The characterization of lung cancer: molecular and pathway analysis

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Consultancy fees from
- Abbott, Amgen, AstraZeneca, BMS, EOS, GSK, Lilly, Merck-Serono, MSD, Pfizer, Roche-Genentech, Sanofi.

My talk will focus on NSCLC
Overview

- Lung cancer from histology to biology
- Molecular classification of adenocarcinomas
- Molecular classification of squamous cell carcinomas
- Towards an integrative approach
  - clonal architecture, new targets and resistance mechanisms
  - DNA repair complexity and related biomarkers
  - biomarkers of activity of immunotherapies
LUNG CANCER
Incidence = 1,600,000/yr
Mortality = 1,370,000/yr
⇒ Lethality ≈ 85%
What is lung cancer? The old perception
Current definition of cancer

- a tumor
- an organ
- a pathological sample

= A definition from the XIXth century

Significantly mutated pathways in adenocarcinoma of the lung

Ding et al. Nature 455, 1069, 2008
## Classification of Non-squamous NSCLC

<table>
<thead>
<tr>
<th>Classification</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-squamous‡</strong></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma 30–50%*</td>
<td>• Malignant epithelial tumors with glandular differentiation</td>
</tr>
<tr>
<td></td>
<td>• Subtypes are acinar, papillary, bronchoalveolar carcinoma (BAC), and solid adenocarcinoma with mucin production</td>
</tr>
<tr>
<td></td>
<td>• Usually peripherally located</td>
</tr>
<tr>
<td>Large cell carcinoma 10%*</td>
<td>• Involves large cells (subtypes are giant cell, clear cell) with large nuclei</td>
</tr>
<tr>
<td></td>
<td>• No evidence of squamous or glandular differentiation</td>
</tr>
<tr>
<td></td>
<td>• Usually peripherally located</td>
</tr>
<tr>
<td><strong>Squamous</strong></td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma 30%†</td>
<td>• Involves cells of the squamous epithelium</td>
</tr>
<tr>
<td></td>
<td>• Usually centrally located</td>
</tr>
</tbody>
</table>

*Image from www.surgical-pathology.com; †Image from http://www.lmp.ualberta.ca/resources/pathoimages/PC-S.htm; ‡Other less common subtypes of non-squamous NSCLC include adenosquamous carcinoma and sarcomatoid carcinoma.*

In daily practice, histology remains the main classifier of NSCLC.
NSCLC

80%
no clear driver or oncogenic event

20%
NSCLC
Oncogene Addicted

LARGE CELL

cisplatin + pemetrexed

ADENOCARCINOMAS
doublet + bevacizumab

SQUAMOUS
platinum doublet

ALK, ROS translocated

crizotinib

EGFR mutated

gefitinib, erlotinib afatinib
Selecting the right therapy

Cancer Patient

Tumor type

Therapy

Wrong match

The right drug for the tumor type

Wrong match

Oncologist Selects therapy based on experience histology and tumor site

Modified from D Weaver, On-Q-ity
Driver-pathways will increase in number notably because of the reduced cost of sequencing.

MacConaill L E, Garraway L A JCO 2010;28:5219-5228
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Many previous large-scale sequencing efforts in adenocarcinoma:

Ding et al 2008
623 genes in 188 Lung Adeno

Lee et al 2010
WGS of a single Lung Adeno T-N pair

Kan et al 2010
1507 genes in 57 Lung Adeno

Ju et al 2011
WGS of a single Lung Adeno T-N pair
Incidence of driver mutations in adenocarcinoma

Mutations found in 67% (IGR experience)

- No mutation detected 33%
- KRAS (28%)
- EGFR (13%)
- STK11 (10%)
- ALK - EML4 (2%)
- NRAS (2%)
- BRAF (2%)
- PDK1 (2%)
- HER2 (1%)
- KDR (1%)
- MET (1%)
- PI3K (1%)
- TOP1 (1%)
- FGFR4 (1%)
- ALK amplification (2%)

