Molecular testing is entrenched in the workup and management of hematological malignancies.

Biomarkers in Hematological Malignancies: A Review of Molecular Testing in Hematopathology
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Background: Molecular interrogation of genetic information has transformed our understanding of disease and is now routinely integrated into the workup and monitoring of hematological malignancies. In this article, a brief but comprehensive review is presented of state-of-the-art testing in hematological disease.

Methods: The primary medical literature and standard textbooks in the field were queried and reviewed to assess current practices and trends for molecular testing in hematopathology by disease.

Results: Pertinent materials were summarized under appropriate disease categories.

Conclusion: Molecular testing is well entrenched in the diagnostic and therapeutic pathways for hematological malignancies, with rapid growth and insights emerging following the integration of next-generation sequencing into the clinical workflow.

Introduction
Perhaps in no other field of oncology is the routine use of molecular markers more integrated into the diagnostic, prognostic, and therapeutic workup of disease as in the realm of hematological malignancies. Molecular diagnostics is a burgeoning field in the era of personalized medicine, with high-volume laboratories running 10,000 molecular tests or more every year, many of which are for the workup of leukemia and lymphoma. Molecular testing has wide applicability in hematology, guiding diagnosis (eg, TCR gene rearrangement to establish T-cell clonality), subclassification (eg, recurrent cytogenetic translocations in acute myeloid leukemia [AML]), prognosis (eg, Philadelphia chromosome-positive [Ph+] in acute lymphoblastic leukemia [ALL]), and minimal residual disease testing (eg, BCR-ABL transcripts in chronic myelogenous leukemia [CML]).

Myeloid Neoplasms
Myeloproliferative Neoplasms
Chronic Myelogenous Leukemia: The Ph chromosome in CML was discovered in 1960. t(9;22) (q34;q11) juxtaposes most of ABL1 to 5′ regions of BCR, resulting in constitutively increased kinase activity and neoplastic transformation. Although the breakpoint for ABL1 is mostly conserved, occurring in the intron preceding exon 2, the breakpoints in BCR are more variable and typically occur in either...
the major or minor breakpoint regions (M- or m-bpr). M-bpr fusions result in a p210 fusion protein, which is the form typically found in Ph+ CML. A sizeable number of patients with Ph+ B-cell acute lymphoblastic leukemia (B-ALL; 40% of adults and 10% of children) also harbor the p210 product. Conversely, m-bpr translocations result in a p190 fusion found in most Ph+ B-ALL cases but rarely in Ph+ CML.7 Uncommonly, BCR breakpoints fall in the microregion (μ-bcr), resulting in the p230 fusion product associated with chronic neutrophilic leukemia (CNL).8,9 Detection of t(9;22) is most commonly performed by either cytogenetics, fluorescence in situ hybridization (FISH), or reverse transcription–polymerase chain reaction (RT-PCR; amplification of the transcript product); the latter is used for minimal residual disease testing.

At diagnosis, conventional cytogenetics can detect t(9;22) in 95% of cases of CML; however, an additional 2.5% of cases with submicroscopic translocations can be recovered by applying molecular methods.6 If the results of both cytogenetics and FISH are negative, then an alternative diagnosis should be considered.

Assaying for t(9;22) can be used to monitor the therapeutic response of imatinib mesylate and for relapse surveillance (using quantitative polymerase chain reaction [qPCR] methods, particularly screening for positive results 6–12 months after transplantation).6 However, the importance of the BCR-ABL1 fusion gene in CML is in its role as a paradigm for targeted cancer therapy; patients with CML may receive imatinib as first-line therapy as established by data from the International Randomized Study of Interferon and STI571 trial.9 Baseline values from this trial are also used as the basis of the international reporting scale (IS). The IS allows for the standardization and comparison between laboratories with regard to BCR-ABL levels.10

Response to therapy can be classified as complete hematological response, complete cytogenetic response, and molecular response based on levels of fusion transcripts by RT-qPCR. A major molecular response is defined as a 3-log reduction compared with baseline (or ≤ 0.1% IS), and a complete molecular response is defined as a 4.5-log reduction or more from baseline.9,11 A lack of response may indicate acquired resistance. In such cases, Bcr-Abl kinase domain mutations (> 100 types documented) can be found in one-half of refractory cases and are an indication to adjust therapy by integrating a second-generation tyrosine kinase inhibitor into treatment.12

