ALK and Other Gene Fusions in Lung Cancer

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DISCLOSURE

• I have no financial relationships to disclose.
Gene Fusions in Lung Cancer

- ALK
- ROS1
- RET
- Others
Methods for Detecting Gene Fusions

• Traditional cytogenetic-based methods
• Modified NIH 3T3 cell transformation assays
• Outlier clinical response to therapy
• Outlier patterns: FISH, chromogenic in situ hybridization (CISH), mRNA or protein (IHC) expression
• Massively parallel paired-end sequencing
• Transcriptome sequencing

Modified from Kaye, Mol Cancer Ther 2009
Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer

Manabu Soda1,2, Young Lim Choi1, Munehiro Enomoto1,2, Shuji Takada3, Yoshihiro Yamashita1, Shunpei Ishikawa5, Shin-ichiro Fujiwara1, Hideki Watanabe1, Kentaro Kurashina1, Hisashi Hatanaka1, Masashi Bando2, Shoji Ohno2, Yuichi Ishikawa6, Hiroyuki Aburatani5,7, Toshiro Niki3, Yasunori Sohara4, Yukihiko Sugiyama2 & Hiroyuki Mano1,7

NIH 3T3 cell transformation assay-AC 67yr, smoker

echinoderm microtubule-associated protein-like 4 (EML4) anaplastic lymphoma kinase (ALK)

Soda et al, Nature 2007
Critical Domains for \textit{EML4-ALK} Transforming Activity

Expression of *EML4-ALK* variant 1 in NSCLCs

Reverse transcription-PCR

ALK Kinase and Growth Inhibition

**EML4-ALK** in Caucasians and NSCLC cell lines

~3% of 305 primary NSCLC [U.S. and Korean] and 83 NSCLC cell lines

- Exon arrays and validation with RT-PCR/FISH

Kovunen et al, CCR, 2008
ALK Kinase Inhibition \textit{In vitro} and \textit{In vivo}

- PC-9 (EGFR del)E746_A750
- H2228 (EML4-ALK variant 3),
- H3122 (EML4-ALK variant 1)
- DFCI032 (EML4-ALK variant 1)
- A549 (KRAS G12S)

Kovunen et al, \textit{CCR}, 2008
A mouse model for *EML4-ALK*-positive lung cancer

Manabu Soda\textsuperscript{a,b}, Shuji Takada\textsuperscript{a}, Kengo Takeuchi\textsuperscript{a}, Young Lim Choi\textsuperscript{a}, Munehiro Enomoto\textsuperscript{a}, Toshihide Ueno\textsuperscript{a}, Hidenori Haruta\textsuperscript{a}, Toru Hamada\textsuperscript{a}, Yoshihiro Yamashita\textsuperscript{a}, Yuichi Ishikawa\textsuperscript{c}, Yukihiro Sugiyama\textsuperscript{b}, and Hiroyuki Mano\textsuperscript{a,d,1}

ALK kinase inhibitor: 10nM
2,4-pyrimidinediamine derivative

*PNAS* 2008
EML4-ALK Fusion Detection by FISH

>600 Adenos/SCC: 2.7% with EML4-ALK fusions - ALK amplification

Perner et al, Neoplasia 2008
Frequent ALK rearrangement and TTF-1/p63 co-expression in lung adenocarcinoma with signet-ring cell component

Akihiko Yoshida\textsuperscript{a,b}, Koji Tsuta\textsuperscript{a}, Shun-ichi Watanabe\textsuperscript{c}, Ikuo Sekine\textsuperscript{d}, Masashi Fukayama\textsuperscript{b}, Hitoshi Tsuda\textsuperscript{a}, Koh Furuta\textsuperscript{a}, Tatsuhiro Shibata\textsuperscript{e,*}

Yoshida et al, Lung Cancer 2011
Histopathological Characteristics

- *EML4-ALK* is present in 3-7% of NSCLC cases.
- Adenocarcinoma histology, non- or light smokers.
- No ethnic differences for NSCLCs with *EML4-ALK*.
- *EML4-ALK* and *EGFR* or *KRAS* mutations are mutually exclusive; fewer *p53* mutations.
- A solid, signet-ring cell pattern or mucinous cribriform pattern often present with *EML4-ALK*.

KIF5B-ALK, a Novel Fusion Oncokine Identified by an Immunohistochemistry-based Diagnostic System for ALK-positive Lung Cancer

Takuechi et al, CCR 2009
ALK Fusion Partners and *EML4-ALK* Variants

Sasaki et al, *Eur J Cancer* 2010
Crizotinib

The response rate for crizotinib in patients with ALK fusion NSCLCs is 57%, with a disease control rate of up to 90% (Kwak et al, *NEJM* 2010).

