14 Myeloproliferative Neoplasms, Myelodysplastic/Myeloproliferative Neoplasms, and Myelodysplastic Syndromes

Julie Li and Gang Zheng

List of Frequently Asked Questions

1. How many *BCR-ABL1* fusion variants are there in chronic myeloid leukemia (CML)?
2. Which molecular genetic techniques are commonly used for the diagnosis of chronic myeloid leukemia (CML)?
3. What cytogenetic abnormalities are commonly seen in accelerated/blast-phase chronic myeloid leukemia (CML)?
4. How is "complete response" to tyrosine kinase inhibitor (TKI) therapy defined in chronic myeloid leukemia (CML)?
5. What tests are useful to monitor tyrosine kinase inhibitor (TKI) therapy in patients with chronic myeloid leukemia (CML)?
6. What is the major cause of resistance to tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML)? What are the therapeutic options for CML?
7. What are the common molecular alterations in the *BCR-ABL1*-negative myeloproliferative neoplasms?
8. Which molecular genetic abnormality is frequently associated with chronic neutrophilic leukemia (CNL)?
9. How to distinguish chronic neutrophilic leukemia (CNL) from chronic myeloid leukemia (CML)?
10. What molecular changes are associated with systemic mastocytosis?
11. What is the underlying molecular abnormality of *PDGFRA* rearrangement?
12. What are the common underlying genetic abnormalities involved in chronic myelomonocytic leukemia (CMML)?
13. What are the prognostic significance of the genetic changes in chronic myelomonocytic leukemia (CMML)?
14. In the 2017 WHO classification, chronic myelomonocytic leukemia (CMML) is further categorized into "proliferative CMML" and "dysplastic CMML." What are the molecular genetic differences between these two types?
15. Which genetic abnormalities are relatively common in atypical chronic myeloid leukemia (aCML), *BCR-ABL1*-negative?
16. What are the major features to differentiate atypical chronic myeloid leukemia (aCML), *BCR-ABL1*-negative, from chronic neutrophilic leukemia (CNL)?
17. Which genes are most commonly mutated in juvenile myelomonocytic leukemia (JMML)?
18. What are the common molecular genetic abnormalities that are associated with myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T)?
19. What are the disease-defining chromosomal abnormalities in myelodysplastic syndrome (MDS)?
20. What are the typical clinicopathological findings in patients with myelodysplastic syndrome (MDS) with isolated del(5q)?
21. What are the common molecular abnormalities associated with MDS?
22. How to differentiate idiopathic cytopenia of undetermined significance (ICUS), clonal hematopoiesis of indeterminate potential (CHIP), clonal cytopenia of undetermined significance (CCUS), and myelodysplastic syndrome (MDS) from each other?

J. Li (✉)
Pathology and Laboratory Medicine Department, Boston University/Boston Medical Center, Boston, MA, USA
e-mail: Julie.li@bmc.org

