Let's Get Started!

Program Objectives

- Discuss incorporation of new molecular markers into the diagnostic algorithms for myelodysplastic syndromes and myeloproliferative neoplasms
- Understand how the clonal hierarchy may influence myeloproliferative neoplasms
- Understand the benefits and pitfalls in the use of next-generation sequencing (NGS) for the workup of low-grade MDS
Primary Versus Secondary Driver Mutations

- Primary
  - necessary for neoplasm
  - strongly associated with disease type
  - can be sole

- Secondary
  - superimposed on primary
  - can be multiple
  - may be nonrandom
  - non-specific
  - important in tumor progression
  - some may be neutral
  - have lineage promiscuity

Myelodysplasia

"The myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell diseases characterized by cytopenia(s), dysplasia, ineffective hematopoiesis, and increased risk of development of acute myeloid leukemia."

2008 WHO Classification of Myelodysplastic Syndrome

- Refractory cytopenia with unilineage dysplasia (RCUD)
- Refractory anemia (RA), refractory neutropenia (RN), refractory thrombocytopenia (RT)
- Refractory anemia with ring sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia (RCMD)
- Refractory anemia with excess blasts-1 (RAEB-1)
- Refractory anemia with excess blasts-2 (RAEB-2)
- MDS associated with isolated del(5q)
- Myelodysplastic syndrome- unclassified (MDS-U)
Risk Stratification in MDS

Before 2013, risk in MDS was determined by the international prognostic scoring system (IPSS):
- BM % blasts
- Karyotype group
- Hematologic parameters (hemoglobin, platelets, ANC)

Now numerous publications proposing supplementing risk stratification of MDS with mutation profiling.

Mutational Landscape in MDS

Early MDS somatic mutations:
- Epigenetic regulators (DNMT3A, TET2, IDH1/2)
- Spliceosome mutations (SF3B1, SRSF2, U2AF1, ZRSR2)
- Epigenetic/spliceosome mutations precede chromosome changes
  - Early to late stages
  - Later stages of MDS and AML
- G1-S phase mutations such as TP53, ATM
- Differentiation factors (RUNX1, BCOR)
- Disease specific associations
  - SF3B1 mutations and ring sideroblasts
  - NRAS/RAS/IDH2 in CMML
  - CSF3R in chronic neutrophilic leukemia
  - TP53 in 20% of 5q- which heralds resistance to lenalidomide

Case 1

- 93 yo male with macrocytic anemia

Complete Blood Count:
- RBC: 2.85 millions/μL, Hgb: 9.7 g/dL, Hct: 29.5%, MCV: 103.5 fl, MCH: 34.2 pg, MCHC: 33.0 g/dL, RDW: 23.0%
- Platelet: 201 thousand/μL
- WBC: 5.4 thousand/μL, N: 55.8%, L: 33.0%, M: 9.3%
Case 1: Morphology

- Hypercellular Marrow for Age (70%)
- Erythroid Megaloblastoid
- Changes with Increased Ring Sideroblasts (51%)
- Dysmegakaryopoiesis, and No Increased in Blasts

Case 1: Cytogenetics

- Clinical Indication: Rule out chromosome abnormality
- Method: GTG
- Cells Counted: 20
- Band Level: 400
- Cells Analyzed: 20
- Cells Karyotyped: 2
- RESULT: 46,XY[20]

Case 1: Molecular

- Myelodysplastic Syndrome Panel
- Specimen Source: Cell Pellet (Bone Marrow Aspirate)
- Sequencing Result: Positive
- Mutations detected in the ASXL1 and TET2 genes below consistent with the presence of a clonal hematopoietic disorder.
- Similar mutations have previously been reported in myelodysplastic syndromes. Complete with clinical and morphologic features for final classification.

Gene Mutated: ASXL1
- Mutation Site: Gly646fs*12
- Mutation Type: fs/STOP
- Mutation Level: Heterozygous
- Exon: E12
- Nucleotide Change: c.1934dupG

Gene Mutated: TET2
- Mutation Site: Glu524fs*43
- Mutation Type: fs/STOP
- Mutation Level: Heterozygous
- Exon: E03
- Nucleotide Change: c.1568dupG

Diagnosis: Refractory Cytopenia with Multi-lineage Dysplasia
Case 2

- Thirty-three y/o female with fever of unknown origin; and anemia and neutropenia.