(Sasaki and Janne *CCR*, 2011)
Crizotinib Resistance in NSCLC

(Sasaki and Janne CCR, 2011)
Crizotinib Resistance in NSCLC

- Efficacy of crizotinib limited by the emergence of multiple resistant events:
  - mutations involving either gatekeeper residue (L1196M) or residues away from crizotinib binding (L1152R, C1156Y, and F1174L) (Choi et al, NEJM, 2010).
  - also see L1196M, G1269A ALK kinase domain mutations (Doebele et al CCR 2012).
- Emergence of ALK gene fusion negative tumors.
- ALK gene copy number changes.
- KRAS, EGFR mutation and HER Family signaling (Tanizaki et al CCR, 2012)
Crizotinib Resistance (cont.)

(Doebele et al CCR 2012)
ALK and Heat shock protein 90 (HSP90) inhibitors

• EML4-ALK associates with HSP90 thus inhibitors can lead to degradation of EML4-ALK.

• Cell lines bearing the crizotinib-resistance mutations (L1196M and F1174L) remain equally sensitive to HSP90 inhibitors.

• Next generation ALK inhibitors with these combination therapies may be effective.
Global Survey of Phosphotyrosine Signaling Identifies Oncogenic Kinases in Lung Cancer

Klarisa Rikova, Ailian Guo, Qingfu Zeng, Anthony Possemato, Jian Yu, Herbert Haack, Julie Nardone, Kimberly Lee, Cynthia Reeves, Yu Li, Yerong Hu, Zhiping Tan, Matthew Stokes, Laura Sullivan, Jeffrey Mitchell, Randy Wetzel, Joan MacNeill, Jian Min Ren, Jin Yuan, Corey E. Bakalarski, Judit Villen, Jon M. Kornhauser, Bradley Smith, Daqiang Li, Xinmin Zhou, Steven P. Gygi, Ting-Lei Gu, Roberto D. Polakiewicz, John Rush, and Michael J. Comb

SLC34A2-ROS Fusion in HCC78 cell line

CD74-ROS Fusion in patient CS042

<1%

Cell 2007
**ROS1 Gene Fusions in Lung Cancer**

1529 lung cancers: IHC and FISH
13 ROS1 fusions

## Histopathology and genotypes of ROS1 IHC positive NSCLC

<table>
<thead>
<tr>
<th>Tumor ID</th>
<th>Diagnosis</th>
<th>Histologic pattern (%)</th>
<th>IHC Score</th>
<th>ROS1 FISH</th>
<th>ROS1 fusion</th>
<th>EGFR mutation status</th>
</tr>
</thead>
<tbody>
<tr>
<td>147</td>
<td>Adenocarcinoma</td>
<td>BAC (40), papillary (30), Acinar (20), Solid (10)</td>
<td>3+ cytoplasmic</td>
<td>+</td>
<td>SLC34A2-ROS1</td>
<td>L858R</td>
</tr>
<tr>
<td>306</td>
<td>Adenocarcinoma</td>
<td>Acinar (70), papillary (20), and solid (10)</td>
<td>3+ cytoplasmic</td>
<td>+</td>
<td>CD74-ROS1</td>
<td>−</td>
</tr>
<tr>
<td>570</td>
<td>Adenocarcinoma</td>
<td>Acinar (90), BAC (5), micropapillary (5)</td>
<td>3+ cytoplasmic, punctae</td>
<td>+</td>
<td>CD74-ROS1</td>
<td>−</td>
</tr>
<tr>
<td>760</td>
<td>Adenocarcinoma</td>
<td>Signet cells</td>
<td>3+ cytoplasmic, membrane</td>
<td>+</td>
<td>Insufficient material</td>
<td>Insufficient material</td>
</tr>
<tr>
<td>400037</td>
<td>Adenocarcinoma</td>
<td>Acinar</td>
<td>2+ cytoplasmic, punctae</td>
<td>+</td>
<td>CD74-ROS1</td>
<td>−</td>
</tr>
<tr>
<td>575</td>
<td>Large Cell</td>
<td>Solid (80), Acinar (10), BAC (10)</td>
<td>2+ cytoplasmic</td>
<td>Not scoreable</td>
<td>Unknown</td>
<td>−</td>
</tr>
<tr>
<td>668</td>
<td>Adenocarcinoma</td>
<td>Solid (80), Acinar (10), BAC (10)</td>
<td>1+ cytoplasmic</td>
<td>+</td>
<td>CD74-ROS1</td>
<td>−</td>
</tr>
<tr>
<td>702</td>
<td>Adenocarcinoma</td>
<td>Papillary (40), Acinar (30), Solid (30)</td>
<td>1+ cytoplasmic</td>
<td>+</td>
<td>SLC34A2-ROS1</td>
<td>E746-A750del</td>
</tr>
<tr>
<td>749</td>
<td>Adenocarcinoma</td>
<td>Solid (80), Acinar (20)</td>
<td>1+ vesicular</td>
<td>+</td>
<td>FIG-ROS1 (S)</td>
<td>−</td>
</tr>
</tbody>
</table>

