NON-SMALL CELL LUNG CANCER: 70% PRESENT IN ADVANCED STAGE
2015 Classification: Impact on Management of Advanced Lung Cancer Patients

- Criteria/terminology for small bx/cytology
- More accurate histologic subtyping
- Strategic management of small tissues
- Streamlining workflow for molecular testing
- Need for local multidisciplinary team
THERAPEUTIC ADVANCES IMPACTED NEED FOR MORE ACCURATE HISTOLOGIC DIAGNOSIS AND MOLECULAR TESTING

- Predictive of response
  - *EGFR* mutation (adenocarcinoma) – TKI’s
  - Adenocarcinoma or NSCC-NOS – pemetrexed
  - *ALK* fusion (adenocarcinoma) - crizotinib

- Predictive of toxicity
  - Bevacizumab – contraindicated in life-threatening hemorrhage in squamous carcinoma
CLASSIFICATION OF LUNG CANCER NOW REQUIRES GENETIC TESTING

- EGFR: 17%
- HER2: 2%
- KRAS: 21%
- RET fusions: 2%
- ROS1 fusions: 2%
- ALK fusions: 2%
- BRAF V600E: 1%
- MET exon 14: 3%

Other: 50%

MSK-IMPACT data, May 2015

Courtesy of Greg Riely
# MOLECULAR TARGETED THERAPY

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR</strong></td>
<td>Erlotinib</td>
</tr>
<tr>
<td></td>
<td>Afatinib</td>
</tr>
<tr>
<td><strong>ALK fusions</strong></td>
<td>Crizotinib</td>
</tr>
<tr>
<td></td>
<td>Ceritinib</td>
</tr>
<tr>
<td><strong>BRAF V600E</strong></td>
<td>Dabrafenib</td>
</tr>
<tr>
<td><strong>ROS1</strong> fusions</td>
<td>Crizotinib</td>
</tr>
<tr>
<td><strong>RET</strong> fusions</td>
<td>Cabozantinib</td>
</tr>
<tr>
<td><strong>MET</strong> splice site Exon 14 mutations</td>
<td>Cabozantinib (and crizotinib)</td>
</tr>
</tbody>
</table>

*Courtesy of Greg Riely*
Response to EGFR TKI

Day 1

Day 5
TARGETABLE GENETIC CHANGES IN SQUAMOUS CELL CARCINOMA

- PTEN
- FGFR1 AMP (20%)
- PIK3CA
- AKT1
- DDR2
- UNKNOWN (34%)

Evolution of molecular testing of lung adenocarcinomas at MSKCC

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

- 2006
  - Sequenom MassARRAY®
  - Multiplex mass-spect. genotyping for hot-spot mutations in 8 oncogenes
  - (EGFR, KRAS, BRAF, MEK1, NRAS, HER2, PIK3CA, AKT1)
    - + EGFR Exon 19 Δ

- 2009
  - EGFR
  - + KRAS
  - Sanger Sequencing
  - + EGFR Ex 19 Δ
  - + ALK FISH

- 2012
  - Sequenom MassARRAY®
    - + EGFR Ex 19 Δ
    - + ALK FISH + IHC pre-screen
    - + RET FISH
    - + ROS1 FISH

- 2013
  - 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)
      - “rapid EGFR/ALK”:
        - ALK-D5F3 IHC
        - EGFR-L858R IHC
        - EGFR Ex 19

- April 2014

-Courtesy of Natasha Rekhtman
Initial Therapy of Advanced Adenoca or NSCLC-NOS

- Adenocarcinoma
  - Large cell ca
  - NSCLC-NOS

- EGFR Mutation
  - Exon 19 del
  - Exon 21 L858R, L861X
  - Exon 18 G719A/S

- Neg EGFR mut
  - Pos ALK fusion

- Neg EGFR mut
  - Neg ALK fusion

- Unknown EGFR Mutation & ALK Status

- Erlotinib/Gefitinib
  - ±
  - Pem/Bev/Cis

- Crizotinib

- Pemetrexed
  - Bevacizumab
  - Cisplatin

Modified from Mark Kris, Thoracic Oncology, MSKCC
Initial Therapy of Advanced Adenocarcina or NSCLC-NOS

- Adenocarcinoma

Could add ROS1 fusion, BRAF mutation, RET fusion, MET splice site exon14 mutation to this algorithm

