Update of WHO and Molecular Classifications in Myelodysplastic syndromes

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Pivotal role of morphology in diagnosis and prognostication of MDS

WHO classification of MDS

• Refractory Cytopenia with **Unilineage Dysplasia (RCUD)**
  
  (*mainly refractory anemia*)

• Refractory Anemia with **Ring Sideroblasts (RARS)**

• Refractory Cytopenia with **Multilineage Dysplasia (RCMD)**

• Refractory Anemia with **Excess Blasts (RAEB type I and II)**

• Myelodysplastic Syndrome with Isolated **del(5q)**

*Swerdlow et al (Editors). IARC 2008*
Overall survival according to erythroid, granulocytic and megakaryocytic morphological score value

Della Porta et al. 2014 May 20. doi: 10.1038/leu.2014.161
Prognostic relevance of dysplasia: number of lineages involved

1 dysplastic lineage (mainly erythroid)

2 dysplastic lineages

3 dysplastic lineages

Della Porta et al. 2014 May 20. doi: 10.1038/leu.2014.161
Outcome of MDS according to WHO classification

MDS with isolate del(5q): distinct nosologic entity caused by haploinsufficiency of genes mapping on the deleted region

Loss of a micro RNA and thrombocytosis

Coordinate loss of a microRNA and protein-coding gene cooperate in the pathogenesis of 5q- syndrome

Activation of p53 and apoptosis of immature red cells
Pellagatti et al. Blood. 2010 Apr 1;115(13):2721-3
Dutt et al. Blood. 2011 Mar 3;117(9):2567-76

Haploinsufficiency of RPS14 phenocopies the disease in normal hematopoietic progenitor cells

Lenalidomide induces ubiquitination and degradation of CSNK1A1 in MDS with del(5q)

- Lenalidomide induces the ubiquitination and consequent degradation of CSNK1A1.
- del(5q) cells have only one copy of *CSNK1A1*, so they are selectively depleted over wild-type cells.
MDS prognostic scoring systems

• **WPSS**
  – WHO classification (ring sideroblasts, multilineage dysplasia, excess blasts)
  – IPSS cytogenetics
  – severity of anemia (transfusion requirement)

• **IPSS-R**
  – degree of cytopenia (Hb, ANC, PLT)
  – excess blasts (≤2%, 3-4%, 5-10%, >10%)
  – revised cytogenetics
A study of the International Working Group for Prognosis in Myelodysplasia (IWG-PM) on 5326 untreated MDS patients

Della Porta et al. 2014, unpublished results
Somatic gene mutations in patients with MDS

Genes mutated in ≥10% of MDS pts: SF3B1, TET2, SRSF2, ASXL1, DNMT3A, RUNX1

Most patients have somatic mutations of RNA splicing and/or DNA methylation

17/20 most frequently mutated genes are common to both studies (Papaemmanuil et al & Haferlach et al)
The blood cells of individuals with solid tumors contain mutations that may represent premalignant events that cause clonal hematopoietic expansion.

The Cancer Genome Atlas (TCGA)

Genetic basis of myelodysplastic syndromes

1. Occurrence of a founding driver mutation in an immature hematopoietic stem cell with capacity for self-renewal: the mutation, typically involving a gene of RNA splicing or DNA methylation, provides selective advantage and determines local clonal expansion.

2. Because of their clonal advantage, mutated hematopoietic stem cells actively follow migratory pathways and progressively settle in other bone marrow districts, and eventually achieve full clonal dominance in the body. Clonal cells carry also hundreds of background or passenger mutations that have been captured at the start of clonal proliferation. At the end, nearly all bone marrow cells are clonally derived and carry the founding driver mutation plus additional passenger mutations.

3. The development of clinically apparent disease may or may not require cooperating mutations, and is primarily caused by abnormal differentiation/maturation of clonal hematopoietic progenitors and precursors. Skewed proliferation and/or defective maturation and/or excessive apoptosis most often lead to dysplasia, ineffective hematopoiesis and peripheral blood cytopenia, but leukocytosis or thrombocytosis may be observed with some mutant driver genes.

4. The acquisition by clonal cells of subclonal driver mutations, typically involving genes of chromatin modification or transcription regulation or signal transduction, involves the formation of subclones of hematopoietic cells with further impaired differentiation/maturation capacity. As a result, the proportion of blast cells progressively increases until the development of overt AML. This condition is not monoclonal in the strict sense, but is instead a mosaic of clones/generomes characterized by different sets of somatic mutations (concept of intraclonal or intratumoral heterogeneity).

Frequencies and distribution of spliceosome pathway gene mutations in myeloid neoplasms

The SF3B1 protein is a core component of the U2 snRNP, which recognizes the 3′ splice site at intron–exon junctions.

