MOLECULAR PATHOLOGY OF HEMATOLOGIC MALIGNANCIES

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Abstract

Hematological malignancies represent a heterogeneous group of diseases that may have overlapping clinical manifestations. Successful and optimal management depends on early and accurate diagnosis of the disease. Differential diagnosis therefore requires methods of morphology, immunohistochemistry, flow cytometry, and also molecular and cytogenetic examinations. Molecular diagnostic techniques are becoming more accurate and sophisticated, which is why nowadays hematopathological diagnosis relies heavily on molecular and cytogenetic analyses. They are beneficial not only for diagnosis, but also for evaluating prognostic and risk markers, as well as treatment monitoring. This article presents an overview of selected hematological malignancies – mature lymphoid neoplasms, multiple myelomas, myeloproliferative neoplasms, myelodysplastic syndromes, and acute myeloid leukemias; their molecular pathology, risk and prognostic markers. Understanding of the biologic basis leds to a targeted therapy development for the treatment of these diseases.

Key words: hematologic malignancies, molecular pathology, recurrent mutations, genetic alterations

INTRODUCTION

Hematologic malignancies are myeloid and lymphatic tumors caused by a disruption of normal hematopoietic function (1). This heterogenous group of diseases differs in cellular origin and clinical manifestation. Classification system combines clinical, pathologic, and molecular features of the diseases. In general, they can be divided into leukemias and lymphomas. While leukemias involve peripheral blood and are composed of immature hematopoietic elements lymphomas are composed of B-cells, T-cells or natural killer cells of varying degrees of maturity that mainly affect solid tissues or lymph nodes (2).

Cytogenetics and targeted molecular assays are now routine and necessary for the diagnosis and prognostication of most myeloid neoplasms. Molecular methods to detect specific mutations include sequencing methods (Sanger or Next-Generation Sequencing (NGS) gene panels) or modified polymerase chain reaction (PCR) techniques using genomic DNA. Most hematologic neoplasms require the analysis of chromosomal abnormalities to detect gene fusions and rearrangements by karyotype and/or fluorescence in situ hybridization (FISH) or RNA-based PCR assays may be performed, which does not require amplification of large intronic regions. After the initiation of the treatment, quantitative PCR (qPCR) or digital droplet PCR (ddPCR) assay may be used to asses for response to therapy, measured by the decrease in fusion protein transcript level. Measurable residual disease (MRD) is an important biomarker that is used for prognostic, predictive, monitoring, and efficacy-response assessments (3).

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Mature lymphoid neoplasms

These neoplasms can be classified as Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). B-cell NHL is far more common than T-cell or NK-cell NHL and comprises approximately 80 % of lymphomas in Europe. Usually they are very heterogenous in their etiology and pathogenesis, but many are derived from the germinal center reaction (2).

Chronic lymphatic leukemia

CLL/SLL (Small lymphocytic lymphoma) is defined as a monoclonal lymphoproliferative disease characterized by the proliferation and accumulation of morphologically mature but immunologically dysfunctional B-cell lymphocytes (4).

The most frequently mutated genes in CLL are *NOTCH1* (10–15%), *SF3B1* (10%), *TP53* (5–10%), *ATM* (10–15%), and *MYD88* (3–8%) . Immunoglobulin genes are frequently rearranged and with somatic hypermutation in 50–60%. Based on the mutation status of the immunoglobulin heavy-chain variable region (*IGHV*) two subtypes of CLL were described considered as mutated *IGHV* (M-CLL) and as unmutated *IGHV* (U-CLL). U-CLL cells are associated with an aggressive disease as compared to M-CLL cells exhibit a good prognosis with low-risk genetic alterations. Cytogenetic markers are used because the acquired chromosomal abnormalities are observed in approximately 80% of individuals with CLL. Cytogenetic markers can be used to categorize patients into prognostic groups. Patients with normal cytogenetics have a median survival of approximatelly 111 months. Deletion 13q14.3 (median survival 133 months) is the most common chromosomal abnormality occurring in 40–60% of patients. Deletion 11q22.3 (median survival 79 months) is present in up to 20 % patients and 11q – patients have an aggressive clinical course characterized by a bulky lymphadenopathy and a shorter progression free survival (PFS). Deletions within the chromosome 17p13 (a median survival of 32 months) locus have been reported in 4 to 16% of the cases of CLL and show a poor survival due to an advanced disease at diagnosis, a short time to the first treatment, and a high risk of chemorefractoriness to alkylating agents and purine analogues. *TP53* mutations can be seen in the absence of deletion 17p13 in at least 20% of the cases. Trisomy 12 (a median survival of 114 months) defines a subgroup of CLL with a more frequent atypical morphology including prolymphocytes and intermediate to poor prognosis (5,6). Table 1 summarizes risk stratification in CLL.