Planchard et al, European Lung Cancer Conference 2012
The “targeted therapome” in lung adenocarcinoma

ERBB2 3%
ALK fusion 4-6%
ROS fusion ~1%
RET fusion ~1%
STK11 20%
TP53 60%
KEAP1 10%

Courtesy M Meyerson

Pao and Chmielecki, Nature Reviews Cancer 2011
Actionable alterations leading to clinical benefit

**Erlotinib in NSCLC EGFR mut+**

- Deletion exon 19
- Mutation exon 21

**Crizotinib in NSCLC ALK+**

- 30% decrease

Ref: Rosell Lancet Oncol 12

Ref: Kwak NEJM 10
Missing key driver oncogenes in lung adenocarcinoma

48% lack a known “driver” oncogene mutation

38% also lack an additional focal amplification in a “driver” gene

Courtesy M Meyerson
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The Cancer Genome Atlas (TCGA): Complete cancer genome description

25 forms of cancer

- glioblastoma multiforme (brain)
- squamous carcinoma (lung)
- serous cystadenocarcinoma (ovarian)

Multiple data types

- Clinical diagnosis
- Treatment history
- Histologic diagnosis
- Pathologic report/images
- Tissue anatomic site
- Surgical history
- Gene expression/RNA sequence
- Chromosomal copy number
- Loss of heterozygosity
- Methylation patterns
- miRNA expression
- DNA sequence
- RPPA (protein)
- Subset for Mass Spec

Biospecimen Core Resource with more than 150 Tissue Source Sites

- 6 Cancer Genomic Characterization Centers
- 3 Genome Sequencing Centers
- 7 Genome Data Analysis Centers

Data Coordinating Center

Courtesy M Meyerson
Focal copy number alterations in squamous cell lung carcinoma

Amplification

Deletion

The Cancer Genome Atlas (TCGA) initiative

Courtesy M Meyerson

Nature 2012, Sept 9
FGFR1 as a relevant target in squamous cell carcinoma

The Clinical Lung Cancer Genome Project

Weiss J et al. Sci Transl Med 2010
FGR1 amplification

- 10% of SCC by CGH
- 20% of SCC by FISH (high levels > 8 copies)
- specific cell lines with FGR1 amplification sensitive to PD170374

The Clinical Lung Cancer Genome Project

Weiss J et al. Sci Transl Med 2010
Targeting FGFR family

Selective-FGFR inhibitors
- BJG398
- AZD 4547
- JNJ-42756493

Non Selective-FGFR inhibitors
- EOS3810
- Dovitinib (TKI258)

Target genes
- Negative feedback. For example, through SPRY, SEF, MAPK3 and MKP1
- Downstream transcription factors. For example, FOS, JUN and PEA3

Courtesy N Auger

Turner N, Nature Reviews Cancer, 2010
Lung SCC at 100 mg BJG398: PR

- Confirmed PR at D56 – patient presently in 7th cycle of treatment

Wolf et al. AACR 2012
SCC has a very high rate of somatic mutations
Top mutated genes in SCC

The Cancer Genome Atlas (TCGA) initiative

Courtesy M Meyerson

Nature 2012, Sept 9
Oxidative response and differentiation pathway alterations in lung SCC

The Cancer Genome Atlas (TCGA) initiative

Nature 2012, Sept 9
40 to 60% of lung SCCs have a possible therapeutic target—more if we include CDKN2A alterations—in a disease with no targeted therapies today.

Targets will need to be validated

In the clinical setting

FGFR1/2, PIK3CA and DDR2 inhibitor trials are ongoing.
Frequencies of potentially actionable/targetable genetic abnormalities present in SCC of the lung
Lung Cancer Patient in the near future

Lung nodule/metastases

Tumor Biopsy/ blood

MOLECULAR PORTRAIT

Diagnostic

Cancer? Yes/No

Prognosis

Chemotherapy sensitivity

Targeted therapy sensitivity

Need for treatment?

Which treatment and when?

