2011 ASCP Annual Meeting
Molecular Pathology in the Community Laboratory Setting

Presented by: Dan M. Hyder, M.D.
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Dr. Hyder has no conflicts of interest to disclose which relate to material that will be presented in this course.
Genetic Engineering and Biotechnology News

Sky Could Be the Limit for DNA Testing

Exponential Market Growth Depends on Ability to Overcome Utility and Reimbursement Issues

Bruce Carlson, BioMarket Trends: Feb 15, 2009 (Vol. 29, No. 4)
Does massively parallel DNA resequencing signify the end of histopathology as we know it?

Samuel AJR Aparicio, David G Huntsman
The Journal of Pathology
Special Issue: Genes, Genomes and Disease
Volume 220, Issue 2, Pages 307-315, January 2010
Laboratory Services

- Hematopathology
- Cytogenetics
- Hemostasis and Thrombosis
- Pharmacogenomics/pharmacogenetics

MOLECULAR COMPONENT FOR ALL
Course Objectives

• Review factors impacting decisions to bring molecular testing in house

• Discuss local factors, resources and platforms to facilitate implementation of molecular testing

• Review validation protocols for in house molecular assays
Questions from Community Laboratories

- Specialized training?
- What tests to do, methods to use?
- Do I need PhD help?
- Molecular lab design?
- How different from regular lab testing?
- ASR and RUO assays?
- Federal/state regs?
- Can I trust vendors?
- Make/buy decisions?
- Economic issues?
- Drivers
- Dealing with genetic testing?
THESE THREE LEAD ON THIS PREPARATION

Coriolanus 1.2

DNA
According as I gave directions
King Henry VI Part II 3.2

RNA
Come, cousin you shall be the messenger.
King Henry VI Part III, 7.1

Protein
There are the players.
Hamlet 2.2
NON-AMPLIFICATION TECHNIQUES

ISH = in Situ Hybridization

AMPLIFICATION TECHNIQUES

PCR and real time PCR
<table>
<thead>
<tr>
<th>Method</th>
<th>Analytic Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytogenetics</td>
<td>$5 \times 10^{-2}$</td>
</tr>
<tr>
<td>FISH</td>
<td>$1-5 \times 10^{-2}$</td>
</tr>
<tr>
<td>Southern Blot</td>
<td>$5 - 10 \times 10^{-2}$</td>
</tr>
<tr>
<td>PCR</td>
<td>$10^{-4} - 10^{-6}$</td>
</tr>
</tbody>
</table>
## Diagnostic Sensitivity for Detection of Follicular Lymphoma

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse field electrophoresis</td>
<td>90%</td>
</tr>
<tr>
<td>FISH</td>
<td>90%</td>
</tr>
<tr>
<td>Southern Blot</td>
<td>80%</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>75%</td>
</tr>
<tr>
<td>PCR</td>
<td>70%</td>
</tr>
</tbody>
</table>
Decision Factors

Motivation – why offer the test?

♦ Clinical
♦ Economic / market
Essential References

- CAP Molecular Pathology Checklist, 7/11/2011 Revision
- Molecular Diagnostic Methods for Genetic Disease; Approved Guideline, 2nd Edition, MM1 2006, Clinical and Laboratory Standards Institute
- Molecular Diagnostic Methods for Infectious Disease; Approved Guideline, 2nd Edition MM3-A2 2006
- Nucleic Acid Amplification Assays for Molecular Hematopathology; Approved Guideline (MM5-A), NCCLS Standards and Guidelines, 2003
- Quantitative Molecular Methods for Infectious Diseases; Approved Guideline (MM5-A), NCCLS Standards and Guidelines, 2003
- Fluorescence In Situ Hybridization (FISH) Methods for Medical Genetics; Approved Guideline (MM7-A), NCCLS Standards and Guidelines, 2004
- Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods; Approved Guideline (MM13-A), NCCLS Standards and Guidelines, 2005
- Proficiency Testing (External Quality Assessment) for Molecular Methods; Approved Guideline (MM14-A), CLSI Standards and Guidelines, 2005
Exceptions to using the Molecular Checklist:

- **FISH**
  - Cytogenetics
  - Anatomic Pathology

- **BRISH**
  - Anatomic Pathology

- Infectious diseases (FDA approved assays)
  - Microbiology
Important References


Decision Factors

- Technological Considerations – what’s the best method?
  - FDA vs. non-FDA cleared
  - Platform
  - Intellectual property
Assay VERIFICATION Requirements
FDA cleared Tests