**Atypical Chronic Myelogenous Leukemia and Chronic Neutrophilic Leukemia:** The clonality of CNL has been well established given the prior detections of 20q-, 11q-, and JAK2 V617F mutation in this disease, but they are not disease specific.13–15 Deep sequencing has identified CSF3R mutations in CNL and atypical CML in 59% of patients,36 and these findings were subsequently documented in all World Health Organization (WHO)–defined CNL cases, possibly prompting a future revision of the WHO diagnostic criteria.17

**Polycythemia Vera, Essential Thrombocytosis, and Primary Myelofibrosis:** JAK2 codes for an intracellular tyrosine kinase and provides signaling for growth factor receptors, including the erythropoietin receptor. The JAK2 V617F mutation was discovered in 2005 and was shown to be present in 95% of polycythemia vera cases and approximately 50% to 65% of cases of essential thrombocytosis (ET) and primary myelofibrosis (PMF).18 In addition, the JAK2 V617F mutation can also be seen in nearly one-half of cases of refractory anemia with ring sideroblasts associated with marked thrombocytosis.19,20 In cases of polycythemia vera in which the JAK2 V617F mutation is not detected, the remaining 5% of patients may harbor mutations in exon 12 of JAK2.20 Similarly, in ET and PMF cases lacking the JAK2 V617F mutation, an assessment of MPL is indicated, given that 5% or more of patients with PMF and even fewer patients with ET (1%) will show an aberration in this gene (W515K/L).20–22 Exon 10 c-MPL mutations have also been reported in ET or PMF (5%).23 The JAK2 V617F mutation can be detected via targeted PCR followed by sequencing of the amplicon. Other methods include restriction digest of PCR-amplified products followed by separation by capillary electrophoresis, allele-specific PCR using probe-based gene expression analysis, real-time PCR, pyrosequencing, and melting curve analysis.24–25

Despite the discovery of JAK2 and MPL mutations, until recently many ET and PMF cases did not have a unique genetic basis (ie, JAK2, MPL) until 2 independent groups identified CALR mutations in this patient subset. CALR mutation, which comprises insertions (ins) and deletions (del) leading to a frameshift, are found in 20% to 25% of ET and PMF cases and tend to cluster in exon 9.26–28 Commercial testing utilizes sequencing and fragment length analysis.

Recently, the Dynamic International Prognostic Scoring System Plus listed unfavorable karyotype as a risk factor for predicting survival in primary myelofibrosis.29

**Mastocytosis:** Activating point mutations in KIT are highly associated with mastocytosis and can be detected in more than 95% of cases of systemic mastocytosis using real-time qPCR, allele-specific oligonucleotide PCR, or direct sequencing.30,31 KIT mutations result in the ligand-independent activation of the c-kit tyrosine kinase. The most common mutation in systemic mastocytosis is the D816V variant seen in 68% of cases of mastocytosis; however, in certain subsets (eg, aggressive systemic mastocytosis), its incidence may exceed 80%.32 The presence of this variant constitutes a minor criterion for the diagnosis of systemic.
mastocytosis. Other KIT variants have been described (< 5%) and are more likely to be detected in the context of cutaneous mastocytosis rather than systemic mastocytosis.\textsuperscript{20} Patients with the D816V variant are resistant to imatinib.\textsuperscript{20,35}

**Myeloid and Lymphoid Neoplasms With Eosinophilia and Abnormalities of PDGFRA, PDGFRB, or FGFR1**

A unique group of myeloid and lymphoid neoplasms are defined by aberrant tyrosine kinase activity due to translocations involving PDGFRA, PDGFRB, or FGFR1, all of which are characteristically associated with eosinophilia. A workup for abnormalities in these genes should be considered in cases of eosinophilia with end-organ damage or in which secondary reactive eosinophilia has been excluded.

