Genetics in Hematologic Disorders: Implications of Recurring Chromosome Abnormalities and Gene Mutations

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Introduction

Hematologic malignancies are characterized by numerous chromosome abnormalities, including translocations and inversions, gains and losses, deletions and duplications, and various genomic imbalances and gene mutations. Conventional chromosome analysis, fluorescence in situ hybridization (FISH) test, genomic microarray, and next-generation sequencing (NGS) are the specific techniques used to detect disease-related chromosome aberrations, genomic imbalances, and gene mutations. Many chromosome abnormalities are closely associated, and sometimes uniquely, with morphologically and clinically distinct subtypes of leukemia and lymphoma, and are one of the most reliable predictors of disease prognosis. The detection of these chromosome abnormalities assists not only in establishing diagnosis but also provides important prognostic information and risk stratification and helps decide efficient treatment based on genetic targets, as well as monitor treatment response and disease progression [1, 2].

Techniques

Chromosome abnormalities and gene mutations may be detected by conventional chromosome analysis, FISH, genomic microarray, and NGS techniques. Each technique has special requirements in experiments and various advantages and limitations of quality and applications (Table 23.1).

Table 23.1 Comparison of cytogenetics, FISH, microarray, and next-generation sequencing techniques

Techniques	Cytogenetics	FISH	Microarray	NGS
Resolution	+	++	+++	++++
Sensitivity	+	+++	++	++
Copy neutral LOH	–	–	+++	+/–
Cell division	+	–	–	–
Balanced translocations/ inversions	+	+	–	+
Multiple clones	+	–/+	–	+/–
Screen for novel lesions	+	–	+	+

Chromosomal Analysis (Karyotyping)

Chromosomal analysis requires fresh and viable cells from bone marrow aspirate, peripheral blood, lymph nodes, or tumor tissues. It starts with short-term cultures, followed by arresting cells at the metaphase phase of the cell cycle, hypotonic treatment and fixation, dropping and banding metaphase cells, and careful analysis for numerical and structural chromosome abnormalities. Acute leukemia cells often grow actively and divide spontaneously in short-term culturing. For low-grade B- or T-cell leukemia or lymphoma, specific mitogens, such as PHA or pokeweed, and IL-2/CpG oligonucleotide may be used in promoting cells to grow and divide in cell culturing. *Advantages:* (1) Whole genome level analysis to check all chromosome abnormalities, which may identify novel chromosome aberrations. (2) Precise analysis at single cell level to reveal clonal evolution and heterogenicity. (3) Chromosome abnormalities observed may reflect tumor cell proliferation and biology. *Limitations:* (1) Analysis at low-level chromosome band resolution. (2) The analysis success heavily relies upon cell division and thus is not feasible in specimens with non-dividing, or fixed cells. (3) It requires extensive skills and knowledge in analysis and high chromosome preparation quality.

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Fluorescence In Situ Hybridization (FISH) Testing

FISH test uses specific DNA probes to detect various chromosome and gene aberrations, including numerical and structural chromosome abnormalities, such as translocation, inversion, deletion and gain, amplification, etc., in a large number of metaphase and interphase cells as well as tissue sections. In comparison with chromosome analysis, FISH technique has a high sensitivity, specificity, and resolution.

Two main types of FISH probes are routinely used in clinical services. (1) Centromere-specific probes can reveal chromosome gain or loss, such as −7 in AML and MDS and +12 in CLL. (2) Locus-specific probes, such as dual fusion probes for *BCR* and *ABL1* to detect t(9;22) in CML and B-cell ALL or *MLL* (now called *KMT2A*) break-apart probes for various 11q23 translocations in leukemia, such as t(4;11) in B-ALL and t(9;11) in AML. A dual fusion set with two probes for both involved genes allows a precise detection of specific chromosome translocations or inversion, whereas a break-apart probe set with two probes that cover the 5′ and 3′ regions of the relevant gene locus, respectively, shows all translocations with a breakpoint region franking by the probes but cannot determine partner chromosome or genes involved.

Advantages: (1) It is relatively straightforward in procedure and analysis and generally has a quick turnaround time of 1–2 days. (2) FISH can be applied to both dividing (metaphase) and nondividing (interphase) cells; therefore it is suitable in diseases with low mitotic index, such as CLL, myeloma, and in tissue preparations, cytospin prep, peripheral blood smear, touch prep, and formalin-fixed and paraffin-embedded (FFPE) tissue section. (3) FISH analysis is at single-cell level and thus may be combined with immunophenotyping or morphological assessment and allows simultaneous visualization of abnormal FISH signal patterns and cell lineage features, which is particularly helpful in patients with coexistence of lymphoid and myeloid neoplasm, such as CLL and MDS in elderly patients. (4) FISH may be applied to enriched or sorted cell population by flow cytometry techniques, such as multiple myeloma, various lymphomas, or myeloid populations. This significantly increases the sensitivity of FISH tests, allowing determining minimal residual disease status precisely, as well as in lymph nodes or other FFPE tissues with scattered tumor cells. *Limitations:* (1) It applies only to the targeted regions and genes where probes bind to, and thus, it is a targeted test, not whole genome analysis. (2) Due to relatively large probe sizes, FISH may not detect small deletions or inversion within the franking regions.

Genomic Microarray Testing

Advantages: Microarray technique employs thousand to million probes over the entire genome to detect genomic imbalances, such as gain or loss of relevant chromosome regions in tumor cells. Genomic microarray with single nucleotide polymorphism (SNP) probes also reveals copy-neutral loss of heterozygosity (CN-LOH). Microarray tests are particularly useful in revealing whole genomic abnormalities and CN-LOH features in solid tumors in FFPE preparations or in those without dividing cells. The analysis also defines a precise size of deletion or gain region and genes involved. *Limitations:* (1) Balanced chromosome translocations, inversions, or insertions cannot be detected. (2) The sensitivity of the test is approximately only 10–20%; therefore, it cannot reproducibly detect small clones below 20% of tumor content in a specimen.

Next-Generation Sequencing (NGS) Techniques

NGS analysis of targeted gene fusions and mutations rapidly becomes available for routine clinical diagnostic service of leukemia and lymphoma and allows better risk stratification and selection of targeted therapies. *Advantages*: (1) It may precisely detect recurring leukemia-specific gene fusion transcripts or (2) identify novel gene fusions that define specific leukemia entities, such as BCR-ABL1-like B-cell acute lymphoblastic leukemia. (3) It has higher sensitivity and is more comprehensive than single-cell mutations tests by traditional Sanger sequencing and thus may apply for detecting minimal residual disease and the analysis of multiple gene mutations in a panel reaction. *Limitations*: (1) It requires specific and expensive sequencing equipment; (2) data analysis and interpretation heavily depend on bioinformatic pipelines and experience; (3) copy number gain or loss cannot be definitely detected, partly depending on tumor content, such as 20% or above.

Selection of Karyotyping, FISH, and/or Microarray Tests in Clinical Diagnostics

Selection of one or more of these tests above depends on disease types, disease status, specific targets, and history of previous analysis results. The goal is to appropriately use one or more tests to maximally detect and clarify chromosome abnormalities that are critical for diagnosis, prognosis, and monitoring treatment responses and, at the same time, to avoid redundant or non-informative follow-up tests and cost.

Chromosome analysis should be performed as initial screening for chromosome abnormalities in all newly diagnosed or suspected acute and chronic myeloid neoplasia and in acute lymphoblastic leukemia. FISH tests at the front line are helpful in certain diseases with specific diagnostic or prognostic targets, such as FISH tests for t(9;22) in CML and B-ALL and for t(15;17) in APL [3]. FISH is critical in leukemia with recurring cryptic chromosome abnormalities, such as *CHIC2* deletion/*PDGFRA* in hematological neoplasia with eosinophilia and t(12;21) *ETV6/RUNX1* fusion in B-ALL. FISH may be necessary to clarify specific chromosome abnormalities in samples with suboptimal chromosome quality and incomplete chromosome analysis. For lymphoma patients, FISH for specific targets would be more helpful than chromosome analysis in determining a precise diagnosis and prognosis, such as t(11;14) in suspected MCL, deletion of 11q (*ATM*) and 17p(*TP53*) in CLL panel, as well as *MYC* translocations in high-grade B-cell lymphomas [4].

For follow-up samples with residual disease or in remission, FISH for the known chromosome abnormalities from a diagnostic specimen is more sensitive and objective than chromosome analysis in determining the clone size. FISH is also very precise in determining engraftment status in patients who received sex-mismatched allogeneic hematopoietic cell transplantation. Chromosome analysis should be repeated in all samples with relapse or in progression in order to analyze all chromosomes for potential clonal evolution and novel chromosome abnormalities.

Microarray tests will provide a high resolution of whole genomic level detection and therefore is particularly useful for those diseases with recurring chromosome gain or loss, such as MDS, CLL, and myeloma, and may replace multiple FISH tests in these diseases. However, FISH may be critical to detect an early small clone of certain targets that are significant in prognosis, such as *TP53* deletion in myeloma and CLL. In addition, microarray test cannot detect chromosome translocations and inversions that are detectable by chromosome analysis, FISH or reverse PCR, or NGS tests.

Significance of Detection of Cytogenetic Abnormalities

The detection of specific chromosome translocations and gene fusions helps establish a precise diagnosis of leukemia and lymphoma, and the detection of recurring gene mutations in hematological neoplasia provides important prognostic information and guides the selection of targeted genetic-based therapies [1, 3, 4, 5].

(a) Establish a specific diagnosis, such as t(9;22) in CML, t(15;17) in APL, and t(11;14) in mantle cell lymphoma.
(b) Determine disease prognosis, i.e., t(8;21), inv(16)/t(16;16) and t(15;17), and t(12;21), and hyperdiploid clones are associated with favorable prognosis AML and B-cell ALL, respectively, whereas complex karyotype, particularly with loss of chromosome 17 or deletion of *TP53*, is indicative of poor prognosis in AML, and t(9;22) is a strong predictor of high risk in B-ALL.
(c) Monitor treatment response and disease progression when new chromosome abnormalities or clonal heterogeneity and subclones occur. FISH analysis provides a quick and precise engraftment status in patients after sex-mismatched stem cell transplant.
(d) Help select appropriate treatment options based on genetic abnormalities. Certain chromosomal changes may predict for response (or nonresponse) to specific therapies, i.e., tyrosine kinase inhibitors, such as imatinib in CML with t(9;22) and early treatment with ATAR for patients with APL and t(15;17).
(e) Understand the pathogenesis of the disease and identify genes that are involved in leukemogenesis.

Understanding the Cytogenetic Report

Definition of Clonality According to the International System for Human Cytogenetic Nomenclature (ISCN), [6] an abnormal clone is established with (1) two or more cells with the same structural rearrangement, such as translocations, deletions, or inversions or with gain of the same chromosome or (2) three cells with loss of the same chromosome. However, a single cell with a known abnormality from previous samples, or confirmed by a second test, such as FISH, also confirms the presence of an abnormal clone.

Commonly Used Cytogenetic Terms and Definition (ISCN) A karyotype is described first by the total number of chromosomes and the sex chromosome component, followed by abnormalities of autosomal chromosomes listed in numerical order irrespective of aberration type. Chromosome abnormalities are written in order of the involved chromosomes in the first set of parentheses and the breakpoints in a second set, followed by the total number of the analyzed cells with the clone. A typical karyotype in a male patient with CML is written as 46,XY,t(9;22)(q34.1;q11.2)[20]. The most commonly used terms in conventional chromosome studies are listed in Table 23.2 with explanation and examples of abnormalities.