Complete Blood Count:
- RBC: 3.83 millions/μL, Hgb: 10.7 g/dL, Hct: 30.9%, MCV: 81 fL, MCH: 28 pg, MCHC: 35 g/dL, RDW: 14.5%
- Platelet: 161 thousand/μL,
- WBC: 2.9 thousand/μL, N: 53%, L: 20%, M: 6%

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Case 2: Morphology

- Blasts: 1 (0-3)
- Promyelocytes: 0 (2-8)
- Myelocytes: 4 (5-20)
- Metamyelocytes: 5 (13-32)
- Neutrophils: 19 (7-30)
- Eosinophils: 2 (0-4)
- Basophils: 0 (0-1)
- Lymphocytes: 15 (3-17)
- Plasma Cells: 1 (0-2)
- Monocytes: 1 (0-5)
- Pronormoblasts: 10 (1-8)
- Normoblasts: 42 (7-32)
- M:E Ratio: 0.62 (3-4)

- Adequacy: Good
- Erythroid cells: Adequate maturation
- Erythroid Dysplasia: Moderate megaloblastoid maturation and occasional erythroblasts with irregular nuclear contours
- Myeloid: Adequate maturation
- Myeloid Dysplasia: None
- Megakaryocytes: Adequate maturation
- Megakaryocytes Dysplasia: Mild

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Case 2: Cytogenetics

Clinical Indication: Anemia, leukopenia
Method: GTG
Cells Counted: 20
Band Level: 400
Cells Analyzed: 20
Cells Karyotyped: 2
RESULT: 46,XX[20]

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Case 2: Molecular

Cytopenia/MDS Mutation Panel

POSSIBLE MUTATION

Sequence alteration detected in the TET2 gene below that would result in a change in amino acid sequence. This alteration most likely represents an acquired/somatic mutation associated with a hematopoietic neoplasm or possibly a rare normal sequence polymorphism.

- TET2 L34F
- Heterozygous
- Somatic vs. SNP
- E03 c.100C>T

Case 2: Diagnosis

- BONE MARROW, SITE UNSPECIFIED, ASPIRATE SMEAR, TOUCH PREPARATION, AND CORE BIOPSY:
- NORMOCYTOPLASMIC MARROW (70%) WITH TRILINEAGE HEMATOPOIESIS, ERYTHROID AND MESENTERIC CYTOKINESIS, HYPERPLASIA, DYSERYTHROPOIESIS, AND NUMEROUS GRANULOMATOUS, SEE COMMENT.

- Comment: The TET2 sequence variant L34F has been identified previously in patients with myeloid malignancies (1,2). However, in each case, the variant appears to germline, suggesting that it is likely a SNP. Thus, there is no concrete evidence of a clonal hematopoietic neoplasm.

References

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Case 3

- 88 yo female with leukocytosis, anemia, and thrombocytopenia

Complete Blood Count:
- Hgb: 10.5.7 g/dL
- Platelet: 79 thousand/uL
- WBC: 16.2 thousand/uL, mild monocytosis
FISH confirms loss of CTNNA1 locus

OLIGO/SNP Array

Molecular Analysis

- ASXL1 frameshift/stop mutation (Y591*, 41% of reads)
- EZH2 point mutation (R690H, 85%)
- Two RUNX1 frameshift/stop mutations (c.474dupT, -19% and c.424_425ins11bp, -7%) and two
- TET2 frameshift/stop mutation (c.1510_1513delAAAA, 77% and R1465*, 10%).

Does a Mutation = a Neoplasm?

- Late in 2014, two large series reported that clonal hematopoiesis is extremely common in elderly populations and can be identified by sequencing.
- A high percentage of these have mutations in three genes commonly implicated in myeloid disease, DMNT3A, TET2, and ASXL1.
- Clonal hematopoiesis is a strong predictor of subsequent hematologic cancer and shortened survival.

References:
Clonal Hematopoiesis of Indeterminate Potential (CHIP)

- Absence of definitive morphologic evidence of a hematologic neoplasm
- Does not meet criteria for PNH, MGUS, or MBL
- Presence of a somatic mutation associated with hematologic neoplasm at an allele frequency of >2%
- Odds of progression to overt neoplasia are approximately 0.5-1%/yr

Myeloproliferative Neoplasms

Mutation Prevalence in Different Types of Myeloproliferative Neoplasm (MPN)

