What are the practical issues in FLT3 testing as it relates to PCR vs. NGS?

Alexander Perl, MD
Associate Professor of Medicine
Abramson Cancer Center
University of Pennsylvania
Philadelphia, Pennsylvania

Welcome to *Managing AML*, I am Dr. Alexander Perl. I would like to briefly review some of the practical issues in FLT3 testing as it relates to polymerase chain reaction (PCR) versus next-generation sequencing (NGS). Now ideally both of these tests are sent from patients at an initial diagnosis of AML, and it is important both for diagnostic and prognostic information to detect a FLT3 mutation, because this can actually guide therapy. In particular, in patients with a FLT3 mutation, we now have data that adding a drug to standard induction improves survival. That drug is called midostaurin and it is an inhibitor of FLT3 kinase among other kinases; it is a multi-kinase inhibitor. We know that patients who were treated on the study that showed this benefit in overall survival were detected by a PCR reaction. The advantage of the PCR is that it comes back quickly. The PCR can turn around in just a matter of a few days whereas next-generation sequencing can sometimes take several weeks to get a result back. The sensitivity of PCR, at least for the most common mutations in FLT3, seems to be better than next-generation sequencing as well. The reason is that larger internal tandem duplication mutations can be missed by next-generation sequencing, or it may be hard to actually map within the gene such that there could be discrepancies between the two tests. Both for the question of getting the right diagnosis and directing therapy, and also making sure you have not missed the presence of an important mutation, it is important that PCR is sent.

One other thing that is important in terms of that turnaround time is the study that showed the benefit of midostaurin, added the midostaurin on day 8 which, as I have mentioned, is important to get a result prior to that time to know to add the drug. If it takes three weeks to get a mutation test back by next-generation sequencing, the midostaurin was only given for two weeks and suddenly you are outside the window where we know it works. For that reason, among others, it is important to actually send both tests at diagnosis. There are some advantages of next-generation sequencing in that you will actually get a fingerprint of the FLT3 mutations, in particular for the internal tandem duplications or ITDs, and that can give you additional information about perhaps how many clones are present or possibly the ability to pick up very low levels of disease. In reality, for diagnostic purposes, largely we prefer using PCR to detect the FLT3-ITD. For the detection of other FLT3 mutations, the two tests are fairly interchangeable, though I would point out that certain mutations in FLT3 can be missed by PCR and still picked up by next-generation sequencing, especially if there is a mutation that is a point mutation somewhere other than the identified hotspot in the activation loop at D835. This being said, the prognostic importance of those other mutations is a little less established. The most important ones for guiding therapy we think are the internal tandem duplications – which have a negative prognostic effect in AML – and also the TKD mutations at D835, which we know respond to midostaurin. Thank you for listening.