Burkitt lymphoma (BL)

BL is an aggressive B-cell lymphoma characterized by a high degree of proliferation of the malignant cells and deregulation of the c-*myc* gene. Typically monomorphic proliferation of a medium sized transformed germinal center related B-cells is present. The diagnosis of BL is based on morphologic findings, immunophenotyping results, and cytogenetic features. BL has a characteristic chromosome abnormality of translocation at chromosome 8q24 involving *MYC* usually with chromosome 14q32 involving *IGH* (table 1). Variant translocations occur with the lambda light chain gene (*IGL*) at chromosome 22q11 or the kappa gene (*IGK*) at chromosome 2q12 in up to 16% of cases (2). However, BL can have overlapping morphologic and immunophenotypic features, and the characteristic t(8;14) translocation with diffuse large-B-cell lymphoma (DLBCL) (7). Thus, translocations involving *MYC* are characteristic but not specific for BL (2). The most frequent genetic events in pediatric BL cases up to 90% represent somatic single-nucleotide variants, insertions, and deletions (SNV/indels) of the *ID3-TCF3-CCND3* pathway. In adults BL with a significantly lower frequency of only 63% (8).

Diffuse Large B-Cell Lymphoma (DLBCL)

DLBCL represent a group of aggressive B-cell lymphomas with underlying genetic diversity and variable clinical presentations. Based on cell-of-origin several subtypes of DLBCL were identified: GCB – Germinal center B-cell, ABC – activated B-cell, PMBL – primary

mediastinal B-cell, and 15–20% of cases are unclassified. The 2016 revision of the World Health Organization (WHO) classification recognized and introduced a new entity, high-grade B-cell lymphoma (HGBCL), defined by the presence of *MYC* and *BCL2* and/or *BCL6* rearrangements and is present in approximately 8% of DLBCL (table 1). Approximately 10–15% of patients with untreated DLBCL have a rearrangaement of the *MYC* oncogene (9).

Mantle cell lymphoma (MCL)

MCL is a B-cell neoplasm characterized by the expansion of mature B cells frequently co-expressing CD5 that tend to widely spread in bone marrow, blood, lymphoid tissues, and extranodal sites. The tumor cells carry the t(11;14)(q13;q32) that leads to the constitutive overexpression of cyclin D1. Cryptic rearrangements of IG regulatory regions could be an alternative oncogenic mechanism in a minor subgroup of patients (10). *TP53* gene aberrations (mutations or deletions) are a well-established high-risk factor in MCL and were associated with an activated MYC pathway, hyperproliferation, deletion of 9p, and worse clinical prognosis (table 1). At diagnosis, the frequency of *TP53* mutations is about 11%–25%; the frequency increases to 45% at relapse. The presence of both *TP53* deletion (detected by FISH) and *TP53* mutations (detected by DNA sequencing) was associated with the worst survival. *TP53* gene mutations may co-exist with other aberrations such as *NOTCH1* mutation (71%), deletion of *CDKN2A* (del9p21) (31%), and deletion of *TP53* (del17p13) (31%). Lack of *SOX-11* with mutated *IGHV* identified a subset of MCL patients with a favorable prognosis. Patients with *IGHV* mutation may exhibit a better outcome compared to those with unmutated *IGHV*. A complex karyotype, defined as having three or more chromosomal abnormalities in addition to t(11;14), is generally considered as a high-risk factor (11).