In addition, the interpretation of chromosome abnormalities and their correlation with morphology and association with prognosis is important. Complex karyotype and monosomal karyotypes are associated with unfavorable

Table 23.2 Common cytogenetic and FISH karyotype terms and definitions according to the International System of Human Cytogenetic Nomenclature (2016) [6]

Terms/ symbols	Definitions	Examples
+ or −	Gain or loss of chromosomes	+12, −7
add	Addition of unknown material	add(5)(q31)
del	Deletion of chromosome material	del(13)(q12q14)
der	Derivative chromosome of structural abnormalities	Der(7)add(7)(p11.2)del(7)(q11.2q32)
dic	Dicentric chromosome	Dic(5;17)(q11.2;p11.2)
dup	Duplication of chromosome bands	dup(1)(q12q41)
idem	Identical to the stem clone	46,XY,t(9;22)(q34;q11.2)[2]/47,idem,+8[18]
i	Isochromosome	i(17)(q10)
inv	Inversion within chromosome arm or both arms	Inv(16)(p13.1q22), inv(3)(q21q26.2)
ins	Insertion	Ins(12;4)(q13;q21q31)
mar	Marker chromosome	mar1, mar2
r	Ring chromosome	r(7)(p11.2q22)
t	Translocation of two or more chromosomes	t(9;22), t(11;14)
cp	Composite karyotype of multiple various aberrations (not all cells have all abnormalities)	47~49,XY,+8,+11,+18[cp5]
nuc ish	Interphase FISH test	Nuc ish (ABL1,BCR)x3 (ABL1 con BCRx2)[190/200] i.e., t(9;22) in CML
sep	Separation of break-apart probes	(MLLx2)(5′MLL sep 3′MLLx1)[40/200] i.e., 11q23/MLL translocation positive
con	Signal fusion	See above

prognosis in AML and MDS, more often in therapy-related myeloid neoplasia. The definition of complex karyotype refers to clones with three or more independent chromosome abnormalities; these complex clones often carry marker chromosomes with unknown origin of the centromere, ring chromosome, and derivative chromosome with complex abnormalities. A monosomal karyotype is defined as loss of at least two autosomal chromosomes or loss of one autosomal chromosome and one or more structural chromosomal abnormalities, excluding ring and marker chromosomes.

The chapter below describes the most common chromosome abnormalities and gene mutations in various hematological diseases and discusses their significance in disease diagnosis and biology and impact in assessing prognosis and therapeutic choices.

Table 23.3 Recurring chromosome abnormalities that define unique chronic myeloid neoplasia

Chromosome abnormalities	Genes involved	MPN
t(9;22)(q34;q11.2)	BCR/ABL1	CML
t(5;12)(q33;p13)	PDGFRB/ ETV6	CMML
−7	?	JMML
del(4q12)/t(4q)	FIP1L1/ PDGFRA	Myeloid and lymphoid neoplasia with eosinophilia
t(5q32), multiple	PDGFRB	
t(8p11.2), multiple	FGFR1	
t(8;9)(p22;p24.1)	PCM1/JAK2	

CML chronic myeloid leukemia, *CMML* chronic myelomonocytic leukemia, *JMML* juvenile myelomonocytic leukemia

Chronic Myeloid Leukemia (CML) and t(9;22)/ Philadelphia Chromosome

CML is the best model for our understanding of the mechanisms of genetic abnormalities in leukemogenesis. CML was the first cancer to be associated with a recurring chromosome abnormality, i.e., Philadelphia chromosome in 1960 [7], and later defined as t(9;22) in 1973 [8]. CML was also the first disease that was found to result in a novel *BCR/ABL1* fusion gene at molecular level in the early 1980s [9]. Furthermore, CML serves as the first cancer with an efficient genetic-based treatment that directly targets the genes responsible for the pathogenesis of the disease, i.e., imatinib in1996 [10].

The t(9;22) is the hallmark of CML, and results in the *BCR/ABL1* fusions, and is detectable by chromosome analysis in >90% of CML patients (Table 23.3) [5]. In the remaining 2–10% of patients with CML, a variant of the t(9;22), including three-way translocations, is observed. In some rare cases, the insertion of the *BCR* gene into the *ABL1* gene or vice versa occurs and can be detected only by FISH or molecular tests. The *BCR/ABL1* fusion is a constitutively active tyrosine kinase, which is implicated in the origin of CML and is the primary genetic target for tyrosine kinase inhibitor, such as imatinib.

When CML progresses, additional chromosome abnormalities, including gain of Ph chromosome, +8, i(17q), and +19, are most common. The presence of these additional chromosome abnormalities is considered CML in acceleration. The t(3;21) and inv(3q)/t(3;3) are also frequently observed in CML in progression that results in the overexpression of the EVI1 gene (3q26.2). With imatinib and other TK inhibitors as the frontline drugs, the outcome of CML has been significantly improved. Chromosome analysis, FISH tests, and qualitative PCR for monitoring the t(9;22) and BCR/ABL1 transcript level remain critical in CML.

Table 23.4 Recurring chromosome abnormalities defining unique AML according to WHO 2016 classification and their prognostic significance

Chromosome abnormalities	Genes involved	Clinical prognosis
t(8;21)(q22;q22)	RUNX1 (AML1)/ RUNX1T1 (ETO)	Favorable
inv(16)(p13.1q22)/t(16;16)(p13.1;q22)	CBFB/MYH11	Favorable
t(15;17)(q24;q21)	PML/RARA	Favorable
t(9;11)(p22;q23)	KMT2A (MLL)/ MLLT3 (AF9)	Intermediate
t(6;9)(p23;q34)	DEK/NUP214	Poor
inv(3)(q21q26.2)/t(3;3)(q21;q26.2)	GATA2, MECOM	Poor
t(1;22)(p13;q13)	RBM15/MKL1	Poor
t(9;22)(q34;q11.2)	BCR/ABL1	Poor

Table 23.5 Prognostic significance of recurring chromosome abnormalities in AML according to Grimwade et al. [11]

Favorable (10%)
t(15;17)
t(8;21)
inv(16)/t(16;16)
Intermediate (70%)
Normal karyotype (40%)
t(9;11)
Other entities not as favorable or adverse
Adverse (20%)
Inv(3)/t(3;3), other 3q abnormalities (excluding t(3;5)),
add(5q)/del(5q)/−5,
add(7q)/del(7q)/−7,
add(17p)/del(17p)/−17,
11q23 (MLL) translocations, including t(6;11), t(10;11),
t(9;22),
Complex karyotype (three or more unrelated chromosome abnormalities)

Acute Myeloid Leukemia (AML)

With combination of morphology, immunophenotype, genetics, and clinical features, many subtypes of acute and chronic leukemias are classified in the WHO classification system (Table 23.4) [3]. Chromosomal abnormalities are detectable in 50–60% of de novo AML and up to 92% of therapy-related myeloid neoplasia. t(15;17), t(8;21), inv(16)/t(16;16), and 11q23/*MLL*(*KMT2A*) translocations are unique chromosome translocations and inversions in AML, whereas deletion or loss of chromosomes 5, 7, 17, +8, del(20q), del(9q), and del(12p) are the most common recurring chromosomal deletions and gains. In addition, t(6;9), inv(3)/t(3;3), t(1;22), and t(9;22) are also included in the WHO classification system as AML with recurrent genetic abnormalities. Detection of t(8;21), inv(16)/t(16;16), and t(15;17) establishes a diagnosis of AML with these unique chromosome abnormalities, regardless the blast cell percentage in the bone marrow or peripheral blood specimens. These balanced translocations or inversions often result in a chimeric fusion gene encoding a novel protein that participates in leukemogenesis of AML and are associated with various prognoses (Table 23.5) [11].

CBF Leukemia with t(8;21)or inv(16)/t(16;16)

The core-binding factor (CBF) leukemias are defined as AML with either t(8;21) or inv(16)/t(16;16) that involves the core-binding factor (CBF) unit A (*RUNX1/AML1*) and unit B (*CBFB*), respectively. The core-binding transcription factor (CBF) complex binds directly to an enhancer core motif at the transcriptional regulatory regions of several genes, including IL3, GM-CSF, the CSF1 receptor, myeloperoxidase, and neutrophil elastase. These genes are critical to hematopoietic stem and progenitor cell growth, differentiation, and function.

The t(8;21) is one of the most common chromosome translocations in children and adult patient with AML. This translocation results in a chimeric fusion of the *RUNX1* (also called *AML1*) gene (21q22) with the *RUNX1T1 (ETO)* gene (8q22) (Fig. 23.1). The RUNX1 protein is one of the transcription factor family members with homology to the pair-rule Drosophila gene runt.

inv(16) or t(16;16) is another recurring chromosome abnormality in AML often associated with monocytic and granulocytic differentiation and with abnormal eosinophils in the bone marrow. The inversion results in a novel chimeric fusion between the *CBFB* gene at 16q22, and the *MYH11* gene at 16p13.1 (Fig. 23.2). Both novel fusion proteins resulting from t(8;21) or inv(16)/t(16;16) disrupt the normal function of the CBF complex and result in the repression of transcription.

CBF leukemia is generally associated with a favorable prognosis with high remission and long-term survival. In AML with t(8;21), loss of a sex chromosome (−X or −Y) or a del(9q) is frequently observed as secondary chromosome abnormality with no significant impact on the favorable prognosis. In AML with inv(16)/t(16;16), gain of chromosome 22 is frequent and may be associated with a more favorable prognosis than those with inv(16)/t(16;16) only. However, C-KIT mutations are not rare in CBF AML and may lead to less favorable outcome.

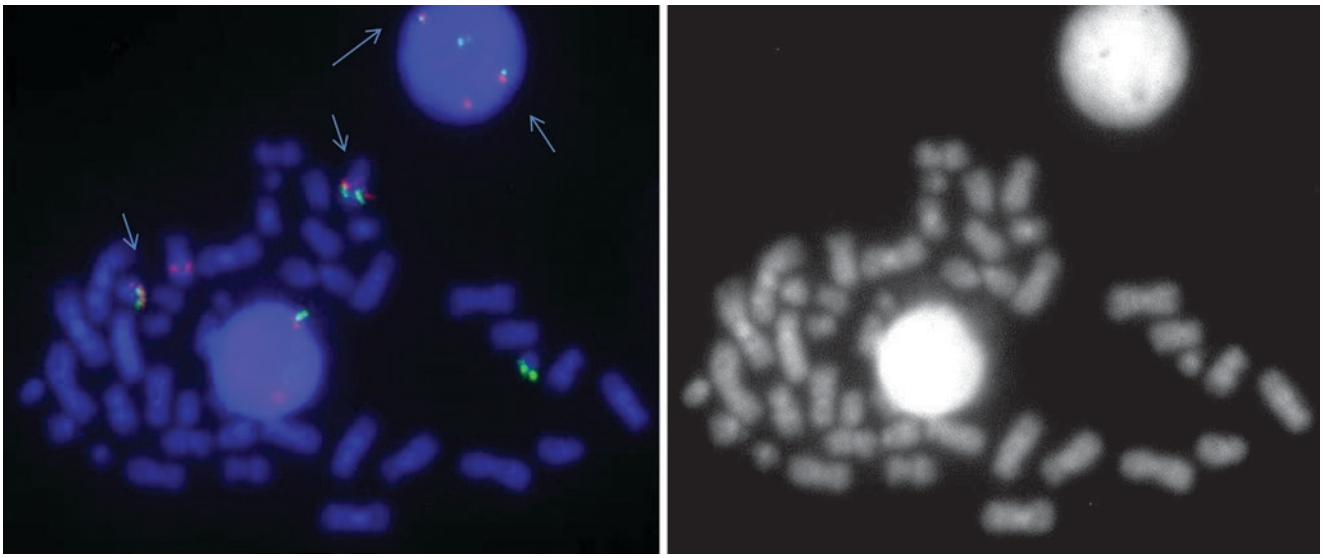


Fig. 23.1 FISH analysis confirms t(8;21) that results in the RUNX1/RUNX1T1 fusion in a patient with AML and t(8;21). FISH analysis employs dual color dual fusion probes with RUNX1 (21q22, labeled in green) and RUNX1T1 (ETO, 8q22, labeled in orange) and shows two fusions (arrow) on the derivative chromosomes 8 and 22, respectively. Normal single orange (RUNX1T1) and green (RUNX1) signals reside on normal chromosomes 8 and 21, respectively. The dual fusion signals are observed in interphase cells, too (arrows). The DAPI counterstaining (right) displays individual chromosomes concisely

