Enhancing Assessment of Myeloid Leukemia in the Era of Precision Medicine

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Neoplastic Hematopathology Update 2018
Disclosures

I do not have anything to disclose.
The 20 Most Commonly Diagnosed Cancers Worldwide

- **Lung** (13%) 1,608,055
- **Female Breast** (11%) 1,384,155
- **Colorectum** (10%) 1,235,108
- **Stomach** (8%) 988,602
- **Prostate** (7%) 899,102
- **Liver** (6%) 749,744
- **Cervix** (4%) 530,232
- **Oesophagus** (4%) 481,645
- **Bladder** (3%) 382,660
- **Non-Hodgkin Lymphoma** (3%) 356,431
- **Leukaemia** (3%) 350,434
- **Uterus** (2%) 288,387
- **Pancreas** (2%) 278,684
- **Kidney** (2%) 273,518
- **Lip and Oral Cavity** (2%) 263,020
- **Brain and CNS** (2%) 237,913
- **Ovary** (2%) 224,747
- **Thyroid** (2%) 213,179
- **Malignant Melanoma** (2%) 199,627
- **Larynx** (1%) 150,677
- **Other Sites** (12%) 1,566,634

(Number of New Cases)
## Estimated Number of New Hematologic Malignancies and Deaths, US, 2017

<table>
<thead>
<tr>
<th></th>
<th>Estimated New Cases</th>
<th>Estimated Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Both sexes</td>
<td>Male</td>
</tr>
<tr>
<td><strong>All Sites</strong></td>
<td>1,688,780</td>
<td>836,150</td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
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<tr>
<td>Hodgkin lymphoma</td>
<td>80,500</td>
<td>44,730</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>72,240</td>
<td>40,080</td>
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<tr>
<td>Myeloma</td>
<td>30,280</td>
<td>17,490</td>
</tr>
<tr>
<td>Leukemia</td>
<td><strong>62,130</strong></td>
<td><strong>36,290</strong></td>
</tr>
<tr>
<td>Acute lymphocytic</td>
<td>5,970</td>
<td>3,350</td>
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<tr>
<td>Chronic lymphocytic</td>
<td>20,110</td>
<td>12,310</td>
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<tr>
<td>Acute myeloid</td>
<td>21,380</td>
<td>11,960</td>
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<tr>
<td>Chronic myeloid</td>
<td>8,950</td>
<td>5,230</td>
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</table>

**Source:** Estimated new cases are based on 1999-2013 incidence data reported by the North American Association of Central Cancer Registries (NAACCR). Estimated deaths are based on 2000-2014 US mortality data, National Center for Health Statistics, Centers for Disease Control and Prevention.

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Estimated annual incidence of AML worldwide

- 2.5-3% cases per 100,000 population but higher in US
- Median patient age at diagnosis is 65 years with slight male predominance
- In children aged <15 about 15-20% of all cases of acute leukemia with peak incidence in the first 3-4 years of life
Classification of Leukemia Long time ago!

Acute
- Acute Myeloid Leukemia (AML)
- Acute Lymphoblastic Leukemia (ALL)

Chronic
- Chronic Myeloid Malignancies (CML, etc)
- Chronic Lymphocytic Malignancies (CLL, etc)

Myeloid origin
- AML
- CML

Lymphoid origin
- ALL
- CLL

Classification is easy when there is NO effective treatment.
WHO: Era of formal incorporation of genetic abnormalities in the diagnostic algorithms for AML

- Advances in sequencing technology have enabled the cost-effective detection of tumor genome and transcriptome mutation events
- We are in an era that promises a rational rather than empiric approaches for the treatment of cancer patients.
Philadelphia Chromosome and the Long Way to Discovery of “The Magic Bullet”

- In 1960 the Ph chromosome was discovered by Peter Nowell and David Hungerford.
- In 1973, Janet Rowley identified the mechanism by which the Ph chromosome arises as a translocation.
- In late 90s the mechanism by which STI-571 inhibits the BCR/ABL kinase domain was determined by Jon Kuriyan.
- In 2001 the first BCR/ABL kinase was marketed as imatinib mesylate.
91% missense mutations
8% nonsense changes
<2% splice site or UTR”

