Molecular Diagnostic Pathology for Solid Tumor Oncology in the Era of Personalized Medicine

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Departments of Pathology, Urology and Oncology
Johns Hopkins University
Disclosures

- No Relevant Financial Commercial Relationships to Disclose
OVERVIEW

• Solid Case for Solid Tumor Molecular Dx
• Lung Cancer as a paradigm for “Precision Medicine”
• The Future is even more “Personalized”

Next Generation Sequencing Technology
Nanotechnology: BEAMs and PAREs
A decade later, the revolutionary progress in Human Genomics is reshaping our approach to therapy and diagnosis.

It is estimated that 5-10% of all laboratory tests are DNA/RNA based analyses.
Steps Involved in Genetic Approach to Diagnosis and Treatment of Disease

- Disease with Genetic Component
- Map Gene(s) to Specific Chromosomal Regions
- Identify Gene(s)
  - Molecular Test
    - Diagnostics
    - Prognostics
    - Pharmacogenomics
    - Prevention
  - Gene Therapy
  - Targeted Drug Therapy
- Understand Underlying Biology of Disease

*JAMA, 2001; vol 285(5), p540*
Genomic Landscape
CRCa and Breast Ca

Laura Wood et al Science 2007
Core Signaling Pathways in Human Pancreatic Cancers Revealed by Global Genomic Analyses


SMAD4 Gene Mutations Are Associated with Poor Prognosis in Pancreatic Cancer


Hazard ratio (95% CI) P

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>P</th>
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<tbody>
<tr>
<td>SMAD4 mutant vs wild-type</td>
<td>1.92</td>
<td>(1.20-3.05)</td>
<td>0.006</td>
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<tr>
<td>Metastases vs no metastases</td>
<td>1.00</td>
<td>(0.57-1.75)</td>
<td>&gt;0.99</td>
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<tr>
<td>Positive vs negative margins</td>
<td>1.29</td>
<td>(0.78-2.10)</td>
<td>0.31</td>
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<tr>
<td>Poor vs moderate/well grade</td>
<td>1.08</td>
<td>(0.66-1.77)</td>
<td>0.76</td>
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<tr>
<td>Age</td>
<td>1.03</td>
<td>(1.01-1.05)</td>
<td>0.002</td>
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<tr>
<td>Tumor size ≥3.5 vs &lt;3.5 cm</td>
<td>1.42</td>
<td>(0.89-2.27)</td>
<td>0.14</td>
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</table>
SOLID REASONS FOR ACCELERATION OF DISCOVERY AND DELIVERY OF MOLECULAR DX TESTING

- **Need for Targeted Rx**
  - only limited success in lethal Solid Tumors

- **Case Load Eclipses non-solid tumors**
  - liquid tumors lead in Targeted Rx and in Monitoring Rx Response (BCR-ABL /Gleevec)
### Estimated New Cases

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td>Prostate</td>
<td>238,590</td>
<td>232,340</td>
</tr>
<tr>
<td>Lung &amp; bronchus</td>
<td>118,080</td>
<td>110,110</td>
</tr>
<tr>
<td>Colorectum</td>
<td>73,680</td>
<td>69,140</td>
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<tr>
<td>Urinary bladder</td>
<td>54,610</td>
<td>49,560</td>
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<tr>
<td>Melanoma of the skin</td>
<td>45,060</td>
<td>45,310</td>
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<tr>
<td>Kidney &amp; renal pelvis</td>
<td>40,430</td>
<td>32,140</td>
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<tr>
<td>Non-Hodgkin lymphoma</td>
<td>37,600</td>
<td>31,630</td>
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<tr>
<td>Oral cavity &amp; pharynx</td>
<td>29,620</td>
<td>24,720</td>
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<tr>
<td>Leukemia</td>
<td>27,880</td>
<td>22,480</td>
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<tr>
<td>Pancreas</td>
<td>22,740</td>
<td>22,240</td>
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<tr>
<td>All Sites</td>
<td>854,790</td>
<td>805,500</td>
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### Estimated Deaths

