Molecular Advances in Hematopathology

HOW MOLECULAR METHODS HAVE CHANGED MY PRACTICE
Objectives

• Understand the importance of cytogenetic/molecular studies in hematolymphoid diseases

• Know some of the important molecular updates from the 2016 revised WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues

• Know an ordering algorithm for cytogenetic/molecular tests in both new diagnoses and in evaluation for recurrent hematopathic diseases
What’s changed?

• Technology has made information cheaper!
  ◦ More data
  ◦ More specificity
  ◦ More with less (MRD)

• AML genome sequenced in 2009 at cost of $2M
Myeloid Neoplasms
Evolution of Evaluation of MRD

1950s: MRD by morphology and physical exam (<5% blasts)
1970s: Karyotype, immunohistochemistry
1980s: Multiparameter flow cytometry
1990s: FISH, microarray
2000s: RT-PCR (APL, BCR-ABL, NPM1)
2010s: Gene sequencing
Limited Number of Recurrent Mutations in MDS

Percentage of MDS Cases with Mutation

- TET2
- SF3B1
- ASXL1
- SRSF2
- DNMT3A
- RUNX1
- U2AF1
- TP53
- ZRSR2
- SPP2
- EZH2
- BCR
- JAK2
- CBL
- IDH2
- NF1
- NRAS
- NUP98
- PHF6
- MPL
- IDH1
- KRAS
- ETV6
- SMC3
- GNAS
- ATRX
- FLT3
- KDM6A
- NPM1
- KIT
- RAD21
- PTPN11
- GNA2
- FBXW7
- NOTCH1
- WT1
- GNB1
- IRF1
- U2AF2
- CEBPA

~10 Frequently mutated genes

Long ‘tail’ of less frequently mutated genes

n=1,839 patients

Limited Number of Recurrent Mutations in AML

Exome/WGS (n=200)

AML TCGA Data, NEJM 2013
Recurrent Abnormalities in AML

n=1,540 patients
111 genes sequenced

Papaemmanuil et al, NEJM 2016
Minimal Residual Disease in AMLs

• Molecular risk stratification and evaluation for minimal residual disease relies on identification of abnormalities at diagnosis

• Repeat sequencing at 30 days after 7+3 induction to determine clearance of abnormalities
  ◦ Positive MRD on day 14 after intensive induction chemotherapy is not invariably associated with treatment failure

• Patients with mutation detection at variant allele frequency (VAF) greater than 2.5% at day 30 almost all died within 5 years
Minimal Residual Disease in AMLs

• Doesn’t matter the mutation, detection at day 30 is a bad prognosticator
  ◦ Positive MRD test identifies patients with higher risk of relapse and shorter survival than similarly treated patients in morphological remission who test negative for MRD

Klco et al, JAMA, 2015
WHO 2016 Updates-Myeloid

• New Chapters
  ◦ Mast cells
  ◦ Myeloid/lymphoid neoplasms with eosinophilia and gene rearrangement
  ◦ Myeloid neoplasms with germline predisposition

• MDS Categorization
  ◦ Elimination of “refractory anemia”
  ◦ SF3B1
Case Presentation

• 67 year old with macrocytic anemia, rule out lymphoma and myeloma
  ◦ Iron studies, B12 level, and folate level are within normal limits
  ◦ Flow cytometry: No discrete blast population is identified by CD45 versus side scatter. There is no evidence of B-cell surface light chain restriction or aberrant T-cell immunophenotype. Plasma cells are identified by bright CD38 and CD138 expression and they show no evidence of cytoplasmic light chain restriction.
  ◦ Normal cytogenetics and MDS FISH panel
Ring sideroblasts
One in every 20 erythroid cells
Case Presentation

• Diagnosis
  ◦ Myelodysplastic syndrome with single lineage dysplasia
  vs
  ◦ Myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia
Lymphoid
WHO 2016 Updates-Lymphoid

• High grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements

• Mutations identified
  ◦ Hairy cell leukemia
  ◦ Lymphoplasmacytic lymphoma
Clinical Relevance of Mutational Profiles in Lymphoid Neoplasms