Average 33-66 genes mutated
Most common Mutations in AML

### Diagnosis
- **AML**: NPM1, CEBPA, RUNX1
- **MDS**: SF3B1
- **MPN**: JAK2, CALR, MPL, CSF3R

### Prognosis
- **AML**: FLT3-ITD, DNMT3A, IDH1/2, KIT, TET2, TP53, ASXL1, WT1....
- **MDS**: TP53, EZH2, ETV6, RUNX1, ASXL1, ...
- **MPN**: ASXL1, EZH2, ETV6, TET2, IDH2, SETBP1, SF3B1, SRSF2

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*Plus-minus values are means±SD. Percentages may not total 100 because of rounding. AML denotes acute myeloid leukemia, and FAB French-American-British classification.†Race or ethnic group was self-reported.*
AML Genomic Knowledge

- Affecting DNA methylation: DNMT3A, TET, IDH, ASXL
  - Commonly acquired early and present in the founding clone
- May persist after therapy, lead to clonal expansion during hematologic remission, and eventually lead to relapsed disease.
- Activated signaling: FLT3, RAS, NPM1
  - Secondary events and occur later during leukemogenesis.

The Updated WHO 2016 Classification of AML

- AML, not otherwise specified (NOS)
- AML with recurrent genetic abnormalities
- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- Myeloid sarcoma
- Myeloid proliferations associated with Down syndrome
AML, NOS  WHO 2016

- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute monoblastic/monocytic Leukemia
- Pure Erythroid Leukemia
  - > 50% erythroid precursors & will not have the % of blasts based on nonerythroid precursors.
- Acute Megakaryoblastic Leukemia
- Acute Basophilic Leukemia
- Acute Panmyelosis with Myelofibrosis

Previous Acute Erythroid Leukemia now can be AML with MDS-related features
AML, not otherwise specified (NOS)

AML with recurrent cytogenetic abnormalities

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasm

Myeloid sarcoma

Myeloid proliferations related to Down syn.

Myeloid neoplasm's with Germline predispositions
AML with recurrent cytogenetic abnormalities

1. t(8;21); RUNX1-RUNX1T1 some maturation of neutrophilic line; rare in older patients; AML1/ETO fusion protein; FAB M2; flow cytometry CD19

2. t(15;17); PML-RARA: APL (granular and microgranular variants); t(11;17), t(5;17)

3. inv(16) or t(16;16); CBFB-MYH11 abnormal eosinophilic component; AMML with eos(M4eo)

4. t(9;11); MLLT3- KMT2A (MLL) - monocytic; children

5. t(6;9); DEK-NUP214 Basophilia and multilineage dysplasia

6. Inv3 or t(3;3); GATA2, MECOM;
   - Thrombocytosis, Multilineage dysplasia with small mono or bilobed megakaryocytes, May have a MDS phase but >= 20% blasts required for Dx

7. t(1;22); RBM15-MKL1: Megakaryoblastic, Infants, Intermediate

8. AML with BCR- ABL1 (a provisional entity): Aggressive disease; compared with AML transformed from CML, these patients have less frequent splenomegaly and <2% basophiles in PB
Prognostic Cytogenetics Abnormalities in Adult AML

MRC/NCRI AML Trials: Overall Survival
Ages 16–59

- t(15;17) (n=607)
- t(8;21) (n=421)
- inv(16)/t(16;16) (n=284)
- t(9;11) (n=61)
- t(6;9) (n=42)
- inv(3)/t(3:3) (n=69)
- t(9;22) (n=44*)
- Other t(11q23) (n=60*)
- t(3:5) (n=25*)
- -5/del(5q) (n=258*)
- -7/del(7q) (n=336*)
- AML with other MDS–related (n=343**)

% alive

Years from entry

81%
61%
55%
39%
34%
27%
22%
-11%
10%
6%
3%

AML with Recurrent Genetics Abnormalities

1. AML with *NPM1* – Mutually exclusive with partial tandem duplications of *MLL* and bi*CEBPA*; **good prognosis**

2. AML with *CEBPA*<sup>DM</sup> – only changes prognosis (good) if present as a double mutant

3. AML with *RUNX 1* mutation – older men, poor prognosis (provisional)

- Mutations in 3 genes - *RUNX1, ASXL1, and TP53* - have been added in the risk stratification of the European Leukemia Net recommendations for AML.

References:
1540 patients, intensively treated in prospective trials, were studied using targeted NGS assay of 111 myeloid cancer genes, along with cytogenetic profiles.

