The Importance of Molecular Testing for Therapeutic Decision in Lung Cancer

Professor Tony Mok
Dept of Clinical Oncology
The Chinese University of Hong Kong
Lung Cancer: Phenotype versus Genotype

Non-small cell lung cancer

Adenocarcinoma

Driver Gene

From files of TS Mok

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### Genomic driver in adenocarcinoma: East versus West

<table>
<thead>
<tr>
<th></th>
<th>LCMC</th>
<th>China</th>
<th>Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>17%</td>
<td>40%</td>
<td>50%</td>
</tr>
<tr>
<td>KRAS</td>
<td>22%</td>
<td>7%</td>
<td>15%</td>
</tr>
<tr>
<td>ELM4-ALK</td>
<td>7%</td>
<td>7%</td>
<td>5%</td>
</tr>
<tr>
<td>BRAF</td>
<td>2%</td>
<td>2%</td>
<td>1%</td>
</tr>
<tr>
<td>HER2</td>
<td>1%</td>
<td>NA</td>
<td>3%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>1%</td>
<td>4%</td>
<td>NA</td>
</tr>
<tr>
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<td>NA</td>
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</tr>
<tr>
<td>Nil</td>
<td>46%</td>
<td>29%</td>
<td>22%</td>
</tr>
</tbody>
</table>
Genomic Driver

- Over 50% of all adenocarcinoma harbor genomic driver
- Incidence of genomic driver is variable between ethnic populations
- EGFR mutation is the most prevalence genomic driver
**EGFR Mutations**

- **Activating mutation**
  - Deletion at exon 19
  - Point mutation at exon 21 L858R
- **Concept of oncogenic addiction**
  - Mutation at this sites shift the equilibrium such that it favors the activated states
  - Activated states induce downstream pro-survival and pro-apoptotic activity
  - As result tumor cell depends on EGFR signal for survival

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Lynch TJ *et al.* NEJM 2004; 350(21):2129-2139,
Paez JG *et al.* Science 2004;304:1497-1500. Reprinted with permission from AAAS
## Prospective randomized studies of EGFR TKIs in unselected, chemotherapy pretreated patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient #</th>
<th>Agent</th>
<th>RR</th>
<th>PFS or OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDEAL 1 (1)</td>
<td>210</td>
<td>Gefitinib 250 mg 500 mg</td>
<td>18.4% 19.0%</td>
<td>PFS 2.7 months 2.8 months</td>
</tr>
<tr>
<td>IDEAL 2 (2)</td>
<td>221</td>
<td>Gefitinib 250 mg 500 mg</td>
<td>12.0% 9.0%</td>
<td>OS 7.0 months 6.9 months</td>
</tr>
<tr>
<td>BR-21 (3)</td>
<td>488</td>
<td>Erlotinib</td>
<td>8.9%</td>
<td>PFS 2.2 months</td>
</tr>
<tr>
<td>ISEL (4)</td>
<td>1129</td>
<td>Gefitinib</td>
<td>8.2%</td>
<td>OS 5.6 months</td>
</tr>
</tbody>
</table>

1 Fukuoka M. et al., J Clin Oncol 2003; 21:2237-2246,  
2 Kris MG et al., JAMA 2003; 290:2149-2158,  
4 Thatcher N. et al., Lancet 2005; 366:1527-1537,
## Prospective Studies of Patients with EGFR mutations treated with EGFR TKIs

<table>
<thead>
<tr>
<th>Author</th>
<th>#screened</th>
<th>EGFR mutations</th>
<th>Agent</th>
<th>RR</th>
<th>TTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inoue(^1)</td>
<td>99</td>
<td>16</td>
<td>Gefitinib</td>
<td>75%</td>
<td>9.7 mos</td>
</tr>
<tr>
<td>Rosell(^2)</td>
<td>2105</td>
<td>217</td>
<td>Erlotinib</td>
<td>70%</td>
<td>14.0 mos</td>
</tr>
<tr>
<td>Tamura(^3)</td>
<td>118</td>
<td>32</td>
<td>Gefitinib</td>
<td>75%</td>
<td>ND</td>
</tr>
<tr>
<td>Sutani(^4)</td>
<td>100</td>
<td>38</td>
<td>Gefitinib</td>
<td>78%</td>
<td>9.4 mos</td>
</tr>
<tr>
<td>Morikawa(^5)</td>
<td>123</td>
<td>46</td>
<td>Gefitinib</td>
<td>62%</td>
<td>9.7 mos</td>
</tr>
<tr>
<td>Sequist(^6)</td>
<td>98</td>
<td>31</td>
<td>Gefitinib</td>
<td>55%</td>
<td>11.4 mos</td>
</tr>
</tbody>
</table>