Folicullar lymphoma (FL)

FL is considered as the most common indolent B cell lymphoma. Histologically is characterized by a follicular or nodular pattern of tumor cell growth. More than 85% of FL cases harbor the characteristic t(14;18)(q32;q21), which occurs in pro- or pre-B cells of the bone marrow. Using sensitive techniques, the t(14;18) may be detected in B cells from peripheral blood and/or lymphoid tissues of a large proportion (up to 70%) of healthy individuals although the vast majority of them will never develop FL, indicating that *BCL2* deregulation alone is insufficient for tumorigenesis. In addition to t(14;18), FL has a characteristic genomic profile, with frequent losses of 1p (15–20%), 6q (20–30%), 10q (20%), and 13q (15%), and gains of 1q (25%), 2p (25%), 8q (10%), 12q (20%), and 18q (30%), and trisomies 7 (20%), 18 (20–30%), and chromosome X (20%) (table 1) (12).

Hairy cell leukemia (HCL)

HCL is an uncommon chronic LPD characterized by progressive bone marrow failure due to infiltrating malignant B cells with “hairy-like surface projections” provoking frequent infectious complications (13). HCL comprises the clonal hematologic malignancies of classical (cHCL) and variant (vHCL). The mutations present in each HCL subtype are distinct, with *BRAF*V600E mutations in 100% of cHCLs, whereas 30% of vHCLs harbor activating mutations in *MAP2K1*, encoding the MEK1 kinase just downstream of BRAF. Studies of diverse cancers marked by the *BRAF*V600E mutation suggest that additional alterations are frequently required for tumor initiation and/or progression in *BRAF*V600E-mutant cells. Durham et al. recently detected coexistence of *CDKN1B* and *BRAF*V600E mutations in 16% of cHCLs (14).

Mucosa-associated lymphoid tissue (MALT) lymphoma

MALT lymphomas are a diverse group of lymphoid neoplasms with B-cell origin, occurring in adult patients and usually having an indolent clinical behavior. These lymphomas may arise in different anatomic locations, sharing many clinicopathological characteristics, but

also having substantial variances in the aetiology and genetic alterations. MALT lymphomas can occur at any extranodal site. The most common anatomic sites are the stomach (30%), followed by eye/adnexa (12%), skin (10%), lung (9%), and salivary gland (7%). However, these lymphomas have been described at many other mucosal organs, such as thyroid, liver, small intestine, large intestine, bladder, dura, and many other sites. Chromosomal translocations are recurrent in MALT lymphomas with different prevalence among different sites, being the 4 most common: t(11;18)(q21;q21), t(1;14)(p22;q32), t(14;18)(q32;q21), and t(3;14)(p14.1;q32). All these translocations and their products target the activation of nuclear factor k-light-chain-enhancer of activated B-cells (NF-kB) pathway. MALT lymphomas have highly altered variable heavy chain immunoglobulin (*IGHV*) and variable light chain immunoglobulin (*IGLV*) genes. Beyond translocations, a spectrum of chromosomal numerical abnormalities has been described in MALT lymphomas. The most common numerical alterations found in MALT lymphomas are trisomy of chromosome 3 or 18, although the frequencies at which these trisomies occur vary markedly with the primary site of disease (15).