37 Patterns of comutations segregated AML cases into 11 non-overlapping classes, each with a distinct clinical phenotype and outcome.

Beyond 11 known disease classes, 3 additional, heterogeneous classes emerged:

1. AML with mutations in chromatin and RNA-splicing regulators
2. AML with TP53 mutations and/or chromosomal aneuploidies
3. AML with IDH2R172 mutations (provisionally).
Mutated genes were classified into 1 of 9 functional categories:
1. Transcription factor fusions,
2. NPM1 gene,
3. Tumor suppressor genes,
4. DNA methylation-related genes,
5. Signaling genes,
6. Chromatin-modifying genes,
7. Myeloid transcription factor genes,
8. Cohesin complex genes, and
9. Spliceosome complex genes
### Prognostic Gene Mutations in CN-AML

<table>
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<tr>
<th>GENE</th>
<th>Year of Discovery</th>
<th>Prognostic impact</th>
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<tr>
<td>MLL-PTD</td>
<td>1998</td>
<td>Unfavorable</td>
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<tr>
<td>FLT3-ITD</td>
<td>1999</td>
<td>Unfavorable</td>
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<tr>
<td>FLT3- TKD</td>
<td>2002</td>
<td>?Unfavorable</td>
</tr>
<tr>
<td>CEBPA (single and Biallelic)</td>
<td>2002, 2012</td>
<td>?Favorable, Favorable</td>
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<tr>
<td>NPM1</td>
<td>2005</td>
<td>Favorable</td>
</tr>
<tr>
<td>WT</td>
<td>2007</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>IDH1 /2, RUNX1</td>
<td>2009</td>
<td>?Favorable, Unfavorable</td>
</tr>
<tr>
<td>TET2, ASXL1</td>
<td>2010</td>
<td>Unfavorable, Unfavorable</td>
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<tr>
<td>DNMT3A</td>
<td>2011</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>TP53</td>
<td>2003, 2009, 2013</td>
<td>Unfavorable</td>
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</table>

Dohner H et al. (Blood 2010;115:453–474)
Patel JP et al. (NEJM 2012;366:1079–89)
Grossmann V et al. (Blood 2012;120:2936–72)
Classification of Myeloid Neoplasms with Germ Line Predisposition

<table>
<thead>
<tr>
<th>Classification</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid neoplasms with germ line predisposition without a preexisting disorder or organ dysfunction</td>
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<tr>
<td>AML with germ line CEBPA mutation</td>
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<tr>
<td>Myeloid neoplasms with germ line DDX41 mutation*</td>
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</tr>
<tr>
<td>Myeloid neoplasms with germ line predisposition and preexisting platelet disorders</td>
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<tr>
<td>Myeloid neoplasms with germ line RUNX1 mutation*</td>
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<tr>
<td>Myeloid neoplasms with germ line ANKRD26 mutation*</td>
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<tr>
<td>Myeloid neoplasms with germ line ETV6 mutation*</td>
<td></td>
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<tr>
<td>Myeloid neoplasms with germ line predisposition and other organ dysfunction</td>
<td></td>
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<tr>
<td>Myeloid neoplasms with germ line GATA2 mutation</td>
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</tr>
<tr>
<td>Myeloid neoplasms associated with BM failure syndromes</td>
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<tr>
<td>Myeloid neoplasms associated with telomere biology disorders</td>
<td></td>
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<tr>
<td>JMML associated with neurofibromatosis, Noonan syndrome or</td>
<td></td>
</tr>
<tr>
<td>Noonan syndrome-like disorders</td>
<td></td>
</tr>
<tr>
<td>Myeloid neoplasms associated with Down syndrome*</td>
<td></td>
</tr>
</tbody>
</table>

*Lymphoid neoplasms also reported.