- EGFR Mutation
  - Exon 19 del
  - Exon 21 L858R, L861X
  - Exon 18 G719A/S

- Neg EGFR mut
  - Pos ALK fusion

- Neg EGFR mut
  - Neg ALK fusion

- Unknown EGFR Mutation & ALK Status

- Erlotinib/Gefitinib
  - ±
  - Pem/Bev/Cis

- Crizotinib

- Pemetrexed
  - Bevacizumab
  - Cisplatin

Modified from Mark Kris, Thoracic Oncology, MSKCC
LUNG ADENOCARCINOMA

CLASSIFICATION IN SMALL BIOPSY AND CYTOLOGY SPECIMENS

Because this was never addressed by WHO, by necessity other histologies needed to be addressed
SMALL BIOPSY/CYTOLOGY LUNG CANCER DIAGNOSIS: IN USA OVER 133,000 CASES IN 2016

- 2016: ACS estimates for USA:
  - 224,390 Lung Cancers
    85% NSCLC = 190,731 (15% SCLC)

- 70% Advanced Stage = 133,512
  - Unresectable: Diagnosed by small biopsies/cytology
PHASE III STUDY COMPARING CISPLATIN PLUS GEMCITABINE WITH CISPLATIN & PEMETREXED IN ADVANCED NSCLC

PHASE III STUDY COMPARING CISPLATIN PLUS GEMCITABINE WITH CISPLATIN & PEMETREXED IN ADVANCED NSCLC


IN THIS STUDY APPROXIMATELY 20% OF CASES REPRESENT NSCLC-NOS
PSEUDOSQUAMOUS SOLID ADENOCARCINOMA

EGFR Exon 19 Deletion

TTF-1

Mucicarmine
PSEUDOKERATINIZING ADENOCARCINOMA

TTF-1

p40
## 2015 WHO Terminology for Small Biopsies and Cytology

<table>
<thead>
<tr>
<th>2015 WHO Resections</th>
<th>Small Biopsy/Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADENOCARCINOMA</strong></td>
<td><strong>Morphologic adenocarcinoma patterns clearly present:</strong> Adenocarcinoma, describe identifiable patterns present</td>
</tr>
<tr>
<td>Lepidic</td>
<td></td>
</tr>
<tr>
<td>Acinar</td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td></td>
</tr>
<tr>
<td>Micropapillary</td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td></td>
</tr>
<tr>
<td>No 2004 WHO counterpart – most will be solid adenocarcinomas</td>
<td><strong>Morphologic adenocarcinoma patterns not present (supported by special stains; i.e. TTF-1 +; p40 -):</strong> Non-small cell carcinoma, favor adenocarcinoma</td>
</tr>
<tr>
<td><strong>SQUAMOUS CELL CARCINOMA</strong></td>
<td><strong>Morphologic squamous cell patterns clearly present:</strong> Squamous cell carcinoma</td>
</tr>
<tr>
<td>Keratinizing</td>
<td></td>
</tr>
<tr>
<td>Nonkeratinizing</td>
<td></td>
</tr>
<tr>
<td>Basaloid</td>
<td></td>
</tr>
<tr>
<td>No 2004 WHO counterpart</td>
<td><strong>Morphologic squamous cell patterns not present (supported by stains; i.e. p40+, TTF-1 -):</strong> Non-small cell carcinoma, favor squamous cell carcinoma</td>
</tr>
<tr>
<td><strong>LARGE CELL CARCINOMA</strong></td>
<td>Non-small cell carcinoma, not otherwise specified (NOS)</td>
</tr>
</tbody>
</table>
Nonsmall cell carcinoma, favor squamous cell carcinoma

P40 (TTF-1 was negative)
NONSMALL CELL CARCINOMA, FAVOR ADENOCARCINOMA

TTF-1 (p40 was negative)
IMMUNOHISTOCHEMICAL MARKERS

- **ADENOCARCINOMA (ONE MARKER)**
  - TTF-1 (best), Napsin, PE-10

- **SQUAMOUS CARCINOMA (ONE MARKER)**
  - p40 (best), p63, CK5/6, 34βE12
  - Desmocolin-3 (need more testing)

- Cocktails – nuclear/cytoplastic antibodies
  - Adenoca – TTF-1/Napsin
  - Squamous – p63/CK5/6
LIGHT MICROSCOPY