Multiple myeloma (MM)

MM is the second most common hematologic malignancy. Typical clinical symptoms of MM include bone destruction, hypercalcemia, renal failure, cytopenia, and immune paralysis. Symptomatic multiple myeloma can be preceded by 2 premalignant conditions called monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM), all of which share several genetic features. Translocations can be found in half of MGUS and MM patients. Most translocations involve the IgH locus (14q32), which puts oncogenes under the influence of the powerful IgH enhancer and thus result in upregulation. Translocations involving the immunoglobulin lambda (IgL) locus are present in 10% of patients with newly diagnosed MM and up to 20% in relapsed-refractory MM and are indicative of poor prognosis (table 2) (16). The most common result of IgH translocation is dysregulation of cyclin D (*CCND*). It involves t(11;14), t(12;14), and t(6;14). In about 50% of myeloma patients, mutations induce aberrant signaling in the MAPK/ERK pathway (*NRAS*, *KRAS*, *BRAF* and *EGR1*, and *FGFR3*). About 15% of MM patients show mutations affecting DNA repair pathways like *TP53*, *ATR*, *ATM*, and *ZFHX4* genes, which are associated with a shorter survival. Moreover, in about 20% of MM patients mutations involving the NFκB pathway can be detected. Most MM cases are aneuploid, in which there are frequent gains and losses of complete chromosomes or chromosome arms (17p, 1p, 13q, and 16q). According to the ploidy status, MM is usually categorized in hyperdiploid and non-hyperdiploid MM. The hyperdiploid (H-MM) group, which accounts for 50–60% of all MM cases, is characterized by the presence of trisomies that typically affect the odd chromosomes (17).

Myeloid neoplasms

Myeloid neoplasms are clonal hematopoietic proliferations representing a wide range of clinical, hematologic, genetic, and immunophenotypic properties and with a variable rate of genetic instability and clonal progression. Based on these properties, myeloid neoplasms are divided into 10 broad categories with more than 60 entities. Based on the complete blood count data (CBC) and blood smear morphology, myeloid neoplasms can be segregated into four broad disease categories – Acute myeloid leukemia (AML), Myelodysplastic syndrome (MDS), Myeloproliferative neoplasm (MPN), and Myelodysplastic/myeloproliferative neoplasm (MDS/MPN) (18).

Myeloproliferative neoplasms (MPN)

MPNs are characterized by an excessive production of terminally differentiated blood cells that are fully functional. All MPN entities arise from a single somatically mutated hemato-

poietic stem cell (HSC) that clonally expands and gives rise to virtually all myeloid cells, and B and natural killer (NK) cells. The clonal expansion of the MPN HSC is accompanied by single or multilineage hyperplasia. Somatic mutations are responsible for the clonal expansion of HSCs not only in MPNs, but also in most types of myeloid malignancies (19). Among MPNs, chronic myeloid leukemia is characterized by the presence of Philadelphia chromosome (Ph) resulting from the translocation between chromosomes 9 and 22 [t(9;22)(q34;q11)] leading to BCR/ABL1 gene fusion. The Ph-negative MPNs encompass 3 clinical subtypes: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). In contrast to chronic myeloid leukemia, disease-specific genetic abnormalities have not been detected that distinguish PV, ET, and PMF (20).

Chronic myeloid leukemia (CML)