The cellular ontogeny of these disorders may originate from a pluripotent (lymphoid–myeloid) stem cell. PDGFRB or FGFR1 can be detected with conventional cytogenetic analysis (ie, karyotype); however, the FIP1LI-PDGFRA results in an 800-kb cryptic del(4q12) that houses CHIC2. Typically, it is detected using FISH with a probe spanning CHIC2 or break-apart assay for either of the translocation partners. The translocation can also be detected using RT-PCR.\textsuperscript{20} FIP1LI-PDGFRA disease commonly manifests as chronic eosinophilic leukemia, and FIP1LI-PDGFRB is detected in 10% to 20% of those with idiopathic hypereosinophilia.\textsuperscript{35} Patients have a response to imatinib more than 100 times greater than that seen in BCR-ABL rearrangement.\textsuperscript{20,35}

Neoplasms associated with PDGFRB commonly present as chronic myelomonocytic leukemia. ETV6 is the most common translocation partner, but more than 13 others have been described; patients will be responsive to imatinib.\textsuperscript{54}

Neoplasms associated with FGFR1 can manifest as acute leukemias (myeloid or lymphoid) or as chronic eosinophilic leukemia. Translocation partners include ZNF198, CEP110, FGFR10P1, BCR, TRIM24, MYO18A, HERVK, and FGFR10P2. By contrast to PDGFRB- and PDGFRB-associated neoplasms, these disorders are unresponsive to tyrosine kinase inhibitors.\textsuperscript{20,34}

**Myelodysplastic Syndrome**

Myelodysplastic syndrome (MDS) is a clonal disorder of myeloid cells characterized by morphological dysplasia and ineffective hematopoiesis that manifests as peripheral cytopenia.\textsuperscript{56} Cytogenetic abnormalities are seen in one-half of MDS cases, and they most commonly involve del(5q)/7q or monosomies of the same.\textsuperscript{20,37} TP53 mutations are associated with therapy-related MDS and have a poor prognosis.\textsuperscript{58} Various cytogenetic abnormalities can be considered presumptive evidence of MDS even in the absence of sufficient dysplasia (ie, -5/del[5q], -7/del[7q], +8, \(-Y, \text{del}[20q], \text{isochromosome }[i][17q], -13/\text{del}[13q], \text{del}[11q], \text{del}[12p], \text{del}[9q]), \text{isodicentric }[\text{idic}[Xq13]], \text{and certain balanced translocations involving chromosomes }1, 2, 3, 9, 11, 16, \text{ and } 21).\textsuperscript{20} Various prognostic models are available for MDS. The most widely adopted is the International Prognostic Scoring System (IPSS) and its revised version (IPSS-R), both of which integrate the percentage of blasts in the bone marrow, cytogenetic abnormalities, and number of cytopenias.\textsuperscript{39} In the latter scheme, cytogenetics are placed in 5 tiers: very good (-Y, del[11q]), good (normal, del[5q], del[12p], del[20q], and del[5q] + 1 more), intermediate (del[7q], +8, +19, [i][17q], and others), poor (-7, inversion [inv]3/3q/del[3q], -7/del[7q] + 1 more, and 3 cytogenetic aberrations), and very poor (> 3 abnormalities).\textsuperscript{40} Although gene-expression profiling and single nucleotide polymorphism arrays are powerful tools, they are not routinely employed in the clinical setting. However, somatic mutation in 40 genes has been found in MDS and analysis for these genes can add prognostic value.\textsuperscript{41} By contrast to cytogenetic abnormalities, which are seen in one-half of cases, at least 1 of these “driver” mutations can be found in most cases of MDS.\textsuperscript{42,43} For example, patients with 1 or more mutations in TP53, EZH2, ETV6, RUNX1, or ASXL1 show survival patterns analogous to those in the next higher tier by subgrouping in the IPSS.\textsuperscript{44}

Various epigenetic modifiers (DNA methylation regulators, spliceosome mutations, and histone modifiers TET2, IDH1/2, DNMT3A, EZH2, ASXL1, SF3B1, U2AF1, SRSF2, and ZRSR2), transcription factor genes, and kinase signaling genes have been implicated in MDS, providing a basis for approved therapies and those in development.\textsuperscript{45} However, these aberrations have limitations because their clinical significance is not always clear given their association with poor prognostic clinical features, our lack of knowledge of their interactions with other markers often concurrently detected, intratumoral clonal heterogeneity, and the wide gamut of mutations in any given gene.\textsuperscript{42}

