Evaluation of Epidermal Growth Factor Receptor by FISH on Cytologic Vs Histologic Specimens of Non-small Cell Lung Cancers

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Introduction

- Lung Cancer is one of the most common cause of cancer deaths
- NSCLC accounts for ~ 85% of all lung cancers
- Targeted therapy against EGFR following relapse after platinum based chemotherapy is indicated for patients with advanced NSCLC
EGFR Signaling Pathway

Ligand binding to EGFR induces receptor dimerization & activation of the TK activity
EGFR mutations and increased copy number

- May predict response to EGFR tyrosine kinase inhibitors (EGFR-TKI) in NSCLC

- Mutations are detected by PCR but increased copy number is detected by FISH

- There is a correlation between mutations and increased gene copy number
FISH Testing

- Currently mutation status and FISH testing in NSCLC typically relies on the use of histologic specimens.

- In advanced stage, excisional biopsies may be difficult to obtain.

- Pathological diagnosis may be made solely on the basis of cytology procured from FNA and pleural fluids.

- Hence, it is important to evaluate the accuracy of gene copy number by FISH on cytologic specimens for guidance in therapy.
Goal

- To correlate the EGFR FISH pattern of histologic specimens with the cytologic specimens from the same site
- To validate that FISH results performed on cytology specimens may substitute for FISH performed on histologic specimens
### Materials and Methods

#### 34 Cases Evaluated

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 Adenocarcinomas</td>
<td>18 Stage I</td>
</tr>
<tr>
<td>15 Squamous Cell Carcinoma</td>
<td>9 Stage II</td>
</tr>
<tr>
<td>3 NSCLC</td>
<td>4 Stage III</td>
</tr>
<tr>
<td></td>
<td>3 Stage IV</td>
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</table>

- Touch imprints with corresponding histologic sections from same site
- None of the patients received any therapy prior to surgery
Materials and Methods

- The LSI EGFR 7p12 Spectrum Orange/CEP 7 Spectrum Green dual color probe set (Abbott Laboratories, USA) was used.
- FISH was performed on imprints and histologic sections.
- A minimum of 50 cancer cells were scored.
- FISH scores were correlated with histology and stage of diagnosis by Pearson correlation.
Scoring of Signals

FISH results were defined according to the Cappuzzo* et al’s criteria as follows:

**FISH-positive:** High-polysomy (≥ 4 EGFR/Cep 7 gene copies in ≥40% cells) or Amplification tight EGFR clusters ≥ 15 copies & EGFR gene to Cep 7 ratio of ≥ 2 in 10% of the analyzed cancer cells)

**FISH-negative:** if disomy (2 copies each), trisomy (3 copies each in ≥ 40% of cells), low-polysomy (4 EGFR/Cep 7 gene copies in ≤ 40% cells)

**FISH-deleted:** (EGFR copies < CEP 7 signals)

High-Polysomy
≥ 4 EGFR copies in ≥40% cells

PAP 20X

Imprint

H & E 20X

Histology
Amplification- EGFR gene (O) to Cep 7 (G) ratio $\geq 2$ in 10% of the Cells
Disomy - 2 Orange & 2 Green Signals

PAP 20X

Imprint

H & E 20X

Histology
Low-polysomy
Trisomy - 3 copies each in ≥ 40% of cells

PAP 20X
Imprint

H & E 20X
Histology
Deleted
EGFR copies (O) < CEP 7 (G)

PAP 40X

Imprint

H & E 20X

Histology
Correlation of EGFR by FISH with Cytology, Histology & Stage

<table>
<thead>
<tr>
<th></th>
<th>Cytology</th>
<th>Stage</th>
<th>Histology</th>
<th>Stage</th>
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<tr>
<td>Number</td>
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<td>13</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>II-IV</td>
<td>II-II</td>
<td>3</td>
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<tr>
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Correlation of EGFR by FISH on Cytology with Type & Stage

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<th>ADC</th>
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<th>SCC</th>
<th>Stage</th>
<th>NSCLC</th>
<th>Stage</th>
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<td>I-II</td>
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<td>4</td>
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<td>III-IV</td>
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<tr>
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</table>

ADC: Adenocarcinoma; SCC: Squamous Cell Carcinoma; NSCLC: Non Small Cell Carcinoma; Neg: Negative; Del: Deletion
## Correlation of EGFR by FISH on Histology with Type & Stage

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<tr>
<td><strong>FISH Neg/Del</strong></td>
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ADC: Adenocarcinoma; SCC: Squamous Cell Carcinoma; NSCLC: Non Small Cell Carcinoma; Neg: Negative; Del: Deletion
Results

- EGFR on cytology specimens were highly correlated with EGFR histology specimens (p < 0.0001)

- In the FISH-positive subgroup, cytology but not histology specimens were highly correlated with stage (p < 0.008)
Results

- 2 cases that were FISH positive on histology were FISH negative on cytology

- 2 cases that were FISH positive on cytology were FISH negative on histology

- Deletions of EGFR occurred in PD tumors that presented at high stage
The discrepancies between the histology & cytology may be due:

- Truncated nuclei on sections, leading to lower # of gene signals/nucleus (on cytology nuclei are intact)
- Overlapping nuclei, leading to higher # of gene signals/nucleus
- Variable signal strength or pattern due to uneven sectioning
Conclusions

• This study demonstrated that EGFR gene analysis by FISH for prediction of response to TKI in NSCLCs is feasible in cytological specimens.

• Using the criteria of Cappuzzo et al we showed a high correlation of EGFR FISH between cytology and histology specimens.
Conclusions

- EGFR evaluation by FISH on cytology specimens are easier to interpret and less time consuming to perform.

- Differences in EGFR pattern are most likely due to sampling & technical factors and not interpretation.
Conclusions

- EGFR polysomy and amplification on cytology appears to be correlated with stage.

- The findings reported in this pilot study are encouraging and if validated, will represent a minimally invasive method to evaluate EGFR status on cytologic specimens for initiation of targeted molecular therapy.
Acknowledgements

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