-556 NSCLC: 9 (1.6%) ROS1 fusion positive
-\textit{FIG-ROS1} originally seen in glioblastoma

(Rimkunas et al, \textit{CCR}, 2012)
FIG-ROS1 Fusion

A

FIG-ROS1 Fusion

B

Cell growth inhibition by crizotinib

C

(Rimkunas et al, CCR, 2012)
**SDC4–ROS1 fusion**

447 NSCLC FISH: 1.2% with SDC4-ROS1 fusions

(Davies et al, *CCR*, 2012)
The Oncogenic Lung Cancer Fusion Kinase CD74-ROS Activates a Novel Invasiveness Pathway through E-Syt1 Phosphorylation

Hyun Jung Jun¹, Hannah Johnson⁵, Roderick T. Bronson³, Sebastien de Feraudy⁴, Forest White⁵, and Alain Charest¹.²

Increased phosphorylation of synaptotagmin-like protein E-Syt1 with fusion

(Jun et al CR, 2012)
(Stumpfova and Janne, *CCR*, 2012)
**RET Gene Fusions in NSCLC**

massively parallel whole-genome and transcriptome sequencing

(Ju et al, *Genome Res*, 2011)
RET Fusion Variants

(KIF5B-RET)

(RET)

(CCDC6-RET)

(Takeuchi et al, Nat Med, 2012)
## Summary of KIF5B-RET fusions in 2650 NSCLC

<table>
<thead>
<tr>
<th>Study</th>
<th>Identification and verification method(s)</th>
<th>Ethnicity (n)</th>
<th>Percentage positive for KIF5B-RET</th>
<th>Variants identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kohno et al.</td>
<td>Whole-transcriptome sequencing*</td>
<td>Japanese (30)</td>
<td>3.3</td>
<td>K15;R12, K16;R12, K23;R12, K24;R8</td>
</tr>
<tr>
<td></td>
<td>RT-PCR and Sanger sequencing</td>
<td>Japanese (289)</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>American (80)</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Norwegian (34)</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>433</strong></td>
<td><strong>1.6</strong></td>
<td></td>
</tr>
<tr>
<td>Lipson et al.</td>
<td>Targeted capture and resequencing*</td>
<td>Not specified (24)</td>
<td>4.2</td>
<td></td>
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<tr>
<td></td>
<td>RT-PCR</td>
<td>Caucasian (121)</td>
<td>0.8</td>
<td>K15;R11, K15;R12, K16;R12, K22;R12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Korean (347)</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Japanese (58)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RET IHC and RT-PCR</td>
<td>Caucasian (92)</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>African American (5)</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown (20)</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>667</strong></td>
<td><strong>1.8</strong></td>
<td></td>
</tr>
<tr>
<td>Takeuchi et al.</td>
<td>IHC and FISH screen*</td>
<td>Japanese (1529)</td>
<td>0.9</td>
<td>K15;R12, K16;R12, K22;R12, K23;R12, K24;R11</td>
</tr>
<tr>
<td>Ju et al.</td>
<td>Whole-genome and whole-transcriptome sequencing*</td>
<td>Korean (1)</td>
<td>100.0</td>
<td>K15;R12, K16;R12, K23;R12, K24;R11</td>
</tr>
<tr>
<td></td>
<td>Whole-transcriptome sequencing (screen)</td>
<td>Korean (5)</td>
<td>20.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RT-PCR</td>
<td>Korean (15)</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>21</strong></td>
<td><strong>14.3</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>2,650</strong></td>
<td><strong>1.3</strong></td>
<td></td>
</tr>
</tbody>
</table>


**RET Gene Fusion Adenocarcinomas**

- Younger age, never-smoker status, early lymph node metastases, poor differentiation, and a solid-predominant sub-type.
- Kohno et al, (*Nat Med* 2012) most were well or moderately differentiated AD.
Other Driver Fusions in Lung Cancer?

ALK, ROS1, RET Fusions
Application of robust methodology to discover gene fusions in next generation sequencing data

(Adapted from Maher et al., *PNAS* 2009, and Maher et al., *Nature* 2009)
R3HDM2-NFE2 Fusion

Paired end transcriptome sequencing  
(Wang et al, Nat Biotechnol 2009)
R3HDM2-NFE2 Fusion in Lung Adenocarcinomas

(Wang et al, Nat Biotechnol 2009)
Gene Fusions are Frequent

- Many single or low frequency gene fusions detected.
- Role and impact of gene fusions unclear.
- Potential inactivating as well as driver fusions.
- Challenge is to identify novel tumor drug sensitivities as a consequence of gene fusions.
- Resistance mechanisms will likely necessitate “comprehensive” tumor analyses.
What Underlies Gene Fusion Events?

(Mani and Chinnaiyan, Nat Rev Genetics 2010)
Acknowledgements

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