The Challenges of Precision Medicine: New Advances in Molecular Diagnostic Testing - Impact for Healthcare

Jessica Wang-Rodriguez, MD
Chief, VISN22 Consolidated Pathology and Laboratory Medicine Services
Professor of Pathology
University of California, San Diego
Disclosure Information
Jessica Wang-Rodriguez, MD

Disclosure of Relevant Financial Relationships

I have no financial relationships to disclose.

Disclosure of Off-Label and/or investigative Uses

I will not discuss off label use and/or investigational use in my presentation.
Objectives

• Identify new molecular diagnostic testing strategies that have significant impact on establishing diagnosis in hematologic malignancies.

• Evaluate the utility of these diagnostic techniques within the context of disease entities and the provider’s treatment options.

• Suggest mechanisms whereby VHA can successfully manage the financial burden associated with these new technologies.
VHA Molecular Genetics Workgroup

- Standardization of Tests
- Develop and Establish Practice Guidelines
- Communication within VHA to include e-consults or inter-facility consults, etc.

No federal dollars were used
Hematopathology Molecular Genetic Subcommittee (HMGS)

- Standardize testing nomenclature (as much as feasible)
- Develop practice guidelines for molecular testing for:
  - AML, MDS, MPN, CLL, Lymphoma, and Plasma Cell Neoplasms—for which we will examine literature evidence for “strong” vs. “weak” recommendations
- Develop standardized reporting guidelines for the current VA Molecular Labs
- Identify new tests as they are being reported in the literature or requested by Heme/Onc services and discuss the clinical utility for VA patient population.
- Networking and fact-finding of the current existing VA molecular laboratories and compile test menus, with the potential of forming VA-wide consultation services.
- This committee will ultimately form a “Hemepathology Molecular Genetic Testing” national e-consult service that can assist Path/Lab Chiefs to triage requests and direct proper testing to proper labs.
Members

- Dr. Jessica Wang-Rodriguez (Jessica.Wang-Rodriguez@va.gov) – VISN22
- Dr. Andrea Yunes (andrea.yunes@va.gov) San Antonio
- Dr. Frank Zhao (Frank.Zhao2@va.gov)
- Dr. Ryan Phan (Ryan.Phan@va.gov) Los Angeles
- Dr. Naili Ma (Naili.Ma@va.gov) Syracuse
- Dr. Fady Baddoura (Fady.Baddoura@va.gov)
- Dr. Claudio Mosse (Claudio.Mosse@va.gov) Nashville
- Dr. Mark Lu (Mark.Lu@va.gov) San Francisco
- Dr. Steven Schichman (Steven.Schichman@va.gov) Little Rock
- Dr. Aamir Ehsan (Aamir.Ehsan@va.gov) San Antonio
• “In a recent study, 54% of academic family medicine physicians reported that they were not knowledgeable about genetic tests.” (Fam Med 2013 Apr;45 (4):257-62)
Hematolymphoid Malignancies Testing Methodologies

• Morphology, Flow Cytometry, and Immunohistochemistry (IHC) are standard approaches to diagnosis and offer initial prognostic glimpse of the disease
• Cytogenetics/FISH
• PCR, qPCR
• Sequencing (Whole genome or targeted genes)
General Testing Strategies

• CLL and B cell lymphoproliferative disorders:
  – FISH
  – Chromosome karyotype
  – PCR

• Myeloid MDS/MPN, AML
  – FISH
  – Chromosome karyotype
  – PCR
  – qPCR
  – Sequencing
Next Generation Sequencing

• Advantages:
  – Cheaper and higher-throughput sequencing of the human genome than Sanger sequencing
  – Facilitate discovery of genes
  – Identification of disease-causing mutations for diagnosis, prognosis, and possibly treatment

• Disadvantages:
  – Still expensive and not widely available
  – Inaccuracy in homopolymer regions on certain NGS platforms
  – Data analysis is time-consuming and requires special expertise in bioinformatics
Drowned in next generation sequencing data