<table>
<thead>
<tr>
<th><strong>CLIA</strong></th>
<th><strong>CAP (7/11/2011)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>● Reportable range</td>
<td>🔧 Accuracy</td>
</tr>
<tr>
<td>● Precision</td>
<td>🔧 Precision</td>
</tr>
<tr>
<td>● Accuracy</td>
<td>🔧 Analytic Sensitivity</td>
</tr>
<tr>
<td></td>
<td>🔧 Interferences</td>
</tr>
<tr>
<td></td>
<td>🔧 Reference Range</td>
</tr>
<tr>
<td></td>
<td>🔧 Reportable Range</td>
</tr>
<tr>
<td>CLIA</td>
<td>CAP 7/11/2011</td>
</tr>
<tr>
<td>------------------------------</td>
<td>---------------------------------------------------</td>
</tr>
<tr>
<td>Reportable Range</td>
<td>Diagnostic and Analytical Sensitivity</td>
</tr>
<tr>
<td>Precision</td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td>Diagnostic and Analytical Specificity</td>
</tr>
<tr>
<td>Reference Range</td>
<td>Precision</td>
</tr>
<tr>
<td>Analytical Sensitivity</td>
<td>Linearity (quantitative tests)</td>
</tr>
<tr>
<td>Analytical Specificity</td>
<td>Reportable Range</td>
</tr>
<tr>
<td>Other</td>
<td>Reference Range</td>
</tr>
<tr>
<td></td>
<td>Any other applicable performance characteristics</td>
</tr>
</tbody>
</table>
## Minimum Guidelines for Verification of New Test Systems

*reference: Clinical Microbiology Newsletter 29:12, 2007*

<table>
<thead>
<tr>
<th></th>
<th>FDA Cleared</th>
<th>Modified FDA, ASR, laboratory developed</th>
<th>Time Span</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy – Qualitative</strong></td>
<td>50 positive specimens 50 negative</td>
<td>50 positive specimens 50 negative</td>
<td>At least 10 days</td>
</tr>
<tr>
<td><strong>Accuracy – Quantitative</strong></td>
<td>20 positive specimens in duplicate 50 negative specimens</td>
<td>40 specimens in duplicate 50 negative samples</td>
<td>3 – 4 days</td>
</tr>
<tr>
<td><strong>Precision – Qualitative</strong></td>
<td>Positive and negative controls</td>
<td>Positive and negative control</td>
<td></td>
</tr>
<tr>
<td><strong>Precision – Quantitative</strong></td>
<td>3 replicates at 2 concentrations (within run and between day)</td>
<td>2 concentrations in duplicate run twice per day</td>
<td>5 days and 20 days</td>
</tr>
<tr>
<td><strong>Analytical Sensitivity – limit of detection (LOD)</strong></td>
<td>Not required – consider verifying with 20 results at claimed level</td>
<td>60 measurements to establish 20 low concentrations to verify</td>
<td></td>
</tr>
<tr>
<td><strong>Analytical Specificity</strong></td>
<td>Not required</td>
<td>Evaluate all interfering compounds (same chemical or genetic structure/source)</td>
<td></td>
</tr>
<tr>
<td><strong>Reportable Range – Quantitative</strong></td>
<td>3-4 concentrations measured in duplicate</td>
<td>3-4 concentrations measured in duplicate</td>
<td></td>
</tr>
<tr>
<td><strong>Normal Values</strong></td>
<td>20 specimens per category</td>
<td>120 specimens</td>
<td></td>
</tr>
</tbody>
</table>
List of FDA Cleared Molecular Tests

Association for Molecular Pathology web site

http://www.amp.org/FDATable/FDATable.doc
# Viral Load Test Platforms (FDA Cleared)

<table>
<thead>
<tr>
<th>HIV-1</th>
<th>HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>End Point</strong></td>
<td></td>
</tr>
<tr>
<td>VERSANT® HIV RNA 3.0</td>
<td>VERSANT® HIV RNA 3.0</td>
</tr>
<tr>
<td>NucliSens® HIV-1 QT</td>
<td></td>
</tr>
<tr>
<td>Amplicor HIV-1 Monitor™ v1.5</td>
<td></td>
</tr>
<tr>
<td>COBAS Amplicor HIV-1 Monitor™ v1.5</td>
<td></td>
</tr>
<tr>
<td><strong>Real Time</strong></td>
<td></td>
</tr>
<tr>
<td>Abbott Real Time HIV-1</td>
<td></td>
</tr>
<tr>
<td>COBAS® AmpliPrep/TaqMan HIV-1</td>
<td></td>
</tr>
</tbody>
</table>
Reference/Validation Materials