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<th>Females</th>
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<tbody>
<tr>
<td>Lung &amp; bronchus</td>
<td>87,260</td>
<td>72,220</td>
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<tr>
<td>Prostate</td>
<td>29,720</td>
<td>39,620</td>
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<tr>
<td>Colorectum</td>
<td>26,300</td>
<td>24,530</td>
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<tr>
<td>Pancreas</td>
<td>19,480</td>
<td>18,980</td>
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<tr>
<td>Liver &amp; intrahepatic bile duct</td>
<td>14,890</td>
<td>14,030</td>
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<tr>
<td>Leukemia</td>
<td>13,660</td>
<td>14,030</td>
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<tr>
<td>Esophagus</td>
<td>12,220</td>
<td>10,060</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>10,820</td>
<td>8,190</td>
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<tr>
<td>Non-Hodgkin lymphoma</td>
<td>10,590</td>
<td>8,430</td>
</tr>
<tr>
<td>Kidney &amp; renal pelvis</td>
<td>8,780</td>
<td>6,780</td>
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<tr>
<td>All Sites</td>
<td>306,920</td>
<td>273,430</td>
</tr>
</tbody>
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**Cancer Statistics, 2013**

Rebecca Siegel, MPH; Deepa Naishadham, MA, MS; Ahmedin Jemal, DVM, PhD
Moving Toward Individualized Health and Personalized Care

Hanahan and Weinberg Cell 2011
Targetable Genetic Alterations

- EGFR
- ALK
- VEGF/R
- (KRAS)
- BRAF
- MET
- HER2
- RET
- ROS-1
- PIK3CA
- AKT
- MEK
- NRAS
- MAP2K
- PTEN
- IGF-1R
- DDR2
- FGFR


Categories: 0-250, 100-250
NEXT GENERATION SEQUENCING (NGS):
- Paradigm shift
- Multiple Genes Mol Dx Approach
- Cost Reduction?
- TAT
- $$$$: Investment cost and return opportunities
$1 Billion Drugs R&D cost for small patient number with a given targeted molecular alteration?

1-2% of 220,000 lung cancers in USA (1.5 Million deaths worldwide) is serious $$$$.

The horizontal applicability of the shared target among various tumor types (CML and GIST).
The HER2/neu HERCEPTIN PARADIGM
HER-2/neu (ERBB2)

- Member of class I receptor tyrosine kinase (ERBB) family (EGFR, HER-2/ERBB-2, ERBB-3 and ERBB-4)
- ERBBs regulate cell survival, proliferation and differentiation

- HER-2/neu gene encodes for a 185 kd HER-2 protein (p185)
- HER-2: Ligand binding domain, transmembrane domain and tyrosine kinase domain.
- Ligand: Neuregulin (NRG)
Anti RTK mAbs

Receptor TK
Targeted Rx Strategies

Small Molecules TKI

Activation of cell signaling pathways:
- Ras
- PI3K
- PLC-γ
- MAPK
- AKT
- PKC
- STAT

Transcription of target genes:

NUCLEUS
USE OF CHEMOTHERAPY PLUS A MONOCLONAL ANTIBODY AGAINST HER2 FOR METASTATIC BREAST CANCER THAT OVEREXPRESSES HER2

DENNIS J. SLAMON, M.D., PH.D., BRIAN LEYLAND-JONES, M.D., STEVEN SHAK, M.D., HANK FUCHS, M.D.,
VIRGINIA PATON, PHARM.D., ALEX BAJAMONDE, PH.D., THOMAS FLEMING, PH.D., WOLFGANG EIERMANN, M.D.,
JANET WOLTER, M.D., MARK PEGRAM, M.D., JOSE BASELGA, M.D., AND LARRY NORTON, M.D.*

Efficacy and Safety of Trastuzumab as a Single Agent in First-Line Treatment of HER2-Overexpressing Metastatic Breast Cancer

By Charles L. Vogel, Melody A. Cobleigh, Debu Tripathy, John C. Gutheil, Lyndsay N. Harris, Louis Fehrenbacher,
Dennis J. Slamon, Maureen Murphy, William F. Novotny, Michael Burchmore, Steven Shak, Stanford J. Stewart,
and Michael Press

Slamon et al 2001 NEJM, Vogel et al JCO 2002,
Importance of Standardization of COMPANION TESTING