HELP!
Information Generated by the Next-Generation Sequencing

- **Cell Signaling**
  - FLT3
  - KIT
  - JAK2
  - MPL
  - KRAS/NRAS
  - PTPN11
  - NF1
  - CSF3R

- **Transcription**
  - CEBPA
  - RUNX1
  - GATA1/GATA2
  - PHF6
  - ETV6

- **Splicing**
  - SF3B1
  - SRSF2
  - ZRSR2
  - U2AF1

- **Myeloid Malignancies**

- **Cell Cycle**
  - TP53
  - NPM1

- **Epigenetics**
  - DNMT3A
  - TET2
  - IDH1/IDH2
  - ASXL1
  - EZH2
  - SUZ12
  - KDM6A

- **Cohesin Complex**
  - STAG2
  - SMC1A
  - SMC3
  - RAD21
<table>
<thead>
<tr>
<th>Test</th>
<th>Guideline or Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-gene RT-PCR</td>
<td>Breast cancer</td>
</tr>
<tr>
<td><em>BCR-ABL1</em> translocation, <em>ABL1</em> mutation</td>
<td><em>Ph+</em> acute lymphoblastic leukemia, chronic myelogenous leukemia</td>
</tr>
<tr>
<td><em>ALK</em> rearrangement</td>
<td>Non-small cell lung cancer</td>
</tr>
<tr>
<td><em>BRAF</em> mutation</td>
<td>Non-small cell lung cancer, melanoma, colon cancer, rectal cancer</td>
</tr>
<tr>
<td><em>EGFR</em> mutation</td>
<td>Non-small cell lung cancer</td>
</tr>
<tr>
<td><em>ERBB2</em> amplification/overexpression</td>
<td>Breast cancer, esophageal and esophagogastric junction cancers, gastric cancer</td>
</tr>
<tr>
<td><em>ESR1</em> expression</td>
<td>Breast cancer</td>
</tr>
<tr>
<td><em>KIT</em> mutation</td>
<td>Soft tissue sarcoma: GIST</td>
</tr>
<tr>
<td><em>KRAS</em> mutation</td>
<td>Colon cancer, rectal cancer, non-small cell lung cancer</td>
</tr>
<tr>
<td><em>MGMT</em> promoter methylation</td>
<td>Central nervous system cancers: anaplastic glioma/glioblastoma</td>
</tr>
<tr>
<td><em>MLH1</em>, <em>MSH2</em>, <em>MSH6</em>, <em>PMS2</em> expression and/or mutation, MSI testing</td>
<td>Colon cancer, rectal cancer</td>
</tr>
<tr>
<td><em>PDGFRA</em> mutation</td>
<td>Soft tissue sarcoma: GIST</td>
</tr>
<tr>
<td><em>PGR</em> expression</td>
<td>Breast cancer</td>
</tr>
<tr>
<td><em>ROS1</em> rearrangement</td>
<td>Non-small cell lung cancer</td>
</tr>
</tbody>
</table>

Abbreviations: GIST, gastrointestinal stromal tumor; MSI, microsatellite instability; PDGFRA, platelet-derived growth factor receptor α; *Ph+*, Philadelphia chromosome-positive; RT-PCR, reverse transcription-polymerase chain reaction.

* All biomarker recommendations were downloaded, analyzed, and sorted into categories from material from the Test Detects field. Analysis was performed in early 2014, and records may have updated since then, as Guideline changes have been recorded.