Next-generation sequencing (NGS) technologies, which garner the power of massively parallel sequence generation, enable laboratories to clinically sequence many genes simultaneously, which was previously untenable by traditional sequencing technologies. Commercial testing is available for activated signaling genes (KIT, JAK2, NRAS, CBL, MPL), transcription factors (RUNX1, ETV6), epigenetic genes (IDH1/2, TET2, DNMT3A, EZH2, ASXL1, SETBP1), ribonucleic acid splicing genes (SF3B1, U2AF1, ZRSF2, SRSF2), and tumor suppressors (TP53, NPM1, PHF6), among others. In cases of MDS or MDS/myeloproliferative neoplasms in which the diagnoses are unclear or dysplasia has yet to emerge, detecting a mutation in one of these key genes may be helpful in establishing the diagnosis of a clonal myeloid neoplasm. Although
they are not formally incorporated into prognostic stratification schemas, certain mutations may also carry prognostic implications. \(^{46}\) In addition, robust myeloid testing commercially available (FoundationOne Heme, Cambridge, Massachusetts) can interrogate 405 cancer-related genes, allowing — in theory — for the identification of targetable mutations and patient enrollment in clinical trials. \(^{47}\)

**Acute Myeloid Leukemia**

AML is the most common type of acute leukemia occurring in adults. \(^{48}\) In 2015, an estimated 20,830 new cases of AML will occur in the United States, along with 10,460 deaths. \(^{49}\) AML is a lethal disease and has a 5-year relative survival rate of 24.2%. \(^{50}\) However, outcomes are heterogenous and overall survival rates range from approximately 5% to 70%. \(^{50}\) Thus, a need exists for prognostic markers to predict outcomes and guide therapeutic decision-making. Prognostic markers can be clinical, disease related, and molecular, although the strongest prognostic factor for predicting therapeutic response and survival is cytogenetic subgrouping.

The results of numerous clinical trials across several decades have indicated that overall survival rates can be as long as 11.5 years in favorable patient groups or shorter than 1 year in patients with adverse risk. \(^{51-53}\) Those with favorable risk (5-year survival rate of 50%–80%) include those with t(15;17), t(8;21), inv(16/16q22–22q12), t(1;22)(p13;q13), TP53 deletion, and a complex karyotype, may undergo transplantation after standard induction. \(^{53,56}\)

Determining whether consolidation therapy is appropriate in those with intermediate risk (overall survival rate of 20%–40%) \(^{53}59\) is not as clear. In this cohort, molecular testing for FLT3, NPM1, and CEBPA is informative and has therapeutic implications. For example, detecting FLT3 internal tandem duplication by PCR in patients with normal karyotype AML may lead to consolidation therapy with hematopoietic stem cell transplantation, after which patients may have a 30% likelihood of cure. \(^{55}\) FLT3 codes for a transmembrane signal-transducing protein of the tyrosine receptor kinase family and reveals 2 major abnormalities in AML, ie, internal tandem duplication in the juxtamembrane portion resulting in constitutive activation and a point mutation in Asp835 (the activity loop portion of protein) resulting in dysregulation. FLT3 aberrations are seen in 5% to 10% of AMLs. \(^{57,58}\)

* NPM1 encodes nucleophosmin, a 37 kDa protein. \(^{46}\) NPM1 mutations involve 4 to 11 break-point insertions in exon 12 that lead to the mislocalization of normal nucleophosmin to the cytoplasm via dimerization. \(^{59,60}\) This can be detected by PCR followed by sizing via capillary electrophoresis. NPM1-mutated AML has been designated as a provisional entity in the 2008 WHO classification and is associated with unique morphological (blasts with “cup-like” nuclei) features and a favorable prognosis in normal karyotype AML. \(^{20}\)

CCAAT/enhancer-binding protein \(\alpha\) is a 42 kDa transcription factor whose loss is associated with the oncogenic transformation of myeloid cells due to a loss of differentiation. \(^{61}\) Patients with mutated CEBPA show outcomes similar to those in the favorable cytogenetic subgroup of AML (eg, t[8;21] AML). \(^{62}\) The prognostic value of CEBPA is in the double-mutated subset of patients lacking FLT3 and NPM1 mutations. \(^{63}\)

