Molecular Pathology in Hematopathology

Ryan Phan
VA Los Angeles/UCLA
- Introduction of Molecular Pathology/Cytogenetics techniques
- Utilization of Molecular Pathology in Hematopathology
- Interesting case studies
- RNA seq evaluation for CLL
The expanding roles of Molecular Pathology in Hematopathology Practice

Clinicians send sample to Pathology (BM/blood/tissues)

Path & Lab (Hematopathology)

Diagnosis/Prognosis
Personalized Risk
Prediction
Medication, Dosing

- Morphology
- Immunohistochemical staining
- Flow cytometry
- Cytogenetics
- Molecular testing
Genetic Abnormalities in Hematopoietic Neoplasms

Genetic alterations

- Aberrant mutations
- Insertion/Deletions
- Amplification
- Chromosomal gains or losses
- Translocations

Deregulation

- Proto-oncogenes
- Tumor suppressor genes
- Genes involved in apoptotic regulation
Integrating Molecular Pathology to Hematopathology

Molecular Pathology

- Aberrant mutations
- Insertion/Deletions
- Amplification
- Chromosomal gains or losses
- Translocations

Hematopathology

- Diagnostic tool
- Tumor classifications
- Prognostic markers
- Predictive Markers for Therapies/Rx selections
- Minimal residual disease testing
- Future Directions
Current Strategies to Detect Molecular Alterations

- Karyotyping
- FISH
- Southern Blot
- PCR based- methods
  - Various detection methods
  - Q-PCR and Q-RT-PCR
- Arrays (Expression, CGH, SNP)
- DNA Sequencing
- Next Generation Sequencing
Molecular Cytogenetics

- Conventional karyotyping
  Large indels, amplification, translocations

- In situ hybridization (FISH)
  Large indels, amplification, translocations
Southern Blot Analysis: Clonality Testing

IGH rearrangement

Tumor # 1

<table>
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<tr>
<th></th>
<th>EcoRI</th>
<th>Bgl II</th>
<th>Sal I</th>
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Tumor # 2

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Tumor # 3

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<td>3</td>
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J<sub>H</sub> probe

* GL
PCR based- Analysis: Clonality Testing
Detection of Chromosomal Translocation

**FL t(14;18) (q24;q32)**

- **Normal**
- **Abnormal**

* IgH/BCL2 fusion probes

**Burkitt t(8;14) (q24;q32)**

- **Normal**
- **Abnormal**

* CEP8-Myc/IgH fusion probes
Detection of Chromosomal Translocation

- Only ~85% positive
- t(14;18) does not establish a diagnosis
- t(14;18) negative cases: might have extra copies of chromosomal 18/BCL2 or BCL6 translocation
**Molecular Genetics in CLL/SLL: Prognosis**

**FISH for CLL/SLL**

- Used for prognosis
- 17p13.1 deletion (p53) = poor prognosis
- 11q22.3 deletion (ATM) = poor prognosis
- +12 = Intermediate prognosis (CDK4?)
- 6q23 deletion (Myb) = intermediate prognosis
- Isolated 13q deletion <65%) = good prognosis

![FISH Images](image-url)
CLL/SLL and Somatic hypermutation

- Pre-GC B cells have not undergone SHM and memory B cells have.
- CLL/SLL with SHM has significant better prognosis.
Mutations in Normal Cytogenetic AML: Prognosis

- **FLT-3:**
  - Over-expressed ~20-30% of AML cases
  - Typical mutation is ITD and D835
  - Correlates with **poor prognosis** in younger patient

- **NPM-1:**
  - Most common in AML with normal cytogenetics (~60%)
  - **Good prognosis** (yet no effect if FLT3 mutated)
  - Common insertion
Molecular Genetics in Leukemia: Diagnosis/Rx Response

- BCR/ABL: all CML and a subset of ALL
- PML/RARA: virtually all APL
- Monitoring Rx response
- Prediction of relapse

BCR/ABL t(9;22)(q34;q11)  PML/RARA t(15;17)(q22;q21)

Normal

Abnormal
Molecular Genetics in Leukemia: Rx Response

BCR/ABL

- Selection of Rx response
- Prediction of relapse

> 100 different mutations  green  2-10% of patients
> 10% of patients
DNA Sequencing Strategies

Single Gene Sequencing (Sanger)

Next Generation Sequencing

Massively parallel sequencing

Whole genome

Whole or partial exome/gene panel

Courtesy of Dr Eisen lab
Next Generation Sequencing

Next-Generation Sequencing

Massively parallel sequencing

Whole genome

Whole or partial exome/gene panel

Raw Data Analysis

Image processing and base calling

Whole-Genome Mapping

Alignment to reference genome

Variant Calling

Identification of genetic variation (SNP, Indels, SVs…)

Annotation

Linking variants to biological information
Next Generation Sequencing

Advantages
- High throughput
- Better sensitivity
- Efficient use of limited sample
- Wider range of mutation detection

Challenges
- Evaluation of multi-gene panels
- Establishment of assay characteristics
- Validation
- Clinical implementation
- Results interpretation and reporting
- Billing and reimbursement
- Legal and ethical issues
Molecular/Cytogenetic results must be interpreted in clinical and pathological context
Utilization of Molecular Pathology in Hematopathology
The expanding roles of Molecular Pathology in Hematopathology

Clinicians send sample to Pathology (BM/blood/tissues) → Molecular Pathology & Cytogenetics Testing → Diagnosis/Prognosis, Personalized Risk Prediction, Medication, Dosing

“Just another (Molecular/Cytogenetic) test”
OR
Is there an opportunity to optimize these ancillary tests to assess the patient for the presence of hematologic malignancies?
Molecular Genetics Testing in the US

