# Molecular Diagnosis of Hematopoietic Neoplasms

## 2018 Update

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**KEYWORDS**

• Hematopoietic neoplasms • Molecular testing • Cytogenetic testing
• Genetic aberrations

**KEY POINTS**

- Cytogenetic abnormalities are considered common events in hematologic malignancies.
- These abnormalities generally consist of structural chromosomal abnormalities or gene mutations, which often are integral to the pathogenesis and subsequent evolution of an individual malignancy.
- Improvements made in identifying and interpreting these molecular alterations have resulted in advances in the diagnosis, prognosis, monitoring, and therapy for cancer.
- As a consequence of the increasingly important role of molecular testing in hematologic malignancy management, this article presents an update on the importance and use of molecular tests, detailing the advantages and disadvantages of each test when applicable.

## INTRODUCTION

Several hematologic malignancies are associated with diverse genetic aberrations that range from single base-pair substitution to complete chromosomal abnormalities. Before the development of the current modern molecular and cytogenetic techniques, distinguishing between specific diseases was often time consuming and difficult. In the molecular era, however, cytogenetic and molecular tests are commonplace and critical to diagnose hematologic malignancies. Moreover, such testing also plays a significant role in determining prognosis, therapy, and disease status (remission or

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relapse). The 2016 update of the World Health Organization (WHO) *Classification of Tumours of Haematopoietic and Lymphoid Tissues* integrates new categories of diseases based on molecular signatures as well as established and provisional entities and expands the 2008 edition in this respect.[1–3] These signatures have the potential to improve understanding of the disease process and may lead to diagnostic and therapeutic advances.

## MOLECULAR TESTS USED TO IDENTIFY CLONAL T-CELL AND B-CELL POPULATIONS

### *Immunoglobulin Gene Rearrangement*

Immunoglobulins are B-cell receptors (BCRs) found on B lymphocytes and able to bind antigens with high specificity. At the protein level, each immunoglobulin (antibody) is formed of heavy chains and light chains. Based on size and amino acid composition, the heavy chains are divided into 5 isotypes (classes) represented by the Greek letters $\alpha$, $\delta$, $\varepsilon$, $\gamma$, and $\mu$, which are representative of the immunoglobulins class of heavy chains, IgA, IgD, IgE, IgG, and IgM, respectively. The light chain is much smaller than the heavy chain and consists of 1 of 2 possible isotypes, kappa or lambda, represented by the Greek letters $\kappa$ and $\lambda$, respectively. The Ig contains 2 identical heavy chains and 2 identical light chains. Each chain contains 1 constant region that is similar for each isotype and 1 variable region that is different in amino acid sequence for the same isotype. The variable regions of the heavy and light chains undergo gene rearrangement during B-cell development and maturation. An individual B cell, therefore, produces 1 distinct Ig composed of 1 unique variable region for the heavy chain and another unique variable region for the light chain.

Humans inherit many variable region gene segments called germline genes. The immunoglobulin heavy chain gene locus (IGH@) is located on chromosome 14q32.Genes that encode light chains, however, are located on 1 separate chromosomes. Immunoglobulin kappa locus (IGK@) is located on chromosome 2p11.2 and immunoglobulin lambda locus (IGL@) is located on chromosome 22q11.22. The variable region of the IGH@ contains variable numbers of variable (V), diverse (D), and joining (J) gene regions. The light chains also contain a different number of V and J gene regions but lack the D gene region.[4,5] These genes are vital for generating the diverse number of human antibodies required and encode for more than 100 variable regions that encode for the first 90 to 95 amino acid of the variable region. The rest of the variable region, the last 15 to 20 amino acids, are present further along the chromosome in a linked set of DNA. Chronologically, the heavy chain variable region rearrangement precedes that of the light chain. Successful IGH rearrangement triggers the rearrangement of IGK@ and failure to achieve successful IGK rearrangement subsequently triggers the rearrangement of IGL@. In addition, the recombination of an individual variable region also occurs in an ordered sequence. The heavy chain recombination first occurs between 1 randomly selected D and J gene region followed by the joining of 1 V gene region. Then the constant chain gene is added and similarly the rearrangements of IGK@ and IGL@ start by joining the V and J gene regions to give a VJ complex before the addition of the constant chain gene.[4,5]

### *T-Cell Gene Rearrangement*

Each T-cell receptor (TCR) consists of 2 different chains coupled together. TCRa (TCRA) and TCRb (TCRB) chains are present in approximately 95% of the TCR with the rest formed by TCRg (TCRG) and TCRd (TCRD) chains. The genes encoding the TCRd chain are located within the TCRa gene on chromosome 14q11-12, whereas the TCRb and TCRg genes are located on chromosome 7q32-35 and 7p15,