• Diagnostic criteria to refine entities
• Identification of patient subsets
• Prognostic and predictive significance
• Monitoring disease evolution
• Identification of actionable mutations
Early and Late Mutations in Follicular Lymphoma

- Early driver mutations in chromatin regulator genes
  - CREBBP
  - EZH2
  - KMT2D (MLL2)

- Mutations gained at transformation
  - MYD88
  - TNFAIP3

*Early driver mutations* in chromatin regulator genes *(CREBBP, EZH2 and KMT2D (MLL2)), Gained at transformation : EBF1 and regulators of NF-κB signaling (MYD88 and TNFAIP3)*

Somatic Mutations in LPL

**MYD88**
- 95% of LPLs/Waldenstrom’s
- Myeloid Differentiation Factor 88
- Activates BTK
- Increases cell survival

**CXCR4**
- 25-40% of LPLs/WM
- C-X-C chemokine receptor 4
- Often assoc. with MYD88
- More active disease
- Less lymphadenopathy
- More resistant to treatment

**BTK**
- Bruton’s Tyrosine Kinase
- Additional mutations prior to progression
- Ibrutinib
Caution with MYD88

Seen in 95% of LPLs/Waldenstrom’s

BUT ALSO SEEN IN...

70% of DLBCL-Leg Type

30% of DLBCL, ABC Subtype

8% of Follicular Lymphoma

6% of Marginal Zone Lymphoma

3% of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma
### Recurrent Mutated Pathways in Small B-Cell Lymphomas

<table>
<thead>
<tr>
<th>Pathway</th>
<th>U-CLL</th>
<th>M-CLL</th>
<th>MCL</th>
<th>FL</th>
<th>LPL</th>
<th>MZL</th>
<th>HCL</th>
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<tbody>
<tr>
<td>DNA Damage</td>
<td>+</td>
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<tr>
<td>SF3B1</td>
<td>+</td>
<td>+/-</td>
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<td>NOTCH1/2</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
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<tr>
<td>Chromatin remodeling</td>
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<td>+</td>
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<td>BCR-Signaling</td>
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<td>+</td>
<td>+/-</td>
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<td>NFkB</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>MYD88</td>
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<td>+/-</td>
<td>+</td>
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<tr>
<td>MAPK</td>
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What To Do?
CAP Guidelines for AML

• Published in December 2016

• Panel of experts and advisors in hematology and hematopathology
  ◦ EP: 7 pathologists, 1 hematologist, 1 hem/onc, 1 method consultant
  ◦ AP: 1 pt advocate, 1 cytogeneticist, 3 heme/onc, 1 med onc, 2 hemepath