Laboratory costs ~4% of healthcare costs; but are increasing at rapid rate

Molecular Genetics is 15-25% of total; Fastest growth area

Approximately 20-40% of laboratory testing is unnecessary

Courtesy of CAP and Drs Hanson & Kurtin (Mayo Clinic) (2015)
Realities of Today’s Clinical Practice

The “knowledge gap”:
- Various levels of understanding of how to use today’s laboratory assays amongst clinicians
- Clinical knowledge, when a test is ordered, is incomplete. Clinician is compelled to order “everything upfront” as it may be the only chance to get that information!
- Knowledge gap between clinicians and laboratory
- Knowledge gap between science and therapies
- Laboratories do not provide guidance for the appropriate use of assays in various diseases
- New tests emerge without an effort to understand how these tests should be utilized in the context of other existing assays

Ordering process:
- Requisition forms provide no help
- “Fill in the blank” vs. check boxes
- Overbundling of laboratory tests
- No processes to review test requests
- No processes to sequentially add or delete tests after initial results are determined
Some “test requests” that we’ve seen …

“Just another molecular cytogenetics request…”

- B cell lymphoma FISH in a rule out MDS case
- JAK2 on both PB and BM specimens
- MDS FISH in a lymphoma staging bone marrow with no hx of previous treatment
- Karyotyping, lymphoma FISH and PCR of IG gene rearrangement on a morphologically positive FL staging BM
- MM FISH studies on a BM specimen that show no monotypic plasma cell present.
Utilization of Molecular Cytogenetics Testing

Clinicians send sample to Pathology (BM/blood/tissues)

Molecular Pathology & Cytogenetics Laboratory

There must be an opportunity to optimize molecular test utilization
Collaboration:
• Between laboratory divisions
• Flow cytometry
• Hematopathology
• Anatomic Pathology
• Clinicians

Practicing:
• Build a test formulary
• Review requests for genetic and molecular testing
  – Request clinical history and path results
  – Consult with Hem/Onc fellows/clinicians
  – Work closely with pathology residents/attending
  – Hold test requests that “don’t make sense”
  – Request H&E and IHC slides if necessary
  – Convince clinicians to cancel tests if clinically and/or pathologically not indicated

• Create send-and-hold and sequential testing approaches
• Create and use an algorithmic approach
• Unbundle test panels
• Use the data
• Use/update literature
• Reduce repeat testing
**Clinical Cytogenetics Requisition form**

**Department of Pathology & Laboratory Medicine**
VAMC Greater Los Angeles Healthcare System
11301 Wilshire Blvd, BM 1245, Los Angeles, CA 90073
Phone: (310) 478-3711 x41049 or x41400, Fax: (310) 269-4916

### Patient Information
- **Name (Last, First):** [Redacted]
- **Sex:** [Redacted]
- **SSN:** [Redacted]
- **Date of Birth:** [Redacted]

### Specimen Information
- **Collection Date:** [Redacted]
- **Time:** [Redacted]
- **Specimen Submitted:**
  - Bone Marrow (BM)
  - Core biopsy (BM)
  - Blood
  - WBC
  - FNA
  - Urine
  - Fluid
- **Lymph Node:** [Redacted]
- **Tumor/Tissue Type:** [Redacted]

### Physician Information
- **Ordering Clinician:** [Redacted]
- **Phone/Page:** [Redacted]
- **Facility:**
  - VA Los Angeles
  - [Redacted]
- **Received by Cytogenetics lab on:** [Redacted]

### Clinical Information
- **Pathology report must be included:** Indicated
- **New Patient:** No
- **Previous Studies:** [Redacted]
- **Indication:**
  - ALL
  - CLL
  - MM
  - Lymphoma
- **Clinical Information:**
  - MDS
  - AML
  - Other

### TEST REQUESTED:
- **FISH**
- **FISH and KARYOTYPE**
- **KARYOTYPE**

### FISH Panels (Mutation/Probes/Chromosomal(s) of focus)
- **ALL (Adult):** [Redacted]
- **CML:** [Redacted]
- **CML Blast Crisis:** [Redacted]
- **B220 (B-ALL):** [Redacted]
- **A536:** [Redacted]
- **CML:** [Redacted]
- **MM (Diagostic):** [Redacted]
- **MM (Prognostic):** [Redacted]

### Solid Tumors
- **HER-2 Test:** [Redacted]
- **Bcr-Abl:** [Redacted]
- **ETV6-TEL:** [Redacted]

### Other MNP (SMY only)
- **Bcr-Abl:** [Redacted]
- **Phenotype:** [Redacted]

### Individual Probes
- **15q22:** [Redacted]
- **17p13:** [Redacted]

### Specimen Instructions
- **Bone Marrow:** 2-5ml of the 1st draw into transport media and sent to lab immediately. This is the sample of choice for leukemia diagnosis and follow-up. Minimum volume for Karyotyping and additional 5ml for FISH tests are required.
- **BM Core Biopsy:** Send only if BM aspirate is obtained. Collect and placed in the provided transport media.
- **Peripheral blood:** Draw 5ml into sterile green-top sodium heparin vacutainer and mix by inverting. (Note: BM is preferred as karyotyping of leukemia blasts is usually only successful if blasts are above 30%)
- **Tissue:** Collect the non-necrotic tumor in the provided transport media or sterile saline and send immediately to the lab.
- **Type:** Submit paraffin blocks (H&E is required)
- **Notes:** Collect 5-10ml blood or 10-15ml diluted urine. Send specimen immediately or less than 48hrs from remote locations.