The Philadelphia chromosome, originating from a balanced reciprocal translocation *t* (9; 22) (*q*34; *q*11), is present in more than 90% cases of CML. This translocation causes the fusion of the Abelson murine leukemia (*ABL*) proto-oncogene on chromosome 9 with the interrupted end of the breakpoint cluster region (*BCR*) of chromosome 22. The chimeric gene encodes a protein with a high tyrosine kinase activity which acts as a tumorigenic factor. These alterations results in an excessive production of granulocytes in the bone marrow causing both splenomegaly and hyperleukocytosis. In the course of CML progression, additional chromosomal abnormalities appear in particular during accelerated and blastic phase and can cause genetic instability. These abnormalities which are found in the Ph+ cells are classified into major and minor. Major pathway additional abnormalities include trisomy 8, additional Ph derivation (+der (22) *t* (9; 22), isochromosome 17 (*i* (17) (*q*10)), trisomy 19, and others. Minor pathway additional abnormalities is less common and not sufficiently studied. It includes aneuploidies −7, −17, +17, +21, and −Y and one balanced structural abnormality *t* (3; 21) (*q*26; *q*22). In patients with Ph− cells, additional abnormalities found could be a reciprocal translocation *t* (6; 9) (*p*21; *q*34.1), a chromosomal marker (+mar), a trisomy 8, and others. On the other hand, the absence of the Ph chromosome and the presence of −7 (monosomy 7) contribute to the evolution towards a myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) (21). The main group of treatment for CML consists of tyrosine kinase inhibitors (TKI), which represent targeted therapy. BCR-ABL kinase is present only in leukemic cells and its complete blockade by TKI leads to apoptosis. The unique genetic feature of the BCR-ABL gene allows the quantification of the treatment response by quantifying the expression of the fusion gene and analyzing the number of Ph chromosome-positive metaphases. Molecular response is assessed by qPCR based on assessment of the ratio of BCR-ABL to a control gene, most commonly ABL. If the BCR-ABL/ABL ratio is ≤ 0.1% International Scale (IS) (≥ 3 log reduction of BCR-ABL transcript) patient has achieved a major molecular response (MMR).

MR4 (≥ 4 log reduction; ≤ 0.01%)

MR4,5 (≥ 4 ,5 log reduction; ≤ 0.0032%)

MR5 (≥ 5 log reduction; ≤ 0.001%).

It is therefore important at the time of diagnosis to carry out an examination to determine the presence and determination of the amount of transcripts.

Ph- negative myeloproliferative neoplasms

A major characteristic of Ph-negative MPNs is an increased signaling through the Janus kinase (JAK) signal transducer and activator of transcription (STAT) pathway as well as through the phosphatidylinositol 3-kinase (PI3K)-AKT (also known as protein kinase B) pathway in erythroid and myeloid cells. The most significant evidence of molecular pathology was reported in 2005 with the identification of the somatic mutation *JAK2*-V617F. This mutation in *JAK2* exon 14 gene occurs in approximately 95% of patients with PV and about 60% of those with PMF and ET.

Somatic activating mutations in the MPL virus oncogene *(MPL)* were identified in patients with *JAK2*-nonmutated ET and PMF but not in patients with PV. The *MPL* gene is located on chromosome 1p34, encodes the thrombopoietin receptor and is a key factor for growth and survival of megakaryocytes. Acquired mutations at codon W515 constitutively activate the thrombopoietin receptor by cytokine-independent activation of the downstream JAK-STAT pathway. Recurrent pathogenic mutations include the common W515L and W515K and the rare W515A, W515R and W515S mutations. The 2 most recurrent mutations W515L and W515K are found in approximately 15% of *JAK2*-V617F-nonmutated MPN that is 5% of ET and up to 10% of PMF. Alternative mutations have also been reported in rare cases including V501A, S505C, A506T, V507I, G509C, L510P, R514K, and R519T, although the pathogenic significance of some of these mutations is not clear. The median overall survival of patients was approximately 9 years in both *MPL*-mutated and *JAK2*-mutated PMF (20).

Mutations in calreticulin (*CALR*) are also found in approximately 25–35% of patients with ET and 35–40% of those with MF (21). *CALR* is not known to have a direct role in cytokine signaling, hematopoiesis, or cell fate decisions, and therefore the mechanism(s) by which *CALR* mutations result in megakaryocytic proliferation and an ET/MF phenotype were not initially apparent (22). It is clear that the primary mechanism of *CALR*-driven transformation lies in its interaction with MPL, which triggers JAK2-dependent signaling pathways, although it remains to be seen whether mutant *CALR* may also act through other pathways, such as Ca^{2+} signaling or transcriptional regulation (table3) (23).

