

Molecular Testing in Hematologic Malignancies

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Presenter's disclosure of conflicts of interest is found at the end of this article.

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Abstract

Molecular testing technologies have been increasingly integrated into clinical research and are essential tools in the care of patients with hematologic malignancies. During this session at JADPRO Live Virtual 2021, Laura J. Zitella, MS, RN, ACNP-BC, AOCN®, reviewed key concepts for diagnostic testing with a focus on molecular testing (including cytogenetics, FISH, PCR, and next-generation sequencing) and discussed how to interpret the results for hematologic malignancies to inform prognosis as well as treatment.

As molecular testing technologies are increasingly integrated into clinical research and patient care, they have become essential tools for the treatment of hematologic malignancies. According to Laura J. Zitella, MS, RN, ACNP-BC, AOCN®, nurse practitioner and Associate Clinical Professor at the University of California San Francisco, advances in the understanding of the molecular basis of hematologic malignancies has translated directly into improved diagnostic methods, prognostic tools, and therapeutics.

During JADPRO Live Virtual 2021, Ms. Zitella shared key concepts of diagnostic testing for hematologic malignancies, including immunohistochemistry, flow cytometry, cytogenetics, fluorescence in situ hybridization (FISH), molecular diagnostics/polymerase chain reac-

tion (PCR), and next-generation sequencing (Figure 1).

MILESTONES IN UNDERSTANDING OF GENES

As Ms. Zitella explained, there have been extraordinary advancements in the understanding of genes over the past century: the first gene map of the fruit fly in 1911, the discovery of the double helix structure of DNA by Watson and Crick in 1953, the development of DNA sequencing techniques by Frederick Sanger in the 1970s, and the completion of the Human Genome Project in 2003.

Over the past two decades, the cost of sequencing one human genome has also decreased dramatically. In 2003, it was estimated to cost \$53 million to generate a single human genome. By 2006, the cost had fallen to \$12 million per genome, and by 2020, the cost was less than \$700.

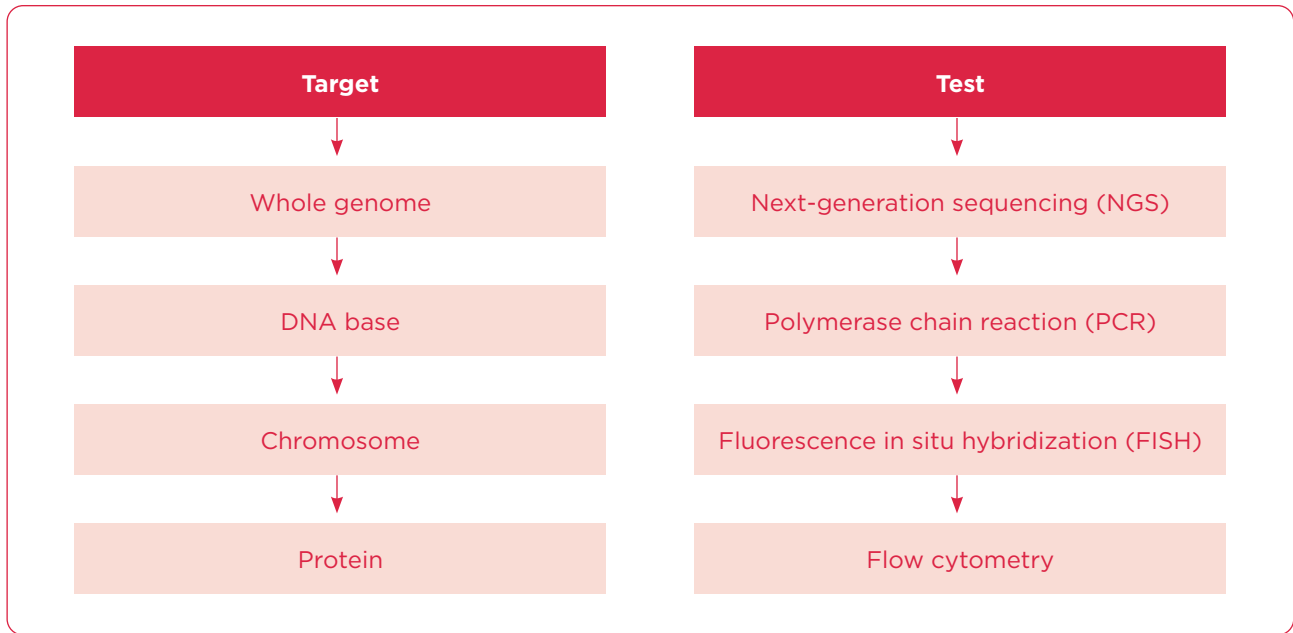


Figure 1. Molecular tests.