Other single gene alterations that may carry important prognostic implications have been identified in AML — many were identified during whole genome sequencing studies — and include DNMT3A, IDH1/2, TET2, WT1, ERG expression, BAALC expression, and MN1 expression, among others. IDH2 is associated with a good prognosis and TET2, ASXL1, and PHF6 confer poor prognoses. However, in the absence of prospective trials and the present controversy regarding them, many of these single genes have not been formally integrated into accepted risk-stratification models. \(^{64}\) In general, investigating the mutation status of these genes is simultaneously obtained using NGS technologies.

Biomarkers are important for subclassifying AML types, and several categories of AML are defined based on the presence or absence of recurrent genetic abnormalities alone, in particular t(8;21)(q22;q22), inv(16)(q22–22q12), t(9;11)(p22;q23), t(6;9)(p23;q34), inv(3), and t(1;22)(p13;q13). \(^{13}\) In some cases, the detection of 1 of these aberrations alone is enough to diagnose AML, even in the absence of the conventional criteria of 20% blasts in the marrow or peripheral blood, such as in the case of t(15;17) and core-binding factor-related leukemias (eg, t[8;21](q22;q22), inv[16]) and possibly inv(3)(t;3). \(^{65,67}\) These translocations can be detected by conventional cytogenetics, FISH, and more novel technologies, including single molecule imaging and NGS. One such type of sequencing uses color-coded barcodes directly hybridized to individual target molecules and then digitally detects them in a multiplexed manner. \(^{68}\)

A comprehensively targeted clinical panel currently on the market uses NGS to interrogate the exons of 405 genes and examines the intronic regions of 31 genes involved in rearrangements as well as complementary DNA (ribonucleic acid) to sequence 265 genes to detect translocations. \(^{69}\)

In some cases, the detection of a translocation...
carries both diagnostic and therapeutic importance. Namely, t(15;17)(q22;q21), which is diagnostic for acute promyelocytic leukemia (AML M3), juxtaposes the 17(q21) retinoic acid receptor α (the receptor for vitamin A involved in cell proliferation and differentiation) to the 15(q22) promyelocytic leukemia zinc finger protein (involved in transcriptional regulation and apoptosis). The chimeric protein blocks differentiation beyond the promyelocytic stage, resulting in acute leukemia. However, treatment with all trans-retinoic acid allows for differentiation and, in combination with cytotoxic chemotherapy, can result in complete remission. Variant translocations involving RARA are seen in fewer than 2% of cases, but they are important given that some patients may not respond to all trans-retinoic acid therapy.20,70

Another example of molecular testing informing therapeutic management involves KIT testing in AML. C-kit mutations are associated with core-binding factor AMLs and may abrogate the favorable prognosis generally associated with this group.71

**Lymphoid Neoplasms**

**Lymphoblastic Leukemia/Lymphoma**

Similar to AML, the detection of certain characteristic translocations further subclasses cases of B-ALL, including t(12;21)(p12;q22) TEL/AML-1, t(1;19)(q23;p13) PBX/E2A, t(9;22)(q34;q11) ABL/BCR, (5;14)(q31;q32) IL3-IGH, and (V;11)(V;q23) V/MLL.20

t(9;22)(q34;q11) is seen in 25% of adult cases and 2% to 4% pediatric cases.72 Patients with this Ph+ translocation carry the worst prognosis of all types of lymphoblastic leukemia73; however, these patients can be treated with adjuvant imatinib, which improves complete remission rates.74

The presence of the translocation also serves as a useful marker for minimal residual disease testing. Molecular methods for detecting the translocation are similar to those used for CML. Notably, when qRT-PCR testing is undertaken, the p190 protein product is typically associated with B-ALL, not the p210 protein typical of CML; in children with ALL, the break point occurs in m-bcr, which generates the p190 protein product in 90% of cases.6 If a p210 protein product is detected, then consideration should be given to a lymphoblastic blast crisis arising in CML.