Myelodysplastic syndrome (MDS)

MDS are a heterogeneous group of hematopoietic precursor cell diseases with altered cell proliferation and maturation characterized by peripheral cytopenia due to ineffective hematopoiesis, dysplasia of one or more cell lineages, and an increased risk of transformation to acute myeloid leukemia (AML). The hallmark of MDS is bone marrow failure due to the growth of somatically mutated clonal hematopoietic stem cells (24). Karyotypic abnormalities are seen in approximately 30–50% of patients with MDS and correlated with prognosis. The most frequent cytogenetic abnormality in MDS is deletion 5q with frequency about 15%. Deletion 5q32-33 is frequently associated with 5q- syndrome and patients with this syndrome have a better overall survival and less risk of transformation to AML. But deletion 5q31is typically present in MDS that arose in connection with previous chemotherapy and has a more aggressive course with a high risk of progression to AML. Monosomy or deletion 7 is connected with a poor prognosis. Approximately 10% of MDS patients have an abnormality of the chromosome 7 either alone or as part of a complex karyotype. Abnormalities of the chromosome 7 occur in up to 50% of patients with MDS arising after the treatment with alkylating agents. Chromosome 7 abnormalities associated with 5q- or transcription factor *RUNX1* mutation are more frequent in these patients compared to other MDS patients, which points to a multistep process of MDS development. Other chromosomal abnormalities such as trisomy 8 and deletion 20 are frequently present in MDS patients. On the other hand, mutations are detectable by next generation sequencing (NGS) in more than 80% of patients with MDS with distinct mutation profiles observed in different MDS subtypes – *TP53, EZH2, ETV6, RUNX1, ASXL1* present in 3–14 % MDS patients connected with a poor prognosis. The mutations of genes that regulate mRNA splicing (*SF3B1,ZRSR2,ZRSF2*) occur in 45-85% of MDS patients. Their result is the synthesis of disturbed proteins, which are involved in the pathogenesis of MDS. With increasing knowledge and new diagnostic possibilities, primarily in the field of genetics, a revised international prognostic scoring system (IPPS-R) was created (table 4). The basis is cytogenetics, percentage representation of blasts and cytopenias (25).

Acute myeloid leukemias (AML)

AML is a heterogenous group of hematopoietic malignancies characterized by a proliferation of immature cells (blasts). Early classification systems were based on the morphologic features of these blasts, while in last two decades, the predominant classification systems by the World Health Organization (WHO) have increasingly incorporated immunophenotypic and genetic characteristics to refine these groupings. The 5th edition of the WHO classification evaluates three categories based on – (1) reccurent genetic abnormalities, (2) myelodysplasia-associated genetic changes, and (3) germline predisposition (26,27).

AML is a lethal disease and has a 5-year relative survival rate of 24.2%. However, the outcomes are heterogenous and the overall survival rates range from 5% to 70%. Thus, a need exists for prognostive markers to predict outcomes and guide therapeutic decision-making. The strongest prognostic factor for predicting therapeutic response and survival is cytogenetic subgrouping. Risk groups in AML were classified into three cathegories according to the 2022 European Leukemia Net (ELN) risk stratification based on genetics (Table 5) (28). Translocations t(15;17), t(8;21), inv16/ t(16;16), a normal karyotype and mutated *NPM*, or a normal karyotype with biallelic *CEBPA* mutations present favorable risk with a 5-year survival rate of 50-80%. But aberations like MLL, inv3, t(6;9), -7/del(7q), -5/del5q, *TP53* deletions and a complex karyotype are of adverse risk with an overall survival rate of 5-20%.

Molecular testing for t(15;17), *FLT3, NPM,* and *CEBPA* is informative and has therapeutic implications. Translocation between choromosomes 15 and 17 involves the *PML* gene and the retinoic acid receptor gene (*RARA*). The resulting fusion protein, PML-RAR-α is oncogene that is typical for acute promyleocytic leukemia (APL). Identification of this genetic alteration led to development of the therapy that specifically target aberrant cells. All-trans retinoic acid (ATRA) binds to fusion protein inside cells and blocks its function. In *FLT3*-mutated AML, midostaurin is added to intensive chemotherapy. Menin inhibitors are being evaluated as a treatment option for patients with *KMT2A* rearrangements or *NMP1* mutations (2, 26).