The Updated WHO 2016 Classification of AML

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- AML with myelodysplasia-related changes
- Therapy-related myeloid neoplasms
- Myeloid sarcoma
- Myeloid proliferations related to Down syn.
AML with myelodysplasia related changes

- Multilineage dysplasia (dyspoiesis):
  - 50% of the cells in 2 non-blast cell lines

- Exclude AML with recurrent genetics abnormalities or prior MDS

- Absence of prior history of therapy
NPM1 and CEBPA mutations and Dyspoiesis:

• Significant MLD found in the presence of NPM1 74/318 (23%) with no prognostic significant in MLD

• MLD found in 28/108 (26%) CEBPA mutated AMLs with no significant survival difference in cases with or without MLD

Falini et al. Blood 2010 - Diaz-Beya M et al Blood 2010

14.7% of NPM1 mutated (+8,+4, -Y, del(9q) and +21) and 26% of bi-CEBPA mutated (del(9q), del(11q), -Y, and +10, and +21) AML cases have abnormal Karyotype

• Revise cytogenetic abnormalities
  – Del9q was removed from de novo cases with MDS related cytogenetic abnormality if there is an NPM1 or CEBPA<sup>dm</sup> (Haferlach C et al Blood 114 2009)
Analyzing of WES data of peripheral blood of 17,182 persons showed somatic mutations that drive clonal expansion of blood cells were a common finding in the elderly and most frequently involved DNMT3A, TET2, or ASXL1.


BCOR Mutations in Myeloid Disorders: One Year Experience at City of Hope

- 238 patients were studied from Sep 2016 to 2017
- Of 120 AML, 54 MDS, 14 MDS/MPN, and 50 MPN cases, BCOR mutations were found in 13 cases (9.1% of AML and 3.7% of MDS cases)
- M:F ratio of 1:1 and a mean age of 63 years
- No clinically significant BCOR mutations were found in the MPN and MDS/MPN cases.
Concurrent mutations with BCOR

- DNMT3A (38%), STAG2 (38%), TET2 (31%), U2AF1 (30%), IDH2 (15.3%), and IDH1 (7.7%)
- No concurrent NPM1 mutations
- 3/13 patients with BCOR mutation died within 6 months of molecular testing: 2 harbored a concurrent DNMT3A and U2AF1 mutations
- One with concurrent U2AF1 had relapsed disease after transplant.
• BCL6 corepressor (BCOR), an X-linked component of the Polycomb group repressive complex 1 (PGRC1).

• BCOR protein functions as a transcriptional suppressor of BCL-6

• When mutated plays a major role in neoplastic transformation of hematopoietic stem cells through upregulation of HOXA genes.
Reporting of the results

• Preliminary report: Morphology or/and flow cytometry report in diagnosis of a new leukemia or relapse
  – Opportunity to get more history

• Initial report: Includes morphology and immunophenotyping

• Final report: Should be an integrated report
  – Include all immunophenotyping, cytogenetics and molecular genetics results to approach the final diagnosis
### Test procedure for a patient with AML

#### 2017 ELN Recommendation

<table>
<thead>
<tr>
<th>Tests to establish the diagnosis</th>
<th>Additional tests/procedures at diagnosis (cont’d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete blood count and differential count</td>
<td>Analysis of comorbidities</td>
</tr>
<tr>
<td>Bone marrow aspirate</td>
<td>Biochemistry, coagulation tests, urine analysis**</td>
</tr>
<tr>
<td>Bone marrow trephine biopsy*</td>
<td>Serum pregnancy test††</td>
</tr>
<tr>
<td>Immunophenotyping</td>
<td>Information on oocyte and sperm cryopreservation‡‡</td>
</tr>
<tr>
<td><strong>Genetic analyses</strong></td>
<td>Eligibility assessment for allogeneic HCT (including HLA typing)*</td>
</tr>
<tr>
<td>Cytogenetics†</td>
<td>Hepatitis A, B, C; HIV-1 testing</td>
</tr>
<tr>
<td>Screening for gene mutations including‡</td>
<td>Chest radiograph, 12-lead electrocardiogram, and echocardiography or MUGA (on indication)</td>
</tr>
<tr>
<td>NPM1, CEBPA, RUNX1, FLT3, TP53, ASXL1</td>
<td>Lumbar puncture⁰</td>
</tr>
<tr>
<td>Screening for gene rearrangements§</td>
<td>Biobanking⁸</td>
</tr>
<tr>
<td>PML-RARA, CBFB-MYH11, RUNX1-RUNX1T1, BCR-ABL1, other fusion genes (if available)</td>
<td>Sensitive assessment of response by RT-qPCR or MFC⁴</td>
</tr>
<tr>
<td><strong>Additional tests/procedures at diagnosis</strong></td>
<td>RT-qPCR⁶,⁷ for NPM1 mutation, CBFB-MYH11, RUNX1-RUNX1T1, BCR-ABL1, other fusion genes (if available)⁶</td>
</tr>
<tr>
<td>Demographics and medical history‖</td>
<td>MFC⁵,⁹</td>
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<tr>
<td>Detailed family history¶</td>
<td></td>
</tr>
<tr>
<td>Patient bleeding history#</td>
<td></td>
</tr>
<tr>
<td>Performance status (ECOG/WHO score)</td>
<td></td>
</tr>
</tbody>
</table>