* Data derived from National Comprehensive Cancer Network.³
## AML

<table>
<thead>
<tr>
<th>Prognosis</th>
<th>Abnormality</th>
<th>Detection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>NPM1 w/o FLT3</td>
<td>PCR/Sequencing</td>
</tr>
<tr>
<td>Poor</td>
<td>FLT3 w/o NPM1</td>
<td>PCR/Sequencing</td>
</tr>
<tr>
<td>Poor</td>
<td>MLL</td>
<td>PCR/Sequencing</td>
</tr>
<tr>
<td>Poor</td>
<td>Kit with t(8;21) or inv(16)</td>
<td>PCR/Sequencing</td>
</tr>
</tbody>
</table>

- t(8;21); t(15;17); inv16; t(11q23)(MLL)
- Karyotype
AML Recommendation

• At diagnosis:
  – Karyotype
  – FLT3, NPM1
  – MLL if ambiguous phenotype
  – FISH for APL if Flow Cytometry is suspicious
  – Do not sequence for additional genes that do not add significant clinical value at this time

• Follow up for relapse:
  – Any of the above positive
  – +cKit Core Binding Factor
## Myeloproliferative Neoplasms

<table>
<thead>
<tr>
<th>Gene</th>
<th>Conditions and Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL</td>
<td>CML: Quant/Qual at dx; Quant at f/u</td>
</tr>
<tr>
<td>JAK2 (V617F)</td>
<td>PV (95-99%)</td>
</tr>
<tr>
<td></td>
<td>ET (50-70%)</td>
</tr>
<tr>
<td></td>
<td>PMF (40-50%)</td>
</tr>
<tr>
<td>MPL</td>
<td>ET (4%)</td>
</tr>
<tr>
<td></td>
<td>PMF (11%)</td>
</tr>
<tr>
<td>CALR</td>
<td>JAK2-/ MPL-</td>
</tr>
<tr>
<td>Mutations in pre-JAK2 Precursors?</td>
<td>EZH2, ASXL1, TET2...</td>
</tr>
</tbody>
</table>
MPN Recommendations

- If highly suspicious for CML, order FISH for bcr-abl (p210); order PDGFR/FGFR if hyper-eosinophilia is present.
- If confirmed CML, order qPCR bcr-abl at initial peripheral blood and bone marrow to establish baseline.
- Follow up can be either with PB or BM to assess 3log reduction to assess treatment response.
- If follow up PB is negative for bcr-abl, order qPCR in bone marrow.
- If morphology suspicious for non-CML MPN cases, order JAK2; calreticulin if JAK2-.
- JAK2 or calreticulin can be used for post bone marrow transplant follow up.
CMML

• No recommendation; possible Nex Gen Sequencing (of ASX1 or TET2) if using for prognostic or treatment options
## MDS and MDS/MPN

<table>
<thead>
<tr>
<th>Prognosis</th>
<th>Abnormality</th>
<th>Detection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>Del 5q, 7q, +8, del 20q</td>
<td>Karyotype/FISH</td>
</tr>
<tr>
<td>Better Prognosis</td>
<td>SF3B1, SRSF2 RNA Splicing</td>
<td>Sequencing</td>
</tr>
<tr>
<td>Poor Prognosis</td>
<td>Mutations in TP53, EZH2, ETV6, RUNX1, ASXL1</td>
<td>Sequencing</td>
</tr>
<tr>
<td>SRSF2-Poor survival</td>
<td>Spliceosome mutations</td>
<td>Sequencing</td>
</tr>
</tbody>
</table>
MDS Recommendations

- Sequencing not useful at this time
- Restrict testing to a good Karyotype and known FISH markers
- FISH if fresh sample for karyotype is not feasible and may be useful for detection of minimal residual disease
### CLL Molecular Abnormalities

<table>
<thead>
<tr>
<th>Prognosis</th>
<th>Abnormality</th>
<th>Detection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>13q del</td>
<td>FISH, Karyotype</td>
</tr>
<tr>
<td></td>
<td>IgVH hypermutation</td>
<td>Sequencing/PCR</td>
</tr>
<tr>
<td>Likely Poor</td>
<td>+12</td>
<td>FISH, Karyotype</td>
</tr>
<tr>
<td>Poor</td>
<td>11q del, 17q del</td>
<td>FISH, Karyotype</td>
</tr>
<tr>
<td></td>
<td>ZAP70, CD38&gt;30%</td>
<td>Flow Cytometry</td>
</tr>
<tr>
<td></td>
<td>IgVH unmutated</td>
<td>Sequencing, PCR</td>
</tr>
<tr>
<td></td>
<td>TP53 or NOTCH1 mutation</td>
<td>Sequencing, PCR</td>
</tr>
</tbody>
</table>