- Standardization of results report: CAP/ASCO consensus
- Which assay to use?
- Triage and confirmation assays
- Central vs local laboratory: variability of results
- Assay sensitivity (cut-offs) and specificity
- Cost and third payer issues
- FDA regulatory issues
Targeted therapies new slide from "Science in Medicine"
Modified from JCI 2007; 117 (10)-2740.
Molecular Diagnostics in Colorectal Cancer
Markowitz S et al NEJM 2009
Targeted Therapy in Colorectal Carcinoma (CRCa)

Cetuximab Monotherapy and Cetuximab plus Irinotecan in Irinotecan-Refractory Metastatic Colorectal Cancer

David Cunningham, M.D., Yves Humblet, M.D., Ph.D., Salvatore Siena, M.D., David Khayat, M.D., Ph.D., Harry Bleiberg, M.D., Ph.D., Armando Santoro, M.D., Danny Bets, M.Sc., Matthias Mueser, M.D., Andreas Harstrick, M.D., Chris Verslype, M.D., Ph.D., Ian Chau, M.B., B.S., and Eric Van Cutsem, M.D., Ph.D.
Anti-EGFR Targeted Rx for CRCa

PFS

OS

Cetuximab

Cetuximab plus irinotecan

Patients Free of Progression (%)

Time to Progression (months)

P = 0.001

HR = 0.54

Patients Surviving (%)

Overall Survival Time (months)

P: NS

KRAS Mutation Status Is Predictive of Response to Cetuximab Therapy in Colorectal Cancer

Astrid Lièvre,1,3 Jean-Baptiste Bachet,3 Delphine Le Corre,1 Valérie Boige,4 Bruno Landi,2 Jean-François Emile,3 Jean-François Côté,1,2 Gorana Tomasic,4 Christophe Penna,3 Michel Ducreux,4 Philippe Rougier,3 Frédérique Penault-Llorca,5 and Pierre Laurent-Puig1,2
KRAS Status Predictive of Response to Anti-EGFR CRCa Rx

Overall survival according to KRAS mutation

- non mutated KRAS
- mutated KRAS

p=0.016

GIST Theranostics
GIST Theranostics

- GIST Mutation status has predictive value for responses to imatinib Rx
  - PDGFRA mutations: No response to imatinib
  - KIT mutations:

<table>
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<tr>
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<th>Exon 11</th>
<th>Exon 9</th>
<th>Wild-Type</th>
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<tr>
<td><strong>Objective response</strong></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>72%</td>
<td>44%</td>
<td>44%</td>
</tr>
<tr>
<td><strong>400 mg</strong></td>
<td>ns</td>
<td>17%</td>
<td>ns</td>
</tr>
<tr>
<td><strong>800 mg</strong></td>
<td>ns</td>
<td>67%</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Time to tumor progression</strong></td>
<td>25 mo</td>
<td>17 mo</td>
<td>13 mo</td>
</tr>
<tr>
<td><strong>Overall survival</strong></td>
<td>60 mo</td>
<td>38 mo</td>
<td>49 mo</td>
</tr>
</tbody>
</table>

Heinrich MC, et al. JCO 2008;26:5360
Genotyping in GISTs is useful as a predictive marker and for selection of the optimal dose of imatinib.

NCCN guidelines support routine mutational analysis for all newly diagnosed intermediate and high-risk patients, as well as for overtly malignant GISTs.

Secondary resistance to IMITANIB: Exons 13,14,17,18.
Molecular Diagnostics in NSCLC

A MODEL FOR FUTURE SOLID TUMOR CANCER MANAGEMENT
EGFR Mutation predicts response to TKI (Gefitinib)

Lynch et al NEJM 2004; 350:2129-39

- 8/9 NSCLC with EGFR mutation Vs 0/7 had evidence of Iressa Rx response
- 8% EGFR mutation rate in 25 tested NSCLC pts

- Women
  - Never smokers
  - Adenocarcinoma; non-squamous carcinoma
  - Somatic mutations in EGFR TK domain
**EGFR and K-ras mutation in NSCLC carcinoma**