The human genome is known to have 6 billion base pairs of DNA distributed in 46 chromosomes, and these chromosomes have different sizes. While chromosome 1 has approximately 2,000 genes, chromosome 21 has approximately 200 genes.

“Incredibly, 99% of genome does not encode proteins, and its function is unknown,” said Ms. Zitella, who noted that the 1% of the genome that encodes for proteins is called the exome.

A gene is a segment of DNA that contains the code to make one protein, and genes are made of introns and exons. Exons are the stretch of DNA that encodes for a protein, and introns are intervening sequences and can influence turning on and off the gene.

THREE MOLECULAR TESTS: CYTOGENETICS, FISH, PCR

Conventional cytogenetics (karyotyping) is a method that examines all the chromosomes in a person and can detect large, structural changes in the chromosome. The test identifies the complete karyotype, including the number of chromosomes, and chromosomal abnormalities such as translocations, inversions, and deletions. One disadvantage of the test is that cells must be cultured for 48 to 96 hours and harvested when actively dividing and in metaphase. Cytogenetics are also unable to detect

small chromosomal abnormalities or genetic variants, and it takes 2 weeks to complete.

Fluorescence in situ hybridization detects specific genes and/or the number of gene copies on chromosomes. The process is much quicker than cytogenetics and typically takes 2 to 3 days to complete. Whereas cytogenetics examines only approximately 20 cells, FISH is generally sensitive to 1 of 200 to 400 cells. The other advantage is that FISH can be performed on both dividing cells and non-dividing cells, so it increases the number of tissues that can be tested. One disadvantage of FISH is that it is specific for one target and does not detect other variants that may be present. Therefore, one must know what one is “FISH-ing” for, and most labs have standard FISH panels to detect pertinent cytogenetic abnormalities associated with a specific malignancy [e.g., a FISH panel for chronic lymphocytic leukemia should include trisomy 12, del(11q), del(13q), del(17p)].

Polymerase chain reaction is a specific way of amplifying DNA to look for a specific variant, and it can be done very quickly (in 1 to 2 days). It has high sensitivity and can detect a change in 1 in 100,000 cells. Like FISH, these cells do not have to be actively dividing to be tested. Whereas conventional cytogenetics can detect changes in any chromosome, PCR is specific for one target and does not detect other variants that might be pres-

ent. Similar to FISH, specific PCR tests must be ordered depending on the disease.

“One of the major advances in molecular diagnosis has been our ability to look at the actual gene sequence rather than only large chromosomal changes,” said Ms. Zitella. “PCR is super important to understand because it is commonly the first step in most DNA analyses and increases the amount of DNA available for analysis for whole or partial genome sequencing.”

As Ms. Zitella explained, PCR can be performed on both DNA and RNA, which makes it helpful in several situations. What’s more, very small amounts of DNA are needed because these DNA sequences are amplified and copied to produce enough DNA to be tested. With RNA, the enzyme reverse transcriptase can also be used to synthesize DNA from the RNA to do the testing. The laboratory technique involves using short DNA sequences called primers to select the portion of the genome to be amplified or increased, and then the temperature of the sample is repeatedly raised or lowered to help a DNA replication enzyme copy target the DNA sequence. This process can produce a billion copies of the target sequence in just a few hours.

VARIANTS

Variants in the DNA sequence may or may not have consequences. An example of the latter is the nonsense variant, which is a genetic alteration that causes the premature termination of a protein. The altered protein may be partially or completely inactivated, resulting in a change or loss of protein function.

With a silent variant, which is also inconsequential, a single base change occurs, but it does not change the amino acid sequence because the change results in the correct amino acid being produced (sometimes multiple codons will encode for the same amino acid).

Conversely, other variants can be very deleterious because they cause abnormal proteins to be produced. An example of this is a missense variant in which a single base change causes a different amino acid to be placed into the protein. According to Ms. Zitella, a frameshift variant, which involves an addition or deletion of one base, is one of the worst variants because it alters the amino

acid position by completely disrupting the reading frame. All amino acids that are produced after this variant are incorrect, she said. Lastly, there can be duplication variants, where a sequence of the DNA is duplicated. An example of a duplication variant is *FLT3*, a serious variant in acute myeloid leukemia that confers a poor prognosis.

