Targeted Treatment and Molecular Biomarkers in Lung Cancer

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Princess Margaret Hospital
University of Toronto
Disclosures

• **Honoraria for Consultancies:**
  – Lilly Canada (Lung cancer histopathology)
  – AstraZeneca Canada (EGFR mutation testing)
  – Boehringer-Ingelheim Canada (EGFR mutation testing)
  – Roche Oncology (EGFR TKI biomarkers)
  – Pfizer (Targeted therapy and ALK testing)

• **Research grants:**
  – Pfizer Canada
  – Ventana Medical Systems
  – Roche Oncology
Topics for Discussion

1. Recent advances in lung cancer treatment and diagnosis
2. Targeted therapy and predictive biomarkers for adenocarcinoma
3. Molecular profile of squamous cell carcinoma
4. Primary tumor xenograft as research model in targeted therapy
2004 WHO Classification of Malignant Lung Cancer

- Squamous cell carcinoma
- Small cell carcinoma
  - combined
- Adenocarcinoma
  - mixed type (>80%)
  - Acinar type
  - Papillary type
  - Bronchioloalveolar carcinoma
  - Solid type
- Large cell carcinoma
  - LCNEC (neuroendocrine)
  - etc.
- Adenosquamous carcinoma
- Sarcomatoid carcinoma
- Carcinoid tumour
  - Typical
  - Atypical
- Salivary gland tumors
  - Mucoepidermoid
  - Adenoid cystic
  - Epithelial-myoepithelial
- Mesenchymal tumours
  - Epithelioid hemangioendothelioma
  - Etc.
Before 2004

• Histological classification underwent minor revisions (1982, 91, 97, 2004)

• Most important to distinguish small cell from non-small cell carcinoma

• Distinction between major subtypes of NSCLC, i.e. adeno, squamous, large cell is not crucial

• Use of non-specific term such as “Non-small cell Not Otherwise Specified (NOS)” has been acceptable
Landmark Discoveries and Studies Leading to Paradigm Shift in Lung Cancer Diagnosis

**EGFR Mutations in Lung Cancer: Correlation with Clinical Response to Gefitinib Therapy**

J. Guillermo Paez, Pasi A. Jänne, Jeffrey C. Lee, Sean Tracy, Heidi Greulich, Stacey Gabriel, Paula Herman, Frederic J. Kaye, Neal Lindeman, Titus J. Boggon, Katsuhiko Naoki, Hidefumi Sasaki, Yoshitaka Fujii, Michael J. Eck, William R. Sellers, Matthew Meyerson

SCIENCE April 29, 2004

Activating Mutations in the Epidermal Growth Factor Receptor Underlying Responsiveness of Non–Small-Cell Lung Cancer to Gefitinib

Thomas J. Lynch, M.D., Daphne W. Bell, Ph.D., Raffaella Sordella, Ph.D., Sarada Gurubhagavatula, M.D., Ross A. Okimoto, B.S., Brian W. Brannigan, B.A., Patricia L. Harris, M.S., Sara M. Haserlat, B.A., Jeffrey G. Supko, Ph.D., Frank G. Haluska, M.D., Ph.D., David N. Louis, M.D., David C. Christiani, M.D., Jeff Settleman, Ph.D., and Daniel A. Haber, M.D., Ph.D.

NEW ENGLAND JOURNAL OF MEDICINE, MAY 20, 2004
Erlotinib in Previously Treated Non–Small-Cell Lung Cancer

Frances A. Shepherd, M.D., José Rodrigues Pereira, M.D., Tudor Ciuleanu, M.D., Eng Huat Tan, M.D., Vera Hirsh, M.D., Sumitra Thongprasert, M.D., Daniel Campos, M.D., Savitree Maoleekoonpiroj, M.D., Michael Smylie, M.B., Ch.B., Renato Martins, M.D., Maximiliano van Kooten, M.D., Mircea Dediu, M.D., Brian Findlay, M.D., Dongsheng Tu, M.D.,相关人员, Mircea Bezjak, M.D., Gary Clark, Ph.D., Pedro Santabárbara, M.D., Ph.D., and Jacky Seymour, M.D., Ph.D., for the National Cancer Institute of Canada Clinical Trials Group