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Clinical Molecular Diagnostics Laboratory (CMDL)

- In operation since 1990s as a clinical reference laboratory: annual volume 2017 ~25,000 cases
- CAP certified, CLIA licensed
- **Service Lines:**
  - Solid Tumors
  - Hematological Malignancies
  - Germline Testing
    - Cancer predisposition
    - Inherited disorders
  - Molecular Microbiology
Molecular Testing at CMDL for Diagnosis, Prognosis, and Treatment

- We provide cutting-edge diagnostic services based on translational research to serve the patients and clinicians at City of Hope, as well as regionally and nationally.

- Hematological Malignancy assays for:
  - Myeloid neoplasms
  - Lymphoid neoplasms
  - Plasma cell neoplasms
• Technologies

Next generation sequencing (NGS), Sanger Sequencing, Real-Time PCR, multiplex ligation-dependent probe amplification (MLPA)
Quantitative Assays:

- **BCR/ABL1** (TAT 1-2 days)
- **PML/RARA, Inv 16, and AML1/ETO** (TAT 1-2 days)
- Mutation analysis (TAT 3-5 working days)
- **FLT3** ITD quantitative with signal ratio and FLT3 TKD
- **MPN assay:** JAK2, CALR, MPL

<table>
<thead>
<tr>
<th>NPM1</th>
<th>CEBPA</th>
<th>IDH1</th>
<th>IDH2</th>
<th>FLT3</th>
<th>KIT</th>
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<tbody>
<tr>
<td>JAK2</td>
<td>CALR</td>
<td>MPL</td>
<td>ABL1</td>
<td>BRAF</td>
<td>TP53</td>
</tr>
</tbody>
</table>

Single Gene Tests
Hematological Molecular Testing: Diagnosis, Prognosis, Treatment, and Discovery

- Entire exon sequencing of all these genes plus,
- RNAseq: Detection of >2000 selected rearrangements (including Ph-like ALL)
- Gene expression profiling (including DLBCL, GCB vs ABC)

<table>
<thead>
<tr>
<th>ASXL1</th>
<th>CCND1</th>
<th>EP300</th>
<th>IDH1</th>
<th>KMT2D</th>
<th>NRAS</th>
<th>SF3B1</th>
<th>WHSC1</th>
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<td>ATM</td>
<td>CCND3</td>
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<td>SETBP1</td>
<td>U2AF1</td>
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- Myeloid neoplasms
- Lymphoid neoplasms
- Plasma cell neoplasms
HopeSeq Heme Fusions: RNAseq assay for detection of gene fusions, mutations, and expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fusion</th>
<th>Splicing</th>
<th>Exon Skipping</th>
<th>Mutation</th>
<th>Expression</th>
<th>Expression Imbalance</th>
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<tr>
<td>CEBPA</td>
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<td>✔</td>
<td></td>
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<tr>
<td>CEBPD</td>
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 gene fusions, mutations, and expression
HopeSeq Heme Fusions: Detection of Known and Novel fusions

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of gene fusions*</th>
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<tr>
<td></td>
<td>Total</td>
<td>Confirmed as</td>
<td>recurrent</td>
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<tr>
<td><strong>Haematological disorders</strong></td>
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<tr>
<td>Undifferentiated and biphenotypic leukaemia</td>
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<tr>
<td>Acute myeloid leukaemia</td>
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<tr>
<td>Myelodysplastic syndromes</td>
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<tr>
<td>Myeloproliferative neoplasms, including chronic myeloid leukaemia</td>
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<tr>
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<td>Plasma cell neoplasms</td>
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<tr>
<td>Mature B cell neoplasms</td>
<td>181</td>
<td>31</td>
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<tr>
<td>Mature T cell and natural killer cell neoplasms</td>
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<td>Hodgkin disease</td>
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“HopeSeq HemeMutationsV2” assay by NGS for Myeloid, Plasma cells, and Lymphoid malignancies: Coming soon