Genetic Testing Reference Materials Coordination Program (GeT-RM)

http://wwwn.cdc.gov/dls/genetics/rmmaterials/default.aspx
<table>
<thead>
<tr>
<th>Category</th>
<th>URL</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residency Training Program for Pathologists</td>
<td><a href="http://www.amp.org/T&amp;E/molegenetics.htm">http://www.amp.org/T&amp;E/molegenetics.htm</a></td>
<td></td>
</tr>
<tr>
<td>Workshops for Pathologists</td>
<td><a href="http://www.amp.org/T&amp;E/moletrain.htm">http://www.amp.org/T&amp;E/moletrain.htm</a></td>
<td></td>
</tr>
<tr>
<td>Molecular Technologist Training Programs</td>
<td><a href="http://www.amp.org/T&amp;E/moletech.htm">http://www.amp.org/T&amp;E/moletech.htm</a></td>
<td></td>
</tr>
</tbody>
</table>
Amplification Techniques

Target Amplification
- Polymerase Chain Reaction (PCR)
- Real Time PCR
- Strand Displacement Amplification (SDA)
- Ligase Chain Reaction (LCR)
- Nucleic Acid Sequence Based Amplification (NASBA)
- Transcription Mediated Amplification (TMA)

Signal Amplification
- Hybrid Capture
- Branched chain DNA (bDNA)
- Invader Technology
PCR

Conventional PCR: requires thermocycler for amplification step and separate detector

Real time PCR: amplification and detection performed simultaneously
Conventional PCR

Thermocycler

amplicons

Detector
Real Time PCR

![Graph showing Real Time PCR cycle number and corresponding values for different phases: baseline, no template, exponential phase, plateau, threshold, and CT.](Image)
Real Time PCR

![Graph showing patient specimens, positive controls, and negative controls against cycle number and Delta Rn values.](image-url)
DNA Melting Curve Analysis

Temperature
Melting Curve Analysis

Fluorescence $-d(F/2)/dT$ vs. Temperature (°C)

- Mut
- WT
- WT/Mut

Negative control
Proprietary Issues

• At least 20% of human genome is patented!!! (4,382 of the 23,883 genes and counting)
• Most heavily patented genes deal with serious human diseases
• A small number of companies hold most of the patents
• Be cautious with LDTs
• Will become a major barrier to deep sequencing
RUO & LDT

“Antibodies, nucleic acid sequences, etc., labeled ‘Research Use Only’ (RUO) purchased from commercial sources may be used in home brew tests if the laboratory has made reasonable effort for FDA-approved/cleared kits, and ASR class reagents. The results of that failed search should be documented by the laboratory director.”

CAP Molecular Pathology Checklist, Page 55, 9/27/07

“A laboratory is considered to have developed a test if the test procedure was created by the laboratory performing the testing, irrespective of whether fundamental research underlying the test was developed elsewhere or reagents (including ASRs), equipment, or technology integral to the test were purchased, adopted, or licensed from another entity.”

CAP Molecular Pathology Checklist, Page 13, 7/11/2011
Unique Aspects of Molecular Pathology Reports


- Type of procedure
- Defined target
- Pertinent details, e.g., IVD, ASR, instrument type
- Disclaimer for non-FDA cleared tests
- Signature (may be electronic) of reporting physician if applicable
- Must use HUGO nomenclature for genes/loci
- Must include morphologic correlation if applicable
- Estimation of mutation detection rate and residual risk of being a carrier (if applicable)
Examples of Tests to Consider Bringing In-house
CF Carrier Screening

- Most frequently performed molecular genetic test.
- Goal to identify couples at risk of having a child with classic cystic fibrosis
COG Update on Carrier Screening for Cystic Fibrosis (ACMG 3/25/2011)

Recommendations

CF carrier screening should be offered to ALL women of reproductive age
CF Carrier Screening
Recommended Mutations
(ACMG/ACOG)

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Allele Frequency</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1077delT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3120+1G&gt;A</td>
<td></td>
<td>A455E</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G85E</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R334W</td>
</tr>
<tr>
<td>1717-1G&gt;A</td>
<td></td>
<td>ΔF508</td>
</tr>
<tr>
<td>3659delC</td>
<td></td>
<td>I148T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R347P</td>
</tr>
<tr>
<td>1898+1G&gt;A</td>
<td></td>
<td>Δ1507</td>
</tr>
<tr>
<td>3849+10kbC&gt;T</td>
<td></td>
<td>N1303K</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R553X</td>
</tr>
<tr>
<td>2184delA</td>
<td></td>
<td>G542X</td>
</tr>
<tr>
<td>621+1G&gt;T</td>
<td></td>
<td>R1162X</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R560T</td>
</tr>
<tr>
<td>2789+5G&gt;A</td>
<td></td>
<td>G551D</td>
</tr>
<tr>
<td>711+1G&gt;T</td>
<td></td>
<td>R117H</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W1282X</td>
</tr>
</tbody>
</table>