CLL Recommendation

- Morphology, standard Flow Cytometry
- FISH \textit{del}(13q14), \textit{del}(11q), Trisomy 12, \textit{del}(17p). IgHV mutation
  - At the time of diagnosis; OR
  - Immediately prior to initiation of therapy
- ZAP 70 is optional (uncertain for the implication to treatment)
- For “high risk” CLL: perform TP53, NOTCH1, and SF3B1 mutations by sequencing based assays (TP53 mutation for treatment by Ibrutinib).
- Treatment of CLL is evolving.
- Should molecular tests be done at diagnosis?
Implications for Treatment

• 2008 guideline, treat only if:
  – Anemia (Hgb<11 g/dl) or thrombocytopenia (platelet <100X109/L)
  – Symptomatic lymphadenopathy or organomegaly

• 2015 German CLL Study Group (phase 3):
  – Ibrutinib vs. observation at early stage CLL
A

Wang, 2011; Quesada, 2011; Puente, 2011 (n=4-105)
Landau, 2013 (n=160)
DFCI-ICGC, 2015 (n=282)

Number of affected samples

No. of sequenced tumor-normal pairs needed for mutation detection

% frequency to which recurrent mutations can be confidently called

Blood.2015; 126 (4):445-453

B

(▼) = 5 patients
(●) = 0 patients

<table>
<thead>
<tr>
<th></th>
<th>MYD88</th>
<th>SF3B1</th>
<th>TP53</th>
<th>ATM</th>
<th>NOTCH1</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFCI Broad Institute</td>
<td>10/111</td>
<td>9/111</td>
<td>6/16</td>
<td>3/111</td>
<td>8/111</td>
</tr>
<tr>
<td>Italy/Columbia</td>
<td>26/637</td>
<td>67/1007</td>
<td>10/59</td>
<td>24/1007</td>
<td>15/59</td>
</tr>
<tr>
<td>MLL</td>
<td>15/628</td>
<td>51/889</td>
<td>80/889</td>
<td>12/151</td>
<td>112/906</td>
</tr>
<tr>
<td>SCALE</td>
<td>15/628</td>
<td>51/889</td>
<td>80/889</td>
<td>12/151</td>
<td>112/906</td>
</tr>
<tr>
<td>GCLLSG CLLB</td>
<td>114/621</td>
<td>72/628</td>
<td>40/229</td>
<td>33/224</td>
<td>62/622</td>
</tr>
<tr>
<td>UK LRF CLL4</td>
<td>73/437</td>
<td>17/97</td>
<td>39/100</td>
<td>49/466</td>
<td>13/67</td>
</tr>
<tr>
<td>GCLLSG CLL2H</td>
<td>21/100</td>
<td>24/100</td>
<td>11/100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UK UKRN</td>
<td>3/114</td>
<td>33/114</td>
<td>29/114</td>
<td>29/114</td>
<td>18/114</td>
</tr>
</tbody>
</table>

Early Front-line R/R
Plasma Cell Dyscrasia
Recommendations

• Flow Cytometry for initial and follow up
• Karyotype at diagnosis only
• FISH panel at diagnosis and follow up
• Sequence based assays not recommended at this time
B-cell Lymphoproliferative Disorders

• At diagnosis, morphology, flow cytometry, IHC, + Ig gene rearrangement by PCR (if flow cytometry and IHC is inclusive)
• MCL- if IHC inconclusive, do FISH for t(11;14)
• Indolent MCL may be post g.c. origin; Ki67 may be helpful for predicting MCL behavior
• Follicular Lymphoma (low grade)- if IHC inconclusive, do FISH for t(14;18)
• In general, FISH more sensitive than Karyotype
• Sequence-based assays not recommended at this time
DLBCL
## DLBCL Molecular Abnormalities