<table>
<thead>
<tr>
<th>ABL1</th>
<th>CCND3</th>
<th>CUX1</th>
<th>GATA2</th>
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<th>NOTCH2</th>
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<tr>
<td>ARID1A</td>
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<td>HRAS</td>
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<td>PAX5</td>
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<td>KRAS</td>
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<td>IDH1</td>
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<td>PDGFRA</td>
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<td>TNFAIP3</td>
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<td>IKZF1</td>
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<td>PIK3CD</td>
<td>SMC3</td>
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<td>MIR 142</td>
<td>PIK3CG</td>
<td>SOCS1</td>
<td>WHSC1</td>
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<td>INPP5D</td>
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<td>IRF4</td>
<td>MYC</td>
<td>PLCG2</td>
<td>SPI1</td>
<td>WT1</td>
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<td>CBL</td>
<td>CRBN</td>
<td>FLT3</td>
<td>JAK1</td>
<td>MYD88</td>
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<td>XPO1</td>
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<td>CREBBP</td>
<td>FOXO1</td>
<td>JAK2</td>
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<td>PRDM1</td>
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<td>ZEB1</td>
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<td>GATA1</td>
<td>JAK3</td>
<td>NOTCH1</td>
<td>PTEN</td>
<td>STAT3</td>
<td>ZRSR2</td>
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</tbody>
</table>
Current COH Hematopathology Approach to AML Work up

Morphologic Features

Flow Cytometric analysis

Cytogenetics and FISH analysis

Molecular studies

Order “Molecular as needed” DNA and RNA will be extracted and hold indefinitely

Test can be ordered Later

Initial gene Panel - TAT: 3-5 days including:
- FLT3 ITD, TKD
- NPM1
- CEBPA
- IDH1
- IDH2
- KIT
- TP53
- BCR/ABL

HopeSeq HemeComplete - TAT: 10-14 days

Up-front NGS panels
### Novel therapies in clinical development in AML

**Protein kinase inhibitors**
- FLT3 inhibitors (midostaurin, quizartinib, gilteritinib, crenolanib)
- KIT inhibitors
- PI3K/AKT/mTOR inhibitors
- Aurora and polo-like kinase inhibitors, CDK4/6 inhibitors, CHK1, WEE1, and MPS1 inhibitors
- SRC and HCK inhibitors

**Epigenetic modulators**
- New DNA methyltransferase inhibitors (SGI-110)
- HDAC inhibitors
- IDH1 and IDH2 inhibitors
- DOT1L inhibitors
- BET-bromodomain inhibitors

**Chemotherapeutic agents**
- CPX-351
- Vosaroxin
- Nucleoside analogs

**Mitochondrial inhibitors**
- Bcl-2, Bcl-xL, and Mcl-1 inhibitors
- Caseinolytic protease inhibitors

**Therapies targeting oncogenic proteins**
- Fusion transcripts targeting
- EVI1 targeting
- NPM1 targeting
- Hedgehog inhibitors

**Antibodies and immunotherapies**
- Monoclonal antibodies against CD33, CD44, CD47, CD123, CLEC12A
- Immunoconjugates (eg, GO, SGN33A)
- BiTEs and DARTs
- CAR T cells or genetically engineered TCR T cells
- Immune checkpoint inhibitors (PD-1/PD-L1, CTLA-4)
- Anti-KIR antibody
- Vaccines (eg, WT1)

**Therapies targeting AML environment**
- CXCR4 and CXCL12 antagonists
- Antiangiogenic therapies

---

Case 1

- 52 year-old female with history of RA who previously received methotrexate and had multiple BM at Iowa and one more recent at VA LV, which was reported to be markedly hypercellular with +2 reticulin fibrosis

- Her Karyotype and FISH was reported normal
Case 1

- Her oncologist did not believe the previous MDS diagnosis

- Longstanding pancytopenia with severe thrombocytopenia and splenomegaly, for which she has regular transfusion. She had a recent splenectomy and is responded to 100mg promacta, hematopoietin agonist.
Case 1: Molecular consultation

- 2 purple top tube of her PB for HopeSeq HemeComplete testing
- Pathology consult, review of his outside marrow.
# Case 1: HopeSeq HemeComplete Results