APL with t(15;17)

t(15;17), the hallmark of APL, results in a fusion gene of the alpha retinoic acid receptor gene (*RARA, 17q21*) and the *PML* gene (15q24). The PML/RARA fusion has low sensitivity to retinoic acid in dissociation of a ubiquitous nuclear protein, i.e., N-CoR, which mediates transcriptional repression and thus leads to persistent transcriptional repression and prevents promyelocyte differentiation. This transcriptional repression can be efficiently overcome by treatment with ATRA, which leads to terminal differentiation of promyelocytes. With early treatment of ATRA, patients with APL have a very favorable prognosis.

There are several variant chromosome translocations of *RARA* in APL, such as the t(5;17) and t(11;17). APL with t(11;17) *PLZF/RARA* fusion does not respond to ATRA. Because APL is life-threatening due to high risk of hemorrhage, an early confirmation of APL is critical by rush FISH analysis within 2–4 h.

t(9;11) and Other KMT2A(MLL)11q23 Translocations

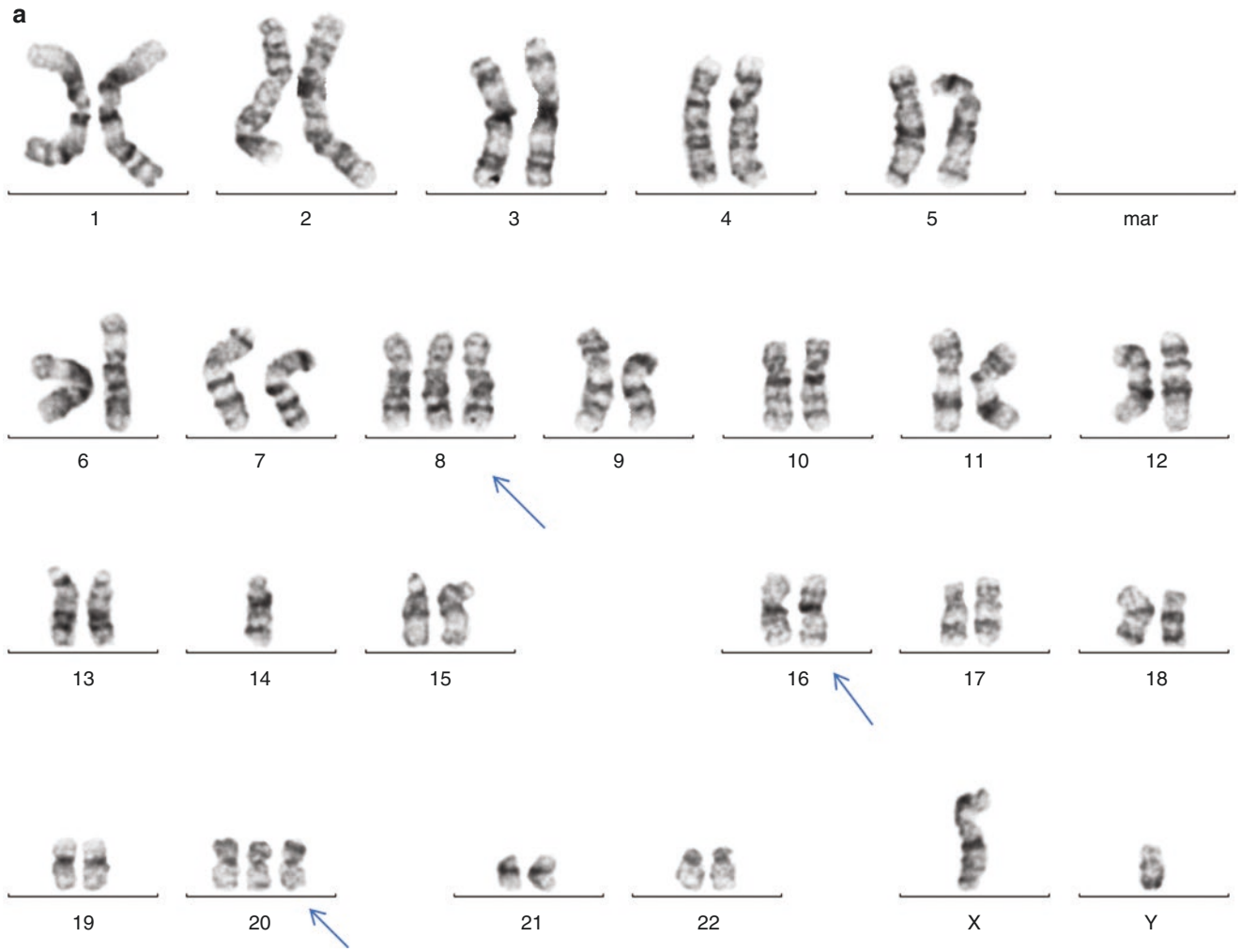
11q23/*KMT2A (MLL)* translocations are among most common chromosome translocations in both AML and ALL. So far, about 200 *KMT2A/MLL* fusion partner genes have been identified. In AML, t(9;11) and other MLL translocations are often associated with monocytic or monoblastic morphology (M5).

These translocations consistently disrupt the *KMT2A* gene (lysine [K]-specific methyltransferase 2A), which contains multiple functional domains, such as AT-hook DNA-binding motif, repression domain, and transcriptional activation domain. The protein has homology to the Drosophila trithorax gene, which regulates embryonic development and tissue differentiation. KMT2A methylates histone H3 lysine 4 (H3K4) and positively regulates expression of various *HOX* genes that are important in hematopoietic and lymphoid cell development. In *MLL* fusion gene, the activation domain at the 3′ region was replaced by the partner gene, thus, leading to loss of the H3K4 methyltransferase activity [12].

t(9;11) is associated with an intermediate prognosis in AML. All other 11q23/*KMT2A* translocations are associated with a poor prognosis in AML, such as t(6;11), t(10;11), and t(11;19). Notably, partial tandem duplication (PTD) of the *KMT2A* gene occurs in up to 90% of AML with +11 and is associated with poor prognosis in AML.

inv(3)/t(3;3)/MECOM/EVI1 Fusion

inv(3) or its variant t(3;3) are relatively rare in both AML and MDS, including therapy-related myeloid neoplasia, and are often associated with thrombocytosis and micromegakaryocytes [3, 13]. It results in the relocation of a distal *GATA2* enhancer closer to *MECOM/EVI1*, which activates *MECOM/EVI1* expression and simultaneously confers *GATA2* functional haploinsufficiency [14, 15]. The *MECOM* gene is a



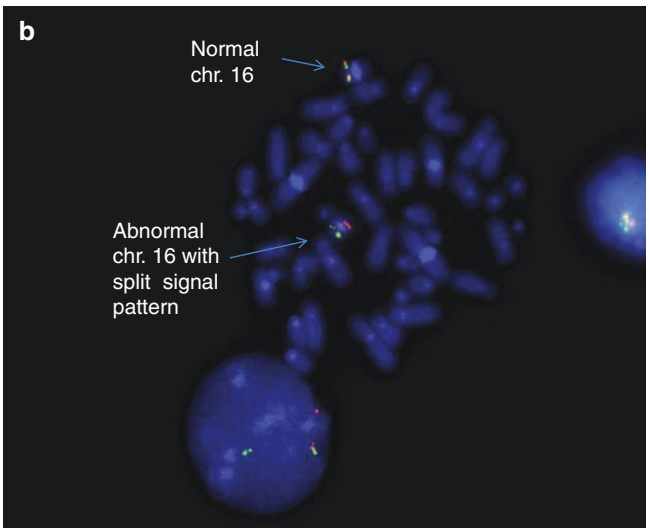


Fig. 23.2 inv(16)/CBFB/MYH11 fusion in a male patient with newly diagnosed AML detected by conventional chromosome analysis, FISH, and NGS fusion tests. (**a**) Karyogram shows a leukemia cell with inv(16) and gain of chromosomes 8 and 20 (arrows). The karyotype of all 20 metaphase cells is written as 48,XY,+8,inv(16)(p13q22),+20[20]. (**b**) FISH analysis using CBFB break-apart probes reveals an abnormal signal pattern with one intact fusion on the normal chromosome 16 and a split signal pattern on the derivative chromosome 16 (arrows). The probe franking the 5′ CBFB is labeled with orange, and the probe covering the 3′ CBFB is labeled with green. A split signal pattern was observed in an interphase cell, too. (**c**) Next-generation sequencing (NGS) of leukemia fusion studies confirms the presence of the CBFB/MYH11 fusion

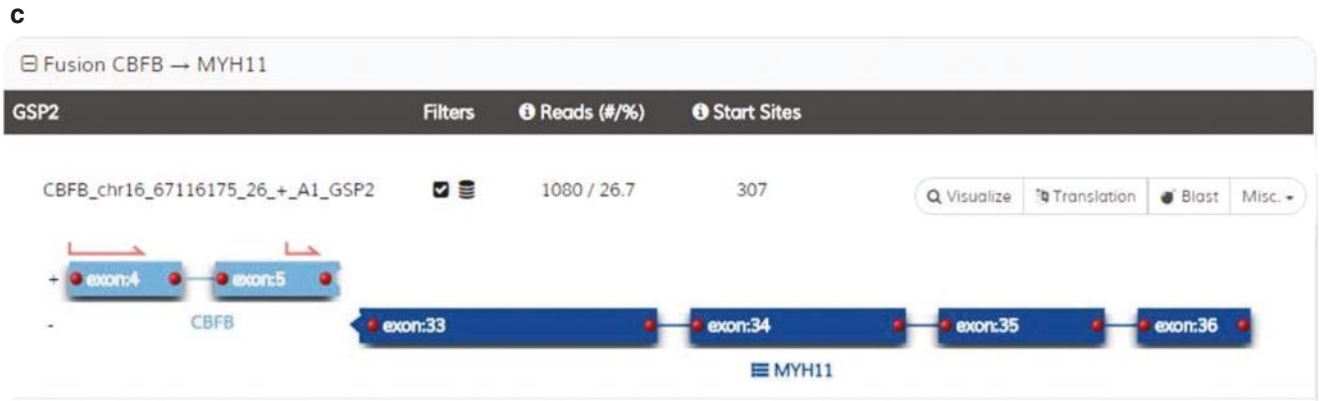


Fig. 23.2 (continued)

zinc-finger transcription factor gene that interacts with many important transcriptional and epigenetic regulators, such as CREBBP, GATA1, GATA2, DNMT3A, and DNMT3B, and thus mediates chromatin modifications and DNA hypermethylation. The activation of MECOM/EVI1 by inv(3)/t(3;3) promotes the proliferation of hematopoietic stem cells. In addition, the *MECOM/EVI1* gene is fused with *RUNX1* in t(3;21), often observed in t-MN and CML in blast crisis, which results in loss of *RUNX1* function and leads to overexpression of *MECOM/EVI1*. Inv(3)/t(3;3) is associated with a poor prognosis in AML and MDS. Loss of chromosome 7 is the most common secondary aberration.