Gefitinib or Carboplatin–Paclitaxel in Pulmonary Adenocarcinoma

Tony S. Mok, M.D., Yi-Long Wu, M.D., F.A.C.S., Sumitra Thongprasert, M.D., Chih-Hsin Yang, M.D., Ph.D., Da-Tong Chu, M.D., Nagahiro Saijo, M.D., Ph.D., Patrapim Sunpaweravong, M.D., Bao-hui Han, M.D., Benjamin Margono, M.D., Ph.D., F.C.C.P., Yukito Ichinose, M.D., Yutaka Nishiwaki, M.D., Ph.D., Yuichiro Ohe, M.D., Ph.D., Jin-Jeng Kang, M.D., Bucyanas Chonsakuluyong, M.D., Haiyi Jiang, M.D., Emma L. Duffield, M.Sc., Claire L. Watkins, M.Sc., Alison A. Armour, F.R.C.R., and Masahiro Fukuoka, M.D., Ph.D.
Personalized Cancer Therapy Based on Biomarkers or Molecular Pathology Information
# New Therapies in NSCLC

<table>
<thead>
<tr>
<th>Drug</th>
<th>Selection Marker</th>
<th>Histology Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib (EGFR)</td>
<td>EGFR mutation (1st line)</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td>Erlotinib (EGFR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crizotinib (ALK)</td>
<td>ALK rearrangement</td>
<td></td>
</tr>
<tr>
<td>Bevacizumab (VEGFR)</td>
<td>Histology</td>
<td>Non-squamous carcinomas</td>
</tr>
<tr>
<td>Pemetrexed (Folate Pathway)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cetuximab (EGFR)</td>
<td>EGFR protein by IHC (potential)</td>
<td>NSCLC</td>
</tr>
</tbody>
</table>
Epidermal Growth Factor Receptor (EGFR/HER/ErbB)


Roskoski Jr, BBRC 2004;319:1-11
# High Frequency of EGFR Expression in Non-small Cell Lung Cancer

<table>
<thead>
<tr>
<th></th>
<th>SQCC</th>
<th>ADC</th>
<th>LCC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rusch (1997)</strong></td>
<td>94%</td>
<td>57%</td>
<td>63%</td>
</tr>
<tr>
<td><strong>Fontanini (1998)</strong></td>
<td>57%</td>
<td>35%</td>
<td>23%</td>
</tr>
<tr>
<td><strong>Hsieh (2000)</strong></td>
<td>92%</td>
<td>53%</td>
<td>60%</td>
</tr>
<tr>
<td><strong>Hirsch (2003)</strong></td>
<td>82%</td>
<td>46%</td>
<td>33%</td>
</tr>
</tbody>
</table>

SQCC: squamous cell ca, ADC: adenoca; LCC: large cell ca
EGFR TK Domain Mutations

EGFR Activation and Signaling

EGFR Mutant Lung Cancer Cells are more Sensitive to EGFR TK Inhibitors

Paez et al: Science 2005;304:1497-1500
EGFR TKI Improves Survival of EGFR Mutant Compared to Wild Type Patients


Fig 3. Kaplan-Meier plot of overall survival according to epidermal growth factor receptor (EGFR) mutation status. MST, median survival time.
EGFR TK Domain Mutations

• Adeno > Squamous (<5%)

• Women > Men

• East Asian (40-50%) > Caucasian (10-20%)

• Never smokers > smokers
EGFR TK Domain Mutations

IPASS (Iressa Pan-ASia Study)

**Patients**
- Chemonaïve
- Age ≥18 years
- Adenocarcinoma histology
- Never or light ex-smokers*
- Life expectancy ≥12 weeks
- PS 0-2
- Measurable stage III-B/IV disease

**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

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*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 et al, Chicago 2008
Gefitinib or Carboplatin–Paclitaxel in Pulmonary Adenocarcinoma

Tony S. Mok, M.D., Yi-Long Wu, M.D., F.A.C.S., Sumitra Thongprasert, M.D., Chih-Hsin Yang, M.D., Ph.D., Da-Tong Chu, M.D., Nagahiro Saijo, M.D., Ph.D., Patrapim Sunpaweravong, M.D., Baohui Han, M.D., Benjamin Margono, M.D., Ph.D., F.C.C.P., Yukito Ichinose, M.D., Yutaka Nishiwaki, M.D., Ph.D., Yuichiro Ohe, M.D., Ph.D., Jin-Ji Yang, M.D., Busamas Chewaskulyong, M.D., Haiyi Jiang, M.D., Emma L. Duffield, M.Sc., Claire L. Watkins, M.Sc., Alison A. Armour, F.R.C.R., and Masahiro Fukuoka, M.D., Ph.D.
Gefitinib Treatment Should be Directed Only to EGFR Mutant Patients

