Enhancing Assessment of Acute Leukemia with Next Generation Sequencing

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Neoplastic Hematopathology Update 2017

Objectives and Financial Disclosure

- List molecular targets useful for diagnosis of myeloid leukemia
- List molecular targets useful for monitoring and risk stratification of myeloid leukemia
- NO CONFLICTS of INTEREST to DISCLOSE

Disclosures

- I have nothing to disclose.

- We are in an era that promises a rational rather than empiric approaches for the treatment of cancer patients.
- Advances in sequencing technology have enabled the cost-effective detection of tumor genome and transcriptome mutation events
The 20 Most Commonly Diagnosed Cancers Worldwide

<table>
<thead>
<tr>
<th>Classification</th>
<th>Estimated Number of New Cases</th>
<th>Estimated Number of Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung (13%)</td>
<td>1,608,015</td>
<td>1,384,155</td>
</tr>
<tr>
<td>Female Breast (11%)</td>
<td>1,233,018</td>
<td>918,502</td>
</tr>
<tr>
<td>Colorectum (10%)</td>
<td>799,794</td>
<td>695,892</td>
</tr>
<tr>
<td>Stomach (8%)</td>
<td>530,123</td>
<td>483,645</td>
</tr>
<tr>
<td>Prostate (7%)</td>
<td>456,431</td>
<td>382,400</td>
</tr>
<tr>
<td>Liver (6%)</td>
<td>281,620</td>
<td>273,118</td>
</tr>
<tr>
<td>Cervix (4%)</td>
<td>263,020</td>
<td>257,913</td>
</tr>
<tr>
<td>Oesophagus (4%)</td>
<td>227,318</td>
<td>207,474</td>
</tr>
<tr>
<td>Bladder (3%)</td>
<td>195,627</td>
<td>186,677</td>
</tr>
<tr>
<td>Non-Hodgkin Lymphoma (3%)</td>
<td>156,414</td>
<td></td>
</tr>
<tr>
<td>Leukemia (3%)</td>
<td>156,414</td>
<td>137,027</td>
</tr>
<tr>
<td>Uterus (2%)</td>
<td>128,367</td>
<td>113,894</td>
</tr>
<tr>
<td>Pancreas (2%)</td>
<td>78,604</td>
<td>72,596</td>
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<tr>
<td>Kidney (2%)</td>
<td>72,596</td>
<td>69,873</td>
</tr>
<tr>
<td>Lip and Oral Cavity (2%)</td>
<td>63,900</td>
<td></td>
</tr>
<tr>
<td>Brain and CNS (2%)</td>
<td>57,392</td>
<td></td>
</tr>
<tr>
<td>Ovary (2%)</td>
<td>54,347</td>
<td>50,724</td>
</tr>
<tr>
<td>Thyroid (2%)</td>
<td>213,179</td>
<td>206,353</td>
</tr>
<tr>
<td>Maligant Melanoma (2%)</td>
<td>195,627</td>
<td></td>
</tr>
<tr>
<td>Other Sites (13%)</td>
<td>196,677</td>
<td>187,603</td>
</tr>
</tbody>
</table>

Classification of Leukemia Long time ago!

- Acute
  - Myeloid origin
  - Lymphoid origin
- Chronic

Classification is easy when there is NO effective treatment

- Broken leg: shoot
- Infected eye: shoot
- Splayed hoof: shoot
French-American-British (FAB) Classification 1976

• Eight major subtypes (M0–M7)
• Based on morphology and cytochemistry

FAB Classification Comparison to WHO Classification of AML, NOS

• M0   AML with minimal differentiation
• M1   AML without maturation
• M2   AML with maturation
• M3   Acute Promyelocytic Leukemia
• M4   Acute Myelomonocytic Leukemia
• M5   Acute Monoblastic/Monocytic L.
• M6   Pure Erythroid Leukemia
• M7   Acute Megakaryoblastic Leukemia

Modern Hematopathology

- Tissue pathology
- Molecular pathology

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

• Phenotype and Genotype are Complementary

Personalized Cancer Medicine

• Every type of cancer has distinct genomic profile that defines clinical behavior and response to treatment in each individual
  – Comprehensive Tumor Genotyping
  – Establish specific molecular ‘signature’
  – Match with appropriate targeted therapy
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.