\(^1\) Inoue A., et al., J Clin Oncol. 2006;24(21):3340-3346  
\(^5\) Morikawa N., et al., J Clin Oncol. 2006;24(185 Suppl): Abstract 7077  
Patients
- Chemonaïve
- Age ≥18 years
- Adenocarcinoma histology
- Never or light ex-smokers*
- Life expectancy ≥12 weeks
- PS 0-2
- Measurable stage IIIIB / IV disease

1:1 randomisation
- Gefitinib (250 mg / day)
- Carboplatin (AUC 5 or 6) / paclitaxel (200 mg / m²) 3 weekly#

Endpoints
Primary
- Progression-free survival (non-inferiority)

Secondary
- Objective response rate
- Overall survival
- Quality of life
- Disease-related symptoms
- Safety and tolerability

Exploratory
- Biomarkers
  - EGFR mutation
  - EGFR-gene-copy number
  - EGFR protein expression

*Never smokers, <100 cigarettes in lifetime; light ex-smokers, stopped ≥15 years ago and smoked ≤10 pack years; #limited to a maximum of 6 cycles
Carboplatin / paclitaxel was offered to gefitinib patients upon progression
PS, performance status; EGFR, epidermal growth factor receptor

Mok TS. et al. NEJM 2009; 361(10):947-957
Mok TS et al., Proc ESMO 2008: Abstract LBA2
Overall population: Progression-free survival

Gefitinib demonstrated superiority relative to carboplatin / paclitaxel in terms of PFS.

HR (95% CI) = 0.741 (0.651, 0.845) p<0.0001

Primary Cox analysis with covariates
HR <1 implies a lower risk of progression on gefitinib

Mok TS et al. NEJM 2009;361:947-957,
With permission of Massachusetts Medical Society
IPASS: EGFR Mutation and Progression-free survival

**EGFR mutation positive**

- Gefitinib
- Carboplatin / paclitaxel
- HR (95% CI) = 0.48

**EGFR mutation negative**

- Gefitinib
- Carboplatin / paclitaxel
- HR (95% CI) = 2.85 (2.05, 3.98)
  - No. events gefitinib: 88 (96.7%)
  - No. events C / P: 70 (82.4%)

**Treatment by subgroup interaction test, p<0.0001**

IT&T population
Cox analysis with covariates

Mok TS et al. NEJM 2009;361:947-957
With permission of Massachusetts Medical Society
# PFS in ITT population (updated analysis 26 Jan 2011)

<table>
<thead>
<tr>
<th>Study</th>
<th>Response Rate</th>
<th>PFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>EURTAC</td>
<td>58.1% vs. 14.9%</td>
<td>9.7 vs. 5.2 months (HR 0.37)</td>
</tr>
<tr>
<td>OPTIMAL</td>
<td>83% vs. 36%</td>
<td>13.1 vs. 4.6 months (HR 0.16)</td>
</tr>
<tr>
<td>NEJ 002</td>
<td>74% vs. 31%</td>
<td>10.8 vs. 5.4 months (HR 0.30)</td>
</tr>
<tr>
<td>WJTOG 3405</td>
<td>62% vs. 31%</td>
<td>9.2 vs. 6.3 months (HR 0.49)</td>
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Randomized studies on first line EGFR TKI in patients with EGFR mutation

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<tr>
<td>Mok et al.</td>
<td>IPASS</td>
<td>132</td>
<td>71.2% vs. 47.3%</td>
<td>9.8 vs. 6.4 months</td>
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<tr>
<td>Lee et al.</td>
<td>First-SIGNAL</td>
<td>27</td>
<td>84.6% vs. 37.5%</td>
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<td>Mitsudomi et al.</td>
<td>WJTOG 3405</td>
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<td>62.1% vs. 32.2%</td>
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<td>Maemondo et al.</td>
<td>NEJGSG002</td>
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<tr>
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<td>OPTIMAL</td>
<td>154</td>
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<tr>
<td>Rosell et al.</td>
<td>EURTAC</td>
<td>175</td>
<td>58% vs. 15%</td>
<td>9.7 vs. 5.2 months</td>
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Total of six randomized studies confirmed that molecular testing for EGFR mutation benefited patients.
Landscape of NSCLC

Advanced NSCLC

 EGFR mutation

 EGFR wild type
 Or unknown
Landscape of NSCLC

Advanced NSCLC

EGFR wild type
Or unknown
### Genomic driver in adenocarcinoma: East versus West

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Advanced NSCLC: EGFR mutation negative

First line chemo  →  Second line chemo  →  3rd/4th line therapy  →  BSC  →  Death

ELM-4 ALK
**EML4–ALK fusion gene**

- **EML4–ALK** fusion gene identified as a key driver of oncogenesis in a subset of NSCLC patients\(^1\)
- **ALK** fusion gene results in formation of cytoplasmic chimeric proteins with constitutive kinase activity\(^1\)
  - Rarer fusion partners for ALK such as KIF5B and TFG have also been reported in NSCLC\(^2\)
- *The ALK* fusion gene appears to be a distinct NSCLC molecular subset susceptible to targeted inhibition

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\(^1\)Soda M. *et al.* Nature 2007;448:561–566


Pfizer, data on file
Transforming activity of EML4-ALK

With permission of Macmillan Publishers Ltd
Who May Harbour ELM4-ALK?