**B EGFR-Mutation–Positive**
Hazard ratio, 0.48 (95% CI, 0.36–0.64) P<0.001
Events: gefitinib, 97 (73.5%); carboplatin plus paclitaxel, 111 (86.0%)

**C EGFR-Mutation–Negative**
Hazard ratio, 2.85 (95% CI, 2.05–3.98) P<0.001
Events: gefitinib, 88 (96.7%); carboplatin plus paclitaxel, 70 (82.4%)
# Phase III studies of EGFR-TKI vs. Platinum doublet in EGFR Mutant Patients

<table>
<thead>
<tr>
<th>Group</th>
<th>EGFR mutation</th>
<th>Primary endpoint</th>
<th>N (TKI vs. CT)</th>
<th>TKI</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>WJOG 3405</td>
<td>EX19, L858R</td>
<td>PFS</td>
<td>172 (HR=0.49)</td>
<td>G</td>
<td>CDDP+DOC</td>
</tr>
<tr>
<td>NEJ 002</td>
<td>EX19, L858R, G719X, L861Q</td>
<td>PFS</td>
<td>320 (HR=0.69)</td>
<td>G</td>
<td>CBDCA+PAC</td>
</tr>
<tr>
<td>EURTARC</td>
<td>EX19, L858R</td>
<td>PFS</td>
<td>174 (HR=0.37)</td>
<td>E</td>
<td>Pt doublet</td>
</tr>
<tr>
<td>Optimal</td>
<td>EX19, L858R, T790M</td>
<td>PFS</td>
<td>165 (HR=0.16)</td>
<td>E</td>
<td>CBDCA+GEM</td>
</tr>
</tbody>
</table>
Current Guideline for EGFR Mutation Testing

• Advanced NSCLC patients being considered for treatment with 1st line gefitinib therapy (chemotherapy naïve)

• Mainly for adenocarcinoma or NSCLC with adeno component – Important to use immunohistochemistry markers for more precise histological diagnosis
EGFR Mutation Testing

• Testing performed in a certified clinical diagnostic laboratory

• Minimum test includes exon 19 deletion and exon 21 L858R mutation; rare sensitizing mutations should also be tested

• Pre-testing analysis include examination of the histology/cytology slide by a pathologist to confirm tumor type and define cellularity and areas for microdissection or coring
Pathology Consideration for Good Practice

2. Tissue specimens should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.

3. To guide therapy for patients with advanced lung adenocarcinoma, each institution should develop a multidisciplinary team that coordinates the optimal approach to obtaining and processing biopsy/cytology specimens to provide expeditious diagnostic and molecular results.

7. Cell blocks should be prepared from cytology samples including pleural fluids.
Selection of Samples for Epidermal Growth Factor Receptor (EGFR) Mutation Analysis in Non-Squamous Non-Small Cell Lung Carcinoma

Carolyn J Shiu1,2, Jesse Babwah1,2, Gilda da Cunha Santos1, Jenna Sykes3, Melanie Pintilie3, Scott L Boerner1, William R Geddie1, Suzanne Kamei-Reid1, Cuihong Wei1, David M Hwang, Ming S Tsao1
1 Department of Laboratory Medicine and Pathology, University Health Network; 2 Department of Laboratory Medicine and Pathobiology, University of Toronto; 3 Department of Biostatistics, University Health Network and Princess Margaret Hospital

Ontario (Canada) province-wide mutation testing program
• 1.5 year period (2010-11)

1614 cases for analysis
1255 histology
359 cytology

92 indeterminate cases (5.7%)
72 histology (5.7%)
20 cytology (5.6%)

1522 successful cases (94.3%)
1183 histology (94.3%)
339 cytology (94.4%)

326 cases with EGFR mutation (21.4%)
235 histology (19.9%)
91 cytology (26.8%)

167 Exon 19 deletions (11.0%)
117 histology (9.9%)
50 cytology (14.7%)

1196 Wild-type EGFR (78.6%)
948 histology (80.1%)
248 cytology (73.2%)

159 Exon 21 L858R (10.4%)
118 histology (10.0%)
41 cytology (12.1%)

Modern Pathol 2012;25 (suppl2): 490A (Abstract 2045)
Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer

Nature 2007 (Aug 2);448:561-566

Manabu Soda¹,², Young Lim Choi¹, Munehiro Enomoto¹,², Shuji Takada¹, 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, Yukihiro Sugiyama² & Hiroyuki Mano¹,⁷

cDNA prepared from lung cancer specimen of a 62 year old smoker man negative for EGFR and KRAS mutation
EML4-ALK Gene Fusion

**EML4-ALK v1**

```
MDGFAGSDL8ISAASLSDVQDRLSALERSRQQVEDEITVLKAAALADVRLRILASEDHVA  60
SVKKSVEKQPKSPPRASVPMSTCITNGSGA2NKPSHTAVLSIAKGEFTLSAAKSTETKKE  120
KPKQFRKKEBESN6Q5QITA6PSPQPSQPLIQHQPFTBSKNATPTGK1KRP6PAEBK  180
SENSWESRSNRLSISPKFLSKVTRADHKVTIQSPQETYKIMFRGQRPMF1  240
PSDVNDIYKTELPPEKLKLE8AYGKYKDRKANVYLLPTGE1Y1FASVVLNVEER  300
TQHYYGHTCVCVCLAILHDKRIRIAATGQIAVVDKDRPLQIPFKVRW6DGVL6TLQGIGL  360
TFERGVGCLDFPSKADSGYLCHTVVDDSNEMTIVWDNQYKAKASIKTTNEVVLAVEFHT  420
DATEILTCGKSHFWhWSGSNLTRQKFGKYEKPFPQVC7FLGNSCVTLDGSGYMVL  480
IWSKTTVEPTPGEPKVRKRGQELQAMMELEPSPEYKLKLRSTGI3MTPYFCAGK  540
TSS8L6EKLRSVRKNTILIJKLHGHLGAFEBVQYGQSVGGNP6SLPQAV4LPEVCSEQDE  600
LDLMLLEAIS1KSNH6QVIRC6VGLSLPFLIEIL1AAGDLKLSPLRTRPFRPSQPSSL  660
AMLNDLVARD1ACCGTLEEHF1HERDIAARNLITC6CGFGVRK1DGFMARD1YRAS  720
YRKKCGAMALPVKWMPEAFEMGTSTKDTW6FCVLMIFSLGMYPSKSHQVELEF  780
VTSSGRRMDPKNCQPGPVYRIMTCWQHZQPEDRPN1AET9KBYCTQPDVINAPLJEBY  840
GLPUE22EKVPFDEPEGVFLLVS6QAA9REERSPAAPFPPTTSGKAAKPTA4EVB  900
SVRFPGPAVEVGHNAAF5SNPSPNLHRVGSRNK6LWNPTYGSNFTKEKKNMP  960
IAKKEUFERGNLLEG5CTVFPVATWRELPSALLPESSLILANKEVPLFKLQHPCGN  1020
VNYGQQQQLPLAATAEAPGAGHYETDLKSKSNMQPQP  1059
```

---

**EML4-ALK variant 1**

```
1  Basic  496  WO  981
```

**ALK**

```
1  1059
```

---

**Vector**

```
3T3
```

**Nude mice**

```
Tumour/injection  0/8  0/8  0/8  8/8  0/8  8/8  2/2
```

---

**Diagram**

[Diagram showing the gene fusion and protein structures]
EML4-ALK Signaling Pathway

The EML4-ALK signaling pathway involves multiple steps and proteins. The pathway starts with ALK activation by a ligand, leading to the formation of an EML4-ALK translocation. This translocation activates PI3K, which then leads to the phosphorylation of AKT. AKT further regulates various cellular processes like cell growth, cell survival, and evasion of apoptosis.

The pathway also includes the RAS-RAF-MEK-ERK signaling cascade, which is downstream of AKT. ALK TKIs (PF-2341066, NVPTAE684) can inhibit ALK, thus preventing the downstream effects of the EML4-ALK translocation and restoring cellular function.
ALK Break Apart FISH

Chr 2

ALK Break Apart FISH

~12 Mb

EML4

e20
EML4-ALK Positive NSCLC Patients are Highly Responsive to Crizotinib

Overall Response Rate = 57%
Disease Control Rate (CR+PR+SD) at 8 weeks = 87%

Kwak EL, et al. NEJM 2010;363:1693-703
(EML4)-ALK Fusion Gene Tumors Occur Mainly in Adenocarcinoma