These have an allele frequency in affected general US populations of at least 0.1%.
CF Carrier Screening
Extended Panels

- Limited additional value for added cost
- Difficult to compare results to other labs
- Unknown penetrance
- Validation requires controls
- Test strategies extend turnaround time
- Still will not catch 100% of mutations
FDA Cleared CF Screening Platforms

FDA Approved Assays

- Luminex xTAG® Cystic Fibrosis Kit

- Hologic InPlex® CF Molecular test
  http://www.implexcf.com/

- GenMark DX – eSensor® Cystic Fibrosis Genotyping Test
  http://www.genmarkdx.com
## CF Validation
### (Coriell Standards)

<table>
<thead>
<tr>
<th>Coriell ID</th>
<th>CF Genotype</th>
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<tbody>
<tr>
<td>NA12444</td>
<td>1717-1G&gt;A</td>
</tr>
<tr>
<td>NA18800</td>
<td>1898+1G&gt;A, ΔF508</td>
</tr>
<tr>
<td>NA18799</td>
<td>218delA, ΔF508</td>
</tr>
<tr>
<td>NA11859</td>
<td>2789+5G&gt;A, 2789+5G&gt;A</td>
</tr>
<tr>
<td>NA07441</td>
<td>3120+1G&gt;A, 621+1G&gt;T</td>
</tr>
<tr>
<td>NA11275</td>
<td>3659delC, ΔF508</td>
</tr>
<tr>
<td>NA07381</td>
<td>3849+10KbC&gt;T, ΔF508</td>
</tr>
<tr>
<td>NA11282</td>
<td>621+1G&gt;T, G85E</td>
</tr>
<tr>
<td>NA11280</td>
<td>711+1G&gt;T, 621+1G&gt;T</td>
</tr>
<tr>
<td>NA11290</td>
<td>A455E, 621+1g&gt;T</td>
</tr>
<tr>
<td>NA11277</td>
<td>Δ1507</td>
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<tr>
<td>NA11497</td>
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<td>NA12785</td>
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<td>R334W</td>
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<td>NA11761</td>
<td>R533X, G551D</td>
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<tr>
<td>NA11284</td>
<td>R560T, ΔF508</td>
</tr>
<tr>
<td>NA11723</td>
<td>W1282X</td>
</tr>
</tbody>
</table>
Consent

- Genetic testing is uniquely regulated.
- Varies by state
  - Most require signed, informed consent prior to performing genetic testing.
- Most labs feel a responsibility to make sure consent has been obtained.
Cervical Cancer Screening

Results Obtained on Cervical Cytology

- Atypical squamous cells of undetermined significance (ASC-US)
  - High-risk HPV negative
    - Repeat Pap test in 12 months
  - High-risk HPV positive
    - Colposcopy

- Atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H)
  - Colposcopy
    - No lesion
    - Lesion found
      - Biopsy
      - Review Pap test and colposcopy to confirm ASC-H
      - Repeat Pap test in 6–12 mo

- Atypical glandular cells and adenocarcinoma in situ (AGC/AIS)
  - Colposcopy with endocervical sampling

- Low-grade squamous intraepithelial lesion (LSIL)
  - Colposcopy

- High-grade squamous intraepithelial lesion (HSIL)
  - Colposcopy
## High Risk HPV

<table>
<thead>
<tr>
<th>Test</th>
<th>FDA Cleared</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiagen Digene HPV Test</td>
<td>3/2003</td>
<td>Hybrid capture (signal amplification)</td>
</tr>
<tr>
<td>Hologic Cervista HPV</td>
<td>3/2009</td>
<td>Invader chemistry (target/signal amplification)</td>
</tr>
<tr>
<td>Roche Cobas HPV</td>
<td>4/2011</td>
<td>PCR (target amplification)</td>
</tr>
</tbody>
</table>
## FDA Cleared Specimens

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>HC2</th>
<th>Cervista</th>
<th>Cobas</th>
</tr>
</thead>
<tbody>
<tr>
<td>ThinPrep (PreservCyt)</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>SurePath</td>
<td>NO</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Digene Media</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Cobas Media</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>

If “NO”, test becomes LDT.
## High Risk HPV

<table>
<thead>
<tr>
<th>HPV</th>
<th>HC2</th>
<th>Cervista</th>
<th>Cobas</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>18</td>
<td>✔</td>
<td>✔</td>
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<td>31</td>
<td>✔</td>
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<tr>
<td>68</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
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</table>
## Platform Comparison