<table>
<thead>
<tr>
<th></th>
<th>EGFR mutation</th>
<th>EGRF wild type</th>
<th>K-ras mutation</th>
<th>K-ras wild type</th>
</tr>
</thead>
<tbody>
<tr>
<td>AdenoCA*</td>
<td>20%</td>
<td>80%</td>
<td>22%</td>
<td>78%</td>
</tr>
<tr>
<td>Non-AdenoCA</td>
<td>13%</td>
<td>87%</td>
<td>5%</td>
<td>95%</td>
</tr>
<tr>
<td>Female</td>
<td>18%</td>
<td>82%</td>
<td>14%</td>
<td>86%</td>
</tr>
<tr>
<td>Male</td>
<td>16%</td>
<td>84%</td>
<td>15%</td>
<td>85%</td>
</tr>
<tr>
<td>Smokers#</td>
<td>14%</td>
<td>86%</td>
<td>16%</td>
<td>84%</td>
</tr>
<tr>
<td>Never smoker</td>
<td><strong>28%</strong></td>
<td>72%</td>
<td>11%</td>
<td>89%</td>
</tr>
<tr>
<td>Asian</td>
<td><strong>38%</strong></td>
<td>62%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>Non-Asian</td>
<td>15%</td>
<td>85%</td>
<td>16%</td>
<td>84%</td>
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</table>

EGFR Mutations Associated with Sensitivity

<table>
<thead>
<tr>
<th>Exon 18</th>
<th>Exon 19</th>
<th>Exon 20</th>
<th>Exon 21</th>
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<tbody>
<tr>
<td><strong>G719C</strong></td>
<td><strong>ΔE746-A750</strong></td>
<td><strong>ΔL747-T751</strong></td>
<td><strong>L858R (40%-45%)</strong></td>
</tr>
<tr>
<td><strong>G719S</strong></td>
<td><strong>ΔE746-T751</strong></td>
<td><strong>ΔL747-T751 (ins P/S)</strong></td>
<td><strong>N826S</strong></td>
</tr>
<tr>
<td><strong>G719A</strong></td>
<td><strong>ΔE746-A750 (ins RP)</strong></td>
<td><strong>ΔL747-T751 (ins P)</strong></td>
<td><strong>A839T</strong></td>
</tr>
<tr>
<td>V689M</td>
<td><strong>ΔE746-T751 (ins A/I)</strong></td>
<td><strong>ΔL747-T751 (ins P/S)</strong></td>
<td><strong>K846R</strong></td>
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<tr>
<td>N700D</td>
<td><strong>ΔE746-S752 (ins A/V)</strong></td>
<td><strong>ΔL747-S752 (ins Q)</strong></td>
<td><strong>L861Q</strong></td>
</tr>
<tr>
<td>E709K/Q</td>
<td><strong>ΔL747-E749 (A750P)</strong></td>
<td><strong>ΔL747-P753</strong></td>
<td><strong>G863D</strong></td>
</tr>
<tr>
<td>S720P</td>
<td><strong>ΔL747-E750 (ins P)</strong></td>
<td><strong>ΔL747-P753 (ins S)</strong></td>
<td></td>
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</table>

- **5%**
- **<1%**
- **40-45%**

45%
EGFR Mutations Associated with Resistance

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<tr>
<th>Exon 18</th>
<th>Exon 19</th>
<th>Exon 20</th>
<th>Exon 21</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

- **D761Y**
- **T790M (50%)**
- D770_N771 (ins NPG)
- D770_N771 (ins SVQ)
- D770_N771 (ins G), N771T
- V759L
- S768I

<1% 5%
Acquired EGFR Rx Resistance

- Second mutation in exon 20 (T790M)
  
  
  
  *Maheswaran et al N Engl J Med 2008*

- Amplification of MET gene (7q31)
  
  *Engelman JA. Science 2007; 316(5827);1039-43*
  
  *Sequist et al 2008 JCO*
Clinical Features and Outcome of Patients With Non–Small-Cell Lung Cancer Who Harbor EML4-ALK

Genetic Alterations in Lung Adenocarcinoma

2010

- KRAS - 25%
- EGFR - 15% (US) 15-65% worldwide
- ALK - 4%
- BRAF - 3%
- PIK3CA - 3%
- HER2 - 1%
- UNKNOWN 50%
Genetic Alterations in Lung Adenocarcinoma

Lung Cancer Mutation Consortium study - LCMC (830 patients) 2011

- KRAS: 25%
- EGFR: 23%
- ALK: 6%
- BRAF: 3%
- PIK3CA: 3%
- MET: 2%
- HER2: 1%
- MEK1: 0.4%
- NRAS: 0.2%
- UNKNOWN: 36.4%
Genetic Alterations in Lung Adenocarcinoma

