ADVANCES IN LLM

Current Developments in the Management of Leukemia, Lymphoma, and Myeloma

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Understanding and Utilizing New Molecular Genetics in MDS

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H&O How are myelodysplastic syndromes (MDS) characterized?

MF MDS are a heterogeneous group of clonal disorders of the hematopoietic system characterized by the presence of a hypercellular bone marrow with dysplastic changes, peripheral cytopenias, and an increased risk of malignant transformation to acute leukemia. These patients have anemia, leukopenia, and/or thrombocytopenia despite their hypercellular bone marrows. In other words, this is a disease of ineffective hematopoiesis, where these increased cell numbers in the bone marrow are not translated into an increased cellularity in the peripheral blood. Eventually, however, through mechanisms that are not yet fully understood, there is a change in the phenotype and the malignant cells gain an advantage and become a proliferative clone that takes over the bone marrow, invades the peripheral blood, and transforms into acute myeloid leukemia (AML).

H&O Why have the molecular mechanisms behind MDS been difficult to identify?

MF A large part of the problem is due to the heterogeneous nature of MDS, both from the clinical and molecular standpoints. Simply making a diagnosis was very difficult for a long time. The unifying characteristic underlying the diagnosis of these disorders was the presence of dysplastic changes, so a big portion of the diagnosis relied on morphologic examination of the bone marrow. However, beyond those dysplastic changes (which require a trained hematopathologist to identify), and the presence of peripheral cytopenias, there was no gold standard diagnostic test that could clearly differentiate a bone marrow MDS from a chronic anemia or chronic disorder.

Along with the clinical and morphologic heterogeneity, another diagnostic hurdle was establishing the clonality of the disorder. A major problem behind this was the low resolution of the methods that were used for a long time, such as the cytogenetic studies we all relied upon. By performing a karyotypic study of the bone marrow, certain chromosomal abnormalities that frequently accompany the diagnosis of MDS could be identified. For instance, the presence of abnormalities in the long arm of chromosome 5 (5q), chromosome 7, or chromosome 20 is suggestive of MDS. For many years, researchers pursued the study of genes affecting these chromosomes as potential causes for the disease. However, not all patients present with abnormalities in these chromosomes and up to 40% of patients present at diagnosis with a normal cytogenetic study, indicating that more than just those genetic abnormalities must be responsible for the development of this disease. Pinpointing the exact mechanism has been very elusive, and while a lot of progress has been made in recent years, to date we do not fully understand the mechanisms behind the development of MDS. It is very unlikely that just one mechanism will explain all forms of the disease.

H&O How has the understanding of the molecular mechanisms behind the development of MDS evolved in recent years?

MF The advent of new technologies in the last decade or so has really improved our understanding of MDS by helping to identify new molecular abnormalities. In

the past, genetic studies of MDS were conducted using a microscope. The use of next-generation sequencing as well as high-density microarrays has allowed us to look into the genome of patients with MDS at base-pair resolution. Through the efforts of many groups, we have identified the presence of recurrent molecular abnormalities that were previously unrecognized. The amount of progress that has been made in the last 3–5 years is almost unbelievable and very encouraging.

H&O What have new technologies uncovered?

MF These technologies have allowed for the discovery of recurrent genetic abnormalities which were not previously known to affect this disease. We could say that 2 types of new mutations have been described. The first group of novel mutations consists of those that target the epigenetic machinery, and they may explain the profoundly aberrant epigenetic profiles that had been previously noted in this disease. The epigenetic machinery of a cell is a complex group of proteins that help to determine the transcriptional program of the cell by making certain genes available to the transcriptional machinery while shutting down other parts of the genome. In the case of mutations affecting epigenetic modifying enzymes, we are finding either loss of function or a change in normal functioning of these proteins, thus affecting the normal regulation of the transcriptional program of the cell. The second group of mutations recently described consists of mutations targeting the mRNA splicing machinery, some of which may be responsible for specific forms of MDS. Through the use of these new technologies, we now know that approximately 80% of patients with MDS have abnormalities in their genome at diagnosis.