By contrast to t(9;22), the reverse demographic appears to be true for t(12;21)(p12;q22): It occurs in 25% of pediatric cases but is rare in adults and associated with a favorable prognosis and curative rates of higher than 90% in children.75 MLL can have various translocation partners, the most common of which is AF4 on chromosome 4. MLL translocations carry a poor prognosis, and this is particularly true in infants.76 The unique characteristic of t(5;14)(q31;q32) IL3/IGH B-ALL, which is rare, is its association with eosinophilia due to the overexpression of IL3.77

**t(1;19)** B-ALL is historically associated with a poor prognosis, but this has changed through the use of intensive chemotherapy regimens.78 Immunophenotypically, the blasts lack CD34 but have aberrant CD9 positivity.79 Based on gene-expression profiling, BCR-ABL1–like B-ALL has been identified as being associated with deletions of IKZF1, CRLF2 rearrangements, and poor outcomes.80 JAK1/2-activating mutations are present in a subset of these patients and may benefit from Janus kinase inhibitor therapy.80 In all children with ALL, cytogenetic testing or flow cytometric analysis of ploidy should be undertaken. Hyperdiploidy (>50 chromosomes) is associated with a better prognosis, whereas hypodiploidy (<44 chromosomes) is associated with a poor prognosis.80,81

Translocations in T-cell ALL (T-ALL) commonly involve 1 of the TCR loci (A, B, G, D). The most common translocation partner includes HOX11 on chromosome 10 (occurring in 10%–30% of cases) or various other transcription factors dysregulated by juxtaposition to 1 of the TCR genes.82 PICALM-MLLT10 and MLL rearrangements are seen in approximately 10% of cases.83

Both B-ALL and mature B-cell non-Hodgkin lymphomas (NHLs) show clonal immunoglobulin (Ig) gene rearrangements, which are helpful in residual disease testing as well as in establishing a malignant diagnosis. For follow-up specimens, screening for clonal peaks identical to those identified at diagnosis can be performed to assess for residual/relapsed minimal residual disease.

B-cell antigen receptors are encoded by IGH (14q32), IGLK (2p11), and IGLL (20q11), coding for the Ig heavy chain, κ light chain, and the λ light chain, respectively. Each contains variable (V), joining (J), and constant regions; IGH contains an additional diversity (D) region. Multiplex PCR that uses primers to target highly conserved framework regions within the V segment are used to generate PCR products, which can then be separated using capillary electrophoresis, which is preferred to Southern blot analysis.84,85

Monoclonal peaks have heights 2 to 3 times that of the background and can be seen in clonal B-cell neoplasms. Repeat peaks in duplicate wells raise confidence that clonal peaks do not represent a PCR artifact. Care must be taken because false-positive results can occur in cases of benign lymphoid hyperplasia, which may be present in the setting of immunodeficiency and autoimmune disease.86,87 Furthermore, lineage infidelity is present with BCR gene rearrangement and is similar to that seen in T-cell lymphoma. Most precursor and 5% to 10% of mature B-cell neoplasms will harbor clonal T-cell gene rearrangements.6 Other various factors may result in false-negative results, including primer failure due to
somatic hypermutation (which can occur at a rate of > 50% in certain lymphoid neoplasms [eg, follicular lymphoma]), complex IGH rearrangements, or DNA of poor quality.\(^6\)\(^8\)

** Mature B-Cell Neoplasms**

Various translocations are associated with B-cell NHLs and their detection helps to establish a diagnosis in these entities. t(14;18) involves BCL2 on chromosome 18 and IGH on chromosome 14. BCL2 is juxtaposed to the J region of the heavy chain. Given that the IGH enhancer element is highly active, bcl2 can become overexpressed. Because bcl2 has antiapoptotic properties, its overexpression will result in neoplasia. This translocation is found in 85% to 90% of cases of follicular lymphoma (a lower percentage occurs in cases of high-grade follicular lymphoma) and 25% of cases of diffuse large B-cell lymphoma (DLBCL).\(^2\)\(^0\)

Because follicular lymphomas may lack demonstrable Ig clonality due to ongoing somatic hypermutation, the use of FISH or PCR for the translocation offers alternative markers to assess for clonality, establish a diagnosis, or both; however, FISH is preferred to PCR as it is more sensitive and specific.\(^8\)\(^9\)