New target

ALK
PI3K
FGFR1
AKT
Other Pathways

...
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Individual tumor heterogeneity: a limitation to MTA

Clone architecture in NSCLC

Meric-Bernstam F, Mills GB Nat Rev Clin Oncol 2012
Surgery T1N1

<table>
<thead>
<tr>
<th>Year</th>
<th>Adrenal gland</th>
<th>Lung</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>Tumor Bank</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>Tumor Bank</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>Tumor Bank</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>Tumor Bank</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Tumor Bank</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tissue

- Whole tumor

Treatment

- Vinorelbine cisplatinum
- Adrenal gland biopsy
- Taxol Carbo bevacizumab
- Pemetrexed
- Erlotinib
How do we move forward for patients with unidentified alterations???

- No mutation detected (33%)
- KRAS (28%)
- EGFR (13%)
- STK11 (10%)
- ALK- EML4 (2%)
- NRAS (2%)
- BRAF (2%)
- PDK1 (2%)
- HER2 (1%)
- KDR (1%)
- MET (1%)
- PI3K (1%)
- TOP1 (1%)
- FGFR4 (1%)
- ALK amplification (2%)

Optimize the use of CT and XRT
Better understand DNA repair
Active immunotherapies
Predictive biomarkers
DNA Repair Pathways Are Critical in Cancer

Normal cells

Cancer cells

Six normal DNA repair pathways

The specific pathway changes determine the best course of chemotherapy and radiation (personalized medicine)

Courtesy of D Weaver (DNAR-On-Q-ity)
DNA repair pathways

<table>
<thead>
<tr>
<th>DNA lesion</th>
<th>BER</th>
<th>NER</th>
<th>DR</th>
<th>MMR</th>
<th>HR</th>
<th>NHEJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-strand break</td>
<td>OGG1/ PARP1, PARP2</td>
<td>XPD, XPC, DDB1/XPE</td>
<td>RNA pol β, CSA, CSB</td>
<td>MSH2, MSH6, MLH1/PMS2</td>
<td>ATM, MRN complex</td>
<td>KU70, KU80</td>
</tr>
<tr>
<td>Single-base damage</td>
<td>XRCC1</td>
<td>ERCC1/XPF</td>
<td>PCNA, Pol δ, Pol ε</td>
<td>EXO1/PCNA/RCF</td>
<td>Pol δ</td>
<td>DNA PKs</td>
</tr>
<tr>
<td>Bulky lesions</td>
<td>Pol β, PCNA, FEN 1</td>
<td>PCNA</td>
<td>Ligase I</td>
<td>Pol δ</td>
<td>Pol δ</td>
<td>Pol μ</td>
</tr>
<tr>
<td>Crosslinks</td>
<td>Ligase III</td>
<td>Ligase I</td>
<td>Ligase I</td>
<td>Ligase I/ Ligase IV</td>
<td>Ligase I</td>
<td>Ligase IV</td>
</tr>
</tbody>
</table>

Postel-Vinay S, NRCAO 2012
Adenocarcinomas express low levels of PARP1 and BRCA1/2 compared to squamous histology

Expression values (normalized) / histology

Adk

Squamous
CHEK1 expression according to histotype

**CHEK1 according to tumor type**
- Bild data (n=111)
- Adeno = 58, Sqamous = 53
  - $p = 2.54 \times 10^{-3}$

**CHEK1 according to tumor type**
- Kim data (n=138)
- Adeno = 62, Sqamous = 76
  - $p = 2.17 \times 10^{-8}$

F Commo
Application of a functional DNA repair assay on fresh lung tumor specimens

**Biopsies:** lung tumors

- Cultivate tumor cells *in vitro* after dissociation of the tissue
- Treat with cisplatin
- Measure DNA repair efficiency of the cells over time (dot blot)