t(6;9)/DEK/NUP214 Fusion

t(6;9) is a rare recurring chromosome translocation in AML and MDS and is often associated with basophilia and dysplastic morphology [16]. The translocation leads to a fusion between the *DEK* gene at 6p23 and *NUP214* at 9q34, which interferes normal nuclear transport. As in APL with t(15;17), and AML with a normal karyotype, FLT3 mutations, particularly FLT3 internal tandem duplications (ITD), are frequent in more than two thirds of patients with t(6;9) and likely contributes to an extremely poor prognosis in AML with t(6;9).

t(1;22)/RBM15/MKL1 Fusion

t(1;22) is a rare and unique chromosome translocation for acute megakaryoblastic leukemia (M7), most often in infants, not with Down syndrome. The translocation results in the *RBM15-MKL1*fusion [17]. The RBM15 and MKL1 are RNA- and DNA-binding motif proteins, respectively, which may modulate in chromatin organization, HOX-induced differentiation, or extracellular signaling pathways. There is no prognostic indication of this translocation.

Features of Massive Chromosomal Imbalances, Complex Karyotype, and Clonal Heterogeneity in AML

Besides the unique balanced chromosome translocation or inversion described above, many AML and high-risk MDS patients show a complex karyotype with multiple clonal evolutions, particularly in old adult patients, AML with myelodysplastic features, AML evolving from MDS or MPN, and therapy-related AML/MDS. These often include chromosome gain or loss and numerous structural abnormalities, such as del(5q)/−5, del(7q)/−7, del(17p)/−17, and +8 (Fig. 23.3). In these patients, mutations are common in genes involving in genetic and epigenetic pathophysiological pathways, such as myeloid transcription factors (RUNX1, CEBPA), activating mutations in signaling proteins (RAS, KIT, CBL, and FLT3), DNA methylation proteins (DNMT3A, TET2, IDH1/2, and SETBP1), chromatin modifiers (ASXL1, EZH2, KDM6A, KMT2A/MLL), and tumor suppressor proteins (TP53, WT1, and PHF6). In particular, TP53 deletion and mutation are among the most significant genetic abnormalities [18]. In these complex clones, chromothripsis seems the mechanisms that mess up complex chromosome aberrations, particularly for marker and ring chromosome and many derivative chromosomes [19]. At chromosome level, *chromothripsis* is featured by multiple chromosomal fragmentations due to a single event chromosome shattering over one or more chromosome regions. These complex genomic imbalances and heterogeneity are associated with very poor prognostic significance and poor response to treatment [22, 23].

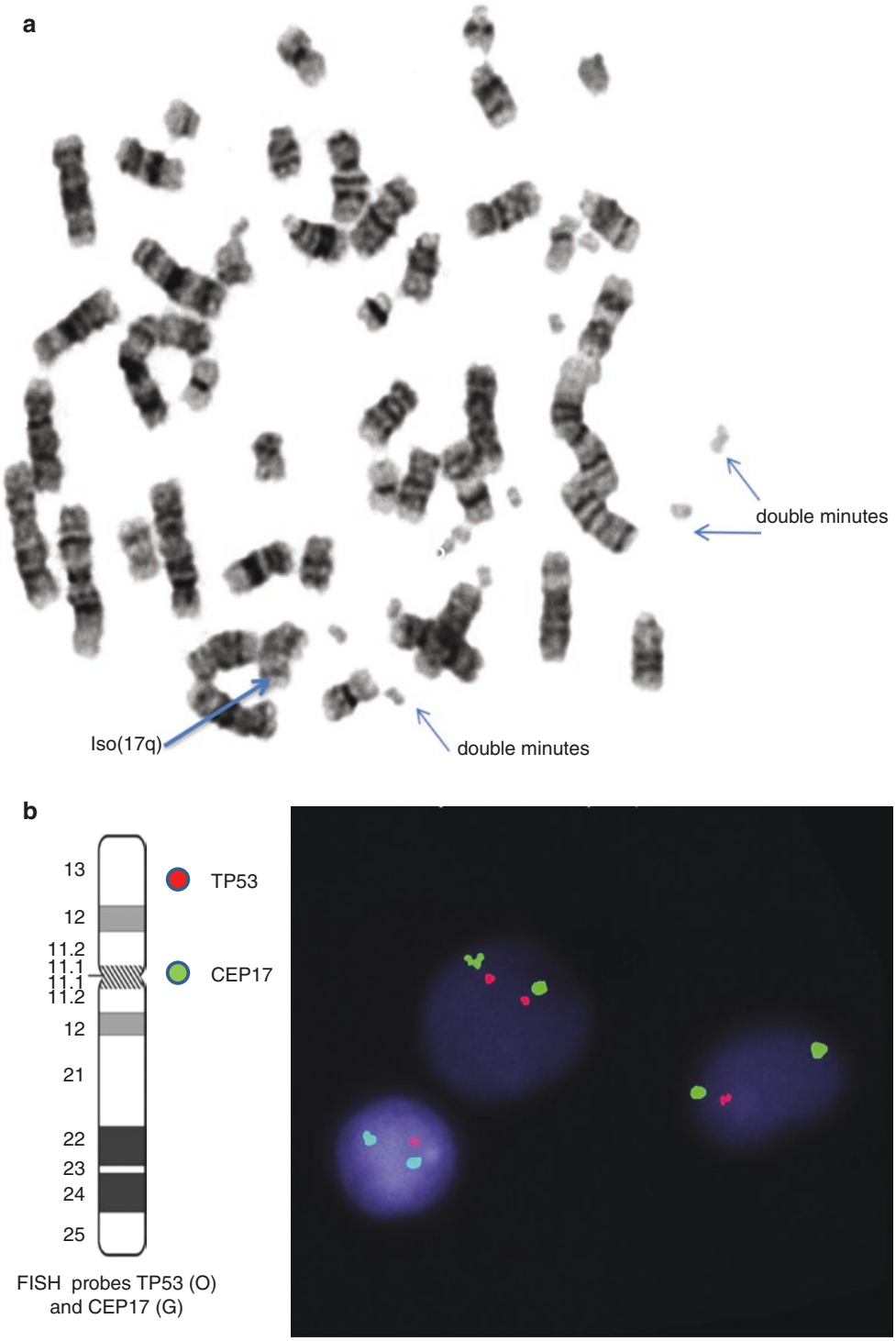


Fig. 23.3 Complex karyotype with loss of the short arm of chromosome 17, double minutes, and MYC amplification in a patient with AML. (**a**) A metaphase spread cell shows complex chromosome abnormalities, including gain of chromosome 4, abnormalities of chromosome 8 and 9, an isochromosome 17q (arrow), and numerous double minutes (arrows). (**b**) FISH analysis using double color probes for TP53 (17p13.1, labeled in orange) and the centromere probe for chromosome 17 (CEP17, labeled with green) (left, schema) showed two green signals and one orange signals in two interphase cells, i.e., loss of one copy of TP53, due to the isochromosome 17. A normal cell shows two orange and two green signals. (**c**) Genomic microarray tests revealed multiple complex chromosomal gains and losses on chromosomes 4, 8, 9, and 17. Amplification at 8q24 was observed, with copy numbers up to 36, including the MYC gene (arrow). (**d**) FISH analysis using MYC break-apart probes shows skyline colorful multiple signals all over the interphase nuclei, confirming the double minutes are MYC amplification in chromosome format, which is associated with poor prognosis in AML

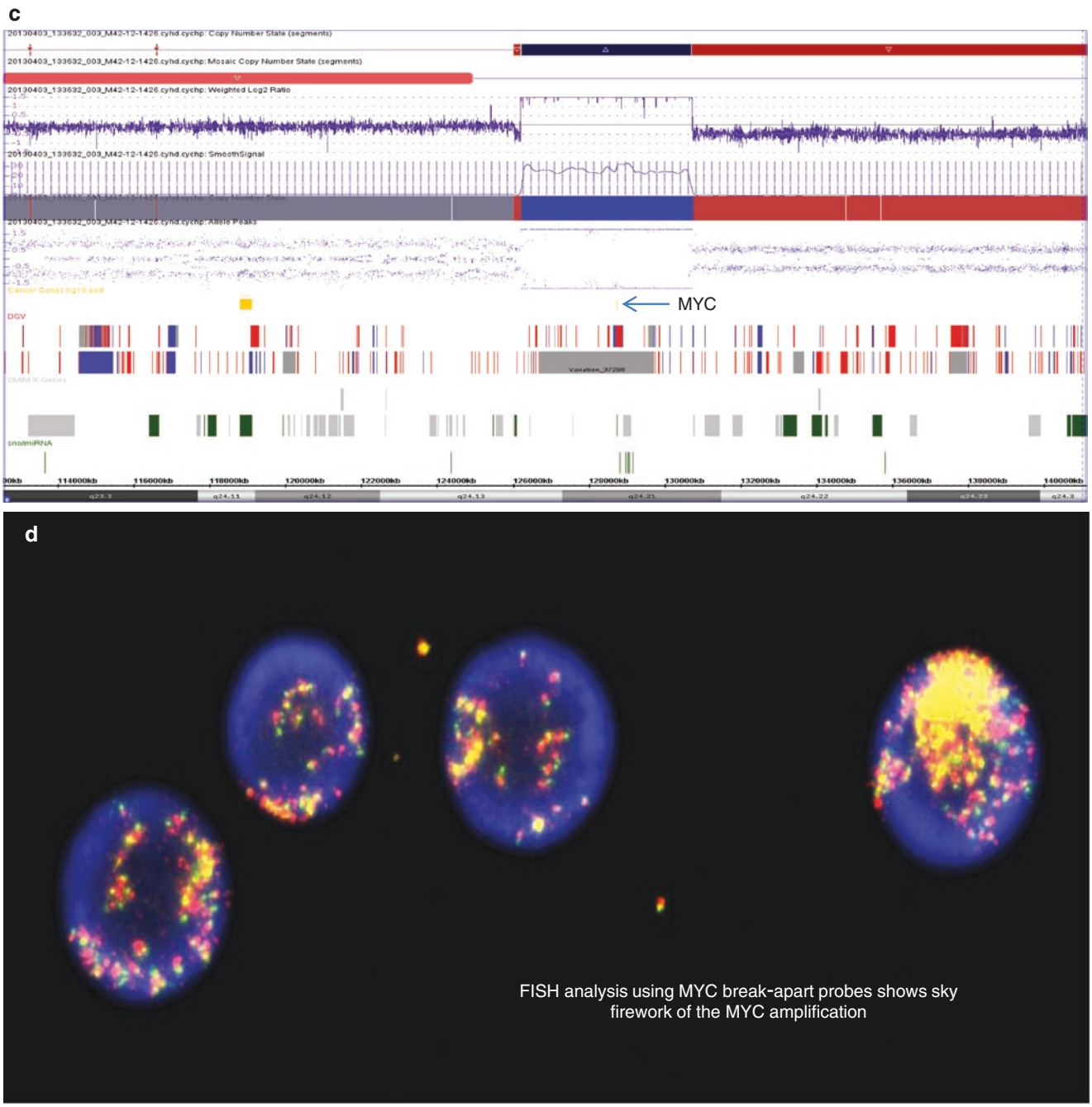


Fig. 23.3 (continued)