- The ALK fusion gene has been identified in 0.6–11.6% of NSCLC\textsuperscript{1,2}
- Any NSCLC patient may have the ALK fusion gene\textsuperscript{3}
- May be more prevalent in Asian population
- However, the ALK fusion gene appears to occur more frequently in:
  - Adenocarcinoma histology (2.4–5.6%)\textsuperscript{4–6}
  - Non- or light smokers\textsuperscript{2,4,5}
  - Rarely (<1%) in those with squamous cell carcinoma\textsuperscript{1,3}
- The ALK fusion gene tends to be mutually exclusive with EGFR and KRAS mutations\textsuperscript{2,4,7}
- Possible correlation between signet ring cell and ALK status\textsuperscript{4}

\textsuperscript{7}Shaw AT, \textit{et al}., J Clin Oncol 2009;27:4247–53
Finding the target

- Four proposed methods of testing
  - Fluorescent in-situ hybridization (FISH)
  - Immunohistochemistry (IHC)
  - Reverse transcriptase polymerase chain reaction (RT-PCR)
  - DNA sequencing

With permission of American Association for Cancer Research
Break-apart FISH assay for ALK fusion*

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

With permission of American Society of Clinical Oncology
The ALK break-apart FISH assay

- The ALK break-apart FISH assay detects ALK fusions in formalin-fixed paraffin-embedded (FFPE) NSCLC tissue specimens.
- Considered ALK positive if >15% cell with break-apart.
- The centromeric (green) and telomeric (red) probes flank the ALK locus.
- Splitting probes of the red and green signals indicates ALK fusion.
- A yellow signal indicates no ALK fusion.

With permission of American Society of Clinical Oncology.
ALK
Intercalater
DAKO FLEX+ system

ALK1 with conventional
ALK1 with sensitive
5A4 with conventional
5A4 with sensitive

EnVision™ System, Dako Denmark
IHC, FISH or PCR?

**Surgically resected samples (n=345)**

- **Multiplex RT-PCR**, covering 80% ALK fusion
- **IHC** using the ab clone 5A4 and highly sensitive detection method (DAKO FLEX+)

<table>
<thead>
<tr>
<th>RT-PCR</th>
<th>ALK(-)</th>
<th>ALK(+)</th>
<th>FISH+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>348</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Mutated</td>
<td>0</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Variant1</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Variant2</td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Variant3a/b</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Yatabe Y. *et al.*, Molecular Target Meeting 2011
Finding the target

Four proposed methods of testing

- Fluorescent in-situ hybridization (FISH)
- Immunohistochemistry (IHC)
- Reverse transcriptase polymerase chain reaction (RT-PCR)
- DNA sequencing

Hirsch F. et al., Clin Cancer Res 2010;16:4909–4911
With permission of American Association for Cancer Research
**Crizotinib: selective inhibitor of ALK and c-MET**

**Cellular selectivity on 10 of 13 relevant hits**

<table>
<thead>
<tr>
<th>Kinase</th>
<th>IC50 (nM) mean*</th>
<th>Selectivity ratio°</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Met</td>
<td>8</td>
<td>–</td>
</tr>
<tr>
<td>ALK</td>
<td>20</td>
<td>2X</td>
</tr>
<tr>
<td>RON</td>
<td>298</td>
<td>34X</td>
</tr>
<tr>
<td></td>
<td>189</td>
<td>22X</td>
</tr>
<tr>
<td>Axl</td>
<td>294</td>
<td>34X</td>
</tr>
<tr>
<td></td>
<td>322</td>
<td>37X</td>
</tr>
<tr>
<td>Tie-2</td>
<td>448</td>
<td>52X</td>
</tr>
<tr>
<td>Trk A</td>
<td>580</td>
<td>67X</td>
</tr>
<tr>
<td>Trk B</td>
<td>399</td>
<td>46X</td>
</tr>
<tr>
<td>Abl</td>
<td>1,159</td>
<td>166X</td>
</tr>
<tr>
<td>IRK</td>
<td>2,887</td>
<td>334X</td>
</tr>
<tr>
<td>Lck</td>
<td>2,741</td>
<td>283X</td>
</tr>
<tr>
<td>Sky</td>
<td>&gt;10,000</td>
<td>&gt;1,000X</td>
</tr>
<tr>
<td>VEGFR2</td>
<td>&gt;10,000</td>
<td>&gt;1,000X</td>
</tr>
<tr>
<td>PDGFRβ</td>
<td>&gt;10,000</td>
<td>&gt;1,000X</td>
</tr>
</tbody>
</table>

