Lung Cancer:
Molecular Biology 2015
Disclosure

• Ownership Interest
  – Foundation for Clinical and Applied Cancer Research
  – Foundation Medicine

• Consultant/Advisory Boards
  – Roche, BI, Pfizer, Novartis, Celldex, AstraZeneca, Astellas, Amgen

• Honoraria from Speakers Bureau
  – Roche, BI, Pfizer, Novartis, AstraZeneca, Amgen, BMS

• Other Commercial Research Support
  – Roche, BI, Novartis, BMS

• Research Grants
  – Roche, BI, Novartis
Only a Fraction of Molecular Aberrations Are Clinically Relevant

Sun

Whole genome

Protein-encoding transcriptome

Jupiter

Earth

Pluto

Predictive abnormalities

Gerber. ASCO 2014.
Why believe?
Prevalence of Somatic Mutations Among Major Cancer Types

Gene Expression Subtypes Integrated With Genomic Alterations

Evolution of Identification of Genomic Alterations in Lung Adenocarcinoma (a Decade After)

1984 - 2003

- No known genotype
- KRAS

2009

- No known genotype
- KRAS
- EGFR
- BRAF
- ALK
- PIK3CA
- HER2

2004

- No known genotype
- KRAS
- EGFR
- NTRK1
- RET
- MET
- ROS1

2014

- No known genotype
- KRAS
- EGFR
- ALK
- HER2
- BRAF
- PIK3CA

Janne. ASCO 2014.
Evolution of Molecular Testing of Lung Adenocarcinoma

2004
- EGFR only
  - Fragment analysis for Exon 19 Δ
  - PCR-RFLP for Exon 21 mutations

2006
- EGFR + KRAS
  - Sanger Sequencing

2009
- Sequenom
  - Multiplex mass-spec. genotyping for hotspot mutations in 8 oncogenes (EGFR, KRAS, BRAF, MEK1, NRAS, HER2, PIK3CA, AKT1)
  - + EGFR Exon 19 Δ

2012
- Sequenom genotyping
  - +EGFR Ex 19 Δ
  - +ALK FISH

2013
- Sequenom genotyping
  - +ALK FISH + IHC pre-screen
  - +RET FISH
  - +ROS1 FISH

2014
- Next-generation sequencing:
  - MSK-IMPACT™
    (Integrated Mutation Profiling of Actionable Cancer Targets)
    Targeted sequencing of 341→410 key cancer genes
    (mutations, small insertions/deletions, CNAs, select rearrangements)

Ladanyi et al. ASCO 2015.
Lung Adenocarcinoma

(numbers based on approximate US annual incidence of 100,000)

Mutually exclusive mutations in kinases and other growth signaling molecules

All targetable (except KRAS)

Even small subsets (e.g. 1%) represent 1000’s of patients.

KRAS 29,000/yr
EGFR 20,000/yr
No known driver oncogene

BRAF – 1500/yr
ERBB2 – 2000/yr
ALK – 4000/yr
ROS1 – 1000/yr
RET – 1000/yr
Others: MEK1, AKT1, NRAS

Ladanyi et al. ASCO 2015.
**Lung Adenocarcinoma**

(numbers based on approximate US annual incidence of 100,000)

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- **RET**: 1000/yr
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Ladanyi et al. ASCO 2015.
Lung Adenocarcinoma
(numbers based on approximate US annual incidence of 100,000)

- KRAS 29,000/yr
- EGFR 20,000/yr
- No known driver oncogene
- SMALL IN-FRAME DELETIONS AND INSERTIONS
- BRAF – 1500/yr
- ERBB2 – 2000/yr
- ALK – 4000/yr
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- RET – 1000/yr
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Ladanyi et al. ASCO 2015.
Lung Adenocarcinoma

(numbers based on approximate US annual incidence of 100,000)

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- **No known driver oncogene**

Gene Fusions:

- **BRAF** – 1500/yr
- **ERBB2** – 2000/yr
- **ALK** – 4000/yr
- **ROS1** – 1000/yr
- **RET** – 1000/yr

Others: **MEK1, AKT1, NRAS**

Ladanyi et al. ASCO 2015.
Lung Adenocarcinoma Molecular Diagnosis: Work Flow
Survival of Pts With Drivers: Targeted Therapy Versus No Targeted Therapy

---

**Group** | **N** | **Median Survival (95% CI)**
--- | --- | ---
Driver, no targeted therapy (A) | 313 | 2.4 years (1.8 to 2.9)
No driver (B) | 361 | 2.1 years (1.8 to 2.5)
Driver, targeted therapy (C) | 264 | 3.5 years (3.2 to 4.6)

---

NSCLC Mutation Consortium. IASLC 2013.
Survival With Five Most Frequent Oncogenic Drivers (n=526)

<table>
<thead>
<tr>
<th>Altered Gene</th>
<th>N</th>
<th>Median Survival (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR (sensitizing)</td>
<td>140</td>
<td>4.0 years (2.7 to 5.4)</td>
</tr>
<tr>
<td>EGFR (other)</td>
<td>50</td>
<td>3.3 years (2.2 to 6.2)</td>
</tr>
<tr>
<td>ALK</td>
<td>73</td>
<td>4.3 years (3.0 to NA)  p=0.001</td>
</tr>
<tr>
<td>KRAS</td>
<td>231</td>
<td>2.4 years (1.9 to 3.6)</td>
</tr>
<tr>
<td>Two Drivers</td>
<td>32</td>
<td>2.0 years (1.6 to 4.6)</td>
</tr>
</tbody>
</table>
Proportion of Each Item Comprising Mean Total Direct Medical Costs

Tumour Heterogeneity

Mayerson et al. IASLC 2013.

Known drivers in COSMIC-identical mutation
Drivers in COSMIC-different mutation
Known driver DNA copy number events
Other shared DNA copy number events

LO2

FGFR1 gain
Chr 5q, 8p, 13, 15, 16 loss

GL

chr3p loss
chr4: CN LOH

FGFR1 loss
ZFHX4
SOX3
MITF

PRDM16

ELN

L2

adenocarcinoma

squamous

copy number:

1
2
3
4
>4
>8

Ubiquitous
Heterogeneous

FGFR1
focal amp in R1 and R2
Complex Rearrangements
<table>
<thead>
<tr>
<th>Fusion Type</th>
<th>#</th>
<th>Frequency</th>
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</thead>
<tbody>
<tr>
<td>ALK</td>
<td>9</td>
<td>4%</td>
</tr>
<tr>
<td>RET</td>
<td>4</td>
<td>2.6%</td>
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<tr>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ROS1</td>
<td>5</td>
<td>3.5%</td>
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<tr>
<td></td>
<td>1</td>
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<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>OTHER</td>
<td>1</td>
<td>1.8%</td>
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<td></td>
</tr>
</tbody>
</table>

Ladanyi et al. ASCO 2015.
ALK
Signaling Via Human ALK

**ALK Ligands**
- Heparin-binding proteins
- Pleiotrophin
- Midkine

**ALK Tyrosine Motifs**
- Insulin receptor substrate-1
- Src homology-2
- Phospholipase C-3

**Cell proliferation**
- Survival
- Migration
- Alterations in cytoskeletal rearrangement

**Apoptosis**

**Pathways**
- JAK/STAT3 pathway
- PI3K/AKT pathway
- PLCγ pathway
- RAS/MAPK pathway

**Signaling Via Human ALK**

[Diagram showing signaling pathways and ligands]
- Gain of function mutations
- 9 variants detected by RT-PCR
- The most common are variants 1, 3 and 6