Myelodysplastic Syndrome (MDS)

In contrast to AML with many unique balanced chromosome translocations and inversion, myelodysplastic syndromes (MDS) are featured by various chromosomal loss, deletion, or gain, such as del(5q)/−5, del(7q)/−7, del(17p)/−17, +8, del(20q), etc., particularly in therapy-related MDS/AML. Only a few balanced chromosome translocations or inversions, such as inv(3)/t(3;3), t(3;21), and t(11;16), may be observed in MDS (Table 23.6) [3]. Chromosome abnormalities become more complex when MDS progress. Although these chromosomal deletion, loss, or gains are not specific for any subtypes of MDS, they carry significant prognostic impact in terms of patient survival and disease progression to AML and death and are critical in risk stratification and treatment selection (Table 23.7) [25]. Several genes in these

Table 23.6 Recurring chromosome abnormalities in MDS

Numerical or unbalanced structural abnormalities	Rare balanced chromosome translocations/inversion
+8	t(1;3)(p36.3;q21.2)
−7/del(7q)	t(2;11)(p21;q23)
del(5q)/t(5q)	inv(3)/t(3;3)(q21;q26.2)
del(20q)	t(3;21)(q26.2;q22)
−Y	t(6;9)(p23;q34)
i(17q)/t(17p)	t(11;16)(q23;p13.3)
−13/del(13q)	
del(11q)	
del(9q)	
idic(Xq)	
del(12p)/t(12p)	

Table 23.7 Prognostic significance of recurring chromosome abnormalities in MDS according to the Revised International Prognostic Scoring System (IPSS-R)

Cytogenetic abnormalities	Prognostic subgroups	Percentage
−Y, del(11q)	Very good	4%
Normal, del(5q), del(12p),del(20q), double with del(5q)	Good	72%
Del(7q),+8,+19,i(17q), any other single or double independent clones two or more unrelated noncomplex clones	Intermediate	13%
−7, inv(3)t(3;3)/del(3q), double with −7/del(7q), complex with 3 abnormalities	Poor	4%
Complex with 4 or more abnormalities	Very poor	7%

genomic lesions in MDS are involved in pathophysiology of various signaling pathway in normal and abnormal stem cell differentiation in leukemogenesis [28, 29].

Del(5q) and 5q- Syndrome

Del(5q) is among the most common chromosome abnormalities in de novo and therapy-related MDS. The deletion size is variable, most covering a large distal region of the long arm, i.e., 5q31 and 5q33. Deletions involving 5q31 are often observed in patients with high-risk and aggressive MDS, or with therapy-related MDS, and in many cases are part of a complex karyotype with *TP53* deletion and/or mutation, whereas a deletion of 5q33 is the hallmarker for the 5q- syndrome in the WHO classification, defined a cytogenetically isolated del(5q) or del(5q) with one other abnormality (excluding −7/del(7q)). 5q- syndrome primarily occurs in older women, age ranging from 65 to 70, and is associated with a relatively good prognosis, low risk of progression to AML and high treatment response to lenalidomide.

Several genes in these 5q deletion regions are implicated in leukemogenesis of MDS and AML, including *RPS14*, *EGR1*, *NPM1*, *APC* and *CTNNA1*, and microRNA *miR-145/146a*. *RPS14* at 5q32 participates in the maturation processing of 18S pre-rRNA, and del(5q) leads to its reduced expression and blockage of differentiation [26]. *APC*, *EGR1*, *NPM1*, and *CTNNA1* often involve in high-risk MDS with complex karyotype and *TP53* deletion or mutations. Various mouse models with deletion or loss of these genes display typical MDS phenotypes, indicative of a cooperative consequence of multiple genes in 5q deletion regions in pathogenesis of MDS.

5q- syndrome is associated with a good prognosis, whereas those with del(5q) as part of a complex clone with other chromosomal changes is associated with advanced MDS and a poor outcome.

−7/del(7q)

Loss of chromosome 7 or, less frequently, del(7q) is recurring abnormality in MDS, particularly in therapy-related MDS. Deletions often involve the middle region (7q22) or the terminal band (7q32–33) of the long arm. The *CUX1* gene at 7q22 likely is a conserved, haploinsufficient tumor suppressor by regulating target genes that promote hematopoiesis through distal enhancers by looping to target promoters [27]. Homozygous mutations of the *EZH2* gene at 7q32 are also frequent in MDS patients and result in loss of its histone methyltransferase activity. −7 is associated with unfavorable prognosis in both AML and MDS, whereas del(7q) as sole abnormality predicts an intermediate prognostic risk in MDS.

Gene Mutations in AML and MDS, Particularly with Normal Karyotypes

In addition to the recurring chromosome translocations, inversion, and chromosomal loss or deletion, numerous high-frequent gene mutations have been identified in AML and MDS, such as *FLT3*, *NPM1*, *CEBPA*, *BAALC*, *WT1*, *IDH1/2*, *KIT*, *and TP53*. Mutations of these genes provide critical prognostic significance and therapeutic selection in AML and MDS, particularly those with normal karyotype. *FLT3* mutations and *WT1* mutations are associated with poor prognosis, whereas *NPM* mutations, without coexistence of *FLT3* mutation, and biallelic mutations of *CEPBA* are indicative of a favorable prognosis in AML [24]. In MDS, most frequently mutated genes are spliceosome genes *SF3B1* and *SRSF2* and *TET2*, *ASXL1*, *DNMT3A*, and *RUNX1* [30]. These mutations often affect epigenetic regulation, i.e., DNA methylation and histone modification, RNA-splicing

machinery, transcription factors, and cytokine signaling pathways, and also carry important independent prognostic significance and associated with progression to AML. TP53 mutations occur in up to 15% of MDS and are more frequent in patients with therapy-related AML/MDS, particularly those with complex karyotype and/or monosomal karyotypes in AML and MDS, and are a strong predictor of poor prognosis [31]. Some of these mutations have been under intensive clinical trials with a promising outcome.

Features of Chromosome Abnormalities in Therapy-Related Myeloid Neoplasia (t-AML/MDS)

Therapy-related myeloid neoplasia (t-MN) is not rare in patients with history of Hodgkin lymphoma, non-Hodgkin lymphoma (NHL), breast cancer and other cancer, rheumatoid arthritis, or organ transplantation who received chemotherapy or immunosuppression treatment for these primary tumors or diseases. Clonal chromosome abnormalities are detected in up to 92% of patients with t-MN.

In general, two different recurring chromosome abnormality patterns exist and are associated with previous chemotherapy (Table 23.8) [20]. One is featured by del(5q)/−5, del(7q)/−7, del(17p)/−17, and/or complex karyotype and TP53 deletion, and many have *NUP98* rearrangement. This group is strongly associated with previous treatment with alkylating agents and/or radiation therapy. The other group is characterized by recurring chromosome translocations involving *MLL* or *RUNX1/AML1* and often noted in patients with previous treatment with DNA topoisomerase II inhibitors, such as VP16. These two categories are also associated with different clinical features and pathology findings (Table 23.8) [21].

Table 23.8 Features of cytogenetic abnormalities in therapy-related myeloid neoplasm associated with previous chemotherapy and disease outcome

Features of different t-MN	Alkylating agents related	Topoisomerase II inhibitor related
Chromosome abnormalities	del(5q)/−5	Translocations of MLL (11q23) and RUNX1 (21q22)
	del(7q)/−7	
	del(17p)/−17	
	Complex	
Preleukemia phase	MDS	Often none
Latency	5–7 years	2–3 years
Morphology	M1, M2, M4	M4, M5 most
Response to chemotherapy	Poor	Good
Long term outcome	Poor	Fair

Clonal Hematopoiesis of Indeterminate Potential (CHIP)

Clonal hematopoiesis of indeterminate potential (CHIP) refers to clonal hematopoiesis with associated somatic mutations, and diagnostic criteria of MDS or other hematologic neoplasms are not met [32]. The incidence of CHIP increases with age, and CHIP is associated with an increased risk for development of hematological cancers. Notably, mutations of *DNMT3A*, *ASXL1*, *TET2*, *SF3B1 and TP53* are common in both CHIP and MDS/AML. Clonal selection of these preexisting mutant clones in hematopoietic stem cells can be driven by oligoclonality associated with normal aging, inflammation, immune destruction, viral infections, defects in the bone marrow niche, and/or dysfunctional hematopoiesis, followed by clonal expansion by various hematopoietic stressors, which eventually progress to MDS and AML [33, 34].

Acute Lymphoblastic Leukemia (ALL)

B-Cell ALL

Chromosome abnormalities, including translocations, inversions, and genomic deletion or gains, occur in more than 80% of B-ALL. These aberrations have a significantly different incidences in pediatric and adults patients with B-ALL, with t(12;21) and hyperdiploid karyotypes most common in pediatric B-ALL, t(4;11) in infants patients, and t(9;22) in adults B-ALL (Table 23.9). As in AML and MDS, chromosome abnormalities play an important role in predicting disease prognosis, responding to chemotherapy, and selecting targeted treatment in B-ALL [35]. In addition, deletions or mutations of genes that regulate lymphocyte development

Table 23.9 Recurring chromosome abnormalities defining unique B-cell ALL according to WHO 2016 classification and their prognostic significance

Chromosome abnormalities	Genes involved	Clinical prognosis
t(9;22)(q34;q11.2)	BCR/ABL1	Poor
t(4;11)(q21;q23)	KMT2A (MLL)/AF4(MLLT2)	Poor
t(12;21)(p13;q22.3)	ETV6/RUNX1	Favorable
t(1;19)(q23;p13.3)	TCF3/PBX1	Intermediate
t(5;14)(q31;q32.3)	IGH/IL3	Poor
iAMP21/add(21q)/der(21q)	RUNX1?	Poor
Hyperdiploid (>50 chromosomes)		Favorable
Hypodiploid (<45) or haploid (1n)		Poor
t(8;14)(q24;q32)/t(2;8)/t(8;22)	IGH/IGK/IGL and MYC	Burkitt leukemia

are common in B-ALL, such as *PAX5*, *IKZF1*, *RB1*, *TP15/TP16 (CDKN2A/B)*, and *TP53* [36].

t(12;21)/ETV6/RUNX1 Fusion

t(12;21) occur in about 25% of children B-ALL and in about 3–4% adult patients with B-ALL. In chromosome analysis, it is cryptic so FISH analysis or RT-PCR is needed to detect. The translocation leads to a novel fusion of the *ETV6/*(*TEL gene* at 12p13.2) with the *RUNX1/AML1* gene at 21q22. RUNX1 is one of the CBF complex (see CBF leukemia with t(8;21)), and the ETV6 protein is a transcriptional repressor. The ETV6-RUNX1 fusion has dominant impact on the normal RUNX1function. The normal *ETV6* allele on the other chromosome 12 is frequently deleted. t(12;21) is associated with a favorable prognosis in B-ALL, with overall long survival and a low relapse risk.