<table>
<thead>
<tr>
<th></th>
<th>HC2</th>
<th>Cervista</th>
<th>Cobas</th>
</tr>
</thead>
<tbody>
<tr>
<td>More established</td>
<td>More established clinical validation (ALT study)</td>
<td>Greater analytic sensitivity</td>
<td>Ongoing clinical trial with 45,000 women</td>
</tr>
<tr>
<td>clinical validation</td>
<td></td>
<td></td>
<td>(ClinicalTrials.gov)</td>
</tr>
<tr>
<td>(ALT study)</td>
<td></td>
<td></td>
<td>Greater analytic sensitivity</td>
</tr>
<tr>
<td>No internal control</td>
<td>No internal control</td>
<td>Internal control</td>
<td>Internal control (globin gene amplification)</td>
</tr>
<tr>
<td>False positives</td>
<td>False positives from cross-reactivity with low-risk HPV</td>
<td>False positives from “triple-positives”</td>
<td>No cross reactivity with low-risk HPV</td>
</tr>
<tr>
<td>from cross-reactivity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with low-risk HPV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More established</td>
<td>More established automation</td>
<td>Less hands-on time</td>
<td>Fully automated</td>
</tr>
<tr>
<td>automation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Gray zone”</td>
<td>“Gray zone”</td>
<td>No “Gray zone”</td>
<td>No “Gray zone”</td>
</tr>
<tr>
<td>Requires 4 ml</td>
<td>Requires 4 ml</td>
<td>Requires 2 ml</td>
<td>Requires 1 ml</td>
</tr>
</tbody>
</table>
CT/NG
FDA Cleared

- Roche COBAS AMPLICOR™
  - Cross reacts with Neisseria subflava and Neisseria cinerea
- Gen-Probe APTIMA COMBO 2®
- Abbott RealTime CT/NG on the m2000
- BD ProbeTec CT/NG
- Qiagen digene HC2 CT/GC
Thrombophilia Screening

- Anti-phospholipid antibodies
- Lupus anticoagulants
- Anithrombin III activity
- Protein C activity
- Protein S free antigen and activity
- Factor V Leiden
- Prothrombin G20210A
- Homocysteine
# Factor V / Factor II / MTHFR Mutation Analysis

<table>
<thead>
<tr>
<th>Factor II</th>
<th>Factor V</th>
<th>MTHFR</th>
<th>FII / FV</th>
<th>FII / FV / MTHFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autogenomics Infiniti</td>
<td>Autogenomics Infiniti</td>
<td></td>
<td>Autogenomics Infiniti</td>
<td></td>
</tr>
<tr>
<td>Nanosphere Verigene</td>
<td>Nanosphere Verigene</td>
<td>Nanosphere Verigene</td>
<td>Nanosphere Verigene</td>
<td>Nanosphere Verigene</td>
</tr>
<tr>
<td>Roche Lightcycler</td>
<td>Roche Lightcycler</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*FDA cleared assays / platforms*
“The Believers”

The present state of generic therapy based on Disease state and histologic site of origin must give way to molecularly-guided, personalized therapies, shifting the risk-benefit ratio of chemotherapeutic intervention toward an overwhelming individualized patient benefit – a goal clearly within our collective technological reach.

It’s easy to tell when an area has run out of ideas. The hype becomes extreme, and technology substitutes for brainpower. The cancer research area has reached this sorry state. The tiniest increase in the survival time of drug-treated cancer patients or median time to progression is touted as a cure, and wildly unrealistic claims about personalized cancer medicine emanate from the highest governmental and academic sources.

Gabor Miklos, GL, Baird, P.J, Curing Cancer: Running on Vapor, GEN: Vol.27, No. 9 May 1, 2007
PATIENT PHARMACOGENOMICS

(Constitutional molecular genetics)
Why is Pharmacogenomics “Potentially” Important?

