Some Key Points

- Molecular Testing has applications in every section of the clinical laboratory.
- The advantages of molecular testing stem from its extreme specificity and sensitivity, as well as its rapidity in producing results.
- Applications in oncology include diagnosis, classification, prognostication, and prediction of response to targeted therapies.
- Clinical Laboratories in the US are regulated under CLIA.
- Laboratories may develop tests that are not FDA approved, under the authority of the CLIA Medical Director.
- Payment for laboratory testing has two major components, coverage and reimbursement.
Assume genome is 50% GC – i.e. A,T,G,C ‘s are equally represented

Consider this 15 bp sequence: aatgcagttaccttttg

- The probability of A in position one is 0.25
- The probability of A in position two is 0.25
- The probability of A in position one and A in position two is $(0.25)(0.25)=0.06$
- The probability of a T following AA is $(0.25)(0.25)(0.25)=0.0156$
- The probability of any specific 15 bp sequence is $(0.25)^{15}=9.3\times10^{-10}$
Consider a PCR reaction with two 15-mer primers

- Primer 1 has $9 \times 10^{-10}$ probability of finding perfect match site
- Primer 2 has $9 \times 10^{-10}$ probability of finding perfect match site
- Combined probability is $81 \times 10^{-20}$
- This is further reduced by the expectation of a specific amplicon size

- If your PCR reaction produces a product of the expected size, it is extremely unlikely that this is due to chance
PCR-RFLP (Restriction Fragment Length Polymorphism)
EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity to gefitinib and erlotinib


*Program in Cancer Biology and Genetics and Departments of Medicine, Surgery, Pathology, and **Radiology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; and ***Genome Sequencing Center, Washington University School of Medicine, 4444 Forest Park Boulevard, St. Louis, MO 63108

Contributed by Harold Varmus, July 19, 2004
Dramatic response to gefitinib

Clinical predictors
- Women
- East Asian
- Never smokers

Histologic predictors
- Adenocarcinomas with BAC features, TTF1+
- None completely reliable
Positions of Mutations Detected in EGFR Tyrosine Kinase Domain in NSCLC

EGF ligand binding  Tyrosine kinase  Autophosphorylation

<table>
<thead>
<tr>
<th>Exon</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
</tr>
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<tbody>
<tr>
<td>TM</td>
<td></td>
<td></td>
<td></td>
<td>K</td>
<td>DFG</td>
<td>Y</td>
<td>Y</td>
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<tr>
<td>718</td>
<td>745</td>
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<td>858</td>
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<tr>
<td>GXGXXG</td>
<td>K</td>
<td>R</td>
<td>H</td>
<td>DFG</td>
<td>L</td>
<td>L</td>
<td>Y</td>
</tr>
</tbody>
</table>

Exon:

- **Paez:**
  - 719: 4/5
  - 757-750: 5/5
  - 858: 1/5

- **Lynch:**
  - 719: 1/5

- **Pao:**
  - 719: 2/5

▲ Tumor with point mutation (amino acid substitution)
★ Tumor with in-frame deletion

EGF = endothelial growth factor; TM = transmembrane.

EGFR MUTATIONS based on MSKCC experience 2009-2010 (1131 cases screened, 258 EGFR mutant, 23%)
How do we test for EGFR mutations?

- Exon 19 deletions
  - PCR product length
  - Sequencing
  - IHC (only good for 15 bp deletion)

- Exon 21 (L858R)
  - PCR RFLP
  - Sequencing

- Exon 20 (T790M)
  - Allele specific PCR
  - Sequencing
Homo sapiens epidermal growth factor receptor (EGFR), RefSeqGene on chromosome 7

NCBI Reference Sequence: NG_007726.1

Exon 19

LREA deletion site shown in green.

Exon 21

Sau96I (GGNCC) site shown in turquoise. Site of L858R (2573T>G) shown in green.
BRAF PCR with FRET Probe Temperature Melt Analysis
Quantitative RT-PCR

Calibration Result: BKV-2/14/2018 1:36 PM

Calibrator Levels

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<th>True</th>
<th>Ave Estimate</th>
<th>% Error</th>
<th>% CV</th>
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<tr>
<td>200</td>
<td>119</td>
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Overview of Molecular Diagnostic Testing

- Landscape of Molecular Diagnostic Tests
- Regulatory Issues
- Coding
- Coverage and Reimbursement
Categories of Molecular Diagnostic Tests

- Infectious Disease
- Identity Testing
- Genetics
- Pharmacogenomics
- Oncology
- Predictive Medicine
Molecular Infectious Disease Testing
High volume tests

- HIV quantitation and genotyping
- HCV screening, quantitation and typing
- GC / Chlamydia screening
- HPV screening and genotyping
Molecular Infectious Disease Testing
Moderate volume screening tests

- Influenza A (H1, H3, 2009 H1N1) and Influenza B
- MRSA
- B. pertussis / B. parapertussis
Molecular Infectious Disease Testing
Lower volume tests