**Diagram:**
- Biopsies: lung tumors
- Dissociation culture
- Dot-blot
- IF
- Viability

**Treatment time (25 µM):**
- 0h
- 1h
- 2h
- 6h
- 16h
- 24h

**Post-treatment time:**
- Repair
- Repair
- Repair

**Cells:** P1, P2, P3
Hanahan
Weinberg
Cell 2011
<table>
<thead>
<tr>
<th>Target</th>
<th>Antibody</th>
<th>Molecule</th>
<th>Company</th>
<th>Development stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-1</td>
<td>BMS-936558/MDX-1106/ONO-4538</td>
<td>Fully human IgG4 mAb</td>
<td>Bristol-Myers Squibb</td>
<td>Phase II multiple tumors</td>
</tr>
<tr>
<td></td>
<td>CT-011</td>
<td>Humanized IgG1 mAb</td>
<td>CureTech</td>
<td>Phase II multiple tumors</td>
</tr>
<tr>
<td></td>
<td>MK-3475</td>
<td>Humanized IgG4 mAb</td>
<td>Merck</td>
<td>Phase I</td>
</tr>
<tr>
<td></td>
<td>AMP-224</td>
<td>B7-DC/IgG1 fusion protein</td>
<td>Amplimmune</td>
<td>Phase I</td>
</tr>
<tr>
<td>PD-L1</td>
<td>MDX-1105/BMS-936559</td>
<td>fully human IgG4 mAb</td>
<td>Bristol-Myers Squibb</td>
<td>Phase I</td>
</tr>
<tr>
<td>PD-L1</td>
<td>MPDL3280A</td>
<td>fully human IgG1 mAb</td>
<td>Genentech</td>
<td>Phase I</td>
</tr>
</tbody>
</table>

Courtesy G Giaccone
## Clinical Activity of PD1 Antibody

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BMS-936558 Dose, mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><em><em>ORR, No. patients</em> (%)</em>*</td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
<td>0 (n=5)</td>
</tr>
<tr>
<td>Non-squamous</td>
<td>0 (n=12)</td>
</tr>
</tbody>
</table>

**3/18 =33% (95%CI 13%-59%)**

**7/56 =12.5% (95%CI 5%-24%)**

<table>
<thead>
<tr>
<th>SD ≥24 wk, No. patients (%)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous</td>
<td>0 (n=5)</td>
<td>0 (n=12)</td>
<td>0 (n=31)</td>
</tr>
<tr>
<td>Non-squamous</td>
<td>1 (8)</td>
<td>2 (15)</td>
<td>2 (6)</td>
</tr>
</tbody>
</table>

**PFSR at 24 wk, (%)**

<table>
<thead>
<tr>
<th></th>
<th>3/18</th>
<th>5/15</th>
<th>2/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous</td>
<td>0 (n=5)</td>
<td>50 (n=12)</td>
<td>43 (n=31)</td>
</tr>
<tr>
<td>Non-squamous</td>
<td>14 (n=12)</td>
<td>37 (n=31)</td>
<td>21 (n=31)</td>
</tr>
</tbody>
</table>

Brahmer et al, ASCO 2012, Abs 7509

Correlation of PD-L1 expression in pretreatment tumor biopsies with clinical outcomes

42 pts include 18 MEL, 10 NSCLC, 7 CRC, 5 RCC, and 2 CRPC.

*2 pts still under evaluation

ASCO 2012, Abs 2501
The path of survival of NSCLC patient

MOLECULAR PORTRAIT

- Sequencing
- CGH, FISH

Specific mutation/amplification

- EGFR → Gefitinib/Erlotinib/Afatinib
- ALK, ROS → Crizotinib
- HER2 → Trastuzumab
- FGFR1 → BJG398, AZD4547, EOS3810

Lung Cancer patient

No driver → Histology-driven Tx

No portrait

Courtesy D Planchard
The path of survival of NSCLC patient

Lung Cancer patient

No driver

Histology-driven Tx

Standard therapy: Ct and XRT

New immune-checkpoints

DNA dysfunctionality analysis

YES

PARPi

CHKi

ATRi

ATMi