- Potential for targeted, cost-effective drug therapy in an environment of escalating health care costs
- Avoid use of a drug with little or no beneficial effect for a specific patient
- Avoid adverse drug reactions
- Avoid searching sequentially for the right drug
- Get to the correct dose of the drug faster
- Goal: The right drug at the right dose to the right patient at the right time
The Problem: drugs don’t work on everyone

**Percentage Non-responders**

- **Hypertension Drugs 10-30%**
  - ACE Inhibitors

- **Heart Failure Drugs 15-25%**
  - Beta Blockers

- **Anti Depressants 20-50%**

- **Cholesterol Drugs 30-70%**
  - Statins

- **Asthma Drugs 40-70%**
  - Beta-2-agonists
Adverse Drug Reactions (ADRs):

- ADRs cause more than 106,000 deaths annually - 4th leading cause of death - ahead of pulmonary disease, diabetes, AIDS, pneumonia, and automobile deaths.
- More than 1 in 9 emergency department visits are due to drug-related adverse events (CMAJ • June 3, 2008; 178 (12))
- ADRs cost $136 BILLION yearly - Greater than total costs of cardiovascular or diabetic care combined.
- ADRs cause 1 out of 5 injuries or deaths per year to hospitalized patients
- Two-thirds of patient visits result in a prescription
- 2.8 BILLION Outpatient prescriptions (10 per person in the United States) filled in 2000
- ADRs increase exponentially with 4 or more medications due to drug-drug interactions

Source: fda.gov
21 CFR 201.57

“…if evidence is available to support the safety and effectiveness of the drug only in selected subgroups of the larger population with a disease, the labeling shall describe the evidence and identify specific tests needed for selection or monitoring of patients who need the drug.”
Warfarin causing ADRs

- **Increased risk of bleeding event INR > 3.0**
  - Risk of bleed increases 42% with each one point rise in the INR
  - 5-fold increase of intracranial hemorrhage in warfarin-treated patients with atrial fibrillation as INR > 4.5

- **Increased risk of thrombosis INR < 2.0**
  - 17-fold increase in stroke as INR fell < 2.0

Ansell JE. Semin Vasc Med 2003;3(3):261-269
Adequacy of Warfarin Control in an Anticoagulation Clinic

Distribution of INR values based on warfarin INR target range of 2 to 3

Adapted from Chiquette E, et al. Arch Intern Med. 1998
What are Economic Benefits of PGx-testing

AEI-Brookings Study

November 2006 study evaluating the effectiveness of formal integration of CYP2C9 testing into warfarin management:

- Avoid 85,000 serious bleeding events
- Avoid 17,000 strokes
- Save an average of $1.1 billion ($100M - $2.2B)

McWilliam et al 2006
<table>
<thead>
<tr>
<th>Pradaxa (dabigatran etexilate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Appears to be more effective than Coumadin (RE-LY trial)</td>
</tr>
<tr>
<td>• More expensive than Coumadin</td>
</tr>
<tr>
<td>• FDA recently recommended Pradaxa to prevent stroke in atrial fibrillation</td>
</tr>
<tr>
<td>Company</td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td>Nanosphere, Inc.</td>
</tr>
<tr>
<td>GenMark DX</td>
</tr>
<tr>
<td>Autogenomics</td>
</tr>
<tr>
<td>ParagonDx, LLC</td>
</tr>
<tr>
<td>TrimGen Corp</td>
</tr>
</tbody>
</table>
Metabolic pathway of Clopidogrel (Plavix)
# CYP2C19 Allelic Variants and Enzyme Activity

## Functional Status

<table>
<thead>
<tr>
<th>Description</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional / normal activity / wild-type</td>
<td>*1</td>
</tr>
<tr>
<td>Increased function / increased activity</td>
<td>*17</td>
</tr>
</tbody>
</table>
Plavix and CYP2C19

- CURE TRIAL for acute coronary syndromes
- Plavix effective regardless of 2C19 status
- Patient adherence to taking drug probably more important than genetic variation (Bove)
# PGx and the four major Cancers

<table>
<thead>
<tr>
<th>Breast Cancer</th>
<th>Colorectal Cancer</th>
<th>Lung Cancer</th>
<th>Prostate Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tamoxifen</strong></td>
<td><strong>Irinotecan</strong></td>
<td><strong>Ifosfamide</strong></td>
<td><strong>Gefitinib</strong></td>
</tr>
<tr>
<td>ABCC11</td>
<td>ABCB1</td>
<td>CYP2C8</td>
<td>ABCB1</td>
</tr>
<tr>
<td>CYP2D6 *</td>
<td>ABCC1</td>
<td>CYP2A6</td>
<td>ABCG2</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>CES2</td>
<td>CYP2B6</td>
<td>CYP2D6</td>
</tr>
<tr>
<td>CYP3A5</td>
<td>CYP3A4</td>
<td>CYP3A4</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>CYP3A5</td>
<td>CYP3A5</td>
<td>CYP2C19</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>SLO1B1</td>
<td>CYP2C9</td>
<td>CYP1A1</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>UGT1A1 *</td>
<td>ALD1A1</td>
<td>CYP3A5</td>
</tr>
<tr>
<td>CYP19A1</td>
<td>UGT1A9</td>
<td>ALDH3A1</td>
<td>CYP2C9</td>
</tr>
<tr>
<td>SULT1A1</td>
<td>UGT1A10</td>
<td>GSTT1</td>
<td>167 SNPs</td>
</tr>
<tr>
<td>176 SNPs</td>
<td>169 SNPs</td>
<td>GSTM1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>135 SNPs</td>
<td></td>
</tr>
</tbody>
</table>