H&O What are the potential advantages of implementing comprehensive molecular analysis of these mutations into clinical practice?

MF When we gain a better understanding of how a disease develops, we can start prognosticating and treating it more effectively. These 2 components of the discovery have different latencies in coming about, largely because developing new therapies takes longer. This newly acquired knowledge is going to change how we diagnose, how we approach our patients in terms of establishing their prognosis, and how we eventually treat these patients in the coming years. It would not be surprising to see a lot of differences in how we risk stratify and treat patients in the next 5–10 years. In fact, there have already been some studies showing the clinical implications of these mutations. Two large studies led by Dr. Benjamin Ebert were published within the last 2 years in the *New England Journal of Medicine* and the *Journal of Clinical Oncology*. Since some of these mutations are not very common, the initial reports from small groups reporting on 30 or 50 patients made it difficult to interpret the actual clinical implications of these mutations. Large studies are therefore key to truly understanding how these mutations affect the behavior of the disease. Such studies help to indicate how we should respond as physicians with the appropriate therapy, whether we need to be more aggressive or less aggressive. Based on these studies, it is becoming clear that there are certain mutations that have a negative impact on patients. Two of these novel mutations that are emerging as negative risk factors are *EZH2* and *ASXL1*. Such studies still require further validation in independent cohorts. However, even in the multivariate analysis of these first 2 studies, these 2 mutations hold true as negative risk factors, particularly in what are known as the low and the intermediate-1 risk groups of MDS. This is beginning to tell us that at least some of these so-called low-risk patients may not in fact be low risk at all, and we might need to consider treating them differently. However, as I mentioned before, this finding still requires independent validation before we can apply it in the clinic. There have also been a few reports suggesting that the presence or absence of certain mutations may have either a positive or negative impact on the response to novel therapies, such as DNA methyltransferase inhibitors. Again, these are isolated reports on small cohorts that will require independent validation. However, if in the near future we design appropriate trials to test this, we will be able to validate the impact of these mutations, and hopefully make positive changes in the way we treat our patients.

H&O What are the limitations preventing the use of such gene sequencing into clinical practice? What efforts are being made?

MF The current limitations preventing the implementation of sequencing of these genes into real-time clinical practice include the high cost, slow turnaround time, and lack of clinical validation. Some mutations do not affect conserved residues and can therefore be all over the gene. For example, abnormalities in *TET2* can target almost any region within exons 4–12 of the gene, all of them resulting in impaired *TET2* function. Given this complexity, it is very difficult to set up an assay for *TET2* abnormalities that is reproducible, approved by the Clinical Laboratory Improvement Amendments (CLIA), and can be performed in a standard diagnostic lab. With mutations that affect conserved residues, one could design a limited polymerase chain reaction (PCR) or another reaction that will capture the presence of that mutation, and those are

therefore easier to incorporate into the diagnostic setting. Efforts to limit the sequencing to panels of target genes that are recurrently affected in MDS may improve all of these limitations. These technologies have yet to be fully CLIA-approved and incorporated into diagnostic and pathology labs, but that will very likely change in the near future. As these technologies become more developed and utilized, I believe there will be clinical trials in place, which will hopefully offer guidance on how to respond to mutations that are present within each patient.

H&O What are the most promising areas of research?

MF This past American Society of Hematology (ASH) meeting offered a glimpse into the beginning stages of developing targeted therapies for these new mutations. There were several groups that reported ways in which to specifically target either epigenetic or RNA splicing mutations. What we would like to see in the future is a tailored therapy where we can treat patients based on their epigenetic and cytogenetic profile. There is also room for research in understanding how these mutations lead to the development of MDS, which will allow us to target these mutations therapeutically and hopefully impact the outcome of patients in a positive way.

Suggested Readings

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