<table>
<thead>
<tr>
<th>Prognosis</th>
<th>Abnormality</th>
<th>Detection Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>GCB variant</td>
<td>IHC</td>
</tr>
<tr>
<td></td>
<td>BCL6 rearrangement</td>
<td>FISH</td>
</tr>
<tr>
<td></td>
<td>BCL6 somatic mutations</td>
<td>Sequencing</td>
</tr>
<tr>
<td>Variable</td>
<td>Ki67</td>
<td>IHC</td>
</tr>
<tr>
<td>Neutral (Rituximab era)</td>
<td>BCL2 rearrangement</td>
<td>FISH</td>
</tr>
<tr>
<td>Poor</td>
<td>CD5, BCL2, CD43</td>
<td>IHC/Flow Cytometry</td>
</tr>
<tr>
<td></td>
<td>ABC variant/ Non-GCB</td>
<td>IHC</td>
</tr>
<tr>
<td></td>
<td>EBV</td>
<td>ISH</td>
</tr>
<tr>
<td></td>
<td>MYC rearrangement</td>
<td>FISH</td>
</tr>
<tr>
<td></td>
<td>Co-express MYC+BCL2</td>
<td>IHC</td>
</tr>
<tr>
<td></td>
<td>Double hit (MYC+BCL2)</td>
<td>FISH</td>
</tr>
<tr>
<td></td>
<td>TP53 somatic mutations</td>
<td>Sequencing</td>
</tr>
</tbody>
</table>

DLBCL Recommendations

• Morphology
• IHC
  – Hans algoritym (CD10, BCL-6, MUM-1) to differentiate between GC and ABC
  – cMyc-IHC, BCL-6 and BCL-2; Ki67
  – CD30 if patients considered for trials of Anti-CD30
• Flow Cytometry
• FISH for Chromosomal Abnormalities
  – BCL2, BCL6, cMYC rearrangements; Double hit (cMYC + BCL2 or BCL6)
  – Expression of cMyc, either by IHC or FISH (protein amplification or translocation), confers worse prognosis
• Not practical for sequencing or expression profiling
• Staging bone marrow- karyotype is not recommended if morphology and flow are negative
ALL Recommendations

• Morphology, Flow Cytometry, and Karyotype
• BCR/ABL qRT-PCR as indicated
• TCR-g if indicated
• MRD by high sensitive Flow Cytometry
• Sequencing not always available and currently not the standard of practice
Minimal Residual Disease Detection Using High-Throughput Sequencing Predicts Clinical Outcome in Patients with Pediatric B-Lineage Acute Lymphoblastic Leukemia

by Marian Harris, Donna S. Neuberg, Jianbiao Zheng, Malek Faham, Stephen E. Sallan, and Lewis B. Silverman

Blood
Volume 124(21):2391-2391
December 6, 2014

©2014 by American Society of Hematology
Kaplan-Meier analysis of event-free survival for A) 30 MRD negative (<10^{-3}) and 5 MRD positive (≥10^{-3}) patients and B) 26 MRD negative (<10^{-4}) and 9 MRD positive (≥10^{-4}) patients.

Marian Harris et al. Blood 2014;124:2391
Challenges of the Molecular Testing

• Cost—where do we stop?
• Few VA labs offer comprehensive molecular testing. Majority of the VA’s send tests out to academic affiliates or reference labs.
• Nomenclature- not all test names are standardized and difficult to compare between tests offered at academic affiliates and reference labs.
Management of the Financial Challenges

• Establish an interfacility consultation services with a panel of VA expert hematopathologists (most likely from this panel) who can diagnose, recommend, and evaluate for the utility of additional testing for prognostic and treatment purposes.

• Recommend standardized LOINC codes for tests such as BCL-ABL1 FISH, BCL-ABL1 Qualitative (RT-PCR), BCL-ABL1 Quantitative (RT-PCR) so to reduce the chance of ordering duplicating tests.
Wish List

• Storing the remaining DNA, whether at the clinical Labs or contracted reference labs that may be a resource in future validation of the tests as they become available