Should we sequence everything?

Applying clinical next-generation sequencing in hematologic malignancies

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Disclosures

• I do not have any relevant financial or other disclosures relevant to the content of this presentation.

• I will not be discussing on- or off-label use of medications.
Objectives

• Understand how the WHO hematologic malignancies classification has evolved to include more genetic information.
• Describe the basics of next-generation sequencing (NGS) technology.
• Understand how to apply NGS panels in the diagnosis and prognosis of acute myeloid leukemia.
• Describe how NGS has led to the definition of new pre-MDS/AML states.
A clinical case

• 53 year old male with no significant past medical history.
• Avid runner (10-20 miles/day) who 4 weeks prior to presentation started having increased dyspnea on exertion and poor energy.
• While on vacation, he had rapidly worsening shortness of breath and presented to an urgent care.
  • WBC 18K with rare blasts and 29% “atypical lymphocytes”
• Required ICU care with intubation for respiratory failure, and was transferred urgently back home to DUMC.
  • WBC leaving OSH: 119K
  • WBC on admission at DUMC: 193K
Acute monocytic leukemia
FAB AML/MDS classification (1976)

- Purely morphologic (M0-M7), depending upon the degree and type of differentiation.
- MDS: RAEB or CMML.

Our case: FAB M5b
WHO classification (1997)

- Cytogenetics comes into play.
  - t(9;22)(BCR/ABL) = chronic myelogenous leukemia
- t(15;17)(PML/RARA) = acute promyelocytic leukemia
- t(8;21)(AML/ETO)
- inv(16) / t(16;16) (CBFB/MYH11)
- 11q23 (MLL) rearrangements

If no abnormalities, fall back on FAB.

Our case: 46,XY (normal karyotype, normal FISH)

Rowley JD Nature 1973
First classification based on sequencing:
AML with mutations in:
- NPM1
- CEBPA
Sequencing performed on our case per WHO 2008

• NPM1: positive for the common 4 base insertion.
• FLT3: negative/no mutations.
• CEBPA: negative/no mutations.

NPM1-positive, FLT3-negative, CEBPA-negative: relatively favorable prognosis.

Standard induction (7+3) and 3 cycles of consolidation

Relapsed 9 months after initial diagnosis
Next generation (massively parallel) sequencing

Millions of sequencing reactions performed and read simultaneously

Deep
- PCR amplification of target panel
- Thousands of reads of each target
- Detection of very small mutant populations

Broad
- Sequencing of the whole exome or genome
- Fewer reads but many more targets
- Detection of a broad range of mutations
Illumina platform

“flow cell”
Illumina platform

Millions of patient DNA molecules
Illumina platform
Illumina platform

Target DNA strand

Sequencing primer
Illumina platform

Target DNA strand

Sequencing primer

Fluorescently-tagged dNTP
Illumina platform

Read (x millions)
Illumina platform

Remove fluor
Illumina platform

+ dNTPs

Duke Pathology
Illumina platform

Read (x millions)
## Clinical next-generation sequencing panels

<table>
<thead>
<tr>
<th>Panel Type</th>
<th>Examples</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hotspot panel</td>
<td>• Mayo OncoHeme</td>
<td>• Target known actionable and clinically relevant mutations.</td>
<td>• Limited number of targets.</td>
</tr>
<tr>
<td></td>
<td>• Genoptix Panel</td>
<td>• Good sensitivity for low-level mutations.</td>
<td>• Do not reliably detect fusions and large genomic deletions/insertions.</td>
</tr>
<tr>
<td></td>
<td>• UNC (Illumina TruSight)</td>
<td>• Relatively inexpensive.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Duke Myeloid Panel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broad targeted panel</td>
<td>FoundationOne Heme</td>
<td>• Cover a wide array of targets, including fusions and large genomic deletions/insertions.</td>
<td>• Questionable utility of broader targeting.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Good sensitivity.</td>
<td>• Relatively expensive.</td>
</tr>
<tr>
<td>Clinical exome</td>
<td>GeneDx</td>
<td>• Very broad coverage</td>
<td>• More applicable for constitutional genetic testing.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Limited sensitivity for low-level mutations.</td>
<td>• Expensive.</td>
</tr>
</tbody>
</table>
## WHO classification of hematologic malignancies (2016)