*measured using ELISA capture method

**Findings for crizotinib**

- **High** probability of ALK and c-Met inhibition at clinically relevant doses
- **Low** probability of relevant inhibition of RON, Axl, Tie-2, or Trk at clinical dose levels
- Did not inhibit phospho-RON (20X) or Tie-2 (52X) *in vivo* at dose levels above which full c-Met inhibition for 24 hours observed
- Important in interpreting MOA

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Pfizer, data on file
Crizotinib in the ALK ATP binding pocket

Pfizer, data on file

Courtesy of Camidge DR. et al. Poster 366 presented at the 35th ESMO, 2010
Crizotinib induces apoptosis in \textit{EML4–ALK}-positive NSCLC cells

Crizotinib demonstrated potent growth-inhibitory activity against H3122 (ALK fusion) cells

\( \text{IC}_{50} = 96 \text{ nM} \)

\( \text{Untreated} \quad 50 \text{ nM} \quad 500 \text{ nM} \)

Activated caspase-3

Pfizer, data on file
Tumor Responses to Crizotinib for Patients with ALK-positive NSCLC

Progressive disease
Stable disease
Confirmed partial response
Confirmed complete response

PR 57%
SD 33%

*Partial response patients with 100% change have non-target disease present

Bang Y. et al. ASCO Annual Meeting 2010, Abstract 3
With permission from American Society of Clinical Oncology
Courtesy Camidge DR. et al. Poster 366 presented at the 35th ESMO, 2010
### Tumor Responses to Crizotinib for Patients with ALK-positive NSCLC

<table>
<thead>
<tr>
<th>Author</th>
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<tr>
<td>Mok et al</td>
<td>IPASS</td>
<td>261</td>
<td>71.2%</td>
<td>9.8 months</td>
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<td>83%</td>
<td>13.1 months</td>
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</tbody>
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*Partial response patients with 100% change have non-target disease present*
Median progression-free survival

Median PFS = 9.2 months (95% CI: 7.6, 10.3)
48% of patients in follow-up for PFS

Median follow-up for PFS: 8.0 months

No. at risk
113
59
17
5
0

Time (months)

PFS probability

--- 95% Hall-Wellner Band

Caption: Courtesy of Camidge DR. et al. Poster 366 presented at the 35th ESMO, 2010
Current crizotinib clinical trials

PROFILE 1007

Key entry criteria
● Positive for ALK by central laboratory
● 1 prior chemotherapy (platinum-based)

PROFILE 1005

Key entry criteria
● Positive for ALK by central laboratory
● Progressive disease in Arm B of study PROFILE 1007
● >1 prior chemotherapy

Crizotinib 250 mg BID \( (n=250) \) administered on a continuous dosing schedule

Pemetrexed 500 mg/m\(^2\) or docetaxel 75 mg/m\(^2\) \( (n=159) \) infused on day 1 of a 21-day cycle

PROFILE 1007: NCT00932893; PROFILE 1005: NCT00932451
Phase 3 study in previously untreated NSCLC: A8081014

**Trial design**
- World-wide, multicenter, randomized, open-label, focused screening

**Endpoints**
- Primary: PFS*
- Secondary: 6- and 12-month OS; OS; ORR*; DCR; DR; Safety; HRQoL; Lung cancer-specific symptoms; General health status; Biomarkers; TTD; HCRU

**Stratification**
- ECOG PS (0/1 vs 2)
- Ethnicity (Asian vs non-Asian)
- Brain metastases

**Key entry criteria**
- Diagnosis of locally advanced/metastatic non-squamous NSCLC; ECOG 0-2
- Positive for ALK
- No prior treatment for advanced disease
- Brain metastases allowed

**Arm A**
- Crizotinib 250 mg BID administered on a continuous dosing schedule
- N=320

**Arm B**
- Pemetrexed/ cisplatin or pemetrexed/ carboplatin
- Day 1 of a 21-day cycle
- N=160

*Based on RECIST v 1.1 and confirmed by independent radiology review

Patients in Arm B who have RECIST-defined PD as determined by the independent radiology review will be allowed to cross over to Arm A

www.clinicaltrials.gov (NCT01154140)
Summary

- Genomic driver is relatively common
- Patients with EGFR mutation will benefit from first line EGFR TKI with higher treatment efficacy comparing to chemotherapy
- Patients with ELM4-ALK may benefit from crizotinib (ongoing phase III first line and 2nd/3rd lines study)
THANK YOU!