<table>
<thead>
<tr>
<th>First author</th>
<th>Adeno (total no.)</th>
<th>Squamous (total no.)</th>
<th>Others (total no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imamura (2008)</td>
<td>3.4% (149)</td>
<td>0% (48)</td>
<td>0 (24)</td>
</tr>
<tr>
<td>Shinamura (2008)</td>
<td>4% (50)</td>
<td>0% (20)</td>
<td>0% (7)</td>
</tr>
<tr>
<td>Takeuchi (2008)</td>
<td>4.3% (253)</td>
<td>0% (71)</td>
<td>0% (19)</td>
</tr>
<tr>
<td>Koivunen (2008)</td>
<td>3.8% (208)</td>
<td>0% (88)</td>
<td>0 (100)</td>
</tr>
<tr>
<td>Rodig (2009)</td>
<td>5.6% (358)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Martlelli (2009)</td>
<td>4.8% (63)</td>
<td>8.3% (48)</td>
<td>22.2% (9)</td>
</tr>
<tr>
<td>Wong (2010)</td>
<td>5.3% (209)</td>
<td>0% (34)</td>
<td>8.7% (23)</td>
</tr>
<tr>
<td>Salido (2010)</td>
<td>4.3% (69)</td>
<td>0% (30)</td>
<td>0% (8)</td>
</tr>
<tr>
<td>Jokoji (2010)</td>
<td>3.1% (254)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Takahashi (2010)</td>
<td>2.4% (211)</td>
<td>0% (75)</td>
<td>0% (27)</td>
</tr>
<tr>
<td>Paik (2011)</td>
<td>6.3% (423)</td>
<td>0% (163)</td>
<td>3.7% (27)</td>
</tr>
<tr>
<td><strong>Tumors studied</strong></td>
<td><strong>2247</strong></td>
<td><strong>577</strong></td>
<td><strong>244</strong></td>
</tr>
</tbody>
</table>
ALK Immunohistochemistry
Screening of Anaplastic Lymphoma Kinase Rearrangement by Immunohistochemistry in Non-small Cell Lung Cancer Correlation with Fluorescence In Situ Hybridization

Jin Ho Paik, MD, PhD,* Gheeyoung Choe, MD, PhD,* Hyojin Kim, MD,* Ji-Young Choe, MD,* Hyun Ju Lee, MD,* Choon-Taek Lee, MD, PhD,† Jong Seok Lee, MD, PhD,† Sanghoon Jheon, MD, PhD,‡ and Jin-Haeng Chung, MD, PhD*

**TABLE 1.** The Relationship between ALK IHC and FISH in the Test Set

<table>
<thead>
<tr>
<th>ALK IHC</th>
<th>ALK FISH</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>413 (91.2)</td>
</tr>
<tr>
<td>1+</td>
<td>0</td>
<td>14 (3.1)</td>
</tr>
<tr>
<td>2+</td>
<td>3 (0.7)</td>
<td>7 (1.5)</td>
</tr>
<tr>
<td>3+</td>
<td>16 (3.5)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>19 (4.2)</td>
<td>434 (95.8)</td>
</tr>
</tbody>
</table>

IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; ALK, anaplastic lymphoma kinase.

J Thoracic Oncol 2011;6:466-72
A transforming *KIF5B* and *RET* gene fusion in lung adenocarcinoma revealed from whole-genome and transcriptome sequencing

Young Seok Ju,¹,² Won-Chul Lee,¹,³ Jong-Yeon Shin,¹,⁴ Seungbok Lee,¹,³ Thomas Bleazard,¹ Jae-Kyung Won,⁵ Young Tae Kim,⁶,⁷ Jong-II Kim,¹,³,⁴,⁸ Jin-Hyoung Kang,⁹ and Jeong-Sun Seo¹,²,³,⁴,⁸,¹⁰

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New Potential Target for RET inhibitors

March 2012

KIF5B-RET fusions in lung adenocarcinoma
Takashi Kohno\(^1,15\), Hitoshi Ichikawa\(^2,15\), Yasushi Totoki\(^3\),
Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies
Doron Lipson\(^1,9\), Marzia Capelletti\(^2,9\), Roman Yelensky\(^1\),
RET, ROS1 and ALK fusions in lung cancer
Kengo Takeuchi\(^1,2\), Manabu Soda\(^3\), Yuki Togashi\(^1,2\),
Molecular Targets for Lung Cancer Treatment

- Adeno
- Squamous
- Small cell
- Large cell & others

- KRAS
- EGFR
- HER2
- BRAF
- PI3KCA
- ROS fusion
- KIF5B-RET
- ALK fusion
- MET-amp
Molecular Targets for Lung Cancer Treatment