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**Molecular Pathology Laboratory VISN22/VA Los Angeles**
Molecular Pathology Laboratory VISN22/VA Los Angeles

Molecular Pathology Requisition form
Department of Pathology & Laboratory Medicine
VA Greater Los Angeles Healthcare System
1111 Wilshire Blvd., rm 1201, Los Angeles, CA 90071
Phone: (310) 478-3711 x40167 or 46580 Fax: (310) 268-0563

<table>
<thead>
<tr>
<th>Patient Information</th>
<th>Specimen Information</th>
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<tbody>
<tr>
<td>Name (Last, First)</td>
<td>M: F</td>
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<tr>
<td>Sex</td>
<td>Age</td>
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<tr>
<td>Collection Date</td>
<td>Time</td>
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<td>Collected by</td>
<td>M.D.</td>
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<td>SN#</td>
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<td>Date of Birth</td>
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<table>
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<tr>
<th>Physician Information</th>
<th>Specimen Type</th>
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<td>Ordering Clinician</td>
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<td>Phone/Page:</td>
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<td>Facility</td>
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<tr>
<td>VA Los Angeles</td>
<td></td>
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<tr>
<td>San Diego, Loma Linda, Long Beach, Las Vegas</td>
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<tr>
<td>Other:</td>
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<table>
<thead>
<tr>
<th>Clinical Information</th>
<th>Indication:</th>
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<tr>
<td>Pathology, and/or Hem/Onc expert must be included</td>
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<tr>
<td>New Patient</td>
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<tr>
<td>Previous Studies:</td>
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TEST REQUESTED: (Must specify Mutation(s) or Gene(s) of focus)
For consultation, contact Molecular Pathology fellows/residents at (310) 478-3711 x40167 or x46580

<table>
<thead>
<tr>
<th>Molecular Oncology Panels (ONC)</th>
<th>Pharmacogenetics Panels (RGX)</th>
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<tbody>
<tr>
<td>Bcr/abl (all 316 genes)</td>
<td>Factor V Leiden (16910-A)</td>
</tr>
<tr>
<td>IG/H@ gene rearrangement</td>
<td>Factor II (Prothrombin) (5020G-A)</td>
</tr>
<tr>
<td>EGFR mutations</td>
<td>PT (1646G) 5' UTR (2210G-A)</td>
</tr>
<tr>
<td>TEG/ER rearrangement</td>
<td>MET (711G&gt;T &amp; 1547A&gt;G)</td>
</tr>
<tr>
<td>MCL/CLL rearrangement</td>
<td>Thrombophilia Panel (Factor V, II, 6MTHFR)</td>
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<tr>
<td>IG/H@ gene rearrangement</td>
<td>IL-23D genotyping</td>
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<tr>
<td>MCL/CLL rearrangement</td>
<td>Hereditary Hemochromatosis</td>
</tr>
<tr>
<td>IG/H@ gene rearrangement</td>
<td>(CA39Y, FY-3D, β-395G)</td>
</tr>
<tr>
<td>MCL/CLL rearrangement</td>
<td>Thipsinic Methyltransferase</td>
</tr>
<tr>
<td>IG/H@ gene rearrangement</td>
<td>(TPMT *1.1,3B/C and 3)</td>
</tr>
<tr>
<td>MCL/CLL rearrangement</td>
<td>Cystic fibrosis (23 mutations &amp; poly T)</td>
</tr>
<tr>
<td>Leukemia/Megakaryocytic</td>
<td>Genotyping &amp; Antiviral Resistance Testing (MM)</td>
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<tr>
<td>BCR-ABL1 RT-PCR (Qualitative)</td>
<td>(to viral load &gt;2000 copies/ml ___ no ___)</td>
</tr>
<tr>
<td>BCR-ABL1 RT-PCR (Quantitative)</td>
<td>HIV-1 RT and Protease Inhibitor Resistance Test</td>
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<tr>
<td>MALT1 (IVS17F) (Quantitative)</td>
<td>(Plasma only)</td>
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<tr>
<td>MALT1 mutations</td>
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<tr>
<td>MALT1 mutations</td>
<td></td>
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<tr>
<td>special request/reflex</td>
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Peripheral Blood/IM: Draw 3-5ml EDTA. Skip immediately to the lab noon temp. Transport refrigerated if delayed >24 hr.
BM Gene biopsy: Collect and place in sterile saline or in transport media if available.
Tissue: Collect non-neoplastic tumor in sterile saline or transport media and transport refrigerated. Frozen (no media) if delayed.
DNA: Collect and place in the provided alcohol-based solution container and transport at room temp.
Plasma: 3-6ml EDTA, centrifuge within 3hrs of collection and freeze minimum 3ml plasma. transport frozen.
SPE: Submit paraffin blocks or 3-5 cuts of tissue each in an Eppendorf tube (N 6 is required)
THE PATIENT

MD requests test

Order is processed

Order is reviewed

Reflex test recommends if necessary

Lab performs test

Test results interpreted

Toolpath report is integrated

MD acts on the results

Molecular Path & Cytogenetics Laboratory
Examples of Send and Hold Approach

- Cytogenetics in lymphoma staging: wait for BM morphology
- T cell gene rearrangement for cytopenia: wait for flow cytometry results
- AML molecular prognostics: wait for cytogenetic results
- FISH in MGUS evaluation: wait for immunohistochemistry and/or flow cytometry results
## Examples Of Heme-Associated Utilization