Genomic Landscapes of Common Human Cancers

Average 33-66 genes mutated
- 91% missense mutations
- 8% nonsense changes
- <2% splice site or UTR

Most common Mutations in AML

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>AML</th>
<th>MDS</th>
<th>MPN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnosis</strong></td>
<td>NPM1, CEBPA, RUNX1</td>
<td>SF3B1</td>
<td>JAK2, CALR, MPL, CSF3R</td>
</tr>
<tr>
<td><strong>Prognosis</strong></td>
<td>FLT3-ITD, DNMT3A, IDH1/2, KIT, TET2, TP53, ASXL1, WT1...</td>
<td>TP53, EZH2, ETN1, RUNX1, ASXL1...</td>
<td>ASXL1, EZH2, ETV1, ETN1, IDH2, SETBP1, SF3B1, SRSF2</td>
</tr>
</tbody>
</table>

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
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 monocytic/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 with recurrent cytogenetic abnormalities

- t(8;21); RUNX1-RUNX1T1: some maturation of neutrophilic line; rare in older patients; AML1/ETO fusion protein; FAB M2; flow cytometry CD19
- t(15;17); PML-RARA: APL (granular and microgranular variants); t(11;17), t(5;17)
- inv(16) or t(16;16); CBFB-MYH11: abnormal eosinophilic component; AMML with eos (M4eo)
- t(9;11); MLLT3-KMT2A (MLL) - monocytic; children;
- t(6;9); DEK-NUP214: Basophilia and multilineage dysplasia

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

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

- 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
AML with recurrent cytogenetic abnormalities – New categories

- AML with **NPM1** – Mutually exclusive with partial tandem duplications of MLL and biCEBPA; **good prognosis**
- AML with **CEBPA<sup>DM</sup>** – only changes prognosis (good) if present as a double mutant
- 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 2017 European Leukemia Net recommendations for AML.


Ten studies covering a total of 6219 subjects indicated bi CEBPA mutations were associated with favorable clinical outcome in patients with AML or in cytogenetically normal AML.

- No significant difference was found between monoallelic CEBPA mutation and wild type CEBPA in patients with AML or CN-AML (**P > 0.05**).


Mutational landscape of AML with normal cytogenetics: Biological and clinical implications

Maria Paola Martelli, Brunangelo Falini, et al.
Institute of Hematology, University of Perugia, Perugia, Italy
Blood Reviews 27 (2013) 13-22

- **FLT3-ITD** loses its prognostic effect in CN-AML patients >60 years and especially those aged >70 years

- On the other hand, **NPM1** mutations maintain their positive effect on clinical outcome in this age group


Genes encoding epigenetic modifiers: DNMT3A, ASXL1, TET2, IDH1, and IDH2

- May persist after therapy, lead to clonal expansion during hematologic remission, and eventually lead to relapsed disease.
- Frequently found to be mutated in healthy elderly individuals along with clonal expansion of hematopoietis (increased risk for the development of hematologic cancers)


CHIP

- 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.


Top 20 Genes in AML

<table>
<thead>
<tr>
<th>Gene name (frequency)</th>
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<tbody>
<tr>
<td>MN1 (33.7%)</td>
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<tr>
<td>JUN (10.9%)</td>
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<tr>
<td>DT40 (14.5%)</td>
</tr>
<tr>
<td>SEK (13.7%)</td>
</tr>
<tr>
<td>TRK (12.8%)</td>
</tr>
<tr>
<td>CHEK2 (8.5%)</td>
</tr>
<tr>
<td>ERX7 (8.4%)</td>
</tr>
<tr>
<td>WT1 (9.1%)</td>
</tr>
<tr>
<td>ATK (18.7%)</td>
</tr>
<tr>
<td>ASXL1 (14.8%)</td>
</tr>
<tr>
<td>TGF (1.5%)</td>
</tr>
<tr>
<td>RAB5A (1.5%)</td>
</tr>
<tr>
<td>PTK2B (3.6%)</td>
</tr>
<tr>
<td>SF3B1 (1.8%)</td>
</tr>
<tr>
<td>STK11 (2.1%)</td>
</tr>
<tr>
<td>GATA2 (1.6%)</td>
</tr>
<tr>
<td>GATA3 (1.1%)</td>
</tr>
<tr>
<td>E2A (1.1%)</td>
</tr>
</tbody>
</table>

Samples with mutation | All samples

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

Management:
For patients with specific recurrent gene mutations, such as BCOR (diffuse large B-cell lymphoma), the del(17p) component of the MLL-SET gene fusion as a PML-RAR bcr1 rearrangement or MLL-MLLT3, certain recurrent gene mutations play a major role in leukemic transformation of hematopoietic stem cells through deregulation of HOXA9. Although the relevance of BCOR mutations in AML, myelodysplastic syndrome (MDS), and myeloproliferative neoplasms (MPN) has been reported in a few studies, the clinical relevance and prognostic implications remain to be defined.

Design: Using whole exome sequencing, we identified recurrent mutations in BCOR, which is a component of the HOXA9-BCOR complex. BCOR mutations were found in 13 patients with AML, including 10 patients with a BCOR mutation and a MDS-like phenotype and 3 patients with a BCOR mutation and a myeloid neoplasm-like phenotype. The BCOR mutations were associated with a poor outcome in patients with AML with BCOR mutations.

Results: In summary, BCOR mutations were identified in 13 patients with AML, including 10 patients with a BCOR mutation and a MDS-like phenotype and 3 patients with a BCOR mutation and a myeloid neoplasm-like phenotype. The BCOR mutations were associated with a poor outcome in patients with AML with BCOR mutations.

Conclusions: Our findings suggest that BCOR mutations are associated with a poor outcome in patients with AML. Further studies are needed to determine the clinical relevance and prognostic implications of BCOR mutations in AML.