- **KRAS**: 25%
- **EGFR**: 23% (LCMC) 15-65% worldwide
- **ALK**: 3-6%
- **BRAF**: 3%
- **PIK3CA**: 3%
- **MET**: 2-5%
- **HER2**: 1%
- **MEK1**: 0.4%
- **NRAS**: 0.2%
- **KIF5B-RET**: 2%
- **ROS1**: 1.7%
- **UNKNOWN**: 32.7%
SCLC
- RB1
- RLF-MYCL1
- MYCL1
- MYCN
- MYC

Squamous carcinoma
- FGFR1
- SOX2
- NFE2L2
- TP63
- NOTCH1

Adenocarcinoma
- TP53
- CDKN2A
- PIK3CA
- PTEN
- KEAP1
- EGFR
- KRAS
- ERBB2
- BRAF
- ALK fusions
- ROS1 fusions
- RET fusions
- STK11
Lung mass

Poorly differentiated adenocarcinoma (TTF-1+, p63+ and p40 negative)
Lung TBBX

Pelvic mass resection

ALK – CLONE 5A4
Abbott ALK FISH
SPECIAL ARTICLE

Molecular Testing Guideline for Selection of Lung Cancer Patients for EGFR and ALK Tyrosine Kinase Inhibitors

Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology

Neal I. Lindeman,* Philip T. Cagle,† Mary Beth Beasley,‡ Dhananjay Arun Chitale,§ Sanja Dacic,‖ Giuseppe Giaccone,‖ Robert Brian Jenkins,⁎ David J. Kwiatkowski,‖† Juan-Sebastian Saldivar,‖‡ Jeremy Squire,‖§ Erik Thunnissen,‖* and Marc Ladanyi||
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Mean ± SD</th>
<th>Percentage</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>EGFR mutation positive</td>
<td>EGFR mutation negative</td>
<td></td>
<td>n (N)</td>
<td>WMD (95% CI)</td>
<td>RR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Response rate, %*</td>
<td>68 ± 11</td>
<td>51 (3644)</td>
<td>5.16</td>
<td>4.41–6.04</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease control rate, %†</td>
<td>86 ± 42</td>
<td>28 (2204)</td>
<td>1.99</td>
<td>1.73–2.29</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to progression/progression-free survival, mo‡</td>
<td>12.0 ± 7.86</td>
<td>3.4 ± 2.59</td>
<td>8.66</td>
<td>6.31–11.00</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median survival time, mo§</td>
<td>23.3 ± 18.4</td>
<td>12.1 ± 13.9</td>
<td>10.66</td>
<td>8.36–12.96</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Different Outcomes in All Stages of Non—Small Cell Lung Cancer Patients With and Without EGFR Mutations, Treated with Tyrosine Kinase Inhibitor
When Should Molecular Testing of Lung Cancers Be Performed?

- EGFR molecular testing should be used to select for EGFR-targeted TKI Rx
  
  *Pts with lung adenocarcinoma should not be excluded on basis of clinical characteristics*

- ALK molecular testing should be used to select for ALK-targeted TKI Rx
  
  *Pts with lung adenocarcinoma should not be excluded on basis of clinical characteristics*
When Should Molecular Testing of Lung Cancers Be Performed?

Setting of lung carcinoma resection specimens

- EGFR and ALK testing is recommended for adenocarcinoma and mixed lung cancers with an adenocarcinoma component, regardless of histologic grade.
- EGFR and ALK testing is not recommended in lung cancers that lack any adenocarcinoma component, such as pure SCC, pure small CC, or large cell carcinoma lacking any IHC evidence of adenocarcinoma.
When Should Molecular Testing of Lung Cancers Be Performed?

Setting of biopsies/cytology

- EGFR and ALK testing may be performed in cases showing squamous or small cell histology but clinical criteria (eg, young age, lack of smoking history)
When Should Molecular Testing of Lung Cancers Be Performed?

- **EGFR/ALK mutation testing at time of Dx** for pts with advanced stage (stage IV) or at time of recurrence or progression

- **EGFR/ALK testing at Dx from patients presenting with** stage I, II, or III is **encouraged** but the decision should be made locally by laboratory/oncology team

- **Tissue should be prioritized** for EGFR and ALK testing
What sample Should Molecular Testing of Lung Cancers Be Performed on?