<table>
<thead>
<tr>
<th>Test requested</th>
<th>Utilization guideline</th>
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<tbody>
<tr>
<td>TCR- blood and marrow</td>
<td>Only in context of T-cell phenotyping studies</td>
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<tr>
<td>BCR- blood and marrow</td>
<td>Almost never useful</td>
</tr>
<tr>
<td>Karyotyping lymphoma staging</td>
<td>Only useful if unexplained cytopenias</td>
</tr>
<tr>
<td>CLL FISH</td>
<td>Only useful in diagnosed CLL</td>
</tr>
<tr>
<td>MM FISH</td>
<td>Not useful if no monotypic plasma cells</td>
</tr>
<tr>
<td>JAK2V617F</td>
<td>Equivalent results in blood and BM</td>
</tr>
<tr>
<td>JAK2 exon 12</td>
<td>Only if JAK2V617F is negative and clinically indicated possible PV</td>
</tr>
<tr>
<td>PML-RARA FISH</td>
<td>Not useful in follow up APL</td>
</tr>
<tr>
<td>MDS FISH</td>
<td>Negative does not rule out MDS</td>
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</table>
Starting the implementation of testing utilization process
Starting the implementation of testing utilization process
Data from Mayo Clinic (Dr. Paul Kurtin)

IGH rearrangements on BM

- Total: 47 cases
  - 14 clonal pattern (positive)
    - Morphology plus flow or IHC positive in all
  - 33 non-clonal pattern (negative)
    - 2 myelodysplastic syndrome
    - 1 acute myeloid leukemia
    - 1 LGL leukemia
    - 29 normal morphology

TCR rearrangements on BM

- Total: 172 cases
  - 20 True positive result
  - 19 False positive results*
  - 12 False negative results
  - 121 Unnecessary studies

*11% as expected

“These tests are dangerous for patient care. Why do you let us order them?”

All Unnecessary
Indications for Clonality Testing

- Atypical lymphoid proliferations
- Equivocal immunophenotype
- Assistantance in establishing lineage
- Multifocal disease
- Monitoring disease (MRD?)
Limitations and Interpretation of Clonality Analysis

- Clonality does not imply malignancy
  - Lymphomatoid papulosis
  - MGUS
  - Multicentric Castleman Disease
→ Clonality does not equal malignancy

- Not all clones are detectable by PCR based assays
→ Lack of detectable clone does not rule out clonality

- Clonal TCR or IG locus does not define lineage
  - Acute leukemias and lymphoblastic lymphoma might rearrange both
  - Lineage infidelity in ALL and immunodeficiency states

Tests only determine clonality and must be interpreted in the clinical and pathologic context
The expanding role of Hematopathologist

MD requests test

Order is processed

Order is reviewed

Reflex test recommends if necessary

Molecular Path & Cytogenetics Laboratory

Lab performs test

Test results interpreted

Hemopath report is integrated

MD acts on the results

THE PATIENT

As far as I can see, Was Missing A Few
Interesting case studies
Case # 1: Lymphoma

Molecular Path lab received:

- Fresh tissue received from tonsil
- Indication: DLBCL vs. Follicular lymphoma
- This is a new patient with no Molecular Cytogenetics record
- The following FISH probes are checked:
  - 14q32 (IGH)
  - t(14;18)(IGH-BCL2)
Case # 1

Clinical Hx:

• 65 year old male with 3-4 weeks of dysphagia
• Med Hx: Prostate CA, multiple skin CAs
• Social Hx: History of smoking and Etoh use

• PE: Exophytic left tonsillar mass

• CBC: within normal limits
Case # 1

Flow cytometry on Bx:

- Monotypic, lambda restricted B-cell population of intermediate to large lymphocytes which
  - Express CD19, CD20, CD10, lambda
  - Negative for CD2, CD3, CD4, CD5, CD7, CD8, kappa
Case # 1

Histologic dx:
- Diffuse large B-cell lymphoma, germinal center type, NOS
  - Sheets of large atypical cells with coarse, clumpy chromatin, minimal cytoplasm and occasional small but prominent central nucleoli
  - 80 to 90% proliferative index
  - Background of karyorrhectic debris

IHC:
- Positive
  CD45, CD10, CD20, PAX 5, BCL6, BCL2
- Negative
  CD3, CD5, CD21, CD23, CD30, CD138, MUM1, Tdt, Cyclin D1
Recommendation by molecular cytogenetics review:

**TEST REQUESTED:**
- FISH
- FISH and KARYOTYPE
- KARYOTYPE

**FISH Panels (Must specify Probe(s) or Chromosome(s) of focus):**

<table>
<thead>
<tr>
<th>ALL (Adult)</th>
<th>CLL</th>
<th>Lymphoma</th>
<th>Solid tumors</th>
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<tbody>
<tr>
<td>t(9;22) (BCR-ABL1)</td>
<td>Trisomy 12 (CEP12)</td>
<td>16q12 (IGH)</td>
<td>HER2 Test (HER-2/neu FISH)</td>
</tr>
<tr>
<td>t(11;22) (MLL1)</td>
<td>13q14/q34 (BCL6)</td>
<td>t(6;23) (c-MYC)</td>
<td>HER2-+/neg 17q12.2-q12 (ERBB2) FISH only</td>
</tr>
<tr>
<td>14q32 (MYC)</td>
<td>del 11q22.3 (ATM)</td>
<td>16q23 (c-MYC)</td>
<td>Lung cancer 2p23 (ALK)</td>
</tr>
<tr>
<td>CML</td>
<td>del 17p13.1 (p53)</td>
<td>8q24 (MYC)</td>
<td>Lung cancer 6q22 (ROS1)</td>
</tr>
<tr>
<td>t(9;22) (BCR-ABL1)</td>
<td>del 6q23 (c-MYC)</td>
<td>8q24 (MYC)</td>
<td>Lung cancer 10q11.2 (RET)</td>
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<tr>
<td>9q34 (AS1)</td>
<td>16q21 (MALT1)</td>
<td>9q34 (AS1)</td>
<td>Lung cancer 7q31.2 (MET)</td>
</tr>
<tr>
<td>9p24 (CEP9)</td>
<td>8q24 (MYC)</td>
<td>9p24 (MYC)</td>
<td>Lung cancer 1q21-22 (NTRK1)</td>
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<tr>
<td>Eosinophilia</td>
<td>16q12 (PDGFRA)</td>
<td>MDS/AML</td>
<td>Ewing sarcoma 22q12 (EWSR1)</td>
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<tr>
<td>4q12 (PDGFRB)</td>
<td>5q31.3 (EGFR)</td>
<td>Other MPD (BM only)</td>
<td>Urothelial (Bladder) (3p21.1q36)</td>
</tr>
<tr>
<td>5p11-12 (FGFR1)</td>
<td>del 5q (5q31)</td>
<td>MDS/AML</td>
<td>Myeloid/lymphoid 12q13 (CHOP)</td>
</tr>
<tr>
<td>11q23 (CEBPA-MYH11)</td>
<td>del 7q (7q31)</td>
<td>MDS/AML</td>
<td>Liposarcoma 16p11 (FUS)</td>
</tr>
<tr>
<td></td>
<td>17q21 (CGBP)</td>
<td>MDS/AML</td>
<td>del 1p36/13q13 (Gliomas/others)</td>
</tr>
<tr>
<td></td>
<td>del 7q31 (7q31)</td>
<td></td>
<td>Trisomies 7, 17 (Papillary RCC)</td>
</tr>
<tr>
<td></td>
<td>8p (CEP8)</td>
<td></td>
<td>Liposarcoma 12q13 (MDM2)</td>
</tr>
</tbody>
</table>

**Individual Probes:**
Case # 1: FISH RESULTS

IGH (14q32)

BCL6 - t(3q27)

IGH-BCL2 - t(14;18)
Case # 1: FISH RESULTS

IGH/MYC/CEP8 - t(8;14)

NORMAL

ABNORMAL

MYC (8q24)
**Case # 1**

**FISH results**

- Clonal 14q32.3 (IGH) - positive (62.5%)
- t(3q27)BCL6 - positive (50%)
- t(14;18)(IGH-BCL2) - positive (60%)
- t(8;14)(IGH-MYC) - negative
- t(8q24 (MYC) - positive (53.5%)

“Triple-hit lymphoma”
Frequency of Additional Genetic Hits in 796 Mature B-Cell Neoplasm Cases with a BCL6/3q27 Rearrangement from the Mitelman Database

<table>
<thead>
<tr>
<th>Additional genetic hit</th>
<th>No. cases (%)</th>
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<tbody>
<tr>
<td>t(8;14)(q24;q32)</td>
<td>2 (0.3)</td>
</tr>
<tr>
<td>t(2;8)(p12;q24)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td><strong>t(8;22)(q24;q11)</strong></td>
<td><strong>10 (1.3)</strong></td>
</tr>
<tr>
<td><strong>t(14;18)(q32;q21)</strong></td>
<td><strong>139 (17.5)</strong></td>
</tr>
<tr>
<td>t(2;18)(p12;q21)</td>
<td>1 (0.1)</td>
</tr>
<tr>
<td>t(18;22)(q21;q11)</td>
<td>3 (0.4)</td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>11 (1.4)</td>
</tr>
<tr>
<td>t(2;11)(p12;q13)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>t(11;22)(q13;q11)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>Dual hit lymphoma</strong></td>
<td><strong>151 (19.0)</strong></td>
</tr>
<tr>
<td><strong>Three hit lymphoma</strong></td>
<td><strong>8 (1.0)</strong></td>
</tr>
</tbody>
</table>

Total 796 (100.0)

No case of 4 hit lymphomas in database

Triple-hit Lymphoma

- Diffuse Large B-cell Lymphoma
- B-cell lymphoma unclassifiable with features intermediate between BL and DLBCL
- Follicular Lymphoma (Transformed)
- Burkitt Lymphoma

- t(14;18) standard in most cases
- Extremely poor prognosis
- Often involve the CNS and have poor response to Rx
- Triple-hit lymphoma median overall survival 3.8 months
Case # 2: AML

Molecular Path lab received:

- Bone marrow aspirate, 24hr post collection
- Indication: AML, D14 post treatment
- Outside patient with no record

- Karyotyping is requested
Case # 2

Clinical hx:

- 72 year old male
- History of prostate cancer, status post hormonal therapy
- Diagnosis of AML with maturation (formerly FAB M2) from outside facility (North Carolina)
- Bone marrow biopsy was performed.

CBC:

- Hemoglobin: 11.3g/dL
- White blood cells: 10.6 x 10^3/μL
- Platelets: 245 x 10^3/μL
- Neutrophils: 18.4% (2000/μL)
- Lymphocytes: 46.7% (4950/μL)
- Monocytes: 4.0% (400/μL)
- Eosinophils: 0.2% (<100/μL)
- Basophils: 0.7% (100/μL)
Peripheral blood:
• 50% blasts, some with Auer rods

Bone marrow aspirate:
• 32% blasts, verified with IHC for CD34

Flow cytometry:
• 26% blasts
• Positive CD7 (partial), CD13, CD15 (subset), CD33, CD34, CD38, CD117, cMPO, HLA-DR

• Negative CD3, CD5, CD10, CD14, CD19, CD22, CD56, CD64, TdT, 79A
Case # 2

FISH
–negative for t(8;21), t(15;17), inv(16), t(11q23),
-5q, -7q, -20q, +8

Karyotype
–No cytogenetic abnormalities observed

Molecular testing:
FLT3 IDT: not detected
FLT3 D835: not detected
NPM-1 mutation: not detected