(30% of NSCLC)
Chromosome 8p12

Frequent and Focal FGFR1 Amplification Associates with Therapeutically Tractable FGFR1 Dependency in Squamous Cell Lung Cancer

Jonathan Weiss, Martin L. Sos, Danila Seidel, Martin Peifer, Thomas Zander, et al

FGFR1 amplification: 15/155 (9.7%)

FGFR inhibitor: PD173074

Mutations in the DDR2 Kinase Gene Identify a Novel Therapeutic Target in Squamous Cell Lung Cancer

11/290 (3.8%) samples screened; 9/277 (3.2%) primary SqCC

Lung squamous cell carcinoma is a common type of lung cancer, causing approximately 400,000 deaths per year worldwide. Genomic alterations in squamous cell lung cancers have not been comprehensively characterized, and no molecularly targeted agents have been specifically developed for its treatment. As part of The Cancer Genome Atlas, here we profile 178 lung squamous cell carcinomas to provide a comprehensive landscape of genomic and epigenomic alterations. We show that the tumour type is characterized by complex genomic alterations, with a mean of 360 exonic mutations, 165 genomic rearrangements, and 323 segments of copy number alteration per tumour. We find statistically recurrent mutations in 11 genes, including mutation of TP53 in nearly all specimens. Previously unreported loss-of-function mutations are seen in the HLA-A class I major histocompatibility gene. Significantly altered pathways included NFE2L2 and KEAPI in 34%, squamous differentiation genes in 44%, phosphatidylinositol-3-OH kinase pathway genes in 47%, and CDKN2A and RB1 in 72% of tumours. We identified a potential therapeutic target in most tumours, offering new avenues of investigation for the treatment of squamous cell lung cancers.

### Potentially Targetable Mutated/Amplified Genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI3KCA</td>
<td>16%</td>
</tr>
<tr>
<td>PTEN</td>
<td>8%</td>
</tr>
<tr>
<td>AKT 1-3</td>
<td>20%</td>
</tr>
<tr>
<td>FGFR 1-3</td>
<td>12%</td>
</tr>
<tr>
<td>EGFR</td>
<td>9%</td>
</tr>
<tr>
<td>ERBB2</td>
<td>4%</td>
</tr>
<tr>
<td>BRAF</td>
<td>4%</td>
</tr>
<tr>
<td>NOTCH</td>
<td>13%</td>
</tr>
<tr>
<td>RAS</td>
<td>6%</td>
</tr>
</tbody>
</table>
NSCLCs are among the most genomically deranged of all cancers
Mutations in Lung SqCCs

HLA-A mutations: possible mechanism for immune avoidance
NFE2L2/KEAP1/CUL3

- Mutations in KEAP1 are loss of function (frequent LOH of 2nd allele)
- Mutations in NRF2 cluster in DLG and ETGE motif -> prevent KEAP1 interaction -> results in NRF2 stabilization and nuclear entry

Shibata et al. *PNAS* 2008

Increased resistance to chemotherapy
Altered Signaling Pathways in Lung Squamous Cell Carcinoma

PI3K/RTK/RAS signaling

69% altered

- **PTEN**: 15%
- **PIK3CA**: 16%
- **STK11**: 2%
- **AKT1**
  - <1%
- **AKT2**: 4%
- **AKT3**: 16%
- **TSC1**: 3%
- **TSC2**: 3%
- **AMPK**
- **MTOR**

**EGFR**: 9%
- **ERBB2**: 4%
- **ERBB3**: 2%
- **FGFR1**: 7%
- **FGFR2**: 3%
- **FGFR3**: 2%
- **KRAS**: 3%
- **HRAS**: 3%
- **NRAS**: <1%
- **RASA1**: 4%
- **NF1**: 11%

Proliferation, cell survival, translation

**RTK** 26%
**RAS** 24%
**PI3K** 47%

Percent of cases (%)

- 50 inactivated
- 0 activated

Activation → inhibition

Alteration pattern
Compared ‘Sanger’ and pyrosequencing against massively parallel sequencing on patients who have been treated by EGFR TKI:

“...... all patients with a confirmed response to EGFR inhibition, only massively parallel sequencing detected all relevant mutations”
Potential New Therapeutic Targets in Lung Adenocarcinoma