**SNPs:**
- Breast Cancer: 176 SNPs
- Colorectal Cancer: 169 SNPs
- Lung Cancer: 135 SNPs
- Prostate Cancer: 167 SNPs
<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>None</th>
<th>Reduced</th>
<th>Increased</th>
</tr>
</thead>
<tbody>
<tr>
<td>*1</td>
<td>*2</td>
<td>*3</td>
<td>*4</td>
<td>*5</td>
</tr>
<tr>
<td></td>
<td>*6</td>
<td>*7</td>
<td>*8</td>
<td>*9</td>
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<tr>
<td></td>
<td>*10</td>
<td>*12</td>
<td>*14</td>
<td>*17</td>
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<td></td>
<td>*29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>*41</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>XN</td>
</tr>
</tbody>
</table>
2D6 Metabolizer phenotype

**Extensive metabolizer** = 2 functional or 1 functional + 1 reduced (eg. *1/*1 or *1/*9 )

**Intermediate** = 1 nonfunctional + 1 functional or decreased (eg. *3/*1 or *3/*41)

**Poor** = 2 nonfunctional (eg. *4/*4 or *6/*7)

**Ultrametabolizer** = multiple copies of functional (eg. *1X13)
### Benefit to CYP2D6 Testing to Predict Tamoxifen Efficiency?

<table>
<thead>
<tr>
<th>Trial</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>BIG 1-98 TRIAL (retrospective)</td>
<td>No Benefit</td>
</tr>
<tr>
<td>ATAC TRIAL (retrospective)</td>
<td>No Benefit</td>
</tr>
<tr>
<td>E3108 TRIAL (prospective analysis)</td>
<td>Pending</td>
</tr>
</tbody>
</table>

### Outstanding Testing Issues

- Is testing medically justified?
- Will the payer pay?
Additional Factors to Consider

- Endoxifen as a drug is being developed
- Measurement of Endoxifen is now available
- ASCO Guidelines DO NOT recommend using CYP2D6
Tumor Pharmacogenomics

- Colon carcinoma
- Lung carcinoma
- Melanoma

(Somatic molecular genetics)
Targeted Therapies

- Erlotinib
- Bevacizumab
- Sunitinib
- Sorafenib
- Panitumumab
- Cetuximab
- Temsirolimus
- Inhibition of programmed cell death (apoptosis)
- Tumor cell proliferation
- Tumor cell invasion/metastasis
- Development of tumor vasculature (angiogenesis)
**EGFR Inhibitors (metastatic colorectal carcinoma)**

**Predictors**

<table>
<thead>
<tr>
<th>Response</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>- EGFR amplification</td>
<td>- KRAS mutation</td>
</tr>
<tr>
<td>- PTEN lack of amplification</td>
<td>- BRAF mutation</td>
</tr>
<tr>
<td>- Epiregulin expression</td>
<td>- PTEN mutation</td>
</tr>
<tr>
<td>- Amphiregulin expression</td>
<td>- PTEN loss of expression</td>
</tr>
</tbody>
</table>
### Frequency of Significant KRAS Mutations

<table>
<thead>
<tr>
<th>Codon</th>
<th>Amino Acid Substitution</th>
<th>Amino Acid Change</th>
<th>Incidence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>Gly12Asp</td>
<td>Aspartate</td>
<td>32.5</td>
</tr>
<tr>
<td>12</td>
<td>Gly12Val</td>
<td>Valine</td>
<td>22.5</td>
</tr>
<tr>
<td>12</td>
<td>Gly12Cys</td>
<td>Cysteine</td>
<td>8.8</td>
</tr>
<tr>
<td>12</td>
<td>Gly12Ser</td>
<td>Serine</td>
<td>7.6</td>
</tr>
<tr>
<td>12</td>
<td>Gly12Ala</td>
<td>Alanine</td>
<td>6.4</td>
</tr>
<tr>
<td>12</td>
<td>Gly12Arg</td>
<td>Arginine</td>
<td>0.9</td>
</tr>
<tr>
<td>13</td>
<td>Gly13Asp</td>
<td>Aspartate</td>
<td>19.5</td>
</tr>
<tr>
<td>Others (61 &amp; 146)</td>
<td></td>
<td></td>
<td>1.8</td>
</tr>
</tbody>
</table>
KRAS Mutation Analysis