GENOMIC SEQUENCING

Genomic sequencing is the process of reading the nucleotides present in DNA or RNA molecule. There are two types of sequencing technologies used today. Sanger sequencing, a first-generation sequencing method, powered the Human Genome Project completed in 2003. The Sanger method sequences one gene at a time. Conversely, next-generation sequencing is a high-throughput methodology that enables parallel sequencing of many genes at the same time, including the entire genome (whole genome), the entire exome (whole exome), or a target panel of select genes of interest.

Next-generation sequencing is a three-step process (Klein & Foroud, 2017). The first step, library preparation, involves the creation of libraries with the use of random fragmentation of DNA. Then, there is amplification of the library using clonal amplification and the PCR method. Finally, there is the sequencing.

“Because of convenience, ease, and cost, we typically will sequence either the exome, which is 1% of the total genome but harbors about 85% of the variants that have an effect on disease, or do a specific targeted panel,” said Ms. Zitella.

Tumor DNA sequencing is a targeted DNA panel that examines subsets of the entire genome for somatic variants (acquired variants that lead to malignancy). The panel of genes is chosen based on disease. Looking for 500 known tumor genes, for example, requires sequencing less than 1% of the genome.

“These panels of genes are the clinical workhorse of molecular testing in hematology,” said Ms. Zitella.

TISSUE AND TERMINOLOGY

For malignancies, the tumor is used for testing. In leukemia or multiple myeloma, fresh samples of bone marrow, peripheral blood, or tissue are needed. With lymphoma, fresh or paraffin blocks of

lymph node tissue are typically tested. Germline sequencing, which looks at inherited variants, can be performed with peripheral blood. For patients with hematologic malignancy that is affecting the peripheral blood, however, a skin biopsy must be used to assess germline variants.

Germline variants are present in the egg or sperm. These are genes that people are born with. Germline variants may be classified as “variants of undetermined significance” in cancer reports or they may be associated with increased risk of cancer. *RUNX1* or *ATM*, for example, are germline variants that are associated with acute myeloid leukemia and myelodysplastic syndromes.

Somatic variants, on the other hand, are variants that are acquired over time. Unlike germline variants, which are found in every cell in the body, somatic variants are only found in certain tissues or cells.

Other important terms to know with respect to variants are “drivers,” “passengers,” and “actionable.” Driver variants are the variants that are felt to drive the malignancy, the sequences that cause cells to be malignant. Passenger variants, on the other hand, are just “along for the ride,” said Ms. Zitella. Passenger variants do not have any known clinical significance, she added, but the classification of a variant may change as new knowledge is acquired. An actionable variant refers to a variant that can be used to make clinical decisions (e.g., when there’s a drug available that targets the variant). To be effective, Ms. Zitella noted that drugs need to target the driver variant, which is driving the malignancy.

The variant allele frequency (VAF) is the percentage of alleles or copies of the gene in the sample with the variant and is useful to estimate the size of the abnormal clone associated with the variant. When you see a variant allele frequency

that is close to 50% or even 100%, said Ms. Zitella, that suggests a germline abnormality rather than an acquired (i.e., somatic) variant.

MEASURABLE RESIDUAL DISEASE

Minimal residual disease, also known as measurable residual disease (MRD), is a concept used to describe detection of low levels of malignant cells after the completion of therapy. Flow cytometry, PCR, and next-generation sequencing can be used to detect measurable residual disease. While flow cytometry and PCR are capable of detecting 1 in 1,000 or 1 in 10,000 cells, next-generation sequencing can detect 1 cell in 100,000 or even 1,000,000 cells (Uchiyama et al., 2020).

“There is a big difference in being able to detect one cell in a thousand vs. one cell in a million,” said Ms. Zitella, who noted that measurable residual disease status has become an important indicator of prolonged survival for hematologic malignancies.

“We now know that the lower the measurable residual disease is, the better the progression-free and overall survival are for most malignancies, but especially for chronic lymphocytic leukemia, acute lymphoblastic leukemia, and multiple myeloma,” Ms. Zitella said. ●

Disclosure

The presenter had no conflicts of interest to disclose.

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