ALK Fusion Splice Variants

TKI sensitivity: v2>v1=v3b>v3a
ALK/ELM4 Is a Potent Oncogenic Driver

Inhibition of ALK leads to dramatic in vivo tumour regression

Series of Patients With Crizotinib-Resistant ALK+ NSCLC

- Re-biopsies of eighteen pts (Katayama, et al) and nineteen pts (Doebele, et al) with acquired ALK TKI resistance
- ALK kinase domain mutations found in almost 30% of samples
- No SCLC transformation described

### Analogous “Gatekeeper” Mutations to Crizotinib

<table>
<thead>
<tr>
<th>ALK Resistance Mutation</th>
<th>Location within the kinase domain</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1196M</td>
<td>Gatekeeper mutation</td>
<td>Katayama, Sakamoto 2011</td>
</tr>
<tr>
<td>C1156Y</td>
<td>N-terminal to the αC-helix</td>
<td>Choi, Katayama 2010, 2011</td>
</tr>
<tr>
<td>L1152R</td>
<td>N-terminal to the αC-helix</td>
<td>Sasaki, 2011</td>
</tr>
<tr>
<td>F1174L</td>
<td>N-terminal to the αC-helix</td>
<td>Bresler 2011, Sasaki 2010</td>
</tr>
<tr>
<td>G1269A</td>
<td>ATP-binding pocket</td>
<td>Doebele, 2012</td>
</tr>
<tr>
<td>S1206Y</td>
<td>Solvent front</td>
<td>Katayama, 2012</td>
</tr>
<tr>
<td>G1202R</td>
<td>Solvent front</td>
<td>Katayama, 2012</td>
</tr>
<tr>
<td>1151Tins</td>
<td>N-terminal to the αC-helix</td>
<td>Katayama, 2012</td>
</tr>
<tr>
<td>D1203N</td>
<td>-</td>
<td>Doebele, 2012</td>
</tr>
<tr>
<td>F1174C</td>
<td>-</td>
<td>Doebele, 2012</td>
</tr>
</tbody>
</table>

Strategies to Overcome AR to Crizotinib

ALK DOMINANT MECHANISM (50%)

ALK NON-DOMINANT MECHANISM (50%)

Resistance mutations

EML4 ALK
L1196M
C1156Y

Second generation ALK inh

AP26113 dual EGFR/ALK inh

HSP90 inh

Inadequate CNS penetration?

New G1202R Mutation Confers Resistance to Second Generation ALK Inhibitors

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>EML4–ALK sequence at crizotinib resistance</th>
<th>EML4–ALK sequence at ceritinib resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGH011</td>
<td>S1206Y</td>
<td>G1202R</td>
</tr>
<tr>
<td>MGH015</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>MGH023</td>
<td>WT</td>
<td>F1174C</td>
</tr>
<tr>
<td>MGH034</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>MGH049</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>MGH051</td>
<td>WT</td>
<td>G1202R</td>
</tr>
<tr>
<td>MGH057</td>
<td>N/A</td>
<td>WT</td>
</tr>
<tr>
<td>MGH061</td>
<td>WT</td>
<td>WT</td>
</tr>
<tr>
<td>JFCR013</td>
<td>N/A</td>
<td>WT</td>
</tr>
<tr>
<td>JFCR021</td>
<td>G1269A (right lung)</td>
<td>F1174Y (left lung) and G1202R (right lung)</td>
</tr>
</tbody>
</table>

MGH011 lung CT scan

Baseline | After 8 weeks of crizotinib | After 34 months of ceritinib | After 12 weeks of ceritinib | After 15 months of ceritinib

EML4–ALK sequence: WT | S1206Y | G1202R

ROS1
ROS1 Population

- Receptor tyrosine kinase of the insulin receptor family (located on chromosome 6q22)
- ROS1 fusions identified as potential driver mutations in a cell line (HCC78; SLC34A2-ROS1) and an NSCLC patient sample (CD74-ROS1)
- HCC78 cell line is among the 10 most sensitive cell lines to ALK inhibitor TAE684
Signalling Pathways Activated by ROS1

