96)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

a. ≥10% tumour cells with any membranous staining
b. Mutation in codon 12, 13 and/or 61

EGFR FISH pattern

EGFR FISH evaluation

EGFR gene deregulation by FISH in NSCLC

FISH interpretation criteria in NSCLC

**EGFR FISH in NSCLC treated with TKIs**

<table>
<thead>
<tr>
<th>Ref</th>
<th>g(l)</th>
<th>TKI</th>
<th>EGFR status</th>
<th>n</th>
<th>FISH+</th>
<th>FISH-</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cappuzzo et al, J Natl Cancer Inst 2008</td>
<td>102</td>
<td>544</td>
<td>38%</td>
<td>2%</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Watanabe et al, JCC 2006</td>
<td>230</td>
<td>636</td>
<td>15%</td>
<td>3%</td>
<td>0.09</td>
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<tr>
<td>Cappuzzo et al, JCC 2007</td>
<td>36</td>
<td>564</td>
<td>10%</td>
<td>9%</td>
<td>0.09</td>
<td></td>
<td></td>
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<tr>
<td>Roa et al, JCC 2006</td>
<td>129</td>
<td>564</td>
<td>47%</td>
<td>9%</td>
<td>0.08</td>
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<tr>
<td>Varella-Garcia et al, J Natl Cancer Inst 2009</td>
<td>54</td>
<td>593</td>
<td>50%</td>
<td>11%</td>
<td>0.09</td>
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<tr>
<td>Izumi et al, J Clin Oncol 2009</td>
<td>44</td>
<td>546</td>
<td>50%</td>
<td>22%</td>
<td>0.09</td>
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<td></td>
</tr>
<tr>
<td>Greco et al, J Clin Oncol 2009</td>
<td>27</td>
<td>546</td>
<td>50%</td>
<td>1%</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Increase EGFR gene copy number predicts response to TKIs
- Less predictive power in Japanese patients

**EGFR gene copy number and mutation analyses**

- **EGFR gene copy number**
  - FISH+ status (UCCC criteria<sup>1,2</sup>)
    1. ≥4 EGFR copies (≥40% of nuclei)
    2. EGFR / CEP7 ratio ≥2
    3. Small / large EGFR cluster (≥10% of nuclei)
    4. ≥15 EGFR copies (≥10% of nuclei)

- **Gene amplification**
- **High polysomy**

- **EGFR mutations**
  - DNA extraction, PCR and sequencing

**Predefined EGFR FISH scoring criteria**

- EGFR IHC, EGFR FISH, KRAS mutations, EGFR mutations, EGFR intron 1 CA-repeat polymorphisms

**Correlation between EGFR mutation status and EGFR gene copy number alterations**

- Presence of EGFR mutations correlated with high percentage of cells having ≥4 EGFR gene copies

**EGFR mutations and relation to other biomarkers**

- Imbalance of EGFR mutation+ cases may influence other biomarker analyses
- EGFR mutation higher in rate, but not restricted to EGFR FISH+
- Lower EGFR mutation rate in KRAS mutation+ or squamous histology

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Denis Soulères, Tudor Ciuleanu, Lila Steimak, Renaud Whitlom, Paul Delmar, Kerstin Rohr, Wolfram Brugger, Federico Cappuzzo, on behalf of the SATURN investigators

PFS according to EGFR FISH (UCCC amplification criterion) and EGFR mutations

When restricted to EGFR wild-type, little difference between PFS benefit in FISH+ vs FISH− suggests that EGFR mutation+ drives greater benefit observed in FISH+ group (quantitative interaction)

Conclusions (I)

- Significant association between EGFR mutations and EGFR gene amplification
- Benefit from erlotinib therapy observed in all patients but greatest in EGFR mutation+ (quantitative interaction)
- Greater PFS benefit in EGFR FISH+ may be due to higher proportion of EGFR mutations in this group
- Multi-variate analyses essential for biomarker investigations (both for PFS and OS)


- 77/386 (20%) cases were FISH+ according to UC scoring criteria
- KRAS+ in 23% of FISH+ cases, and in 32% of FISH− cases (not significant)
- Higher EGFR/CEP7 ratio in EGFR mutated tumor compared to KRAS mutated
- EGFR/CEP7>5 suggest EGFR mutation
- EGFR/CEP7 2 to 5 suggests KRAS mutation

EML4-ALK: new target in NSCLC?

- Fusions between echinoderm microtubule-associated protein-like 4 (EML4) and anaplastic lymphoma kinase (ALK)
- 3%-7% of lung tumors harbor EML4-ALK fusions (??)
- Adenocarcinomas with acinar histology
- Significant relation between EML4-ALK fusions and smoking (more frequent in never smoking or light smoker)
- Lack of EGFR or KRAS mutations
- Younger age
- Resistant to anti-EGFR therapies (TKI-inhibitors)
- ALK alterations may be clinically responsive to pharmacologic ALK inhibition
- Detection by IHC, FISH, or RT-PCR
- Role of EML4-ALK in solid tumors remains unclear!

Wong DW et al, Cancer 2009; Martelli MP, Am J Pathol 2009; Serfalacci W Hum Pathol 2010;Boland JM, Hum Pathol 2009; Mano H Am J Pathol 2010

Suggested algorithm for molecular testing for patients with NSCLC (adenocarcinomas) before target therapy

Horn L, Pao W. JCO 2009, 27:4232
Conclusions (II)

- EGFR mutational status is a better predictive marker for EGFR TKI therapies than EGFR FISH analysis
- EGFR FISH may be a valid alternative to molecular studies in biopsies with limited neoplastic tissue
- Genomic analysis of tumor biopsies will gain significance for genotype-guided treatment of NSCLC