MPN Driver Genes and the Importance of Molecular Genetic Testing

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Learning Objective

Describe underlying genetic alterations that can affect a patient's diagnosis and direct individual management strategies

Introduction

Mutational testing is an integral part of the management of myeloproliferative neoplasms (MPNs), as results from this assessment can both guide treatment selection and provide prognostic information. In this article, Dr. Aaron Gerds discusses best practice regarding the utility of mutational testing in clinical practice when treating patients with MPNs.

Is molecular genetic testing for mutations the gold standard in the diagnosis of a myeloproliferative neoplasm (MPN)? What do the guidelines say?

Mutation testing is a key part of diagnosing MPN and is particularly helpful for ruling in MPN. Take the example of a patient with a mild thrombocytosis, for whom there may or may not be a reactive reason, in whom the iron levels are borderline deficient. If molecular testing identifies a *JAK2* V617F mutation, then you know for certain that it is an MPN. Molecular testing may not be as useful in ruling out potential diagnoses based on test parameters as patients may have triple-negative disease, but it is useful for ruling in MPN.

Certainly, if you see a classic MPN driver mutation, then an MPN is very likely. In the pattern of the diagnostic classification system,[1] it is helpful as a clinician to test your differential and narrow in on your diagnosis.

When do the guidelines specifically state to do molecular testing, and by which method?

Current guidelines state specifically to test for the driver mutations, and by the method that is available at your center.[2] Many centers do not have access to extensive molecular panels that test 30, 40, 50, or 120 genes. You may only be able to do very targeted testing, specifically for the three driver mutations—*JAK2*, *MPL*, and *CALR*. The guidelines do provide some flexibility and recommend to test

for the driver mutations at a minimum. However, if you have access to a broader panel that assesses for multiple mutations, then that is preferred.

In addition to availability, the choice of testing method may depend on insurance coverage. In certain areas, insurers will not pay for large panels, whereas in other areas, they will provide coverage. Similarly, some centers have philanthropy funds or different plans brokered with the lab for panel testing, whereas other centers may not.

What is the prognostic significance of the mutational profile?

We certainly want to identify the driver mutation. For example, some reports suggest that patients with primary myelofibrosis who lack one of the three classic driver mutations, the so-called "triple-negative" patients, have an adverse prognosis compared to patients who do.[3-6] As an additional example, patients with essential thrombocythemia due to *CALR* mutations have a different thrombosis risk than those who have *JAK2* V617F mutations.[7] Moreover, if one of the classic three driver mutations in Philadelphia chromosome-negative MPN is not identified, you may want to consider an alternate diagnosis, such as chronic myelomonocytic leukemia, myelodysplastic syndrome/MPN overlap syndromes, or an entirely different diagnosis.

While identifying driver mutations has prognostic value, these diseases are not "one-hit-wonders," in that multiple mutations generally occur within these malignant cells. For example, approximately one-fourth of patients with essential thrombocythemia (ET) have a second or third mutation, and about 35% of patients with polycythemia (PV) and ET have a second, third, or fourth mutation.[8,9]

What would constitute high-risk MPN based on the genetic testing?

Even in this amazing technological era, in which genomic information to characterize MPNs is robust and mutation testing on patient samples is readily available, we still rely heavily on clinical variables. Although the Mutation-Enhanced International Prognostic System (MIPSS70) does include mutation testing for high-risk mutations, the majority of the prognostic scale is dependent on clinical variables: the age of the patient, the blood count, the amount of fibrosis in the marrow, and history of thrombosis.[10] That is, the mutation testing adds to our ability to prognosticate, but it does not definitively confirm a diagnosis of high-risk MPN. You must look at the entire clinical picture, in addition to the mutation.

A group of mutations across the MPNs confer high risk, regardless of the subtype. These include mutations in *IDH2*, *EZH2*, *TP53* (which are generally associated with worse outcomes in all cancers), and *U2AF1*.[11-13] In addition, splices on mutations such as *SF3B1* and *SH2B3* are associated with an unfavorable prognosis, particularly in ET.[12,14] In both PV and myelofibrosis, a mutation in *ASXL1* has been shown to predict poor outcome, as has an *SRSF2* mutation.[12] In some studies, *CBL* mutation has shown to be an adverse prognostic marker.

Generally, these additional mutations are identified through multigene panels, ie, next-generation sequencing (NGS). Even the basic 20- or 30-gene panels will often include these genes because they represent recurrent mutations not only in MPNs, but also in other myeloid disorders, such as

myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Laboratories will likely have a myeloid panel, which will contain genes that have recurrent mutations in myeloid disorders, and a lymphoid panel, which assess for a slightly different set of genes that have recurrent mutations in lymphoid disorders, such as acute lymphoblastic leukemia (ALL).

Are there other reasons to identify nondriver mutations? Does it have a role in the selection of therapy or intensifying therapy?