- CMV detection
- CMS quantitation
- VZV detection
- HSV 1 & 2 detection
- HSV 1 & 2 quantitation
- RSV detection
- Adenovirus detection
- Parvovirus detection
- Enterovirus detection
- S. aureus screening
- C. difficile detection
- Malaria detection and typing
- M. tuberculosis detection
- Mycobacterial speciation
- Sequenced based ID
- Respiratory pathogen panel
- GI pathogen panel
Molecular Infectious Disease Testing
Antibiotic resistance tests

Methicillin
Vancomycin
Ganciclovir
Rifampin
ESBL
Carbapenem resistant Enterobacteriaceae
Identity Testing

- Transfusion Medicine
  - RBC and platelet antigen typing
- HLA typing
  - Transplantation
  - Disease association
- Forensics
- Paternity
Pharmacogenomic Applications

- Warfarin: CYP 2C9 / VKOR
- Plavix: CYP 2C19
- Irinotecan: UGT1A1
- Abacovir: HLA-B*5710
- SSRI: CYP2D6
Molecular Applications in Genetics

- Common heritable conditions
  - Sickle cell disease
  - Hemoglobinopathies
  - Hemochromatosis
  - Thrombophilia

- Heritable diseases
  - WES – diagnostic odysseys

- Inherited predisposition

- Prenatal testing
  - CF screening
  - SMA screening
  - Inherited disease panels
  - Chromosomal abnormalities
Molecular Testing in Oncology

- Hematologic malignancies
  - Classification
  - Prognostication
  - Therapy selection
  - Monitoring
    - Minimal Residual Disease

- Inherited predisposition
  - BRCA1 and 2, FAP, Lynch, et al.
Molecular Testing in Oncology

- Solid tumor
  - Single analyte
    - Detection and diagnosis
    - Prognosis
    - Therapy
    - Resistance
- Liquid biopsy
- Panels
Molecular Testing in Oncology

- Solid Tumor
  - Classification
  - Prognosis
  - Targeted therapies
- Colon Cancer
  - NSCLC
  - CNS tumors
  - GIST
  - Malignant melanoma
Predictive Medicine

- Cardiovascular disease
- Alzheimer’s Disease
Regulatory Issues – All clinical laboratories in the US are certified by CMS under CLIA

- CLIA – Clinical Laboratory Improvement Act of 1988
  - Specifies personnel requirements
  - Quality Assurance and Proficiency Testing Standards
  - Laboratory Inspection
  - SOP and validation requirements

- Deemed Agents
  - CAP – College of American Pathologists
  - NY CLEP – New York State Clinical Laboratory Evaluation Program
CLIA vs. FDA

- FDA evaluates medical devices for safety and effectiveness
- FDA monitors Severe Adverse Events, but not ongoing Quality Assurance

- The Laboratory Medical Director, under CLIA, is responsible for day-to-day QC and proficiency testing

- The laboratory, under the direction of the Medical Director, can develop, validate, and implement tests for clinical use – Laboratory Developed Tests (LDT)
Regulatory Issues

- CLIA vs FDA
  - Lab tests as medical procedures or as medical devices

- LDT – Laboratory Developed Test

- Companion Diagnostics

- Complementary Diagnostics

- FDA Cleared and FDA Approved
  - 3rd Party Review
Coding Issues

  - Molecular Pathology Codes
    - Tier 1
    - Tier 2
  - Genomic Sequencing Procedure Codes
    - 81479 – Unlisted Molecular Pathology Procedure
    - MAAA – Multianalyte Assay with Algorithmic Analyses - Category I
    - MAAA – Multianalyte Assay with Algorithmic Analysis - Administrative

- PAMA
  - Proprietary Laboratory Assay (PLA) codes

- Mol Dx
  - Palmetto required
Payer Issues

- Private payers vs. Medicare
  - CMS – Center for Medicare and Medicaid Services

- Coverage Policies
  - Does a procedure or service influence treatment decisions or outcome?
  - In what clinical context is the service performed?
    - Medicare restrictions (age, screening, etc)

- Reimbursement
  - Inpatient vs. outpatient vs. non-patient
Current Reimbursement Environment

- Panels
  - CPT GSP Codes
  - NCD Pending
    - Excludes LDTs
    - Linked to Companion/Complimentary Diagnostics

- PAMA

- Palmetto
  - LCDs vs statements
Future Applications

- Crisper/Cas 9 Gene editing
  - Idiosyncratic testing
  - Immune response editing
  - “engraftment” monitoring

- Immune response prediction
  - TMB vs MSI
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- Molecular Testing has applications in every section of the clinical laboratory.
- The advantages of molecular testing stem from its extreme specificity and sensitivity, as well as its rapidity in producing results.
- Applications in oncology include diagnosis, classification, prognostication, and prediction of response to targeted therapies.
- Molecular genetic testing can also inform about inherited predisposition to certain malignancies.
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