DLBCL, in addition to BCL2, can have translocations of BCL6 and MYC (10% of cases).\(^9\)\(^0\) When a MYC translocation is detected along with other specific translocations (usually BCL2 and BCL6) in an intermediate to large B-cell lymphoma, its presence qualifies as a “double hit” lymphoma, which may be categorized under the rubric of large B-cell lymphoma with features intermediate between DLBCL and Burkitt lymphoma.\(^9\)\(^1\)

Typically, MYC gene rearrangements are associated with Burkitt lymphoma, but they can also be present in plasmablastic lymphomas (50% of the time) and, rarely, in follicular lymphoma and primary central nervous system DLBCL.\(^9\)\(^2\)\(^9\)\(^3\) Burkitt lymphoma is characterized by t(8;14) involving MYC and IGH and will less commonly show translocations involving light chain loci (\(\kappa\) or \(\lambda\)).\(^9\)\(^4\)\(^9\)\(^6\) BCL6 translocations can be seen in follicular lymphoma, DLBCL, and are frequently identified in primary cutaneous leg-type DLBCL.\(^9\)\(^7\)

Nearly all cases of mantle cell lymphoma carry t(11;14)(q13;q32) CCND1-IGH, which can be assessed by FISH, and is preferred over PCR-based methodologies that demonstrate lower sensitivity rates (50%–60%); this is because of the large number of dispersed break points at 11q13.\(^9\)\(^8\)

Translocation of CCND1 with light chain has also been reported; rarely, cyclin D2 may be translocated, which should be a consideration in cyclin D1+ tumors otherwise characteristic of mantle cell lymphoma.\(^9\)\(^9\)

Various translocations have also been described in lymphoma involving the mucosa-associated lymphoid tissue. Of these, MALT1 and BCL10 translocations are worthy of mention (t[14;18][q32;q21], t[11;18][q21;q21], t[1;14][p22;q32]) because they represent mucosa-associated lymphoid tissue that usually does not respond to Helicobacter pylori eradication.\(^1\)\(^0\)\(^0\)\(^2\)

Although multitudinous, single nucleotide variants and copy number changes have been found in B-cell NHL, sometimes even with reported prognostic significance (eg, NOTCH1 mutations in chronic lymphocytic leukemia [CLL]), in clinical practice testing for these in B-cell NHL has a limited role.\(^1\)\(^0\)\(^3\) A limited 7-gene CLL panel with targets that carry prognostic implications has been launched by Cancer Genetics (Rutherford, New Jersey).

Commonly, when molecular testing is indicated in B-NHL, the genetic aberrations are usually of diagnostic importance. BRAF V600E mutation was originally found in 100% patients with hairy cell leukemia compared with none of the 195 other peripheral B-cell lymphomas/leukemias.\(^1\)\(^0\)\(^4\)\(^1\)\(^0\)\(^6\) The results of subsequent studies have confirmed that the mutation is present in all cases of hairy cell leukemia and is rare in other chronic lymphoproliferative disorders.\(^1\)\(^0\)\(^4\)\(^1\)\(^0\)\(^6\) In lymphoplasmacytic lymphoma, MYD88 mutation has been detected with high frequency (> 90%),\(^1\)\(^0\)\(^7\) and detecting the mutation may be diagnostically useful given the overlap with lymphoplasmacytic lymphoma and other low-grade B-cell lymphomas that may be associated with plasmacytic differentiation, including marginal zone lymphoma, multiple myeloma, and CLL. In these other conditions, the prevalence of the mutation is 3% to 9%.\(^1\)\(^0\)\(^8\) Of note, nearly one-third of activated B-cell-like DLBCL harbors the mutation, and its presence is not useful in the differential with IgM monoclonal gammopathy of undetermined significance.\(^1\)\(^0\)\(^8\) Thus, a correlation with morphology and other ancillary studies is needed.