Started on 7+3 chemotherapy
Case # 2

Impression from Hematopathologist on D14 post Rx:

• Peripheral smear: rare circulating blasts (~1%)

• Aspirate: 17% blasts, verified by IHC for CD34

• Flow: 15% blasts

Diagnosis of residual AML s/p treatment
Case # 2: Karyotyping result

Trisomy 22 (4 out of 20 examined metaphases (20%)
Trisomy 22 in AML

- Rare as a specific primary abnormality
- Was not found associated with other anomalies recurrently found in de novo AML
- Often associated with inv(16)(p13q22) (15-22% AML cases); the existence of trisomy 22 solely is debated

- Grois et al (Cancer Genet Cytogenet 1989): 3 patients with isolated +22 later diagnosed with inv(16) on revision of karyotype
- Xu et al (Oncogene 2008): 10 cases of +22 as sole cytogenetic abnormality; no detectable inv(16) FISH detected inv(16) in 9 of these cases, del(16q22) in the remaining case
• Can be inv(16)(p12;q22), t(16;16)(p13;q22), or del(16)(q22)
• Results in CBFB-MYH11 fusion
• Associated with acute myelomonocytic leukemia with marrow eosinophilia
• WHO 2008: AML with inv(16) (M4)
• However, can be seen in other morphologic types of AML
• Cytogenetics diagnostic can be difficult; there are cases of inv(16) that are missed by conventional cytogenetics
Inv(16)(p13q22)
90% achieve complete remission
- 50% disease free after 5 years
- del(16)(q22) has poorer prognosis than inv(16)
- Impact of secondary genetic lesions
  - KIT mutations $\rightarrow$ lower relapse-free survival
  - FLT3 mutations, +8 $\rightarrow$ decreased overall survival

inv(16) with +22
- Lower WBC counts on initial presentation
- Lower complete remission rates but improved relapse-free survival
- Improved overall survival (when compared to inv(16) solely)
Case # 2: Karyotyping

Detection of Trisomy +22 after induction chemotherapy

Possible cryptic inv(16) present?
Case # 2: Karyotyping

Atlas of Cytogenetics Reference

Case # 2
Case # 2

Recommendation by molecular cytogenetics:

<table>
<thead>
<tr>
<th>Case</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+8 (CEP8) Eosinophilia</td>
</tr>
<tr>
<td>2</td>
<td>4q12 (PDGFRα)</td>
</tr>
<tr>
<td>3</td>
<td>5q33.1 (PDGFBR)</td>
</tr>
<tr>
<td>4</td>
<td>8p11-12 (FGFR1)</td>
</tr>
<tr>
<td>5</td>
<td>inv16 (CBFB-MYH11)</td>
</tr>
<tr>
<td>6</td>
<td>MM (Diagnostic)</td>
</tr>
<tr>
<td>7</td>
<td>14q32 (IGH)</td>
</tr>
<tr>
<td>8</td>
<td>13q14-q34</td>
</tr>
<tr>
<td>9</td>
<td>ploidy 5, 7, 11</td>
</tr>
<tr>
<td>10</td>
<td>MM (Prognostic)</td>
</tr>
<tr>
<td>11</td>
<td>t(11;14) (CCND1/IGH)</td>
</tr>
<tr>
<td>12</td>
<td>t(4;14) (FGFR3-IGH)</td>
</tr>
<tr>
<td>13</td>
<td>t(14;16) (IGH-MAF)</td>
</tr>
<tr>
<td>14</td>
<td>del 17p13.1 (p53)</td>
</tr>
<tr>
<td>15</td>
<td>del 11q22.3 (ATM)</td>
</tr>
<tr>
<td>16</td>
<td>1p12.3/1q21 (CUX1)</td>
</tr>
</tbody>
</table>

- **MDS/AML**
  - del 5a (5q31) (EGR1)
  - del 7q (7q31) (D7S5466)
  - +8 (CEP8)
  - del 20q (20q12) (D20S108)

- **Other MPD (BM only)**
  - inv(16) (CBFB)
  - +8 (CEP8)
  - Monosomy 7 or del 7q
  - del13q
  - del20q
  - t(3q25) (EV11)

- **Individually Probes**
  - 11q22.3 (ATM)
  - 17p13.1 (p53)
  - 14q32 (IGH)
  - 1p36
  - 19q13
  - 16p11 (FUS)
  - t(12;21) (TEL-AML1) (Ped ALL)
  - 8q24 (MYC)

**Special request/reflex/STAT**: FISH reflex to qRT-PCR

**FLT3/NPM1 mutation** (reflex for normal cytogenetics)

**Specimen Collection**: Keep at room temperature. Transport to the Cytogenetics lab as soon as possible and prior to 2PM on Friday.

**Bone Marrow**: 2-5ml of the 1st draw into transport media and sent to lab immediately. This is the sample of choice for leukemia.
Case # 2: FISH Result

Karyotype:

Atlas of Cytogenetics Reference  
Case # 2

**FISH:**
(Inv(16)(CBFβ)
break-apart/rearrangement probes)
Conclusion

• inv(16) or t(16;16) may be missed on cytogenetic studies
• +22 is specific for inv(16)
• +22 without additional abnormalities should raise concern for cryptic inv(16)
• +22 (along with inv(16)) portend a relatively favorable prognosis in patients with AML

How did we sign this case out?

“Karyotyping analysis revealed the presence of trisomy 22. Additional analysis by FISH indicated the presence of inv(16).

Although inv(16) was not detected in on previous cytogenetic studies, the presence of a cryptic inv(16) prior to treatment cannot be ruled out.