CONCLUSION

Most hematologic malignancies are clonal neoplasms and have specific somatic genetic and molecular characteristic which may influence therapeutic response and prognosis. The molecular basis of each tumor type is becoming essential to the diagnosis, in addition to determining therapy and prognosis. Understanding of the molecular pathogenesis of the diseases has improved, new therapeutic approaches have become crucial. Personalized therapeutic approaches assume prominence, emphasizing the need for tailored interventions based on individual patient characteristics. Incorporating cytogenetic changes alongise othe prognostic factors becomes crucial in determining the optimal treatment strategy. With expanding and increasing use of NGS panels, not just for detection of diagnostic gene mutations but also for detection of clonal lymfoid populations, chromosomal fusions, or minimal residual disease.

Table 1 Recurrent mutations and genetic alterations in mature lymphoid neoplasms

BNHL	Molecular alterations	Risk stratification
B-CLL/SLL	del 13q, 11q, 17p13, 6q21, trisomy 12	**Very high-risk disease:** 17p deletion and/or *TP53* mutations **High-risk disease:** *IGHV* unmutated (without 17p deletion and *TP53* mutation) **Standard-risk disease:** *IGHV* mutated (without 17p deletion and *TP53* mutation) (5,6)
Burkitt lymphoma	translocation at 8q24 (*MYC*) with 14q32(*IGH*); translocations at 22q11(*IGL*) or 2p12 (*IGK*)	*MYC*_translocation, including deletion of 13q, a gain of 7q, or complex cytogenetics may portend a worse prognosis Double hit mutations in *ID3*, *CCND3*, and mutations in 18q21 CN-LOH indicate a poor response to therapy and poor prognosis (29)
DLBCL	rearrangements *of IGH, IGK, IGL,MYC*, 3q27, t(14;18)	16q22-q24, 6p21-p25, 12q22-q24, 11q23-q25, 19q13, 1q21-q23, 8q24, and 19p13, and -17 appeared to be associated with a worse prognosis (30)
Follicular lymphoma	t(14;18), abnormalities *BCL6* and 3q27	deletions of 1p, 6q, and 17p, and gains of 7 and 12q are strongly associated with a poor prognosis also correlate with a higher risk of transformation (31)
Hairy cell leukemia	*BRAF* V600E	
MALT lymphoma	t(11;18), t(14;18)(q32;q21), t(3;14)	
Mantle cell lymphoma	t(11;14)(q13;q32)	**Cluster C1—Best prognosis** mutated IGHV, *CCND1* and *TP53*, amplification of 11q13, and active BCR signaling ***Cluster C2***—deletion of 11q, *ATM* mutations, upregulated TNF-α, NF-kB, and DNA repair pathways ***Cluster C3***—mutations in *NOTCH1*, *NSD2*, *SP140*, and *KMT2D*; amplification of 13q; deletion of 6q; and downregulated TNF-α, NF-kB, BCR signaling, and MYC target pathways ***Cluster C4*—Worst prognosis** deletion of 13q, 17p/*TP53*, and 9p; *TP53* mutations, complex copy number abnormalities; upregulated MYC pathways (11)

Abbreviations: del – deletion, DLBCL – diffuse large B-cell lymphoma, IGH – immunoglobulin heavy chain, IGK – kappa gene, IGL – light chain gene, t – translocation

Table 2 Recurrent mutations and genetic alterations in multiple myeloma (16, 17).