- **Primary** tumors or **metastatic** lesions are equally suitable.

- For patients with **multiple primary lung adenocarcinomas**, each tumor may be tested but testing of multiple different areas within a single tumor is not necessary.
How Rapidly Should Test Results Be Available?

- Results should be available within **2 weeks** (10 working days) of receiving the specimen
- **Sent** to outside molecular pathology laboratories within **3 working days** of receiving requests and to intramural molecular pathology laboratories **within 24 hours**
CONCLUSIONS

• Conveyed the excitement generated around the arrival of *Molecular Diagnostics* to the *Solid Tumors* arena

• Tremendous opportunities for the anatomic and molecular pathologists to be part of the “personalized” approach to oncology patient management
In The Near Future?

NGS Based Actionable/Targetable Gene Panels

Personalized Rx Response Monitoring
Basic Steps of NGS Platforms

Template Preparation
- clonally amplified template originating from sheared genomic DNA

Sequencing Methodology
- Cyclic reversible termination (CRT)
- Single-nucleotide addition (SNA)
- Real-time sequencing
- Sequencing by ligation (SBL): DNA ligase

Imaging/Signal Capture
- Bioluminescent signals
- Four-color fluorescent imaging of a single molecular event

Genome Sequence Alignment and Assembly (IT Demands)
a) Illumina/Solexa — Reversible terminators

- Incorporate all four nucleotides, each labeled with a different dye.
- Wash, four-colour imaging.
- Cleave dye and terminating groups, wash.
- Repeat cycles.

b) Illumina/Solexa

- Solid-phase amplification
  - One DNA molecule per cluster
- Bridge amplification
  - Sample preparation DNA (5 µg)
- Template dNTPs and polymerase
  - 100–200 million molecular clusters
Genome Sequence Alignment /Fragment Assembly (IT Demands)

Depth of Coverage to minimize reading errors of short fragments

Wagle et al Cancer Discovery 2012
High-Throughput Detection of Actionable Genomic Alterations in Clinical Tumor Samples by Targeted, Massively Parallel Sequencing

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
<th>Gene</th>
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<tbody>
<tr>
<td>ABL1</td>
<td>CTNB1</td>
<td>IDH2</td>
<td>MYCL1</td>
<td>RET</td>
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<tr>
<td>ABL2</td>
<td>EGFR</td>
<td>IGF1R</td>
<td>MYCN</td>
<td>RICTOR</td>
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<td>AKT1</td>
<td>EPHA3</td>
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PARE (Personalized Analysis of Rearranged Ends)

- **Somatic structural alterations** can be exploited in an individual solid tumor regardless of recurrence rate in the given tumor type or their “Driver” / “Passenger” role.

- Applied Biosystems SOLiD System:
  1.4kb apart mate-paired tags libraries generated, digitally amplified by emulsion PCR on magnetic beads and 25 bp mate-paired tags sequenced using sequencing-by-ligation approach.

- On average, 40 million mate-paired sequence led to identification of 14 interchromosomal/intrachromosomal rearrangements in each sample (3 CRCa and 3 Breast Ca)

- Digital PCR assays designed to detect a specific rearrangement in a given pt plasma.
PARE (Personalized Analysis of Rearranged Ends)

1. Resected tumor
2. Next generation mate-pair sequence analysis
3. Identification of patient specific rearrangements
4. Plasma or other bodily fluids
5. Quantitative measurement of personalized biomarker for tumor monitoring
Conveyed the excitement generated around the arrival of Molecular Diagnostics to the Solid Tumors arena.