In CLL, hypermutation status is assessed by comparing each IGH clonally rearranged gene sequence with a database of germline V-region sequences to determine the expressed V-region gene and the extent and position of somatic mutations. If a difference exists of more than 2%, then the tumor is considered hypermutated and confers a better prognosis.\(^1\)\(^0\)\(^9\)

** Mature T-Cell Lymphoproliferative Disorders**

A total of 95% of T cells express the \(\alpha\)-\(\beta\) receptor and a smaller proportion express the \(\gamma\)-\(\delta\) receptor; both of these receptors contain heterodimeric proteins encoded by TCR genes located on chromosomes 7 and 14.\(^6\)\(^1\)\(^0\)\(^\)\(^\)\(^\) Early in development, the TCR genes undergo somatic rearrangement involving V, D, and J regions (TCRB and TCRD) or V–J rearrangements alone (TCRA and TCRG).

Unlike in B cells, in which Ig light chains (\(\kappa\) and \(\lambda\)) can be assessed for clonality by flow cytometry or immunohistochemistry, establishing the clonal na-
tecture of T cells using these techniques is difficult, thus making TCR gene rearrangement studies valuable. Each T cell bears a unique, rearranged sequence. Under normal circumstances, a range of gene products can be seen given the gamut of polyclonal T cells present. However, if a clonal process is present, then a particular gene rearrangement product should predominate, and it can be detected using Southern blot analysis as a single clonal band.

Although Southern blot analysis is considered the gold standard, it is inefficient and seldom used in modern clinical laboratories for T-cell clonality detection. Drawbacks of Southern blot analysis include its high cost, increased time, large sample requirements, and low sensitivity rates compared with PCR (5%–10% vs 1%).

PCR amplification of TCRG and TCRB gene products followed by gel separation or capillary electrophoresis is employed in the clinical laboratory. PCR testing demonstrates a clonal peak 2 to 3 times larger than the background peaks in T-cell lymphomas. In certain cases, false-negative results may occur if the rearrangement involves the primer site or too few T cells are present for analysis. Positive cases of gene arrangements should not be taken to mean that T-cell lymphoma is present. Such positivity can be seen in cases of B-cell lymphoblastic leukemia (approximately 50% of cases), mature B-cell lymphomas (5%–10%), AML (10%), and non-neoplastic conditions such as autoimmune disorders, certain infectious diseases (Epstein-Barr virus–induced oligoclonal processes), and certain cutaneous lesions (eg, lymphomatoid papulosis).

In ALK-positive anaplastic large cell lymphoma, t(2;5)(p23;q35) juxtaposing ALK and NPM, respectively, is the most frequent genetic translocation (83% of pediatric and 31% of adult cases) present; however, various, less frequently seen partners have also been described, including TPM3 (13%), ATIC, TGS, CLTC, MSN, TPM4, MYH9, and ALO17 (all < 1%). The translocation can be assayed using RT-PCR or break-apart FISH probes.

In T-cell prolymphocytic leukemia, the most common genetic aberration (80%) involves inv(14) juxtaposing the TRA locus at 14q11 to the TCLI A and TCL1B oncogenes. In a subset of cases, a reciprocal translocation (t(14;14) is present; t(X;14)(q28;1q11) has also been described but is less common. Both can be assayed using FISH. Cytogenetics can be used to detect chromosome 8 abnormalities (70%–80%), ATM deletions, as well as del(12p13), all of which can be seen in the setting of T-cell prolymphocytic leukemia.

Hepatosplenic T-cell lymphoma is associated with numerical abnormalities of chromosome 7, and most cases will demonstrate i(7q). As the disease progresses, 2 to 5 copies of i(7)(q10) or derangements in the second chromosome 7 may be present. i(7)(q10) can be detected using FISH.

In adult T-cell leukemia, clonal integration of the human T-lymphotropic virus type 1 viral DNA can be seen. Although it is conceivable to perform testing via Sanger sequencing, it is typically easier to perform serum studies for human T-lymphotropic virus type 1. In enteropathy-associated T-cell lymphoma, amplification of 19q31.3, del(16q12.1), or both have been reported.

**Conclusion**

Molecular testing is well entrenched in the workup and management of hematological malignancies. As sequencing technologies become both more powerful and affordable, they will take on an even larger role in the molecular diagnostics of hematopathology and in the era of precision medicine.

**References**


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