SQUAMOUS CELL CARCINOMA 20-30%

NSCLC-NOS 20-40%

ADENO-CARCINOMA 40-50%

FORMER NSCLC-NOS: 20-40% OF NSCLC

NEW CLASSIFICATION

NSCLC-NOS

Goal <5%
IMMUNOHISTOCHEMISTRY FOR MUTATION/FUSION SPECIFIC ANTIBODIES

- ALK
- EGFR
  - EGFR L858R
  - EGFR E746
- ROS1
ALK Rearranged Adenocarcinoma

ALK IHC (D5F3)

ALK FISH
EGFR EXON 21 L858R MUTATION SPECIFIC AB

EGFR EXON 19 DELETION MUTATION SPECIFIC AB

EGFR MUTATION SPECIFIC ANTIBODIES

- Exon 19 deletion
  - All 20 cases with 15-bp deletion were MS Ab positive (sensitivity 100%, specificity 99%)
  - 35 other than the common 15bp deletion – 49% stained positively (sensitivity 74%)

- EGFR L858R mutation
  - 17/18 cases were positive with MS Ab (sensitivity 95%, specificity 99%); better if use 2+/3+ for positive

NSCLC – FAVOR ADENOCARCINOMA TOUCH PREP CYTOLOGY
Suitability of Thoracic Cytology for New Therapeutic Paradigms in Non-small Cell Lung Carcinoma

High Accuracy of Tumor Subtyping and Feasibility of EGFR and KRAS Molecular Testing

Natasha Rekhtman, MD, PhD,* Suzanne M. Brandt, MD,* Carlie S. Sigel, MD,* Maria A. Friedlander, MPA, CT (ASCP),* Gregory J. Riely, MD, PhD,† William D. Travis, MD,* Maureen F. Zakowski, MD,* and Andre L. Moreira, MD, PhD*

J Thoracic Oncol 6:451-8, 2011
INVASIVE MUCINOUS ADENOCARCINOMA CYTOLOGY DRUNKEN HONEYCOMBING
Molecular Processing: 1 vs 2 Blocks

- **Two Block Setting**
  - Diagnostic IHC, if adeno: TTF-1, ALK (D5F3) and EGFR (L858R)
  - Second block: Run Group Stains: Molecular Lung (4 choices) – USS directly to DMP

- **One Block Setting**
  - Diagnostic IHC: i.e. TTF-1, ER, CDX2,
  - Unstained Recut (20 small bx, 15 resection)
  - Slides returned to fellow – send USS with H&E to DMP with paper form
TISSUE MANAGEMENT

- Each group of thoracic physicians (clinicians, radiologists, surgeons, pathologists, molecular biologists) must develop a strategy to manage tissues
- Obtaining biopsies or cytology samples
- Optimal processing by laboratories/pathologists for diagnosis AND molecular studies
- Pathologists should be the leader of this
COPATH ORDERING SETS FOR MOLECULAR TESTING

- Molecular Lung Adenoca
- Molecular – T790M
- Molecular Lung Squamous cell ca
- Molecular Lung Small cell ca
- CLINICIANS
  - Oncologists
  - Surgeons
  - Pulmonary
  - Interventional Radiology

- CIS
  - Specimen Requisition

- DMP

- Information Technology
  Key to coordinate communication between hospital and pathology computer systems

- Laboratory
  - Histology/Immuno

- Copath

- 2 block cases
  - 1 H&E, 15/20 USS
KEY PRINCIPLES

- Minimize diagnostic stains to maximize tissue for molecular studies
- Molecular testing is reliable on FFPE tissues – even very small samples
- Unstained slides (n=15-20) provide adequate DNA if sufficient tumor
- Cytology fluids (i.e. pleural) – cytospin and make cell block (for IHC/molecular)
NEW DEVELOPMENTS IN ADVANCED LUNG CANCER DIAGNOSIS

- Immunotherapy – PD-L1 Immunohistochemistry
- Cell free DNA analysis (liquid biopsy)
- Poorly differentiated tumors: New entities discovered by genomic analysis
# PD-L1 IHC