Tremendous opportunities for the anatomic and molecular pathologists to be part of the “personalized” approach to oncology patient management.
Genome Sequence Alignment /Fragment Assembly (IT Demands)

Depth of Coverage to minimize reading errors of short fragments

Wagle et al Cancer Discovery 2012
Other genes known to be altered in lung adenocarcinoma

EML4-ALK Rearrangement

– Echinoderm microtubule-associated protein-like 4 (EML4) gene is fused to the anaplastic lymphoma kinase (ALK) gene
– 3-6% of lung adenocarcinoma (ACA)
– Non-smoker younger patients
– Usually high grade tumors (solid, cribriform, signet ring cell morphology)
– Multiple variants
– Responds to crizotinib
inv(2)(p21p23),

- **EML4–ALK fusion gene**
  - **Variant 1**: Exon 13 of EML4 is ligated to exon 19 of ALK
  - **Variant 2**: EML4 E20 to E20 of ALK
  - **Variant 3**: EML4 E6 to E20 of ALK
  - **Variant 5**: EML4 E2 to E20 of ALK
  - **Variant 4**: EML4 E14 to E20 of ALK via an 11-bp sequence of unknown origin to the nucleotide at position 50 of ALK exon 20

Other genes known to be altered in lung adenocarcinoma

BRAF mutation
- B-Raf also known as v-Raf murine sarcoma viral oncogene homolog B1
- the protein is a serine/threonine-protein kinase
- regulates the MAP kinase/ERKs signaling pathway (cell division, differentiation, and secretion)
- 2-3% of lung adenocarcinoma (ACA)
- V600E most common (others G466, G469)
- Targeted therapy in clinical trials
Other genes known to be altered in lung adenocarcinoma

PIK3CA mutation
- 2-3% of primary lung ACA (10% in EGFR TKI resistant tumors)
- At least 50% also have concurrent EGFR, K-ras or ALK alteration
Other genes known to be altered in lung adenocarcinoma

Her2 Exon 20 mutation*

- More frequent in never smokers (5%; p<0.0001)
- In 1.7% of lung ACA (25 of 1478 tumors)*
- Represents 6% of EGFR/KRAS/ALK-negative tumors
- Small insertions in 96% (24/25 tumors)
- Majority (20/24 – 83%) have 12 bp insertion causing duplication of amino acids YVMA at codon 775
- Majority moderately-poorly differentiated tumors (92%)
- No amplification detected (0/11 tumors tested)

Other genes known to be altered in lung adenocarcinoma

ROS1

- Proto-oncogene, belongs to the sevenless subfamily of tyrosine kinase insulin receptor genes.
- The protein is a type I integral membrane protein with tyrosine kinase activity. The protein may function as a growth or differentiation factor receptor
- Gene located 6q22
- Highly expressed in a number of tumors
Other genes known to be altered in lung adenocarcinoma

ROS1 Rearrangement

- 1.7% of lung adenocarcinoma (ACA)
- Usually high grade tumors in younger never smoker patients
- Only adenocarcinoma histology
- Same overall survival of ROS1 positive/negative tumors
- Detected using FISH (brake away probe)
- Responds to crizotinib (Phase I study)
Other genes known to be altered in lung adenocarcinoma

Clinical activity of crizotinib in NSCLC harboring ROS1 gene Rearrangement (Phase I study)#

- Thirteen patients, median age 47 (range 31-72)
- Majority never smoker (12/13)
- Adenocarcinoma histology
- No ALK rearrangement
- Overall response rate 54% (7/13), 6 partial response & 1 complete response at 7-8 weeks, 1 additional partial response at time of data cut-off.
- Disease control rate at 8 weeks 85%

#Shaw AT et al. J Clin Oncol 30, 2012 (suppl; abstr 7508)
Other genes known to be altered in lung adenocarcinoma

Mesenchymal-epithelial transition factor (MET) polysomy/amplification

– Hepatocyte growth factor receptor and encodes tyrosine-kinase activity.
– Located on 7q31
– Seen 2% of tumors at primary diagnosis
– 10% in tumor with acquired resistance to EGFR TKIs
– Not mutually exclusive with T790M mutation
– MET inhibitors in development/clinical trials (some requiring ancillary testing)
Other genes known to be altered in lung adenocarcinoma

**KIF5B-RET Rearrangement**
- 1-2% of lung adenocarcinoma (in 0.8% of European and 2% of Asian ancestry)
- Usually high grade tumors in younger patients
- Never or light smokers
- No EGFR/KRAS/HER/BRAF/EML4-ALK/ROS1 alterations (KIF5B exon 15 can be fused to ALK)
- May respond to multi-kinase RET inhibitors

Genetic Alterations in Squamous cell carcinoma of the lung

- FGFR1 amplification
- Amplification of 3q amplicon (SOX-2, p63, PIKC3a are in amplicon)
- DDR2 mutations