“Short” List of Targeted Agents in Non-Small Cell Lung Cancer

- VEGF targeted agents
- EGFR targeted agents
- mTOR inhibitors
- Proteasome inhibitors
- Cell cycle targeted agents
  - PARP inhibitors
  - CDK inhibitors
  - Novel chemotherapy
  - Proapoptotic agents
- Sundry Kinase Inhibitors:
  - PI3K
  - AKT
  - MAP kinase
  - MEK (Ras, Raf)
  - SRC
  - Aurora kinase
  - Polo-like kinases
  - PKC
- HSP 70, 90 targeted agents
- HIF1-alpha antagonists
- C-met inhibitors
- VEGF targeted agents
- EGFR targeted agents
- mTOR inhibitors
- Proteasome inhibitors
- Cell cycle targeted agents
  - PARP inhibitors
  - CDK inhibitors
  - Novel chemotherapy
  - Proapoptotic agents
- Sundry Kinase Inhibitors:
  - PI3K
  - AKT
  - MAP kinase
  - MEK (Ras, Raf)
  - SRC
  - Aurora kinase
  - Polo-like kinases
  - PKC
- HSP 70, 90 targeted agents
- HIF1-alpha antagonists
- C-met inhibitors
- Vaccine Therapy
Primary Lung Cancer Xenografts Mimic Primary Tumor Histologies

A549 lung adenocarcinoma cell line

Patient Tumor

Primary Xenograft

kidney capsule

Subcutaneous
IMP4 in Lung Cancer
(Integrated Molecular Pathology, Pharmacodynamic, Pharmacogenomic & Proteomics)

Aim 1
1º patient tumor (400)

Aim 2
Clinical Outcome correlation

Aim 3
Target validation

Aim 4
Xenograft tumor models (200)

- mRNA
  • Illumina array

- Genomic DNA
  • NGS, CGH, mutation panel

- Protein
  • Global
  • Targeted

Generate comprehensive molecular database

- Multiplex bioinformatics & biostatistical models for systems biology analysis

Targeted drug testing
Pathway validation

Funding: 2009-14
# Primary NSCLC Xenograft Establishment
(upto End of August)

<table>
<thead>
<tr>
<th>Year</th>
<th>Tumor Implanted</th>
<th>Confirmed Xenograft (&gt;P1)</th>
<th>Confirmed Take Rate</th>
<th>Potential (still in P1)</th>
<th>Potential Take Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>21</td>
<td>5</td>
<td>24%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2006</td>
<td>24</td>
<td>7</td>
<td>29%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2007</td>
<td>52</td>
<td>21</td>
<td>40%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2008</td>
<td>53</td>
<td>22</td>
<td>42%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2009</td>
<td>46</td>
<td>19</td>
<td>41%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2010</td>
<td>85</td>
<td>35</td>
<td>41%</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2011</td>
<td>80</td>
<td>31</td>
<td>39%</td>
<td>7</td>
<td>48%</td>
</tr>
<tr>
<td>2012</td>
<td>69</td>
<td>13</td>
<td>19%</td>
<td>14</td>
<td>39%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>430</strong></td>
<td><strong>153</strong></td>
<td><strong>36%</strong></td>
<td><strong>21</strong></td>
<td><strong>40%</strong></td>
</tr>
</tbody>
</table>
The Ability to Form Primary Tumor Xenografts Is Predictive of Increased Risk of Disease Recurrence in Early-Stage Non–Small Cell Lung Cancer

First 157 patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Xenograft (%)</th>
<th>No Xenograft (%)</th>
<th>P-value (Fisher Exact Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>63 (40%)</td>
<td>94 (60%)</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>29 (30%)</td>
<td>66 (70%)</td>
<td></td>
</tr>
<tr>
<td>Squamous Cell</td>
<td>30 (65%)</td>
<td>16 (35%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other</td>
<td>4 (25%)</td>
<td>12 (75%)</td>
<td></td>
</tr>
<tr>
<td>Differentiation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>2 (10%)</td>
<td>17 (90%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Moderate</td>
<td>26 (38%)</td>
<td>41 (62%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>35 (53%)</td>
<td>31 (47%)</td>
<td></td>
</tr>
</tbody>
</table>
PHL-196: Similar Histologies

Primary Tumor – Mucinous adenocarcinoma

Fragment xeno

Isolated cells xeno
Tumor Engraftment Predictive of Higher Recurrence Rate and Poorer DFS

Clin Cancer Res 2011;17:134-141.