G. Zheng
Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA
e-mail: zheng.gang@mayo.edu

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Frequently Asked Questions

1. **How Many *BCR-ABL1* Fusion Variants Are There in Chronic Myeloid Leukemia (CML)?**
   - Chronic myeloid leukemia (CML) is a myeloproliferative disorder and characterized by a reciprocal chromosomal translocation between the Abelson oncogene (*ABL*) on chromosome 9q34.1 and the breakpoint cluster region (*BCR*) gene on chromosome 22q11.2, also known as Philadelphia chromosome (Ph). BCR-ABL is a constitutively active tyrosine kinase that promotes proliferation through several downstream signaling pathways involving RAS, RAF, JUN kinase, MYC, and STAT [1]. The most common conserved breakpoint in *ABL1* gene occurs in the intron before exon 2 (a2) and rarely downstream of exon 2 (a3) [2, 3]. Breakpoints in *ABL1* are mostly located in the 5′ of the second exon. The different breakpoints in the *BCR* gene result in different sizes of *BCR-ABL1* fusion genes. Three breakpoint cluster regions in the *BCR* gene have been identified: major breakpoint cluster region *(M-bcr)*, minor breakpoint cluster region (*m-bcr*), and micro-breakpoint cluster region (*μ-bcr*) [4, 5].
   - *M*-bcr breakpoints occur downstream of exon 13 (e13) or exon 14 (e14) and result in a p210 fusion protein. The p210 (*M-bcr*) is detected in majority (97–99%) of CML cases and also presents in B-lymphoblastic leukemia/lymphoma (40% of adults and 10% of pediatric B-ALL patients) [6].
   - *m-bcr* breakpoints occur after the exon 1 (e1) of the *BCR* gene and produce a smaller fusion protein p190. CML with p190 (*m-bcr*) is rare (<1%) and mimics chronic myelomonocytic leukemia with increased numbers of monocytes [7]. p190 is mostly associated with Ph-positive B-ALL (60% of adult and 90% of pediatric patients) [6, 8].
   - *μ-bcr* breakpoints occur beyond the exon 19 (e19) of *BCR* in the micro-region and encode a larger oncoprotein p230. The p230 (*μ-bcr*) is rare and associated with cases of neutrophilic CML that display predominant neutrophilic maturation and/or thrombocytosis [9].
2. **Which Molecular Genetic Techniques Are Commonly Used for the Diagnosis of Chronic Myeloid Leukemia (CML)?**
   - The diagnosis of CML is based on the detection of *BCR-ABL1* or Ph chromosome t(9,22) (q34.1;q11.2) [10]. Screening test is often performed using blood specimen with abnormal high granulocyte count in a proper clinical setting. The commonly used diagnostic methods for CML are summarized in Table 14.1.
   - Conventional cytogenetics is still an important tool for the detection of the Ph chromosome, and the bone marrow aspirate is the commonly used specimen. It's routinely performed at the diagnosis which offers baseline information for monitoring clonal evolution. However, conventional cytogenetics has longer turnaround time due to the cell culture, low sensitivity (5–10%), and failure to detect cryptic translocations [11–13].
   - Fluorescence in situ hybridization (FISH) uses specific probes for *BCR-ABL1* gene and can rapidly identify the abnormality with higher sensitivity and detection cryptic translocations. A wide range of specimens can be used, such as peripheral blood, bone marrow, and paraffin-embedded tissue. To be noted, additional chromosomal changes will be missed by FISH [5]. If the Ph chromosome is detected by conventional cytogenetics, FISH is not mandatory and should not replace conventional cytogenetics.
   - Qualitative reverse transcriptase PCR (RT-PCR) measures *BCR-ABL1* transcripts on the mRNA level. Multiplex RT-PCR and nested RT-PCR are useful for detecting atypical *BCR-ABL1* variants [11, 14–16]. Importantly, qualitative RT-PCR (low sensitivity) should not be used for monitoring molecular response during therapy, which requires quantitative RT-PCR.
   - Quantitative RT-PCR (qPCR) is a highly sensitive assay and required for the initial workup to establish the bassline level for *BCR-ABL1* mRNA transcripts. Peripheral blood is more commonly used than bone marrow and makes monitoring less invasive [17, 18]. An international scale (IS) is recommended to standardize *BCR-ABL1* mRNA level across different laboratories and is defined as the ratio of *BCR-ABL1* transcripts to the internal control (such as *ABL1* and *GUSB*) and reported as *BCR-ABL1* percentage on a log scale (10%, 1%, 0.1%, 0.01%, and 0.032% correspond to 1, 2,3, 4, and 4.5 logs, respectively) [19]. To be noted, the low levels of *BCR-ABL1* can be detected in normal individuals, and interpretation should be used with caution as the results do not indicate the disease of CML [20].
3. **What Cytogenetic Abnormalities Are Commonly Seen in Accelerated/Blast-Phase Chronic Myeloid Leukemia (CML)?**
   - The evolution of CML from chronic to accelerated phase (AP-CML) or blast phase (BP-CML) is caused by the development of subclones with new cytogenetic and molecular changes. Conventional cytogenetics is useful to detect the additional abnormalities when suspicious of accelerated or blast phase [23, 24]. The most common secondary karyotypic abnormalities in CML in the advanced stages include:
     - Trisomy 8
     - Isochromosome 17q

Table 14.1 The molecular techniques for CML diagnosis [12, 13, 16, 18, 21, 22]

	Conventional cytogenetics	FISH	Qualitative RT-PCR	Quantitative RT-PCR (qPCR)
Target	Metaphase chromosome Ph chromosome t(9;22)	DNA *BCR-ABL1*	mRNA *BCR-ABL1*	mRNA
Sensitivity	5–10%	0.1–5%	0.1%	0.001–0.01%
Advantages	Provides the baseline karyotype	Rapid, specific probes; detect complex or cryptic translocation	Rapid; sensitive; cryptic variant; determines breakpoints	Very sensitive
Disadvantages	Time and labor intensive; miss complex and cryptic translocations	Does not detect additional chromosomal abnormalities	Inability to detect rare variants; false positivity; lower specificity with RNA cross contamination	Need to standardize across different laboratories; false positivity

FISH, fluorescence in situ hybridization; Qualitative RT-PCR, qualitative reverse transcription polymerase chain reaction; Quantitative RT-PC, quantitative reverse transcription polymerase chain reaction