t(8;14)/MYC/IGH Fusion

t(8;14) or its variants t(2;8) and t(8;22) are the hallmark of Burkitt leukemia and lymphoma, characterized by mature B-cell phenotypes. The translocations result in overexpression of the MYC protein due to the conjugation of the enhancer of *IGH*, *IGK*, or *IGL* to the *MYC* locus and are associated with a poor prognosis in B-ALL.

t(4;11)/AFF1/KMT2A Fusion and Other KMT2A/MLL Translocations

t(4;11) is the most common 11q23/MLL translocations in B-ALL and occurs in up to 60% of infant patients under 1 year old and in about 10% of adult B-ALL; the leukemia cells are often negative for CD10 expression. The t(4;11) results in a novel fusion gene of the *KMT2A* gene to the *AFF1/*AF4 gene at 4q21 and is strongly associated with a poor prognosis in both adults and children patients. The t(4;11) clones occur in uterus in many infant patients. In addition, other recurring 11q23/*MLL* translocations in B-ALL include t(11;19) with the MLL/ELL fusions and t(9;11) with MLL/AF9 fusions.

t(9;22)/BCR/ABL1 Fusion

t(9;22) occurs in about 30% of adult B-ALL patients, increasing to 50% of patients older than 50 and rare in children patients (up to 5%). As in CML, the t(9;22) results in the BCR/ABL1 fusion, with two different fusion transcripts due to different genomic breakpoints in BCR. One rare fusion protein is p210, which is identical in CML, with a break in major bcr region of *BCR*, and the other is p190 with a break in minor-bcr region of *BCR*. Both type fusion proteins lead to constitutive signaling via the RAS pathway of signal transduction and promote leukemogenesis.

t(9;22) is associated with an extremely poor prognosis in B-ALL, with poor responses to chemotherapy and short survival. Combined chemotherapy and tyrosine kinase (TK) inhibitors, such as imatinib, lead to promising improvement of outcome.

t(1;19)/E2A/TCF3 Fusion

t(1;19) is a common chromosome translocation in about 30% of B-ALL in children and rare in adult patients. Chromosome analysis revealed a balanced t(1;19) or, more frequent, unbalanced der(19)t(1;19). The translocation results in the *E2A*(19p13.3) and *TCF3* (1q21) fusion, which is associated with a favorable prognosis.

iAMP21

Intrachromosomal amplification of chromosome 21 (iAMP21) is detected by FISH analysis using probes for *RUNX1* in about 3–5% of B-ALL. Chromosome analysis often shows a complex structural abnormal chromosome 21. Microarray studies identify multiple gains and amplification along most of the long arm, with deletion of the subtelomeric region, likely due to a mechanism of breakage-fusion-bridge cycles, followed by chromothripsis. iAMP21 is associated with a dismal outcome, which may be overcome by intensive treatment of high-risk regimens.

Hyperdiploidy

Hyperdiploid clone with 55–56 chromosomes is frequently detected in about 30% of pediatric B-ALL and in 4–5% of adult B-ALL. A typical pattern of gain of chromosomes is those even numbered chromosomes, particularly chromosomes 4, 10, and 21, etc. Hyperdiploidy clone with more than 50 chromosomes, particularly with +4, +10, and +17, is associated with a good prognosis in B-ALL [37].

Hypodiploidy

Three groups of hypodiploid clones in B-ALL include (1) near-haploidy with 23–29 chromosomes, (2) low hypodiploidy with 33–39 chromosomes, and (3) high hypodiploidy with 42–45 chromosomes [37]. The near-haploid clone often have higher frequent alterations involving tyrosine kinase signaling and Ras signaling, whereas low hypodiploid clones are featured by high incidence of deletion and/or mutation of *TP53*, *IKZF2*, and *RB1*.

B-cell ALL patients with haploid or hypodiploid clones have a poor prognosis. A doubling of a near-haploid or low hypodiploid clone is common on B-ALL and masking as a hyperdiploid clone with a pattern of gains of two copies, rather than a single copy gain, of multiple chromosomes. It is critical to distinguish the true hyperdiploid clone that is associated with a favorable prognosis from masked doubling haploid or low hypodiploid clone. DNA index analysis and FISH tests are helpful, and genomic microarray with allele homo- vs heterozygosity may clarify these cases.

BCR-ABL1 (Ph)-Like B-Cell ALL

Ph-like B-cell ALL has no t(9;22)/BCR/ABL1 fusion but shares a similar gene expression profile to B-cell ALL with t(9;22). It occurs in about 13% of pediatric patients, 21% in adolescents, and up to 38% of adults with B-ALL and is resistant to standard chemotherapy and associated with a poor prognosis [35].

Various gene fusions and mutations occur in Ph-like B-ALL, commonly including *CRLF2* (cytokine receptor-like factor 2) translocations or overexpression, ABL1-class fusions, rearrangements of *EPOR* or *JAK2*. These genes usually involve in B-cell development, proliferation and differentiation, cell cycle regulation, and cell signaling [39]. Overexpression of *CRLF2*, due to a translocation with IGH [38], or fusion with *P2RY8*, resulting from an interstitial deletion in the pseudoautosomal region at the tip of the X and Y chromosomes, occurs in more than a half of Ph-like B-ALL. In the remaining non-CRLF2 Ph-like B-cell ALL cases, numerous translocations and mutation of several tyrosine kinase gene, such as *NUP214-ABL1*, *BCR-JAK2*, *STRB3-JAK2*, *IGH-EPOR*, and *EBF1-PDGFRB* fusion, and frequent mutations in *FLT3*, *CREBBP*, *IL7R*, or *SH2B3* (*LNK*), are common. Mutations of these genes result in constitutive kinase activation and signaling via activation of the ABL1 and JAK-STAT or RAS pathways, which are sensitive to tyrosine kinase inhibitors or JAK kinase inhibitors [40, 41].

T-ALL

Recurring chromosome abnormalities are detectable in about 50–70% of T-ALL, most commonly involving both T-cell receptor (*TCR*) and non-*TCR* gene loci (Table 23.10). The prognostic impact of most of these chromosome abnormalities in T-ALL is not defined yet [3, 42].

TCR Gene-Related Translocations and Rearrangements

Recurring chromosome translocations in T-ALL are those with *TRA/D* (14q11.2), *TRB* (7q34), and *TRG* (7p14). These translocations lead to deregulation, mostly overexpression of the partner genes that often are cell cycle inhibitor or a transcription factor. The most common transcription factor partner genes in these TCR receptor-related translocations are *HOX11* (*TLX1*, 10q24), *HOX11L2* (*TLX2*, 5q35), *MYC* (8q24), *TAL1*(1p32), *RBTN1*(*LMO1*, 11p15), *RBTN2* (*LMO2*, 11p13), *LYL1*(19p13), and *LCK* (1q34) [44].

Non-TCR loci-related chromosome translocations and deletion or gain are common, including deletion of the long arm of chromosome 6, the short arm of chromosome 9 (*CDKN2A/B*, *TP16*), MLL translocations, IGH translocations, t(10;11)/*PICALM-MLLT10* fusion, *NOTCH1and JAK1* mutations, and overexpression of *TLX1* (*HOX11*). Activating mutations of *NOTCH1* occur in more than a half of T-cell ALL, which may cooperate with other mutations in leukemogenesis of T-ALL [43, 45].

Table 23.10 Recurring chromosome abnormalities defining unique T-cell ALL according to WHO 2016 classification

Chromosome abnormalities	Genes involved
inv(7)(p15q34)/t(7;7)(p15;q34)	TRB/HOXA
t(1;14)(p32;q11.2)	TCRD/TAL1(SCL)
t(10;14)(q24;q11.2)	TCRD/TLX1(HOX11)
ins(10;11)(p13;q23q13)	KMT2A/MLLT10(AF10)
t(10;11)(p12;q14)	MLLT10(AF10)/ PICAL(CALM)
amp(NUP214/ABL1)	NUP214/ABL1
MYB amplification	
Hypodiploid (<45) or haploid (1n)	

Mature B- and T-Cell Non-Hodgkin Lymphoma (NHL) and Hodgkin Lymphoma

As in myeloid neoplasms, various recurring chromosome translocation, inversions, and deletion, loss or gain, and amplifications are detected in majority of B- or T-cell lymphoma, by using conventional chromosome analysis, FISH tests, microarray, and NGS studies. In contrast to those in myeloid neoplasms and many of B-cell ALL, these chromosome abnormalities often involve the immunoglobulin (IG) genes, most often the heavy chain gene *IGH* (14q32) or infrequently the light chain loci *IGK* (2p12) and *IGL* (22q11.2) in B-cell lymphomas, and the TCR loci, i.e., *TRA/D* (14q11.2), *TRB* (7q34), and *TRG* (7p14) in T-cell lymphoma. The most common chromosome translocations that are strongly associated with subtypes of B-cell lymphomas are t(14;18)/*IGH/BCL2* fusion in follicular lymphoma, t(8;14)/*IGH/MYC* fusions in Burkitt lymphoma, t(11;14)/*IGH/CCND1* fusions in mantle cell lymphoma (MCL), and t(11;18)/*API2/MATL1* fusion in mucosa-associated lymphoid tissue (MALT) lymphoma (Table 23.11) [46]. In contrast, the recurring chromosome translocations and inversions in T-cell lymphoma are relatively rare, and only a few subtypes of T-cell lymphoma show specific chromosome translocations or inversions, including t(2;5)/*ALK/NPM 1*fusion in ALK-positive anaplastic large T-cell lymphoma (ALTL), inv(14)/t(14;14) *TRA/D/TCL1* fusion in T-cell prolymphocytic leukemia (T-PLL), and isochromosome 7q, i.e., i(7q) in hepatosplenic T-cell lymphoma (Table 23.12).

Notably, none of these chromosome translocations are exclusively associated with any subtypes of lymphoma. t(8;14) or its variants t(2;8) and t(8;22) are the hallmark of Burkitt lymphoma and also frequently observed in diffuse large B-cell lymphoma (DLBCL), multiple myeloma, and CLL. Detection of t(11;14) is diagnostic in MCL and is also the most common chromosome translocation in myeloma. In addition, the emerging of recurring chromosome translocations is common in lymphoma progression or transformations, such as t(8;14) or *BCL6* translocations in DLBCL transforming from follicular lymphoma with t(14;18),

so-called double- or triple-hit high-grade B-cell lymphoma, which is strongly associated with a poor prognosis (Fig. 23.4) [47, 50]. *BCL6* at 3q26 is frequently observed in B-cell lymphoma, with various non-IG loci, such as *MYC*, *PAX5*, etc., which often lead to the replacement of the promoter of *BCL6* and overexpression of the *BCL6* gene [47].

In contrast to the recurring chromosome translocations in AML, which often results in a chimeric fusion gene that produces new fusion proteins with novel functions, most of the chromosome translocations in B- and T-cell lymphoma lead to the juxtaposition of the enhancer of the IG genes or TCR genes with the partner genes, which results in the overexpression of the partner genes, such as t(14;18) and the *IGH/BCL2* fusions [47].