Updated Follow-up

N=109

XG vs. No-XG, HR=5.16, 95% CI: 2.38 - 11.21
p<0.0001

No-XG, n=67, 2y DFS=85%
XG, n=42, 2y DFS=42%

N=153 (all)

XG vs. No-XG, HR=2.89, 95% CI: 1.69 - 4.95
Log-rank p-value=5.3e-05

No-XG, n=94, 3y DFS=76%
XG, n=59, 3y DFS=36%

N=83 (no known somatic mutations)

XG vs. No-XG, HR=2.48, 95% CI: 1.12 - 5.49
Log-rank p-value=0.021

No-XG, n=47, 3y DFS=79%
XG, n=36, 3y DFS=60%
Mechanisms of TKI Resistance

37 patients with re-biopsy of tumor that progress during EGFR TKI therapy

Afatinib (BIBW 2992)

- Afatinib is an irreversible EGFR and HER2 inhibitor with preclinical activity against H1975 (L858R/T790M) \((EC_{50}: 99 \text{ nM})\)

- Designed to irreversibly bind to the ATP binding pocket of EGFR and HER2

- Highly specific for EGFR and HER2
  - EGFR \(IC_{50}: 0.50 \text{ nM}\)
  - HER2 \(IC_{50}: 14 \text{ nM}\)
LUX-Lung 3
Phase 3 trial comparing Afatinib vs Chemotherapy in EGFR mutant Patients

PFS: Common mutations (Del19/L858R)
Independent review – patients with Del19/L858R (n=308)

<table>
<thead>
<tr>
<th></th>
<th>Afatinib n=204</th>
<th>Cis/pem n=104</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS event, n (%)</td>
<td>130 (64)</td>
<td>61 (59)</td>
</tr>
<tr>
<td>Median PFS (months)</td>
<td>13.6</td>
<td>6.9</td>
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<tr>
<td>Hazard ratio</td>
<td>0.47 (0.34–0.65)</td>
<td>p&lt;0.0001</td>
</tr>
</tbody>
</table>

Number at risk
Afatinib 204 169 143 115 75 49 30 10 3 0
Cis/Pem 104 62 35 17 9 6 2 2 0 0

Interruption of TKI Therapy May Lead to Loss of Resistant Mutant Tumor Clones and Restore Sensitivity

<table>
<thead>
<tr>
<th>Histology</th>
<th>Adeno</th>
<th>Adeno</th>
<th>Adeno</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>L858R</td>
<td>L858R</td>
<td>L858R</td>
</tr>
<tr>
<td></td>
<td>TP53</td>
<td>TP53</td>
<td>TP53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EGFR TKI status</th>
<th>Sensitive</th>
<th>Resistant</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor burden</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Chemo</th>
<th>Erlotinib</th>
<th>Chemo</th>
<th>Chemo</th>
<th>Erlotinib*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timeline</td>
<td>2007</td>
<td>2008</td>
<td>2009</td>
<td>2010</td>
<td></td>
</tr>
</tbody>
</table>

Mechanism of Resistance

More complicated than resistant mutant clones or amplification
Induction of DTP and DTEP appears linked to chromatin alteration.

**A Chromatin-Mediated Reversible Drug-Tolerant Persistant State in Cancer Cell Subpopulations**

Sreenath V. Sharma,1 Diana Y. Lee,1 Bihua Li,1 Margaret P. Quinlan,1 Fumiyuki Takahashi,1 Shyamala Maheswaran,1 Uiltan McDermott,1 Nancy Azizian,1 Lee Zou,1 Michael A. Fischbach,1 Kwok-Kin Wong,2 Kathleyn Brandstetter,2 Ben Wittner,1 Sridhar Ramaswamy,1 Marie Classon,1,3,* and Jeff Settleman1,3,*

DTP: Drug-Tolerant Persisters;
DTEP: Drug-Tolerant Expanded Persisters

*Cell 2010;141:1-12*
Conclusions

1. Future pathology practice must be both diagnostic and predictive (of therapeutic outcome)

2. Predictive pathology will mostly be based on molecular markers

3. Molecular profiling will soon become routine in oncologic pathology reporting

4. Future cancer classification system must integrate molecular features of tumors

5. The FUTURE IS ALREADY HERE!
Opportunities in Pathology

MECHANISMS OF DISEASE

Cell/animal models
Human tissues
Clinical trials

Genomic tools
Molecular/cellular Biology
Cellular imaging

DIAGNOSIS

PATIENT CARE
CLINICIAN SCIENTISTS
IN MOLECULAR ONCOLOGIC PATHOLOGY
This is your future
Website: www.molecularpathology.ca