### Myeloproliferative neoplasms (MPN)
- **Chronic myeloid leukemia (CML), BCR-ABL**
- **Chronic neutrophilic leukemia (CNL)**
- **Polycythemia vera (PV)**
- **Primary myelofibrosis (PMF)**
  - PMF, prefibrotic/early stage
  - PMF, overt fibrotic stage
- **Essential thrombocythemia (ET)**
- **Chronic eosinophilic leukemia, not otherwise specified (NOS)**
- **MPN, unclassifiable**
- **Mastocytosis**

### Myelodysplastic syndromes (MDS)
- **MDS with single lineage dysplasia**
- **MDS with ring sideroblasts (MDS-RS)**
  - MDS-RS and single lineage dysplasia
  - MDS-RS and multilineage dysplasia
- **MDS with multilineage dysplasia**
- **MDS with excess blasts**
- **MDS with isolated del(5q)**
- **MDS, unclassifiable**
  - **Provisional entity: Refractory cytopenia of childhood**
- **Myeloid neoplasms with germ line predisposition**

### Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
- **Chronic myelomonocytic leukemia (CMML)**
- **Atypical chronic myeloid leukemia (aCML), BCR-ABL**
- **Juvenile myelomonocytic leukemia (JMML)**
- **MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)**
- **MDS/MPN, unclassifiable**

Mutational analysis is required for, or aids in, diagnosis
Mutational analysis is required for, or aids in, diagnosis
### Variants of *TD17 0025_Mutation_Report1_Filtered*: *Filters Applied*

<table>
<thead>
<tr>
<th>ID</th>
<th>Gene</th>
<th>Variant Frequency</th>
<th>Coverage</th>
<th>Pathogenicity</th>
<th>RS</th>
<th>Ref</th>
<th>Ref AA</th>
<th>All</th>
<th>Alt AA</th>
<th>Type</th>
<th>Exon Number</th>
<th>HGVS Coding</th>
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<tbody>
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</tbody>
</table>

*Back to our patient*
Back to our patient

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutation</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLT3</td>
<td>ITD</td>
<td>Newly detected on relapse – <strong>worsens overall prognosis</strong></td>
</tr>
<tr>
<td>NPM1</td>
<td>4 bp insertion</td>
<td>Known at original diagnosis</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>R882C</td>
<td>Very common hotspot mutation in AML/MDS – unclear prognostic significance</td>
</tr>
<tr>
<td>PTPN11</td>
<td>D61V</td>
<td>Common hotspot mutation in AML – unclear prognostic significance</td>
</tr>
<tr>
<td>IDH1</td>
<td>R132H</td>
<td>Common hotspot mutation in AML – <strong>potentially actionable in clinical trial</strong></td>
</tr>
</tbody>
</table>
AML has multiple mutations and subclones at diagnosis

**Most common mutations:**
- FLT3
- NPM1
- DNMT3A
- IDH1/2

TCGA Network. NEJM 2013;368
AML segregates into different mutational classes with very different prognoses.

Papaemmanuel E et al, *NEJM* 374(23), 2016
AML mutations interact in unexpected ways to influence prognosis
Neoplastic subclones respond differently to therapy

MDS/AML is a multi-step process (like many other cancers)

- **Progenitor Cell**
  - Epigenetic mutation(s)
  - TET2, DNMT3A, IDH1/2

- **Clonal hematopoiesis**
  - Chromatin/spliceosome mutation(s)
  - SF3B1, ASXL1

- **MDS**
  - Signaling/proliferation mutation(s)
  - FLT3, PTPN11, NRAS

- **Pre-leukemic precursor**
  - Transcription factor mutation or fusion/translocation
  - CEBPA, RUNX1, inv(16)