In addition to these recurring chromosome translocations and inversions involving the immunoglobulin (IG) and T-cell receptor (TCR) loci, various recurring chromosomal deletion, loss and gain, or amplifications occur in most of B- and T-cell lymphoma. These include deletion of the long arm of chromosomes 6, 10, 11, and 13, deletion of the short arm of chromosome 17, and gain of the X chromosome and chromosome 3, 7, 12, etc. Amplification of the *MYC* gene (8q24) occurs in certain high-grade B-cell lymphomas. They are

Table 23.11 Recurring chromosome abnormalities in B-cell NHL

Chromosome abnormalities	Genes/chromosomes involved	Associated diseases
t(11;14)(q13;q32)/t(2;11)/t(11;22)	IGH/IGK/IGL and CCND1	MCL
t(14;18)(q32;q21)/t(2;18)/t(18;22)	IGH/IGK/IGL and BCL2	FL, DLBCL
t(3;14)(q27;q32)	IGH and BCL6	DLBCL
t(8;14)(q24;q32)/t(2;8)/t(8;22)	IGH/IGK/IGL and MYC	Burkitt, DLBCL
t(11;18)(q32;q21)	BRIC3/API2 and MALT1	MZBCL
t(1;14)(p22.3;q32)	IGH and BCL10	MZBCL
t(3;14)(p13;q32)	IGH and FOXP1	MZBCL
MYC and BCL2 and/or BCL6 translocations	MYC, BCL2, BCL6	Double- or triple-hit large B-cell lymphoma

MCL mantle cell lymphoma, *FL* follicular lymphoma, *DLBCL* Diffuse large B-cell lymphoma, *Burkitt* Burkitt lymphoma, *MZBCL* marginal zone B-cell lymphoma

Table 23.12 Recurring chromosome abnormalities in T-cell NHL

Chromosome abnormalities	Genes/chromosomes involved	Associated diseases
inv(14)(q11.2q32)/t(14;14)(q11.2;q32)	TRAD/TCL1A	T-PLL
t(2;5)(p23;q35) and variants	ALK/NPM1	ALK+ ALCL
i(7q)	Often with +8	Hepatosplenic T NHL
+3/+5/+X	Unknown	AITL

T-PLL T prolymphocytic leukemia, *ALCL* anaplastic large T-cell lymphoma, *AITL* angioimmunoblastic T-cell lymphoma

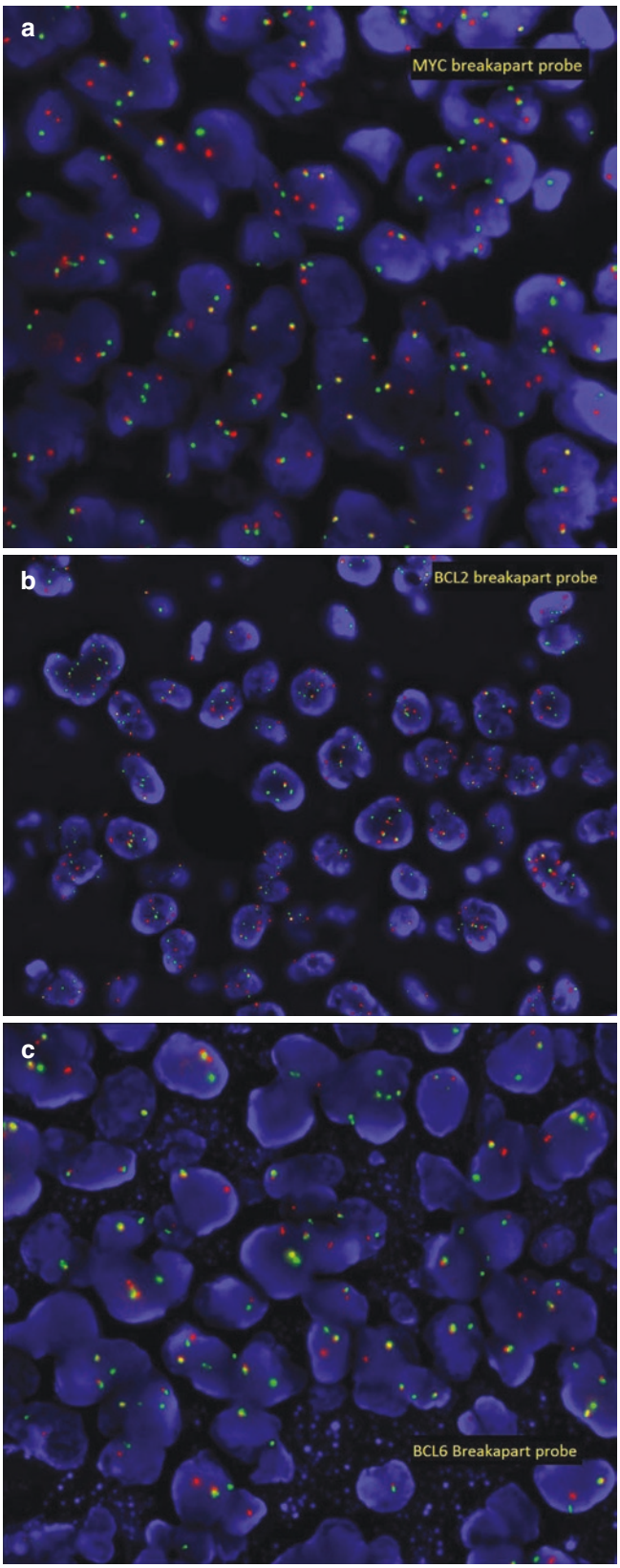


Fig. 23.4 Formalin-fixed paraffin-embedded (FFPE) FISH analysis reveals translocation patterns of MYC, BCL2, and BCL6 in a patient with diffuse large B-cell lymphoma. FISH analysis using break-apart probes for MYC (**a**), BCL2 (**b**), and BCL6 (**c**) reveals a split signal pattern for all three genes in most cells analyzed, indicative of a triple-hit high-grade B-cell lymphoma, which is associated with aggressive clinical course and poor prognosis

observed in various types of lymphomas and more often in advanced disease stages, along with complex and massive genomic abnormalities [49].

Non-Hodgkin Lymphoma

t(14;18)/IGH/BCL2 Fusion in Follicular Lymphoma and DLBCL

The t(14;18) is one of the most common chromosome translocations in B-cell lymphoma and is detected in up to 90% of follicular lymphoma (FL) and in about 30% of DLBCL. It results in a novel fusion of the *IGH* and *BCL2* genes, which leads to the overexpression of BCL2 due to the relocation of the enhancer of the *IGH* gene to the *BCL2* gene region. The *BCL2* protein is an inhibitor of apoptosis. Occasionally, t(2;18) or t(18;22), involving *IGK* or *IGL*, respectively, may occur [47].

In most patients, t(14;18) is accompanied by various additional chromosome abnormalities, particularly during the transformation of follicular lymphoma and in DLBCL. The most common secondary chromosome abnormalities include +X, +1q, +12p and duplication of the derivative chromosome der(18)t(14;18) and del(6q), del(10q). Many of these abnormalities may carry negative impact on the disease prognosis.

t(8;14)/IGH/MYC Fusion in Burkitt Lymphoma and DLBCL

The t(8;14) or its variants t(2;8) and t(8;22) are the hallmark of Burkitt lymphoma and also a common abnormality in DLBCL. In Burkitt lymphoma, t(8;14) is observed in about 85%, t(2;8) in 5% and t(8;22) in 10%. These translocations all result in the *IG/MYC* fusion that leads to the overexpression of MYC, which is a master in regulating cell cycle. In most cases with Burkitt lymphoma, t(8;14) often is sole or a part of a noncomplex clone, in comparison with those with t(8;14) in DLBCL. Additional chromosome abnormalities occur when the disease progresses, with most common +1q, +7, +12, and del(6q), del(17p), etc.

t(11;14)/IGH/CCND1 Fusion in Mantle Cell Lymphoma

t(11;14) is the hallmark of mantle cell lymphoma (MCL) and is critical in differentiating diagnosis with B-cell CLL, when the morphology and immunophenotypes of tumor cells are atypical. It leads to the conjunction of the enhancer of the *IGH* gene with the *CCND1* gene, which results in the expression of CCND1.

Additional chromosome abnormalities may occur in MCL with t(11;14), most common including deletion of 6q, 10q, 13q, and 17p and gain of chromosome 3, 12, etc. These secondary abnormalities are more frequent in disease progression, particularly in its blastoid variant, and have negative impact on prognosis.

t(11;18)/API1/MALT1 Fusion and Other MALT1 Fusion in Mucosa-Associated Lymphoid Tissue (MALT) Lymphoma

The t(11;18) is the primary chromosome translocations in MALT lymphoma and results in the *API2* (also called *BIRC3*)/MALT1 fusion. In addition, immunoglobulin gene-related chromosome translocations are detected in a small percentage of this entity, including t(14;18), which results in the *IGH/MALT1* fusions, and t(1;14) or its variant t(1;22), both involving the BCL10 gene (1p22) with either *IGH* or *IGK* gene. These translocations lead to the activation of the nuclear factor (NF)-kB activation pathway.

inv(14)/t(14;14)/TRAD/TCL1 Fusion in T-Cell PLL

The inv(14)(q11.2q32) or its rare variant t(14;14) is the hallmark of T-PLL, occurring in up to 90% of patients, and results in the *TRAD*(14q11.2)/*TCL1* (14q32) fusions. In rare cases, *TCL1* is fused with other TCR genes, i.e., t(7;14) with the *TRB/TCL1* fusion. In T-PLL without inv(14)/t(14;14), t(X;14) with *TRA/D/MTCP1* fusion is common.

i(7q) in Hepatosplenic T-Cell Lymphoma

An isochromosome of the long arm of chromosome 7, i.e., i(7)(q10), is the characteristic chromosome biomarker for hepatosplenic T-cell lymphoma (HSTL), which is a rare aggressive T-cell lymphoma predominantly in male young patients. Gain of chromosome 8 is the most common secondary chromosome abnormality in this entity.

t(2;5)/ALK/NPM1 Fusion in Anaplastic Large T-Cell Lymphoma (ALTCL)

T(2;5) is the hallmark of the majority (80%) of ALTCL, which is typically CD30 positive. The t(2;5) results in the *ALK*(2p23)/*NPM1*(5q35) fusion, which leads to constitutive tyrosine kinase activity of ALK activation [48]. There are at least seven other *ALK*-related translocations and inversion in ALCL that lead to ALK with other gene fusions, including common t(1;2)/*TPM3/ALK* fusion and rare t(X;2)/*MSN/ALK*

fusion, inv(2)/*ATIC/ALK* fusion, t(2;3)/*ALK/TFG* fusion, t(2;17)/*ALK/CLTC* fusion, t(2;17)/*ALK/ALO17* fusion, and t(2;22)/*ALK/MYH9* fusion. All ALK-positive ALCL have a favorable prognosis.

In the remaining ALCL patients with no *ALK* translocations, two new genetic markers have been identified recently, including the *DUSP22/IRF4* gene in t(6;7), which is associated with a comparable favorable prognosis as ALK-positive ALCL, and the TP63 gene rearrangements, which are associated with poor prognosis.

Hodgkin Lymphoma (HL)

Due to the rarity of the Hodgkin and Reed-Sternberg (H-RS) cells in lymph nodes, conventional chromosome analysis may reveal abnormal clones only in a small number of patients with HL, which often shows a complex karyotype of triploid or tetraploidy with multiple non-specific numerical and structural chromosome abnormalities. No specific chromosome abnormalities or genomic aberrations were identified in HL. However, applying FISH, microarray, and NGS studies, in combination with immunophenotyping of CD30, or microdissection of the H-RS cells has recently discovered several unique genetic aberrations in HL, including novel genomic amplifications, such as *PDL1/PDL2*; chromosome translocations, i.e., *CIITA*; [51] various gene mutations of the NF-kB; and JAK-STAT pathway, as well as epigenetic dysregulation. Some of these novel biomarkers are important therapeutic targets for genetic-based treatment, such as PD1 inhibitory antibody *nivolumab* in patients with refractory classical Hodgkin lymphoma (cHL) and PDL1/PDL2 amplification [52].