*Yoshida et al. Nature. 2011 Sep 11;478(7367):64-9*
Precursor mRNA (pre-mRNA) splicing

Precursor mRNA

RNA splicing process

mRNA

Major U2-dependent spliceosome (snRNPs: U1, U2, U4, U5, and U6)

Donor site

Branch site

Acceptor site

5' Exon GU AG 3' Exon

U2-type intron

Minor U12-dependent spliceosome (snRNPs: U11, U12, U4atac, U5, and U6atac)

Donor site

Branch site

Acceptor site

5' Exon AU/GT AG/AC 3' Exon

U12-type intron

Co-transcriptional RNA splicing and potential outcomes of mutations of genes encoding proteins of the spliceosome

Relationship between somatic SF3B1 mutations and ring sideroblasts

Quantitative enumeration of ring sideroblasts:
- 325 MDS patients

101 (31%) patients with mutation in SF3B1

91 patients >15% ring sideroblasts,
7 patients 1-14%,
3 patients no ring sideroblasts

SF3B1 mutation: positive predictive value for ring sideroblasts 97.7%

Absence of ring sideroblasts: negative predictive value for SF3B1 mutation 97.8%

Relationship between the occurrence of a somatic SF3B1 mutation and the formation of ring sideroblasts in patients with RARS
Comprehensive analysis of aberrant RNA splicing in myelodysplastic syndromes

• RNA sequencing of CD34+ cells revealed 230 splicing events significantly enriched in SF3B1-mutated cases, of which 206 (90%) were caused by misrecognition of 3' splice sites.

• About 50% of these altered 3' splice sites resulted in frameshift, indicating that SF3B1 mutations cause deleterious effects in many genes simultaneously.

• Altered splice sites were found in genes involved in heme biosynthesis, cell cycle progression, and DNA repair.
Novel disease paradigm

Occurrence of SF3B1 mutation in a multipotent hematopoietic stem cell

Misrecognition of 3' splice sites and frameshift in hundreds of genes

Mutation detectable by DNA sequencing

Mutations detectable only by RNA seq

Gain of function at hematopoietic stem cell level

Loss of function at hematopoietic precursor level

Stem cells

Hematopoietic precursors

Ineffective erythropoiesis

Shiozawa et al. ASH 2014, abstract #826
The ability TGF-β superfamily ligand-trapping proteins to alleviate anemia with ineffective erythropoiesis in a mouse model of MDS


Clinical significance of SF3B1 mutation in MDS

RNA splicing factors: SF3B1, SRSF2 and U2AF1

Clinical effect of spliceosome pathway gene mutations in myelodysplastic syndromes

Clinical effect of spliceosome pathway gene mutations in MDS with ring sideroblasts

SF3B1-mutant MDS as a distinct nosologic entity

*Malcovati et al. ASH 2014, abstract #826*
Genetic “predestination”: early founding driver mutations shape the future trajectories of clonal evolution of a cancer through constraints on the repertoire of cooperating subclonal genetic lesions.

Transition from RARS to RARS-T

Genetic “predestination”: early founding driver mutations shape the future trajectories of clonal evolution of a cancer through constraints on the repertoire of cooperating subclonal genetic lesions.
Unsupervised hierarchical clustering analysis of MDS patients

Cluster 1 (SF3B1 mut)
Cluster 2 (NOS)
Cluster 3 (MD)
Cluster 4 (EB)

Overall survival of MDS patients stratified according to genotype and blast excess

P < .001

Progression to AML in MDS patients stratified according to genotype and blast excess

Co-occurrence of *TET2* and *SRSF2* (or *ZRSR2*) mutations is highly specific for myelomonocytic phenotype

**CMML: Monocyte count ≥ 1.5 x 10⁹/L or TET2/SRSF2 co-mutation?**

*Malcovati et al. Blood. 2014 Aug 28;124(9):1513-21*
Somatic mutations of ASXL1, RUNX1 and SETBP1 improve prognostic stratification of CMML

- **TET2** (44%), **SRSF2** (43%), **ASXL1** (34%), **KRAS** (11%), **NRAS** (10%), **CUX1** (10%), **CBL** (9%), **RUNX1** (7%), **SETBP1** (7%), **JAK2** (6%), **SF3B1** (6%), and **U2AF1** (5%)

- Lasso Cox regression model for genetic variable selection. The statistically significant variables were CPSS-specific cytogenetic risk groups (HR=2.49, P=.001), mutations in **ASXL1** (HR=2.77, P=.018), **RUNX1** (HR=5.39, P=.009) and **SETBP1** (HR=3.96, P=.013).

- **CPSS-Mol** performed better than the original CPSS cytogenetic risk classification

_Elena et al. ASH 2014, abstract #1915_
Conclusions

• The identification of somatic mutations of RNA splicing machinery has provided a paradigm shift

• Already established genotype/phenotype relationships include
  – *SF3B1*-mutant MDS
  – *TET2/SRSF2*-comutant MDS/MPN (CMML)

• The time has come for us to develop a genotype-based (molecular) classification of MDS
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