ROS1 Rearrangements in NSCLC

- Present in \( \sim 1\% \) of NSCLC cases (also found in some GBMs and cholangiocarcinomas)
- Enriched in younger never or light smokers with adenocarcinoma histology
- Minimal overlap with other oncogenic drivers

Diagnostic “Break-Apart” FISH Assay for ROS1 Rearrangement

MET
MET Alterations in Lung Adenocarcinoma

TCGA: summary of new driver mutations defined in additional 13% of lung adenoCA

$\Rightarrow$ Only approx 25% remain “driver mutation”-negative
Jeong-Sun Seo, et al. Genome Res. September 2012

- **MET** mutations causing exon 14 skipping: loss of juxtamembrane domain site for CBL-E3-ligase
- reported in about 3-4% of lung adenocA

![Graph showing MET expression and exon skipping](image)

MET Alterations in Lung Adenocarcinoma

DDR2
Mutations in DDR2 were detected in 3-4% of squamous-type NSCLC. DDR2 mutations were shown to:
- be oncogenic
- confer sensitivity to dasatinib in vitro & in vivo
- “gatekeeper” mutation confers resistance

A novel S768R mutation was detected in tumour sample from Index Pt #1.

Dasatinib modeled into ATP-binding site of DDR2.

RET
**RET Rearrangements in Lung Cancer**

- Fusion partners: **KIF5B, CCDC6, NCOA4 & TRIM33**
- Present in **1.2-1.4%** of NSCLC cases (1.2-1.7% of ADC)
- Associated with younger age at presentation, never or light smoking, small size (<3cm) of primary, N2 involvement, poor differentiation (solid pattern) and mucin production
- Possible response to **cabozantinib** and/or other multitargeted TKIs

**Table:**

<table>
<thead>
<tr>
<th>RET Fusions</th>
<th>Never-Smokers Pan-Negative AdenoCAs</th>
<th>Pan-Negative NSCLCs</th>
<th>Unselected NSCLCs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15%</td>
<td>6%</td>
<td>2%</td>
</tr>
</tbody>
</table>

95% CI [3-27%]

n = 5/34

Lipson et al. Nature 2012

Wang et al. CCR 2012

---

Targeting *RET* Fusions: Cabozantinib

**Trial Update** (NCT01639508)

- 8 patients: **ORR 57%** (4 PRs of 7 evaluable)
- NGS performed in 3 patients with PR: no *MET* amplification
KRAS Mutation Subtypes in NSCLC

COSMIC

Never-Smokers

G12C 42%
G12V 20%
G12D 17%
G12A 7%
G12S 5%
G12R 2%
G13D 2%
G13C 3%
other 2%

Smokers

G12C 39%
G12V 21%
G12D 17%
G12A 10%
G12S 3%
G13D 3%
G12F 2%
G12R 1%
G12A 8%
G12S 2%

Yu: ASCO2013

G12C 42%
G12V 19%
G12D 56%
G12A 13%
G12S 2%
HER2
**HER2-Driven Lung Cancers**

- Mutations in the HER2 intracellular domain, predominantly **insertions in exon 20**, occur in **1-2%** of lung cancers (Kris. IASLC 2013 [n=920]; Barlesi. ASCO 2013 [n=10])

- In the Fudan/Vanderbilt series (Li. JTO 2012) HER2 mutations were **4%** (8/224) and **50%** also HER2 amplified

- Mutations also detected in the HER extracellular domain (Greulich. PNAS. 2012)

![Mutations identified (n=25)](chart.png)

NRG1
Neuregulin (NRG1)

- Encodes a protein that is part of the EGF family of proteins
- Diverse functions in the development of the nervous system & multiple roles in embryogenesis
- The protein binds to and activates the erbB family of RTK (erbB2, erbB3 & erbB4), functioning both as heterodimers and monodimers

NRG1 Rearrangements in Lung Cancer

- Fusion partners: CD74 and SLC34A2
- Associated with female gender & never smoking history
- Present in 7-27% of invasive mucinous adenocarcinomas
- Leading to ERBB2/ERBB3 and downstream signalling activation