- CML: t(9;22) translocation, Philadelphia chromosome, BCR-ABL1 fusion
- Polycythemia vera: Essentially all have JAK2 mutations
- Essential thrombocythemia: 40-50% JAK2 V617F mutations, 5-10% MPL mutation
- Primary myelofibrosis: JAK2 V617F or MPL mutations in 40-50%
Testing to determine cause of resistance

Baseline

Level of disease

BCR-ABL1 RT-PCR

Diagnosis

Switch to another kinase inhibitor e.g., dasatinib or nilotinib

other KIs

SCT

CALR: A New Player on the MPN Landscape

- Highly localized mutations in calreticulin (CALR) occur in JAK2-unmutated ET and PMF
- Presented at ASH in December 2013 with simultaneous publication in NEJM by two groups
  - 151 pts (exome), 669 pts (FU): JAK2 neg patients: ET 71%, PMF 56%, not in PV, CML, CMML, AML, lymphoid or solid tumors
  - 1107 pts: 67% ET, 88% PMF, not in PV, 8% RARS-T

CALR: Clinical Correlation

- (largely) mutually-exclusive with MPL and JAK2
- ET: higher platelet counts, less thrombotic consequences
- PMF: less anemia, lower WBC, better outcome
- Independent prognostically of other mutations
- Mechanism involves JAK-STAT signaling
Mutational Landscape in Myeloproliferative Neoplasms

### Genetic Change

**Mutation**
- JAK2 V617F
- CALR exon 9/11 frameshift
- JAK2 exon 12
- MPL exon 10 (calreticulin 505/515)
- CSF3R exons 14, 17

**Translocations**
- BCR-ABL1
- Involving FGFR1
- Involving PDGFRα
- Involving PDGFRβ

### Presentation(s)
- PV, ET, PMF
- ET, PMF
- PV
- ET, PMF
- ET, PMF
- MPNs with granulocytosis
- MPNs with thrombocytosis
- MPN with eosinophilia
- MPN with eosinophilia

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MPNs: Primary Drivers and Secondary Drivers

**Chronic myelomonocytic leukemia**
- JAK2 ET
- JAK2 PMF
- JAK2 PV
- JAK2 ET/PMF

**Mixed MDS-MPN**
- MPN, unspecified

**Calreticulin, MPL**
- JAK2 ET/PMF

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Clonal Hierarchy in MPNs

- **JAK2, then TET2**
  - Younger
  - P.Vera >> ET or PMF
  - Increased risk of thrombosis
  - Increased sensitivity to JAK2 inhibitors
  - More erythroid and megakaryocytic progenitors

- **TET2, then JAK2**
  - Older
  - ET, PMF >> P.Vera
  - Increased expansion of progenitors and stem cells

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Algorithm for MPN

- Leukocytosis associated
  - JAK2 V617F

- Erythrocytosis and/or neutrophilic
  - JAK2 V617F

- Thrombocytosis
  - JAK2 V617F

- Myeloid metaplasia
  - JAK2 V617F

Screen for JAK2

- (+) JAK2 V617F
- (-) JAK2 V617F

EPO level

- Normal EPO
- ↓ EPO

CML

PV

Not PV

PMF

Bone marrow/cytogenetic study

- (+) JAK2 V617F
- (-) JAK2 V617F

Differentiation:

- Bone marrow/cytogenetic study
  - (+) JAK2 V617F
  - (-) JAK2 V617F

ET

CML

PMF

RARS-T

Differential Dx:

ET, CML, PMF, or RARS-T

Differential Dx:

PMF: BM fibrosis
CML: t(9;22)(q34;q11)
MDS/MPN: dysplasia
Carcinoma
Storage diseases

PV

Differential Dx:

CNL, CMML, MDS/MPN

Bone marrow/cytogenetic study

- ↑ monocytes: KRAS or NRAS
- ↑ eosinophilia: PDGFA, PDGFRB, FGFR1

Bone marrow/cytogenetic study

- ET: normal karyotype
- PMF: myelofibrosis
- RARS-T: iron stain positive for RS

Algorithm, internally developed

Summary

- A number of genes have now been identified that are mutation targets in myeloid malignancies
- There is an expanding number of mutations that are being integrated with cytogenetic results to create a more layered risk stratification profile
- Many of the mutations are being identified in “actionable” targets/pathways that will lead to not only prognostic implications but therapeutic ones as well

Questions Remaining To Be Answered

- What role does clonal hierarchy play in the prognostic significance of mutations?
- Not all mutations within a gene are equal
- Biological interaction between mutations not fully understood
- How to integrate molecular mutations into clinical risk models