The presence of inv(16) and trisomy 22 is reportedly associated with lower complete remission rate but improved relapse-free survival.
Gene expression and splicing alterations analyzed by high throughput RNA sequencing of chronic lymphocytic leukemia specimens
CLL: RNA-seq evaluation for expression and splicing alterations

• analyzing expression at the exon level
• alternative splicing variations
• novel transcripts
• fusion genes
• differential transcription start sites
• genomic mutations
Clinical Characteristics of Study Populations

<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>Age, sex, Rai stage, total WBC count (cumm$^3$)</th>
<th>% cells CD38+</th>
<th>Zap-70 status</th>
<th>Normal CD19+ B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>67 M, stage II, 40,000</td>
<td>0%</td>
<td>neg</td>
<td></td>
</tr>
<tr>
<td>B2</td>
<td>88 M, stage III, 90,000</td>
<td>59%</td>
<td>pos</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>62 M, stage I, 96,000</td>
<td>24%</td>
<td>pos</td>
<td></td>
</tr>
<tr>
<td>B4</td>
<td>71 M, stage II, 135,000</td>
<td>8%</td>
<td>pos</td>
<td></td>
</tr>
<tr>
<td>B5</td>
<td>56 M, stage II, 102,000</td>
<td>55%</td>
<td>pos</td>
<td></td>
</tr>
<tr>
<td>CLL6</td>
<td>68 M, stage IV, 320,000</td>
<td>50%</td>
<td>pos</td>
<td></td>
</tr>
<tr>
<td>CLL7</td>
<td>55 M, stage III, 37,000</td>
<td>0%</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>CLL8</td>
<td>61 M, stage 0, 24,000</td>
<td>5%</td>
<td>neg</td>
<td></td>
</tr>
<tr>
<td>CLL9</td>
<td>64 M, stage I, 28,000</td>
<td>0%</td>
<td>pos</td>
<td></td>
</tr>
<tr>
<td>CLL10</td>
<td>78 M, stage III, 98,000</td>
<td>0%</td>
<td>neg</td>
<td></td>
</tr>
</tbody>
</table>

(neg, negative, pos, positive, ND, not done, M-CLL mutated IGH(V), U-CLL non-mutated IGH(V))
Distribution of Sequencing Reads

Distribution of uniquely mapped reads to human genome UCSC_hg19

Distribution of sequencing reads:
- **CDS_Exons**: 42% and 51%
- **5'UTR_Exons**: 3% and 3%
- **3'UTR_Exons**: 20% and 23%
- **Introns**: 20% and 16%
- **Intergenic Regions**: 5% and 7%

Average percentage of sequencing reads:

**Normal**
- CDS_Exons: 42%
- 3'UTR_Exons: 20%
- 5'UTR_Exons: 3%
- Introns: 30%
- Intergenic Regions: 5%

**CLL**
- CDS_Exons: 51%
- 3'UTR_Exons: 23%
- Introns: 16%
- Intergenic Regions: 7%
- 5'UTR_Exons: 3%
Transcriptomic Expression Profile

A

<table>
<thead>
<tr>
<th>Cuffdiff analysis between groups (FDR adjusted q-value &lt; 0.05)</th>
<th>Differentially expressed genes &gt; 2 fold change in expression</th>
<th>Up-regulated in CLL</th>
<th>Down-regulated in CLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal vs. CLL</td>
<td>2091</td>
<td>1231</td>
<td>860</td>
</tr>
<tr>
<td>Normal vs. U-CLL</td>
<td>2425</td>
<td>1332</td>
<td>1093</td>
</tr>
<tr>
<td>Normal vs. M-CLL</td>
<td>1960</td>
<td>1132</td>
<td>828</td>
</tr>
</tbody>
</table>

