Genomic and Molecular Markers for Diagnosis and Prognosis

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Objectives

• Describe mutations and their frequencies in “healthy” populations, aplastic anemia and myelodysplasia

• Discuss the evolving role of the use of mutations in diagnosis and prognosis

• Conflict of interest: None
Definitions

- CHIP – Clonal Hematopoiesis of Indeterminate Potential
- ICUS – Idiopathic Cytopenia of Undetermined Significance
- CCUS – Clonal Cytopenia of Undetermined Significance
- MDS – Myelodysplastic Syndrome (preleukemia)
  - Abnormal Cells >10% of cell lineage
- NGS – Next Generation Sequencing, multiple genes at the same time
- VAF – Variant Allele Frequency, allelic burden, percent of DNA mutated

What is Clonal Hematopoiesis of Indeterminate Potential (CHIP)?

- Absence of definitive morphologic evidence of a hematological neoplasm
- Does not meet diagnostic criteria for PNH, MGUS or MBL
- Presence of a somatic mutation associated with hematological neoplasia at a variant allele frequency of at least 2% (e.g., DNMT3A, TET2, JAK2, SF3B1, ASXL1, TP53, CBL, GNB1, BCOR, U2AF1, CREBBP, CUX1, SRSF2, MLL2, SETD2, SETDB1, GNAS, PPM1D, BCORL1)
- Odds of progression to overt neoplasia are approximately 0.5-1% per year, similar to MGUS

Steensma et al. Blood 2015
What do the Genes do?

- DNA Methylation: TET2, DNMT3A, IDH1/2
- Chromatin/histone modification: ASXL1, EZH2, UTX
- Transcription regulation: RUNX1, BCOR or BCORL1, ETV6, SETBP1, GATA2
- DNA repair/tumor suppressor: TP53
- Signal transduction: JAK2

Zhang et al. Leukemia Research 2014

What Mutations are Seen in Aplastic Anemia?

- 439 Patients
- 36% Somatic Mutations
- Order of Frequency
  - BCOR or BCORL1 9.3%
  - DNMT3A 8.4%
  - PIGA 7.5%
  - ASXL1 6.2%
  - JAK, RUNX1, TP53, Splicing <5% each
- VAF (Allelic Burden): 9.3% vs 30.4% in MDS
- Poor Prognosis: ASXL1, DNMT3A, TP53, RUNX1, CSMD1

Yoshizato NEJM 2015
Single Nucleotide Polymorphism Results in Aplastic Anemia

- 6p: UPD 13%
- -7 1.6% (7 patients)
- Del 13q 0.05% (2 patients)
- Other 2.2% (10 patients)

Adding Mutations & SNP changes show that 47% of Aplastic Anemia patients have clonal hematopoiesis

Yoshizato NEJM 2015

Point Mutations in MDS

- 439 patients samples sequenced
  - 11 genes by Mass Spectrometry
  - 7 genes by Next Generation Sequencing
- 51% had ≥ 1 mutation

- TET2 20%, ASXL1 14%, RUNX1 8.7%, TP53 7.5%, EZH2 6%
- RUNX1, TP53, NRAS associated with Thrombocytopenia
- Poor prognostic mutations: TP53, EZH2, ETV6, RUNX1, ASXL1

Bejar et al. Blood 2011
Comparing Frequency of Somatic Mutations in MDS and ICUS

<table>
<thead>
<tr>
<th></th>
<th>MDS Test Group</th>
<th>MDS Validation Group</th>
<th>Variant Allele Frequency</th>
</tr>
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<tbody>
<tr>
<td>MDS</td>
<td>71%</td>
<td>79%</td>
<td>33%</td>
</tr>
<tr>
<td>ICUS with some dysplasia</td>
<td>62%</td>
<td>45%</td>
<td>30%</td>
</tr>
<tr>
<td>ICUS with no dysplasia</td>
<td>20%</td>
<td>17%</td>
<td>23%</td>
</tr>
</tbody>
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Kwok et al. Blood 2015

Mutations in Preclinical MDS

- 69 patients with nondiagnostic marrow developed progressive MDS or AML in a subsequent diagnostic marrow
- 26 genes studied by NGS
- 91% patients had mutations in pre-diagnostic marrow:
  - TET2 39%, SRSF2 26%, ASXL1 20%,
  - VAF 40%
  - Patients with >3% mutations poor OS,
  - TP53, U2AF1, -Poor prognosis

Cargo et al. Blood 2015
What does the 2016 WHO Say on the Significance of Mutations for Considering MDS?

- “Importantly, acquired clonal mutations identical to those seen in MDS can occur in the hematopoietic cells of apparently healthy older individuals without MDS, so-called clonal hematopoiesis of indeterminate potential (CHIP)”
- The presence of MDS-associated somatic mutations alone is not considered diagnostic of MDS in this classification, even in the patient with unexplained cytopenia, where these mutations may be commonly found”

Arber et al Blood 2016

Overlap in Mutations Occurs in CHIP, AA, and MDS
Status of Myeloid Mutation Testing at Nebraska Medicine

- Current Mutation Testing: JAK2, MPL, CALR, NPM1, FLT3
  - Individual assays by capillary electrophoresis, pyrosequencing, RT-PCR

- Future Myeloid Mutation Panel: 54 genes on MiSeq
  - Under validation in the Molecular Diagnostics lab

<table>
<thead>
<tr>
<th>Gene List in MiSeq Mutation Panel</th>
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<tbody>
<tr>
<td>ABL1 CEBPA HRAS MYD88 SF3B1</td>
</tr>
<tr>
<td>ASXL1 CSF3R IDH1 NOTCH1 SMC1A</td>
</tr>
<tr>
<td>ATRX CUX1 IDH2 NPM1 SMC3</td>
</tr>
<tr>
<td>BCOR DNMT3A IKZF1 NRAS SRSF2</td>
</tr>
<tr>
<td>BCORL1 ETV6 JAK2 PDGFRA STAG2</td>
</tr>
<tr>
<td>BRAF EZH2 JAK3 PHF6 TET2</td>
</tr>
<tr>
<td>CALR FBXW7 KDM6A PTPN11 TP53</td>
</tr>
<tr>
<td>CBL FLT3 KIT PTPN11 U2AF1</td>
</tr>
<tr>
<td>CBLB GATA1 KMT2A RAD21 WT1</td>
</tr>
<tr>
<td>CBLC GATA2 KRAS RUNX1 ZRSR2</td>
</tr>
<tr>
<td>CDKN2A GNAS MPL SETBP1</td>
</tr>
</tbody>
</table>
Observations on Molecular Testing

• Mutation frequency and VAF increase with age. If you perform an NGS test, you are likely to find mutations of various percentages.

• Most mutation panels do not include the PIGA gene, but perhaps they should, in order to cover PNH.

• The recent mutation studies may lead to redefining the definitions of aplastic anemia and myelodysplastic syndrome.

What Criteria Could be Used for Mutations in Diagnosing Myelodysplastic Syndrome?

• “The absence of a mutation as a good predictor of not having MDS”

• Mutation alone without dysplasia or blast increase is not sufficient to diagnose MDS.

• Multiple mutated genes are necessary for a diagnosis of MDS, as single gene mutations are seen in “healthy” patients.

• Usage of a high median variant allele frequency of 30-40% as 10% VAF is in “healthy” population TET2, SRSF2, ASXL1, DNMT3A, IDH1, ZRSR2.

When is it the Right Time to do Molecular Testing?

• When considering congenital disorders.

• When considering paroxysmal nocturnal hemoglobinuria.

• When considering a diagnosis of aplastic anemia vs hypoplastic MDS.

END