PDL1/PDL2 Amplification or Gain in cHL Integrative analysis with array and expression profiling reveals frequent immunoregulatory genes *PDL1/PDL2* amplification or gains as the target of 9p24.1 amplification (Fig. 23.5), including the JAK2 gene, which increased PD-1 ligand expression and further enhanced sensitivity to JAK2 inhibition in cHL and primary mediastinal large B-cell lymphoma (PMLBCL), which reveals the PD-1 pathway and JAK2 as complementary rational therapeutic targets. PDL1 over expression is associated with significantly poor PFS in patients with 9p amplifications [53, 54].

CIITA Abnormalities in HL The major histocompatibility complex (MHC) class II transactivator *CIITA* is frequently rearranged in about 15% of cHL and about 38% of PMLBCL. *CIITA* is often involved in various translocations that result in a novel fusion gene, which decreases MHC class II expression and increases PD-L1 and PD-L2 expression in these two entities (Fig. 23.6).

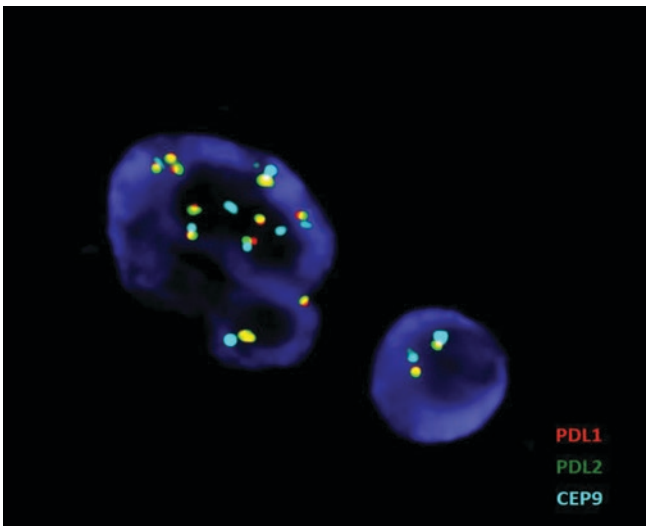


Fig. 23.5 FISH analysis of a patient with cHL showing amplification of PDL1 and PDL2 in a H-RS cell. PDL1 and PDL2 are labeled with orange and green color, respectively. The CEP9 centromere probe is labeled with aqua. The H-RS cell with bizarre nucleus shows multiple copies of PDL1 and PDL2 as well as CEP9

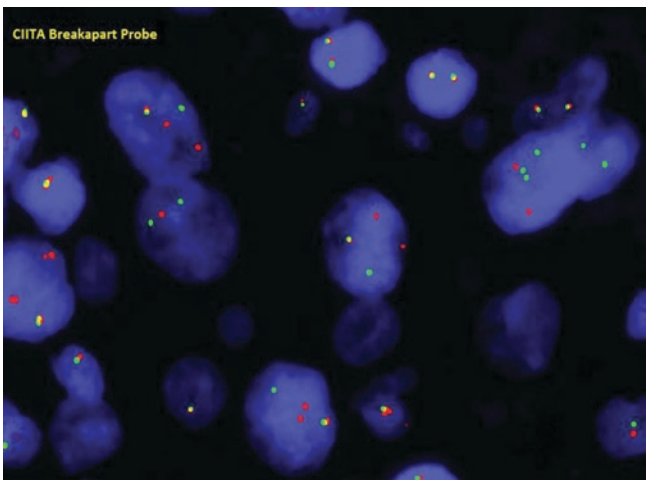


Fig. 23.6 FISH analysis of a patient with cHL showing CIITA rearrangement. The break-apart CIITA FISH probes are labeled in orange and green for the 5′ and 3′ regions, respectively. The abnormal cells show split signal patterns, indicative of a CIITA translocation

Chronic Lymphocytic Leukemia (CLL)

In about 80% of CLL patients, recurring chromosome abnormalities are detectable by conventional chromosome analysis, FISH, or microarray tests. The most common chromosome abnormalities in CLL are -13/del(13q), +12, del(6q)/MYB, del(11q)/*ATM*, and del(17p)/*TP53* (Table 23.13) [55].

Del (13q14) or, less frequent, −13 is the most common abnormality in CLL and can be detected in about a half of CLL cases. A heterozygous deletion is more common than a homozygous deletion. Two different deletions have been

Table 23.13 Recurring chromosome abnormalities in CLL and their prognostic significance

Chromosome abnormalities	Genes involved	Clinical prognosis
Del(13q)/−13	RB, miRNA-15/16	Favorable
+12	?	Intermediate
Del(6q)	MYB (6q23)	Intermediate
Del(11q)	ATM/BIRC3 (11q22.3)	Poor
Del(17p)	TP53(17p13.1)	Poor
Complex	?	Poor
T(14;19) (q32;q13)	IGH/BCL3	Poor

defined based on array studies; a large deletion involves the RB gene and the microRNA cluster of mir15, mir-16, and DLEU1/2 and is associated with an unfavorable prognosis, whereas the small deletion deletes the microRNAs only [57, 58]. The *RB* gene and mir15/16 and DLEU1/2 are negatively involved in the regulation of BCL2 mRNA expression, and thus, deletion of these microRNAs leads to overexpression of BCL2 in CLL. In 15% of CLL patients, +12 is detectable by chromosome and/or FISH tests. As sole abnormality, both del(13q) or −13 and +12 are associated with a relatively favorable prognosis in CLL.

Del(17p) is detected in about 10% of newly diagnosed CLL, and in about 50% of CLL with relapsed or refractory patients, and often results in loss of the TP53 locus. Del(11q), involving the ATM gene, present in about 20% of CLL patients. Both del(17p)/*TP53* and del(11q)/*ATM* are associated with poor prognosis in CLL with no respond to initial treatment well, an early relapse and short overall survival.

In addition to these recurring chromosomal imbalances above, several *IGH* translocations exist in CLL, including most commonly t(14;19), t(2;14), and t(14;18), which result in the fusion of IGH with *BCL3* (19q13), *BCL11A* (2p16), and *BCL2* (18q21) and deregulation of the relevant genes.

Moreover, high frequencies of several gene mutations have been identified in CLL, which involve in cellular signaling pathway, and are associated with poor prognosis. *NOTCH1* mutations occur frequently in CLL patients and are associated with unmutated IGVH, ZAP-70 expression, and a poor prognosis. The *SF3B1* gene is one of the spliceosome family genes that are responsible for splicing messenger RNA [56]. *SF3B1* mutations, particularly point mutations K700E are associated with a poor prognosis in CLL. Overexpression of miR-21 and miR-155 occur in a high frequency of CLL patients. MicroRNA-15a and miR-16-1 result in the upregulation of the BCL2 in CLL often without del(13q) [58].

Multiple Myeloma and Other Plasma Cell Neoplasms

Like other hematological neoplasia, multiple myeloma and other plasma cell neoplasms are featured by recurring chromosome abnormalities. In general, two relatively separated cytogenetic groups are identified; one carries various IGH translocations, and the other contains hyperdiploid clones, both often with recurring deletions of chromosomes 13 and 17 [59]. These chromosome abnormalities are associated with various prognostic significances and provide therapeutic guidelines (Table 23.14). In premalignant plasma cell proliferative disorder, i.e., monoclonal gammopathy of undetermined significance (MGUS), some of these recurring chromosome and genomic abnormalities also occur, indicative of their implication in the initiation of plasma cells neoplasms and in the progression to multiple myeloma [61].

Table 23.14 Recurring chromosome abnormalities in multiple myeloma and plasma cell neoplasia

Chromosome abnormalities	Genes/chromosomes involved	Clinical prognosis
Hyperdiploidy	+3, +5, +7, +9, +11, +15, +19, +21	Good
T(4;14)(p16;q32)	FGFR3/NSD2 and IGH	Poor
T(6;14)(p25;q32)	CCND3 and IGH	Good
T(11;14)(q13;q32)	CCND1 and IGH	Good
T(14;16)(q32;q23)	IGH and MAF	Poor
T(14;20)(q32;q12)	IGH and MAFB	Poor
T(8;14)(q24;q32)	MYC and IGH	Poor
Loss of 1p	TP15	Poor
Gain of 1q	CKS1B	Poor
Del(13q)/−13	RB	Poor
Del(17p)/−17	TP53	Poor

Similar to CLL, chromosome analysis in multiple myeloma is often limited due to low mitotic index of plasma cells in short-term culturing and only detects chromosome abnormalities in about 20% of myeloma patients. Genomic microarray tests on enrich plasma cell population are the choice of test for diagnostic myeloma specimen, along with FISH for IGH-related translocations. FISH tests are sensitive in monitoring specific abnormalities in follow-up samples and in detecting early progression such as del(17p) in small clones.

1. *IGH translocations*: There are five primary IGH translocations in myeloma, including t(4;14)/*FGFR3/IGH* fusion, t(6;14)/*CCND3/IGH* fusion, t(11;14)/*CCND1/IGH* fusion, t(14;16)/*MAF/IGH* fusion, and t(14;20)/*MAFB/IGH* fusion [59]. In addition, t(8;14)/MYC/IGH fusion often occur as secondary chromosome translocation in some myeloma patients. t(11;14) is the most common chromosome translocation in myeloma [60]. These IGH translocations result in the juxtaposition of the IGH active promoter region and overexpress to the partner genes, including transcription factors, growth factor receptors, and cell cycle mediators, and thus promote plasma cell growth and replication.
2. *Hyperdiploid clones*: In about a half of myeloma patients, a hyperdiploid clone is detected, typically with gain of the odd-numbered chromosomes, i.e., chromosomes 3, 5, 7,

9, 11, 15, 19, and 21. Hyperdiploid clones also result in overexpression of various genes on these chromosomes and promote cell proliferation.

3. *Del(17p/TP53) and other genomic gain or loss*: within these two major cytogenetic groups above, del(17p), including the *TP53* gene, and del(13q)/-13, as well as deletion and gain or amplification of the short arm and the long arm of chromosome 1, respectively, are recurring chromosome abnormalities in myeloma. In particular, del(17p/*TP53*) is more often detected in advanced disease stages and plasma cell leukemia and is strongly associated with poor treatment response and rapid disease progression [59].

Summary

Recurring chromosome translocations, inversions, deletion, loss or gains, and genomic imbalances are common in leukemia and lymphoma. Many of these genetic abnormalities are unique and significant for a precise diagnosis, risk stratification, and also importantly for predicting treatment outcome and prognosis. With novel molecular techniques, particularly next-generation sequencing, more genetic biomarkers are being discovered, many of which are genetic-based therapeutic targets that allow various clinical trials with promising novel treatment options, such as immune checkpoint inhibitors targeting PDL1/PDL2 in cHL. This progress benefiting from new genetic findings enables us to better understand the genetic mechanisms of relevant genes and novel fusions in various leukemo- and lymphogenesis and define new disease entities and allow us to study on treatment resistance in many patients even with novel genetic-based treatment.

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