Overlapped DEG (differentially expressed genes) between the three groups

B
## Top 20 up/down-regulated genes in CLL vs. normal B cells

<table>
<thead>
<tr>
<th>Genes</th>
<th>Description</th>
<th>Fold change</th>
<th>q-value</th>
<th>Genes</th>
<th>Description</th>
<th>Fold change</th>
<th>q-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSTL1</td>
<td>follistatin-like 1</td>
<td>1360±4372</td>
<td>0.0090</td>
<td>SYN3</td>
<td>synapsin III</td>
<td>-270</td>
<td>0.0039</td>
</tr>
<tr>
<td>MMP9</td>
<td>matrix metalloproteinase 9 (gelatinase B, 92kDa)</td>
<td>1006±1501</td>
<td>0.0323</td>
<td>DSP</td>
<td>desmoplakin</td>
<td>-179</td>
<td>0.0222</td>
</tr>
<tr>
<td>TMOD</td>
<td>thrombomodulin</td>
<td>789±1041</td>
<td>0.0088</td>
<td>FBLN2</td>
<td>fibulin 2</td>
<td>-134</td>
<td>0.0074</td>
</tr>
<tr>
<td>CXCL5</td>
<td>chemokine (C-X-C motif) ligand 5</td>
<td>593±1008</td>
<td>0.0043</td>
<td>ENAM</td>
<td>enamelin</td>
<td>-117</td>
<td>0.0103</td>
</tr>
<tr>
<td>ADTRP</td>
<td>androgen-dependent TPPI-regulating protein</td>
<td>586±485</td>
<td>0.0031</td>
<td>HDC</td>
<td>histidine decarboxylase</td>
<td>-103</td>
<td>0.0270</td>
</tr>
<tr>
<td>KSR2</td>
<td>kinase suppressor of ras 2</td>
<td>528±750</td>
<td>0.0232</td>
<td>CD1A</td>
<td>CD1a molecule</td>
<td>-99</td>
<td>0.0311</td>
</tr>
<tr>
<td>THBS1</td>
<td>thrombospondin 1</td>
<td>506±640</td>
<td>0.0008</td>
<td>MYO1B</td>
<td>myosin III</td>
<td>-73</td>
<td>0.0112</td>
</tr>
<tr>
<td>TGFBR3</td>
<td>transforming growth factor, beta receptor III</td>
<td>459±205</td>
<td>0.0302</td>
<td>LOC100505738 or MIR4458</td>
<td>uncharacterized LOC100505738 or MIR4458</td>
<td>-66</td>
<td>0.0008</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>cytochrome P450, family 1, subfamily B, polypeptide 1</td>
<td>423±512</td>
<td>0.0191</td>
<td>SLC45A3</td>
<td>solute carrier family 45, member 3</td>
<td>-47</td>
<td>0.0008</td>
</tr>
<tr>
<td>IL6</td>
<td>interleukin 8</td>
<td>411±462</td>
<td>0.0015</td>
<td>MMRN1</td>
<td>multimerin 1</td>
<td>-46</td>
<td>0.0090</td>
</tr>
<tr>
<td>CD300E</td>
<td>CD300e molecule</td>
<td>401±650</td>
<td>0.0251</td>
<td>PPFIBPI</td>
<td>PTPR/P interacting protein, binding protein 1 (extracellular beta 1)</td>
<td>-45</td>
<td>0.0031</td>
</tr>
<tr>
<td>PRF1</td>
<td>perforin 1 (pore forming protein)</td>
<td>388±265</td>
<td>0.0106</td>
<td>ZNF618</td>
<td>zinc finger protein 618</td>
<td>-44</td>
<td>0.0015</td>
</tr>
<tr>
<td>GIMAP7</td>
<td>GTPase, IMAP family member 7</td>
<td>324±305</td>
<td>0.0323</td>
<td>UACA</td>
<td>ureal autoantigens with coiled-coil domains and ankyrin repeats</td>
<td>-44</td>
<td>0.0083</td>
</tr>
<tr>
<td>CTLA4</td>
<td>cytotoxic T-lymphocyte-associated protein 4</td>
<td>276±255</td>
<td>0.0025</td>
<td>AHNAK2</td>
<td>AHNAK nucleoprotein 2</td>
<td>-44</td>
<td>0.0008</td>
</tr>
<tr>
<td>CD8A</td>
<td>CD8a molecule</td>
<td>274±257</td>
<td>0.0173</td>
<td>GATA2</td>
<td>GATA binding protein 2</td>
<td>-39</td>
<td>0.0121</td>
</tr>
<tr>
<td>NRP1</td>
<td>neuropilin 1</td>
<td>263±475</td>
<td>0.0488</td>
<td>PARM1</td>
<td>prostate androgen-regulated mucin-like protein 1</td>
<td>-39</td>
<td>0.0008</td>
</tr>
<tr>
<td>SFTPB</td>
<td>surfactant protein B</td>
<td>261±477</td>
<td>0.0052</td>
<td>CR1</td>
<td>complement component (3b/4b) receptor 1</td>
<td>-39</td>
<td>0.0008</td>
</tr>
<tr>
<td>TNFRSF1A</td>
<td>tumor necrosis factor receptor superfamily, member 1A</td>
<td>240±140</td>
<td>0.0052</td>
<td>CABYR</td>
<td>calcium-binding tyrosine-(Y)-phosphorylation regulated</td>
<td>-39</td>
<td>0.0264</td>
</tr>
<tr>
<td>BBB</td>
<td>hemoglobin, beta</td>
<td>234±553</td>
<td>0.0020</td>
<td>LOC100506178</td>
<td>uncharacterized LOC100506178</td>
<td>uncharacterized LOC100506178</td>
<td>-36</td>
</tr>
<tr>
<td>CYBRD1</td>
<td>cytochrome b reductase 1</td>
<td>216±254</td>
<td>0.0020</td>
<td>FFAR1</td>
<td>free fatty acid receptor 1</td>
<td>-34</td>
<td>0.0224</td>
</tr>
</tbody>
</table>
Transcriptomic comparison of M-CLL vs. U-CLL

<table>
<thead>
<tr>
<th>Cuffdiff analysis between groups (FDR adjusted q-value &lt; 0.05)</th>
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<th>Up-regulated in U-CLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-CLL vs. U-CLL</td>
<td>679</td>
<td>320</td>
<td>359</td>
</tr>
</tbody>
</table>

A

B

qRT-PCR (selected genes)

C

D

E

M-CLL vs. U-CLL

679

320

359

IGLL5

TFEC

T
Alternative Splicing Events in B vs. CLL

A

Alternative splicing events (MATS analysis)

- Skipped exon (SE)
- Alternative 5’ splice site (A5SS)
- Alternative 3’ splice site (A3SS)
- Mutually exclusive exons (MXE)
- Retained intron (RI)

B

Alternative splicing events in B and CLL specimens

<table>
<thead>
<tr>
<th>Event Type</th>
<th>Total number of events</th>
<th>Significant events</th>
<th>B</th>
<th>CLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>SE</td>
<td>40974</td>
<td>128</td>
<td>78</td>
<td>50</td>
</tr>
<tr>
<td>A5SS</td>
<td>6845</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>A3SS</td>
<td>2222</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>MXE</td>
<td>3339</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>RI</td>
<td>2764</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Acknowledgement

VISN22/ VA Los Angeles MolecularPathology & Cytogenetic lab (all technologists and technicians)

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Riccardo Dalla-Favera, MD (Columbia)

Paul J. Kurtin, MD (Mayo Clinic)