Genetic abnormalities	Affected genes	Frequency	Prognosis
t(4;14)	*FGFR3, MMSET*	11–15%	High risk
t(6;14)	*CCND3*	1–2%	Standard risk
t(11;14)	*CCND1*	15%	Intedmerdiate risk
t(14;16)	*MAF*	3–5%	High risk
t(14;20)	*MAFB*	1%	High risk
Del 1q	*FAM46C, CDC14A, MTF2, CDKN2C*	30%	Shorter survival
Gain of 1q	*CKS1B, MUC1, MCL1, ANP32E, BCL9, PSMD, PDZK1*	50% NDMM	Poor prognosis
Del 13q	*RB1*	45%	del(13q) – independent favorable impact on OS monosomy 13 – shorter OS
Del 17p	*TP53*	5–12% NDMM	Shorter survival
KRAS		~ 50%	Neutral
NRAS		~ 50%	Worse outcome
BRAF		~ 50%	Negative influence on survival
EGR1		~ 50%	Favorable effect on outcomes

Abbreviations: del – deletion, NDMM - newly diagnosed MM; OS – overall survival; t - translocation

Table 3 Recurrent mutations and genetic alterations in Ph negative myeloproliferative neoplasms (19)

Gene	Location	Mutation	Protein function	Frequency	Consequence
JAK2	9p24	*JAK2*V617F	tyrosine kinase associated with cytokine receptors	95% PV 50–60% PMF 50–60% ET	increased RBC, WBC, PLT production
		JAK2 exon 12		3% PV	
MPL	1p34	MPL515L/K/A/R MPLS505N	TPOR	2–3% ET	increased PLT production
		other missense mutations		3–5% PMF	
CALR	19p13	indel exon 9	Mutant: activator MPL	20–25% ET 25–30% PMF	increased PLT production

Abbreviations: RBC – red blood cells, ET – Essential thrombocytemia, PLT – platelets, PMF – Primary myelofibrosis, PV – Polycytemia vera, TPOR – trombopoietin receptor, WBC – white blood cells

Table 4 Recurrent mutations and genetic alterations in Myelodysplastic syndrome (24,25)

Cytogenetic alterations	Risk category
del(11q), -Y	Very good
del(5q), del(12p), del(20q), double including del(5q), normal karyotype	Good
del(7q), + 8, + 19, isochromosome i(17q), any other single or double independent clones	Intermediate
-7, inv(3)/t(3q)/del(3q), double including – 7/del(7q), complex karyotype: > 3 alterations	Poor
complex karyotype: > 3 alterations	Very poor
Biallelic *TP53*	Very poor
SF3B1	Favorable risk
EZH2, ASXL1	Lower risk
RUNX1, NRAS	Associated with chromosome 7 abnormalities

Abbreviations: del – deletion, inv – inversion, t – translocation

Table 5 Risk groups in AML according to the European Leukemia Net risk stratification

Favorable risk	Intermediate risk	Adverse risk
t(8;21)(q22;q22.1)/ *RUNX1::RUNX1T1*	*FLT-ITD* (regardless of allelic ratio or *NPM1* mutation	t(6;9)(p23;q34.1)/ *DEK::NUP214*
inv(16)(p13.1;q22)	t(9;11)(p21.3;q23.3)/ *MLLT3::KMT2A*	t(v;11q23.3/*KMT2A* rearranged
t(16;16)(p13.1;q22)/ *CBFB::MYH1*	cytogenetic and/or molecular abnormalities not classified as favorable or adverse	t(9;22)(q34.1;q11.2)/ *BCR::ABL1*
mutated *NPM1* without *FLT3-ITD*		(8;16)(p11;p13)/ *KAT6A:CREBBP*
bZIP in-frame mutated *CEBPA*		inv(3)(q21.3;q26.2) or t(3;3)(q21.3;q26.2)/ *GATA2,MECOM (EVI1)*
		t(3q26.2;v) *MECOM (EVI1)* rearranged
		monosomy 5 or del(5q)
		monosomy 7
		monosomy 17/abn(17p)
		complex karyotype > 3 unrelated chromosomal abnormalities
		mutated *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1* or *ZRSF2*
		mutated *TP53* (variant allele frequency ≥ 10%)

(26)

Abbreviations: abn – abnormal, del – deletion, inv – inversion, t – translocation

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