<table>
<thead>
<tr>
<th>Genomic Alterations Detected</th>
<th>Allele Frequency (%)</th>
<th>FDA-Approved Therapies in patient’s tumor*</th>
<th>FDA-Approved Therapies in other tumor type*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>CBL</em> (c.1151G&gt;A; p.C384Y)</td>
<td>96%</td>
<td>None</td>
<td>None</td>
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<tr>
<td><em>CREBBP</em> (c.5554C&gt;T; p.Q1852*)</td>
<td>49%</td>
<td>None</td>
<td>None</td>
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<tr>
<td><em>DNMT3A</em> (c.2645G&gt;A; p.R882H)</td>
<td>49%</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td><em>RUNX1</em> (c.1429_1430dup; p.W477Cfs*118)</td>
<td>40%</td>
<td>None</td>
<td>None</td>
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<tr>
<td><em>ASXL1</em> (c.1934dup; p.G646Wfs*12)</td>
<td>39%</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
Case 2

- 53 year old female with relapsed AML 6 months post allogeneic stem cell transplantation with matched unrelated donor

- Initial AML negative for FLT3, NPM1, IDH1, IDH2, KIT, CEBPA mutations
  - Cytogenetics 47,XX, +21 [10]/48, sl, +8 [10]

- Relapse AML negative for FLT3, IDH1, IDH2 mutations

- Bone marrow: HopeSeq HemeComplete
## Case 2: NGS Assay Results

<table>
<thead>
<tr>
<th>Genomic Alterations Detected</th>
<th>Allele Frequency (%)</th>
<th>FDA-Approved Therapies in patient’s tumor</th>
<th>FDA-Approved Therapies in other tumor type</th>
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<tbody>
<tr>
<td><strong>CSF3R</strong> (p.T618I)</td>
<td>25%</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td><strong>PTPN11</strong> (p.G503A)</td>
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<tr>
<td><strong>RUNX1</strong> (p.113L)</td>
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<td><strong>RUNX1</strong> (p.S369*)</td>
<td>18%</td>
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<tr>
<td><strong>SRSF2</strong> (p.P95_R102del)</td>
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<td>None</td>
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</table>
Case 2

- **CSF3R c.1853C>T; p.T618I**
  - Encodes the receptor for colony-stimulating factor 3 (signaling through the JAK-STAT pathway)
  - T618I missense mutation is most common
  - Mutations seen in chronic neutrophilic leukemia (CNL)
  - May responsive to JAK inhibitors
Case 2

- **SRSF2 c.284_307del; p.P95_R102 del**
  - Encodes member of spliceosome involved in mRNA processing
  - In-frame deletion at codons 95-102

<table>
<thead>
<tr>
<th>ClinicalTrials.gov #</th>
<th>Title</th>
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<tr>
<td>NCT02841540</td>
<td>Phase 1 Trial to Evaluate the Safety, Pharmacokinetics and Pharmacodynamics of Splicing Modulator H3B-8800 for Subjects with Myelodysplastic Syndromes, Acute Myeloid Leukemia, and Chronic Myelomonocytic Leukemia</td>
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</table>

Spliceosome hotspot mutations in SF3B1, SRSF2, U2AF1, and/or ZRSR2, and **SRSF2 deletion including amino acid P95**.
Is There still a Role for Microscopic Evaluation in the Leukemia Diagnosis?

• Morphologic exam might be the quickest way for establishing a diagnosis
  – Example: Quick diagnosis of acute promyelocytic leukemia to start ATRA

• Evaluation of cellularity, maturation patterns, stromal changes

• Establish baseline for evaluation of treatment efficacy

• Exclude relevant differential diagnoses
Modern Hematopathology

Tissue pathology → Molecular pathology

Microscopy → Immunophenotyping → Karyotyping & FISH → PCR & Sanger → NGS

• Phenotype and Genotype are Complementary
NGS data analysis requires integration of clinical, pathological, and genetic interpretations:

Multidisciplinary Genomic Tumor Board
Things you can count on:

- Death
- Income tax
- A new leukemia classification based on upcoming molecular findings
Acknowledgment

Dr. Dennis Weisenburger
Dr. Patricia Aoun
Dr. Raju Pillai
Dr. Milhan Telatar
Dr. Carrie Louie
Dr. Javier Stella Arias
Dr. Hooi Yew
Dr. Vanina Tomasian
And our entire team of dedicated curators and clinical laboratory scientists at CMDL

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