Molecular Pathology
Prognostic and Diagnostic Genomic Markers for Myeloid Neoplasms

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KEYWORDS

• Next-generation sequencing • Myeloid neoplasms • Genomic alterations • Mutations
• Copy number variation

Key points

- The same genetic alterations are shared among diverse types of myeloid neoplasms.
- Complex combination of genetic alterations is the rule, not the exception.
- Genetic alterations in the same pathway tend to be mutually exclusive.
- Clonal heterogeneity is a way of life for myeloid neoplasms.
- When multiple alterations are present, temporal sequence of acquisition of genetic alterations varies among different individuals and may have prognostic significance.

ABSTRACT

Application of next-generation sequencing (NGS) on myeloid neoplasms has expanded our knowledge of genomic alterations in this group of diseases. Genomic alterations in myeloid neoplasms are complex, heterogeneous, and not specific to a disease entity. NGS-based panel testing of myeloid neoplasms can complement existing diagnostic modalities and is gaining acceptance in the clinics and diagnostic laboratories. Prospective, randomized trials to evaluate the prognostic significance of genomic markers in myeloid neoplasms are under way in academic medical centers.

OVERVIEW

The term myeloid neoplasm refers to a group of neoplastic diseases that include acute myeloid leukemia (AML) and its frequent predecessors, myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN). Myeloid neoplasms originate from the hematopoietic stem/progenitor cells (HSCs).[1] Multistep accumulation of genetic alterations in HSCs progressively confer growth advantages to certain clones and lead to a state of "clonal hematopoiesis" that can either exist transiently or last for many years. Further acquisition of "driver" type of genetic alterations, coupled with epigenetic and environmental changes, eventually enables the uncontrolled outgrowth of cells with reduced capacity to differentiate into more mature hematopoietic elements.[2]

KNOWLEDGE DERIVED FROM NEXT-GENERATION SEQUENCING TESTING OF MYELOID NEOPLASMS

With the advance in next-generation sequencing (NGS)[3] and its application to analyzing patient samples, our understanding of the genetic

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alterations of myeloid neoplasms has expanded significantly in the past few years.[4,5] The diagnosis and classification of myeloid neoplasms is likely to evolve over the next few years when this new information is incorporated into the classification scheme. Although the impact of genetic alterations on diagnosis, treatment, and prognosis is still in its early stages, the themes discussed in the following sections have emerged from the vast amount of new information gained so far.

THE SAME GENETIC ALTERATIONS ARE SHARED AMONG DIVERSE TYPES OF MYELOID NEOPLASMS

For example, when it was first described, *JAK2* V617F was once thought to be a specific marker for MPN.[6] Since then, *JAK2* V617F has been detected in almost every type of myeloid neoplasm, including most patients with polycythemia vera (PCV), a significant portion of patients (30%–70%) with essential thrombocythemia (ET), myelofibrosis (PMF), and refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T), and a small percentage (<10%) in almost all types of myeloid neoplasms.[7–9] Moreover, a small percentage of patients with AML with no prior history of MPN have a *JAK2* V617F mutation and *JAK2* V617F is a frequent finding among older individuals with normal complete blood count, a phenomenon called "age-related clonal hematopoiesis" (ARCH).[10,11] Similar findings apply to almost all other frequently mutated genes in myeloid neoplasms. No one gene is specific to a diagnostic entity and no gene is mutually exclusive with a specific diagnosis. The strongest genotype-phenotype associations (**Table 1**) are seen between splicing factor 3b subunit 1 (*SF3B1*) mutations and the presence of ring sideroblasts and *KIT* D816V with mastocytosis,[12,13] but again, *SF3B1* and *KIT* mutations are also seen in many other types of myeloid neoplasms (**Fig. 1**).

COMPLEX COMBINATION OF GENETIC ALTERATIONS IS THE RULE, NOT THE EXCEPTION

Early in the disease course, patients may present with one or a few mutations but frequently acquire more mutations as the disease progresses. Total number of mutations present in a patient may be indicative of the duration and severity of the disease. Specific co-mutation patterns, either in the form of content or temporal sequence, have not been consistently described across specific entities, with a few notable exceptions.

Table 1
Phenotype-genotype associations between genomic alterations and myeloid neoplasms

Gene	Disease/Phenotype
JAK2 V617F and exon 12 deletions	PV, ET, and PMF
CALR exon 9 frameshift	PV, ET, and PMF
MPL W515 missense	PV, ET, and PMF
JAK2 + *SF3B1*	RARS-T
SF3B1	Ring sideroblasts/RARS
RUNX1	Thrombocytopenia
TET2 + *SRSF2*	Monocytosis
U2AF1	Male predominance
TP53	5q-, 7q-, complex karyotype
CSF3R	Chronic neutrophilic leukemia
KIT D816V	Mastocytosis

Abbreviations: ET, essential thrombocytopenia; PMF, primary myelofibrosis; PV, polycythemia Vera; RARS, refractory anemia with ring sideroblast; RARS-T, refractory anemia with ring sideroblasts associated with thrombocytosis.

Co-mutation of *TET2* and *SRSF2*, for example, has emerged as the most common pair of alterations for chronic myelomonocytic leukemia (CMML)[14,15] and *JAK2* and *SF3B1* combination is seen in more than 50% of patients with RARS-T.[8] Concurrent *DNMT3A*, *NPM1* mutation and *FLT3*-ITD is a common finding in de novo AML.[16] However, even when these co-mutations are present, they are rarely the only changes in a patient. One notable exception is in patients with tumor protein p53 (*TP53*) mutations who generally have relatively few co-mutated genes. The genomic instability associated with *TP53* loss may be sufficient to drive leukemogenesis (**Fig. 2**).

GENETIC ALTERATIONS IN THE SAME PATHWAY TEND TO BE MUTUALLY EXCLUSIVE

Patients with mutations in one splicing factor gene (*SF3B1*, *SRSF2*, *ZRSR2*, *U2AF1*) rarely acquire mutations in other splicing factors and patients with isocitrate dehydrogenase 1 (*IDH1*) mutation typically lack *IDH2* mutation.[17–20] When mutations in the same pathways are observed in the same patient, it is more likely that they are present in different subclones rather in the same cells (see the following section). Presumably, tumoral evolution does not select for mutations in other genes within shared pathways, as they do not offer additional growth or survival advantage.