NRG1 Rearrangements Are Seen Exclusively in Invasive Mucinous Adenocarcinomas

Molecular alterations identified in invasive mucinous adenocarcinoma

- KRAS: 31%
- NRG1: 59%
- None or other: 9.5%

MEK1
MAP2K1 (MEK1) Mutations Define a Distinct Subset of Lung Adenocarcinoma Associated With Smoking

MAP2K1 (MEK1) hotspot mutations as the primary driver in \(\frac{26}{5330}\) lung adenoCa patients (0.5%) tested at two institutions (MSKCC: 18, VICC: 8).
Immune Biomarkers
POPLAR: PD-L1 Expression Subgroups

Interim OS

Subgroup (% of enrolled patients)
- TC3 or IC3 (16%)
- TC2/3 or IC2/3 (37%)
- TC1/2/3 or IC1/2/3 (68%)
- TC0 and IC0 (32%)

ITT (N = 287)

Hazard Ratio$^a$

$^a$Unstratified HR for subgroups and stratified HR for ITT.
Data cut-off Jan 30, 2015.

Kerr et al. ASCO 2015.
PD-L1 Expression on TC and IC Is a Potential Predictive Biomarker for Atezolizumab in NSCLC

**Intrinsic** PD-L1 expression in tumor cells (TC)

**Adaptive** PD-L1 expression in tumor-infiltrating immune cells (IC)

PD-L1 expression levels and TC/IC overlap in POPLAR

- **SP142 IHC assay** is sensitive and specific for PD-L1 expression on both TC and IC.
- Distinct TC and IC sub-populations exist at each of four cutoff levels\(^a\) (Gettinger et al., ASCO 2015).
- PD-L1 expression on TC and IC was independently predictive of response (Horn et al., ASCO 2015).

:\(^a\)TC scored as percentage of tumor cells and IC scored as percentage of tumor area. TC3 or IC3 = TC ≥ 50% or IC ≥ 10% PD-L1+; TC2/3 or IC2/3 = TC or IC ≥ 5% PD-L1+; TC1/2/3 or IC1/2/3 = TC or IC ≥ 1% PD-L1+; TC0 and IC0 = TC and IC < 1% PD-L1+, respectively.
**PDL1 Expression in Paired EGFR Mutant Biopsies**

**Gainor et al. Abst #8013 ASCO 2015**

**Minimal change in PDL1 (-) EGFR mutant pts post TKI therapy**

Rise in PDL1 around 5%

Kerr et al. ASCO 2015.

Gainor et al. ASCO 2015.
Response to Anti-PDL1: Proliferating CD8+ T Cells Localised in Tumour

• High pre-treatment CTLA4 marks presence of activated T cells

Responders:

a. Markers of pre-existing immunity/T cells suppressed by PDL1
b. Reinvigorated by anti-PDL1 antibody treatment
Tumour Staining for PD-L1 and Response to PD1 Checkpoint Blockade

Phase I solid tumour and melanoma trials

Lung cancer trials

Percent Response

Topalian Nivolumab Phase I trial all tumors NEJM 2012
Grosso Nivolumab Phase I trial Melanoma cohort ASCO 2013
Herbst MPDL3280a Phase I trial all tumors ASCO 2013
Robert Nivolumab Phase 3 trial Melanoma NEJM 2015
Antonia Nivolumab Phase I trial NSCLC cohort WCLC 2013
Gettinger Nivolumab CA012 Phase I 1st Line in NSCLC ASCO 2015
Rizvi Nivolumab CA063 Phase 2 in SCC NSCLC Lancet Oncology 2015

PDL1 Positive ORR  PDL1 Negative ORR

Chow. ASCO 2015.
PDL1 Status and Response to MPDL380a (Anti-PDL1)