• Recommendations derived from strength of evidence and feedback during a public comment period
<table>
<thead>
<tr>
<th>Designation</th>
<th>Recommendation</th>
<th>Rationale</th>
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</thead>
<tbody>
<tr>
<td>Strong recommendation</td>
<td>Recommend for, or against, a particular practice. (Can include “must” or “should.”)</td>
<td>Supported by convincing or adequate quality of evidence and clear benefit that outweighs harms.</td>
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<tr>
<td>Recommendation</td>
<td>Recommend for, or against, a particular practice. (Can include “should” or “may.”)</td>
<td>Some limitations in quality of evidence, balance of benefits and harms, values, or costs, but panel concluded that there is sufficient evidence and/or benefit to inform a recommendation.</td>
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<tr>
<td>Expert consensus opinion</td>
<td>Recommend for, or against, a particular practice. (Can include “should” or “may.”)</td>
<td>Serious limitations in quality of evidence, balance of benefits and harms, values or costs, but panel consensus was that a statement was necessary.</td>
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<tr>
<td>No recommendation</td>
<td>No recommendation for, or against, a practice.</td>
<td>Insufficient evidence or agreement of the balance of benefits and harms, values, or costs to provide a recommendation.</td>
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<tr>
<td>Guideline</td>
<td>Designation</td>
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<tr>
<td>1. Clinical data/patient history provided by treating clinician</td>
<td>Strong</td>
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<td>2. PE and imaging data provided by treating clinician</td>
<td>Recommendation</td>
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<tr>
<td>3. Pathologist review of CBC and diff</td>
<td>Strong</td>
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<td>4. Review of bone marrow aspirate and core biopsy</td>
<td>Strong</td>
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<td>5. Flow cytometry, cytogenetics, molecular studies</td>
<td>Strong</td>
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<td>6. Cytochemical stains (PAS, MPO, NSE)</td>
<td>Consensus</td>
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<td>7. Molecular/genetic studies on nucleic acid,cropreserved,FFPE tissue</td>
<td>Recommendation</td>
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<td>8. Pathologist review of CSF analysis/differential in ALLs</td>
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<tr>
<td>9. Pathologist review of CSF analysis/differential in AMLs</td>
<td>Consensus</td>
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<tr>
<td>10. Use of flow cytometry in analyzing CSF</td>
<td>Recommendation</td>
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<td>11. Tissue evaluation in patients with extramedullary disease</td>
<td>Strong</td>
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<tr>
<td>12. Ensure thorough flow cytometry/cytogenetics/molecular for MRD</td>
<td>Strong</td>
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<td>13. Pediatric B-ALL: t(12;21), t(9;22), KMT2A (MLL), iAMP21, +4, +10</td>
<td>Strong</td>
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<td>14. Adult B-ALL: t(9;22)</td>
<td>Strong</td>
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<tr>
<td>15. Testing for PAX5, JAK1, JAK2, IKZF1, NOTCH1, FBXW7, CRLF2</td>
<td>Recommendation</td>
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<tr>
<td>Guideline</td>
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<tr>
<td>16  FLT3 testing in AML</td>
<td>Strong</td>
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<td>17  KIT mutation analysis in adults with CBF-AML</td>
<td>Strong</td>
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<td>18  In suspected APL, rapid t(15;17) detection and testing for DIC</td>
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<td>19  AML patients should be tested for NMPM1, CEBPA, RUNX1</td>
<td>Strong</td>
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<tr>
<td>20  Testing for methylation, microRNA/gene expression in acute leukemias</td>
<td>No recs</td>
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<tr>
<td>21  Mixed phenotype AMLs should have t(9;22) and KMT2A (MLL) testing</td>
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<td>22  All testing performed in labs with regulatory compliance</td>
<td>Strong</td>
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<tr>
<td>23  Patients requiring immediate referral to institution with expertise, initial institution should, defer invasive procedures to avoid duplicate procedures, associated patient discomfort, and additional costs</td>
<td>Strong</td>
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<tr>
<td>24  If patient is referred, provide all labs, slides, flow/CG data, pending tests</td>
<td>Strong</td>
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<td>25  Initial report should include laboratory, morphologic, IPT data, on which the diagnosis along with a list of any pending tests.</td>
<td>Strong</td>
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<td>26  Ensure all tests are entered into the medical record</td>
<td>Strong</td>
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<tr>
<td>27  Use the current WHO terminology</td>
<td>Strong</td>
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</table>
MUSC Evaluation in New Leukemias

• AMLs
  ◦ Peripheral Blood: morphology, flow cytometry
  ◦ Bone Marrow: morphology, flow cytometry, stat FISH t(15;17), karyotype, microarray, next gen sequencing panel

• ALLs
  ◦ Peripheral Blood: morphology, flow cytometry
  ◦ Bone Marrow: morphology, flow cytometry, stat FISH t(9;22) and KMT2A, karyotype, additional FISH, microarray
  ◦ CSF: morphology, +/- flow cytometry
MUSC Evaluation in Residual Leukemia

• AMLs
  ◦ Peripheral Blood: morphology
  ◦ Bone Marrow: morphology, flow cytometry, +/- FISH, karyotype, next gen sequencing panel

• ALLs
  ◦ Peripheral Blood: morphology
  ◦ Bone Marrow: morphology, flow cytometry, karyotype, +/- FISH
  ◦ CSF: morphology, +/- flow cytometry
MUSC Evaluation of Lymphomas

• Morphology and immunohistochemistry
• Flow cytometry
• Karyotype
• FISH
• Molecular testing: BRAF V600E, MYD88
Acknowledgements

• Daynna Wolff, PhD
• Iya Znoyko, PhD
• W. Bailey Glen, PhD
• Tara Ellingham
Thank you!!!