- **AML**
  - Signaling/proliferation mutation(s)

Another clinical case

• 72 year old female with slowly progressive, mild fatigue.
• Past medical history significant for hyperlipidemia and type 2 diabetes.
• Repeated CBCs show mild anemia and thrombocytopenia (Hgb 10.5 g/dL, Platelets 110 K/mm3).
• Thorough workup, including iron studies, autoimmune disease workup, B12, folate, copper, etc, etc., all negative.
• Bone marrow biopsy unremarkable – some mild erythroid atypia but not diagnostic.
• **NGS panel sent: DNMT3A R882H mutation detected**
Hematopoietic driver mutations occur with increasing frequency with age

Jaiswal et al, NEJM 2014;371(26)
Mutations occur mostly in “early” AML drivers

Jaiswal et al, NEJM 2014;371(26)
Mutations confer greatly increased risk of developing hematologic malignancies

Jaiswal et al, NEJM 2014;371(26)
Cardiovascular mortality is increased with mutations

**Cause specific mortality**

<table>
<thead>
<tr>
<th>Cause</th>
<th>HR (CI 95%)</th>
<th>Events/No. at risk</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>0.9 (0.4-2.2)</td>
<td>5/213</td>
<td>0.86</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>1.4 (0.9-2.3)</td>
<td>19/213</td>
<td>0.15</td>
</tr>
<tr>
<td>VAF ≥ 0.10</td>
<td>1.9 (1.1-3.5)</td>
<td>12/93</td>
<td>0.033</td>
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<tr>
<td>VAF &lt; 0.10</td>
<td>1.0 (0.5-2.3)</td>
<td>7/120</td>
<td>0.97</td>
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<tr>
<td>Other/un adjudicated</td>
<td>1.0 (0.7-1.6)</td>
<td>23/161</td>
<td>0.79</td>
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</tbody>
</table>

Why? Studies are ongoing...
Could be related to disordered inflammation

Jaiswal et al, NEJM 2014;371(26)
## Pre-MDS states?

<table>
<thead>
<tr>
<th>Category</th>
<th>Clonality</th>
<th>Cytopenias</th>
<th>Risk of progression to MDS/AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clonal hematopoiesis of indeterminate potential (CHIP)</td>
<td>Yes</td>
<td>No</td>
<td>0.5-1.0% per year</td>
</tr>
<tr>
<td>Clonal cytopenias of undetermined significance (CCUS)</td>
<td>Yes</td>
<td>Yes</td>
<td>?higher?</td>
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</tbody>
</table>
How frequent are CHIP and CCUS?

• 22-33% of patients with unexplained cytopenias not meeting MDS criteria had at least one clonal mutation (two different studies).

• >85% of MDS patients have at least one detectable mutation in common genes.

Steensma DP et al, Blood 2015;126:9-16
Hall J et al, Blood 2014;124(21)
Kwok B et al, Blood 2014;124(21)
Papaemmanuil E et al, Blood 2013;122(22):3616
Should we sequence everything?
Take-home points.

• Next-generation sequencing allows for efficient testing of many genes simultaneously.

• Acute myeloid leukemia:
  • Eliminates most single gene tests, saving cost and perhaps time.
  • Allows detection of novel mutations that may be useful for clinical trials now or in the future.
  • Allow for more accurate separation into prognostic groups.

• Acute lymphoblastic leukemia (did not discuss):
  • Similar panels may be helpful but not as widely implemented as AML.
Should we sequence everything?
Take-home points.

- **MDS:**
  - Could be useful for more definitive diagnosis when you **have morphologic evidence and the karyotype and FISH are normal.**

- **CCUS:**
  - Cytopenias without morphologic or clinical explanation seem the most reasonable to test. There is at least a 1% risk of transformation per year.

- **CHIP:**
  - How will you detect these if the patients are not cytopenic?
  - There is a low level of risk of transformation (0.5-1%) and the risk of CHIP increases with age.
  - Jury is out.