There are a couple of reasons to assess for nondriver mutations, and as mentioned, the main one is prognostic value. Secondly, in cases of triple-negative MPN, in which a *JAK2*, *CALR*, or *MPL* mutation is not present, it will help establish clonality. If you suspect an MPN, or at least a myeloid malignancy, finding a mutation in *ASXL1* or *TET2* on a multigene panel will help establish clonality. In this case, you could narrow the diagnosis to a myeloid malignancy versus a reactive or secondary process.

Not only is identifying nondriver mutations helpful for prognosis and diagnosis, but we are starting to branch out into therapeutics—using the molecular data to target an individual's disease, ie, precision medicine. Clinical trials are now examining the utility of targeted agents for treating these less frequent mutations. Perhaps what is furthest along is the MPN-RC 119 study, assessing the effect of targeting the *IDH2* pathway in MPN. Only about 5% of patients with an MPN will have a mutation in either *IDH1* or *IDH2*, but isocitrate dehydrogenase (IDH) inhibitors are already available, and we are looking to incorporate them into treatment.[15,16]

As of now, there is no evidence that mutation analysis is driving therapy intensity, outside of selecting some patients for allogeneic hematopoietic cell transplantation. For patients who have a high-risk molecular type, are young and fit, and have been on a Janus kinase (JAK) inhibitor for a while, you may consider transplantation sooner rather than later if their disease appears to be progressing.

Approximately how many patients won't have a driver mutation, and what do you do with those patients?

Not very many. Few patients with MPN have no driver mutations. In patients with ET, somewhere in the neighborhood of 15% or fewer will not have a driver mutation, or any mutation at all.[5] In PV, it's 5% or less, and in myelofibrosis, 10% or less. The management of patients with MPN with no clonality is challenging. In those cases in which an MPN is suspected without identifying a driver mutation, we perform the entire work-up, including a bone marrow biopsy. At that point, if the mutation analysis comes back with no clonality at all, I will reassess whether this truly is a primary bone marrow process or a secondary issue. Is there evidence of a rheumatologic disorder or chronic infection that might be driving the clinical picture? If it is not evident, you rely on the rest of the diagnostic criteria.

In looking closely at the World Health Organization (WHO) diagnostic criteria, although clonality helps close the case very succinctly, the criteria allow for not identifying clonality and still approach the disease as a particular MPN to institute appropriate management.

How is the risk of thrombosis affected by the driver gene identified? Is one particularly worse than another?

In myelofibrosis, the overall rates of thrombosis are not as high as in other MPNs. In PV, almost all patients have a *JAK2* mutation, particularly *JAK2* V617F, so it is difficult to know the risk that other mutations impart. The interesting case is ET, in which roughly half of the patients will have a *JAK2* V617F mutation and upwards of one-third will have a mutation in *CALR*. A higher risk of thrombosis is associated with having a *JAK2* V617F mutation versus all others.[17,18] The reason for this is unclear. Some studies have shown that the *JAK2* mutation can be detected inside of endothelial cells of patients with MPN and promotes the release of endothelial P-selectin.[19,20] Others have looked at various thrombotic factors, such as tissue factor.[21] A few have examined platelet microparticles as the reason that patients with *JAK2* V617F mutations are more likely to have blood clots.[22] This is an active area of exploration in MPN.

The revised International Prognostic Score for Thrombosis in Essential Thrombocythemia (IPSET), used to stratify risk in patients with ET, identifies the presence of a *JAK2* V617F mutation as a high-risk feature for thrombosis.[23]

Outside of a clinical trial, what about measuring mutant allele burdens in a patient at treatment?

This question comes up often in clinic. It's a controversial area, even within the realm of MPN experts and people who dedicate their careers to learning more about these diseases and treating patients with these diseases. Reduction of mutant allele burden is an exciting prospect: the thought that if we can reduce the amount of clone, or the amount of disease in a patient, we are somehow setting the clock back on their disease. The truth of the matter is we do not know this for sure. No study has been able to associate reduction in the mutant allele burden with a tangible clinical outcome such as progression-free survival or overall survival, so no conclusive evidence exists that reducing the mutant allele burden of the driver mutation will have an impact on other measurable clinical outcomes. Therefore, its role in everyday clinical practice is very limited.

Reduction in mutant allele burden is often a key outcome in clinical trials, but quite frankly, this is because it is one way for us to measure disease modification. Until we develop a better marker of disease modification, we will continue to look at it.

What has been learned from molecular testing about the genes involved in familial myeloid malignancies?

Familial myeloid malignancies were recognized as a separate disease entity in the revised WHO classification. There are clear instances of familial MPN, or at least a familial MPN disposition syndrome.

As we learn more about recurring mutations in these diseases, we recognize patterns. We know of *RUNX1*, as one example, as a mutation, or at least a polymorphism, that can be inherited in families and can increase the risk for myeloid disorders.[24] In some cases, in obtaining a multigene panel, you will identify cases of familial disposition syndrome, which could be important for patients and their family.

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