Ghandi et al. AACR 2014/15.
PD-L1 Expression in Non–Small-Cell Lung Cancers: Pembrolizumab

- Score of less than 1%
- Score of 1 to 49%
- Score >50%

CheckMate 017

OS by PD-L1 Expression

Spigel. ASCO 2015.
Mutation Burden, Clinical Response, and Factors Contributing to Mutation Burden

![Bar chart showing mutation burden and clinical response](chart)

- **Study ID**: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34
- **Cohort**: V, D, D, V, D, V, D, D, V, D, D, V, D, D, D, V, V, V, V, V, D, V, D, D, D
- **Objective Response**: PR, PR, PD, SD, PR, PR, PD, SD, PR, PR, SD, PD, PD, PD, SD, PD, PD, PD, SD, PD, PD, SD, PR, PD, PD, PD
- **PFS (months)**: 8, 14, 2, 8, 14, 16, 2, 4, 15, 13, 4, 8, 3, 3, 10, 27, 10, 8, 2, 2, 1, 2, 2, 4, 6, 8, 4, 2, 2, 6
- **Ongoing response**: ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ ++ +
Mutational Landscape and Response to Pembrolizumab in NSCLC

PFS
High burden (n=17) vs. low burden (n = 17)

High mutation burden >200 mutations
(HR 0.19, 95% CI 0.08-0.47 log-rank P = 0.0004)

Cut off: 200 mutations

Low mutation burden <200 mutations

Chow. ASCO 2015.

LET'S WORK
ONCOLOGY FROM BOEHRINGER INGELHEIM
Boehringer Ingelheim
Molecular Smoking Signature Is Significantly Associated With Improved PFS in NSCLC Patients Treated With Pembrolizumab

PFS in tumours characterised as TH by molecular smoking signature classifier compared to TL tumours (HR 0.15; 95% CI, 0.06-0.39; log-rank $P=0.0001$)
Frequently Altered Genes in Small-Cell Lung Cancer

26,406 somatic mutations

30% (7,977) of these mutations were protein-altering

- 7,154 missense
- 536 nonsense
- 12 stop loss
- 243 essential splice site
- 32 protein-altering insertion and/or deletion (indel)
- 2,674 synonymous
- 11,460 intronic
- 4,295 other types

~53% of the somatic mutations identified are likely to have functional consequences

Hot spots
- TP53 (75–90%)
- RB1 (60–90%)
- PTEN (2–4%)
- PI3K
- EGFR
- KRAS

Amplification
- MYC family members
- EGFR
- BCL2
- Loss of RASSF1A, PTEN and FHIT

SOX2 as a Frequently Amplified Gene in Small-Cell Lung Cancer

- The mutations in SOX family members were mutually exclusive.
- SOX2 in particular is a key factor in the maintenance of pluripotency and self-renewal of stem cells.
- High levels of amplification (copy number of ≥4) of SOX2 in ~27%.
- Expression of SOX2 was strongly correlated with increased gene copy number and with clinical stage.

Somatic Driver Mutations of Small-Cell Lung Cancer

Extremely high mutation rate of 7.4 ± 1 protein-changing mutations per million base pairs

Recently Reported Candidate Driver Genes in SCLC

- Genetic alterations in PI3K pathway were detected in 40% (alterations were mutually exclusive)
- MYC is a potential modulator of SCLC

<table>
<thead>
<tr>
<th>Gene</th>
<th>This study (n=51)</th>
<th>Peifer et al(^1) (n=27)</th>
<th>Rudin et al(^2) (n=30)</th>
<th>Peifer+Rudin+This study (n=108)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mutated case</td>
<td>Frequency (%)</td>
<td>Mutated case</td>
<td>Mutated case</td>
</tr>
<tr>
<td>TP53</td>
<td>41</td>
<td>80.4</td>
<td>24</td>
<td>25</td>
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<tr>
<td>RB1</td>
<td>21</td>
<td>41.2</td>
<td>18</td>
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<td>SLIT2</td>
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