The Updated WHO 2016 Classification of AML

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- 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
- Revise cytogenetic abnormalities
  - Del 9q is an MDS related entity only in the absence of NPM1 mutations
  - NPM1 commonly associated with del9q and is likely not adverse in this setting

AML with NPM1 and CEBPA Mutations, and Abnormal Karyotype:

- 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
- Del9q currently is an MDS related entity only in the absence of NPM1 or CEBPA mutations due to no prognostic significance (Haferlach C et al Blood 114 2009)
- Removed from de novo cases with MDS related cytogenetic abnormality if there is an NPM1 or CEBPA
**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

**Clinical Molecular Diagnostics Laboratory (CMDL)**

- In operation since 1990s as a clinical reference laboratory: annual volume 2017 ~24,000 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

**CMDL Current NGS Hematological Malignancies TAT (10-14 days)**

- **OncoHeme Complete:** 96 genes
  - Entire exon sequencing of 73 genes
  - Fusions: Detection of >500 selected rearrangements
- **HopeSeq Heme Complete:** 300 genes
  - Entire exon sequencing of >100 genes
  - Fusions: Detection of >2000 selected rearrangements (including Ph-like ALL)
  - Gene expression profiling (including DLBCL, GCB vs ABC)
Quantitative Assays Recently updated:
• BCR/ABL1 (TAT 1-2 days)
• PML/RARA, Inv 16, and AML1/ETO (TAT 1-2 days)
• Mutation analysis (TAT 3-5 days)

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

Case
• 71 year-old male with history of “MDS” since 2014 and deletion chromosome 1
• Transferred to COH form an OSH where her received hydrea and steroids for autoimmune hemolytic anemia
• PBS: WBC:50,000 K/ul; Hb:9 g/dL; Platelets:12 K/uL

Other Hematological Malignancies Assays
• Other PCR assays
  – Immunoglobulin heavy and light chain gene rearrangements with BIOMED-2 primers
  – T-cell receptor gamma and beta chain gene rearrangements with BIOMED-2 primers
    – Inv 16
    – t(8;21)

Peripheral blood and Bone marrow was sent for evaluation
• PBS: WBC:38,100 K/ul; Hb:7.8 g/dL, MCV:103.8; Platelets: 24 K/uL Diff: 66% blasts
Diagnosis

Bone marrow biopsy final diagnosis:

- **Acute myeloid Leukemia (77% BLASTS)** extensively involving a markedly hypercellular bone marrow (95%)

Fast TAT Single Gene Heme Tests Results

- NPM1, IDH1, IDH2, KIT, CEBPA, mutations and BCR-ABL fusion: Not Detected

MOLECULAR-ONCOHEME MUTATIONS REPORT

<table>
<thead>
<tr>
<th>Tumor Type: ACUTE MYELOID LEUKEMIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomic Alteration (Detected)</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>SF3B1 (p.K702E)</td>
</tr>
<tr>
<td>NPM1 (p.R882H)</td>
</tr>
<tr>
<td>RUNX1 (p.E299D)</td>
</tr>
<tr>
<td>FLT3 (p.D835H)</td>
</tr>
<tr>
<td>FLT3 (p.E896G/V897I)</td>
</tr>
</tbody>
</table>

- FLT3 TYROSINE KINASE DOMAIN (TKD) MUTATION CONFIRMED BY PCR (see table for NGS result)
- FLT3 INTERNAL TANDEM DUPLEXION (ITD) CONFIRMED BY PCR WITH A SIGNAL RATIO OF 0.43 (see table for NGS result)

- Initial bone marrow biopsy from 3 years ago reported as MDS-RS but no molecular studies were performed.

- Presence of SF3B1 mutation in MDS-RS reported to be associated with better prognosis, however cases with RUNX1 mutation appear to be associated with shorter survival and higher rate of AML transformation.
Recommendation for AML Genetics Classifications

- Karyotype analysis should be performed on all diagnostic samples
- Molecular genetic studies should be ordered based on assessment of morphology, immunophenotype, and Karyotype analysis or up-front

Current COH Hematopathology Approach for Myeloid Disorder Work up

Morphologic Features
Flow Cytometric analysis
Cytogenetics and FISH analysis
Molecular studies

- HopeSeq 300 gene Panel including: FLT3 ITD, TKD, NPM1, CEBPA, IDH1, IDH2, KIT, DNMT3A, TET2, RUNX1, MLL, WT1, ASXL1, BCOR, GATA2, MIR142, TP53 alterations, ERG expression, MECOM expression
- Order “Molecular as needed” DNA and RNA will be extracted and hold indefinitely
- Test can be ordered Later
Up-front NGS panels

Era of Precision Medicine

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 lymphoma/ leukemia classification based on new molecular findings
Acknowledgment

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Maria Cuellar, MS
Vanina Tomasian, MS
Dongging Gu, MS

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And our entire team of dedicated curators and clinical laboratory scientists!