- Sanger Sequencing
- Pyrosequencing
- Allele Specific Real Time PCR
- Single nucleotide extension
- Allele specific PCR with melt analysis
- Short oligonucleotide mass analysis
- Chip based assays
- Strip assay
On **August 17, 2011** the U. S. Food and Drug Administration approved vemurafenib tablets (ZELBORAF™, Hoffmann-La Roche Inc.) for the treatment of patients with unresectable or metastatic melanoma with the BRAF V600E mutation as detected by an FDA-approved test.
The approval was based primarily on an international, randomized, open-label trial in patients with previously untreated metastatic or unresectable melanoma with the BRAF V600E mutation as detected by Cobas® 4800 BRAF V600 Mutation Test (Roche Molecular Systems, Inc.). This companion diagnostic test was Approved by the FDA concurrently with vemurafenib’s approval.

Only FDA cleared test for this purpose
Binding of Vemurafenib to mutated BRAF V600E
FEAR NO FISH
NON-AMPLIFICATION TECHNIQUES

ISH (in Situ Hybridization)

FISH (fluorescence)

CISH (chromogenic)

SISH (silver)
**FISH (Fluorescent In Situ Hybridization)**

1. Gene
2. DNAase (random cut)
3. Dig-dUTP (or Biotin-dUTP)
4. dCTP + dATP + dGTP
5. Denaturation (75°C)
6. Hybridization (on slides)
7. Antibodies anti-Dig (or Avidin) linked with a fluorophor
8. Epifluorescent Microscopy
   - The gene is located
Types of FISH

**Interphase FISH:**

- Used to determine the chromosome number of one or more chromosomes (CEP)
- Used to detect some specific chromosome rearrangements that are characteristic for certain genetic disorders
- Primary advantage is that it can be performed very rapidly (usually < 24 hours) because cell growth is not required
Types of FISH

**Metaphase FISH**

- Used primarily to detect specific micro deletions beyond the resolution of routine Cytogenetics
- Identify extra material of unknown origin
- Helps in cases where it is difficult to determine from routine Cytogenetics if a chromosome has a simple deletion or is involved in a subtle or complex rearrangement
- Used to detect some of the specific chromosome rearrangements seen in certain cancers
Probe Types

Chromosome enumeration probes (CEP’s)
Allow one to count the number of copies of a chromosome in a cell

Locus specific indicator probes (LSI’s)
Deletion or gain/amplification at specific gene region or fusion of gene regions

Whole Chromosome Paints (WCP’s)
LSI Probes

Normal

Deleted

Translocation

Amplification
Assay Types

Enumeration

Fusion

Specialty…ex. Telomere
UroVysion (Abbott)
FDA cleared

UroVysion Probes
CEP 3 red
CEP 7 green
CEP 17 aqua
LSI 9p21 (p16) gold
DAPI Screening...can you identify the tumor cells?
Examples of FISH (Fluorescence in situ Hybridization) for the HER-2/neu (17q11.2-q12) locus

Breast tumor with NO HER-2/neu amplification

Breast tumor with High Level of HER-2/neu amplification
Her2 SISH (Not Amplified)

Red = chromosome 17
Black = Her2
Her2 SISH (Amplified)

Red = chromosome 17  Black = Her2
<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Assay Name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FISH</strong></td>
<td>Pathvysion Her2 DNA probe kit</td>
<td>(Abbott)</td>
</tr>
<tr>
<td></td>
<td>Her2 FISH PharmDx kit</td>
<td>(Dako)</td>
</tr>
<tr>
<td><strong>CISH</strong></td>
<td>SPOT-light Her2 CISH</td>
<td>(Invitrogen)</td>
</tr>
<tr>
<td><strong>SISH</strong></td>
<td>Inform Dual ISH</td>
<td>(Ventana)</td>
</tr>
</tbody>
</table>
## Assay Types

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>YES!</th>
<th>NO!</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enumeration</td>
<td>YES!</td>
<td></td>
</tr>
<tr>
<td>Fusion</td>
<td></td>
<td>NO!</td>
</tr>
<tr>
<td>Specialty...ex. Telomere</td>
<td></td>
<td>NO!</td>
</tr>
</tbody>
</table>
Automation

Vendors

Genetix
BioView
Applied Spectral Imaging
Combine focal planes to see all signals
Most community laboratories can justify bringing in house most, if not all, of the molecular assays discussed as well as many others, **BUT** you must do it correctly to ensure patient safety.
Thank you for your participation and please submit your course evaluation form.