## Programmed Death Ligand-1 Inhibitors

<table>
<thead>
<tr>
<th>Drug</th>
<th>Company</th>
<th>FDA Approval</th>
<th>mAb/Platform</th>
<th>Scoring Criteria</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pembrolizumab</td>
<td>Merck (Kenilworth, New Jersey)</td>
<td>FDA approved for NSCLC</td>
<td>22C3 (DAKO pharmDx)/Link 48 Autostainer (Dako, Carpenteria, California)</td>
<td>≥50% tumor cells</td>
<td>Companion diagnostic&lt;sup&gt;a&lt;/sup&gt; (as of October 2015)</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>Bristol-Myers Squibb (New York, New York)</td>
<td>FDA approved for squamous and nonsquamous NSCLC</td>
<td>28-8 (DAKO pharmDx)/Link 48 Autostainer</td>
<td>≥1% tumor cells</td>
<td>Complementary diagnostic&lt;sup&gt;b&lt;/sup&gt; (as of October 2015); predictive only in nonsquamous carcinomas</td>
</tr>
<tr>
<td>Atezolizumab</td>
<td>Roche (Basel, Switzerland)</td>
<td>Expected in 2016</td>
<td>SP142 (Ventana, Tucson, Arizona)</td>
<td>Tumor cells and/or tumor-infiltrating immune cells ≥25% tumor cells</td>
<td>In development</td>
</tr>
<tr>
<td>Durvalumab</td>
<td>Astra-Zeneca (London, United Kingdom)</td>
<td>Expected in 2016</td>
<td>SP263 (Ventana)</td>
<td></td>
<td>In development</td>
</tr>
</tbody>
</table>

<sup>a</sup> Companion diagnostic<sup>b</sup>
PD-L1 IHC: CHALLENGES

- Four different IHC clones, staining platforms & scoring criteria
- Limited tissue – cannot perform all assays after genomic testing
- Heterogeneity of staining
- Need for standardization of testing and interpretation of results
- Lack of data on cytology specimens
## CELL FREE DNA ASSAYS

### Performance Characteristics of Published Mutant Cell-Free DNA Assays Relative to Tumor Tissue Genotyping

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Study</th>
<th>Technology</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Concordance, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bai et al, 2009</td>
<td>Advanced NSCLC, first line</td>
<td>Denaturing HPLC</td>
<td>82</td>
<td>89</td>
<td>79.9</td>
</tr>
<tr>
<td>Kimura et al, 2007</td>
<td>Pts tx with gefitinib</td>
<td>Scorpion ARMS</td>
<td>78.9</td>
<td>97</td>
<td>92.9</td>
</tr>
<tr>
<td>Goto et al, 2012</td>
<td>IPASS (subgroup)</td>
<td>Scorpion ARMS</td>
<td>43.1</td>
<td>100</td>
<td>66.7</td>
</tr>
<tr>
<td>Douillard et al, 2014</td>
<td>First-line gefitinib</td>
<td>Scorpion ARMS</td>
<td>65.7</td>
<td>99.8</td>
<td>94.3</td>
</tr>
<tr>
<td>Kim et al, 2013</td>
<td>Pts tx with gefitinib</td>
<td>PCR with PNA clamps</td>
<td>15</td>
<td>100</td>
<td>27.5</td>
</tr>
<tr>
<td>Couraud et al, 2014</td>
<td>Lung cancer from never smokers</td>
<td>Multiplex PCR</td>
<td>58</td>
<td>87</td>
<td>81–97</td>
</tr>
<tr>
<td>Oxnard et al, 2014</td>
<td>Advanced NSCLC</td>
<td>Droplet digital PCR</td>
<td>~65–80</td>
<td>100</td>
<td>Not provided</td>
</tr>
<tr>
<td>Mok et al, 2015</td>
<td>FASTACT-2; advanced NSCLC</td>
<td>Cobas 4800 blood test</td>
<td>75</td>
<td>96</td>
<td>88</td>
</tr>
<tr>
<td>Lee et al, 2016</td>
<td>NSCLC Pts tx with EGFR TKIs</td>
<td>Droplet digital PCR</td>
<td>71–77</td>
<td>100</td>
<td>86–88</td>
</tr>
<tr>
<td>Newman et al, 2014</td>
<td>NSCLC</td>
<td>CAPP-Seq (NGS)</td>
<td>50–100</td>
<td>96</td>
<td>Not provided</td>
</tr>
<tr>
<td>Wei et al, 2014</td>
<td>Saliva specimens from NSCLC patients</td>
<td>EFIRM</td>
<td>94–96</td>
<td>82–100</td>
<td>Not provided</td>
</tr>
</tbody>
</table>

–Sholl L et al: Arch Path Lab Med 2016; epub
CELL FREE DNA ANALYSIS

- Detection of circulating tumor cells – new technology with some potential
- FDA approved CellSearch System for circulating tumor cell detection
- In patients with a genomically defined solid tumor – may be clinically useful
- However, not validated for lung cancer diagnosis and its lower sensitivity could delay diagnosis compared to tissue biopsy