  - Trisomy 19
  - Secondary Ph
  - Abnormalities of 3q26.2
  - Complex karyotype
- The additional chromosomal abnormalities (ACAs) are further subgrouped by occurring frequency into "major route" (trisomy 8, iso17q, a second Ph or trisomy 19) and "minor route" (−7, −17, +17. +21, -Y and abnormalities of 3q26) [25]. The presence of major route ACA at diagnosis has been associated with poor prognosis [26, 27]. Wang et al. proposed prognostic risk stratification based on the survival and prognosis with TKI therapy and divided ACAs into two groups. Group 1 includes trisomy 8, -Y, and a second Ph and is associated with good prognosis, whereas Group 2 includes i(17)(q10), −7/del7q, and 3q26.2 rearrangements with poor prognosis [28]. The patients with ACAs need to be monitored carefully for the evidence of therapy failure.

4. **How Is "Complete Response" to Tyrosine Kinase Inhibitor (TKI) Therapy Defined in Chronic Myeloid Leukemia (CML)?**
   - TKIs can competitively bind to the ATP-binding pocket of the ABL1 tyrosine kinase domain (TKD) so that the downstream cascade signaling pathway is halted. TKI therapy is considered the standard first-line treatment for the patients with newly diagnosed chronic-phase CML (CP-CML). Complete response to TKI therapy is determined by three different measurements.
   - Complete hematologic response (CHR) includes WBC < 10×10^9/L, platelets <450×10^9/L, the absence of immature granulocytes in peripheral blood, and impalpable spleen.
   - Complete cytogenetic response (CCyR) is defined as the absence of Ph chromosomes, which correlates with *BCR-ABL1* ≤1%. The goal of TKI therapy is to achieve a CCyR (≤1% *BCR-ABL1* IS) within 12 months after first-line TKI therapy and to prevent disease progression to AP-CML or BP-CML.
   - The major molecular response (MMR) is defined as *BCR-ABL1* (IS) ≤0.1% or 3-log reduction in *BCR-ABL1* mRNA from the standardized baselines, if qPCR (IS) is not available. The deep molecular response (DMR) is defined as MR 4.0 (*BCR-ABL1* IS ≤0.01%) or MR 4.5 (*BCR-ABL1* IS ≤0.0032%) [29, 30].
5. **What Tests Are Useful to Monitor Tyrosine Kinase Inhibitor (TKI) Therapy in the Patients with Chronic Myeloid Leukemia (CML)?**
   - Cytogenetic analysis is valuable to assess the degree of cytogenetic response and possible clonal evolution if there is disease progression or relapse.
   - Quantitative RT-PCR (qPCR) is recommended for all patients after initiating TKI therapy. The majority of CML patients achieve major or even deep molecular remissions with TKI therapy. To be noted, qPCR is the only method to monitor response after the patient has achieved in complete cytogenetic response (CCyR).
   - *BCR-ABL* kinase domain mutational analysis provides additional guidance in the selection of subsequent TKI therapy for patients who do not respond well with TKIs. 1-log increase in *BCR-ABL1* transcript levels without loss of MMR should prompt bone marrow evaluation for loss of complete cytogenetic response (CCyR). The recommended tests to monitor the response to TKI are listed in Table 14.2.
6. **What Is the Major Cause of Resistance to Tyrosine Kinase Inhibitors (TKIs) in CML? What Are the Therapeutic Options for CML?**
   - The introduction of tyrosine kinase inhibitors (TKIs) decreased mortality rates in CML. Point mutations in the BCR-ABL1 kinase domain are the major causes for the resistance of TKIs and associated with poor prognosis and higher risk of disease progression.

Currently, there are five TKIs available: imatinib (first generation); dasatinib, nilotinib, and bosutinib (second generation); and ponatinib (third generation). The T315I mutation confers complete resistance to imatinib, dasatinib, nilotinib, and bosutinib [28–30]. See Table 14.3.

Table 14.2 Tests recommended for monitoring response to TKI therapy

Test	Recommendations
Bone marrow cytogenetics	Failure to reach response milestones Any sign of loss of response (defined as hematologic or cytogenetic relapse)
qPCR using IS	Every 3 months after initiating treatment. After *BCR-ABL1* (IS) ≤ 1% (>0.1%–1%) has been achieved, every 3 months for 2 years and every 3–6 months thereafter If there is 1-log increases in *BCR-ABL1* transcript levels with MMR, qPCR should be repeated in 1–3 months
BCR-ABL kinase domain mutation analysis	Chronic phase Failure to reach response milestones Any sign of loss of response (defined as hematologic or cytogenetic relapse) 1-log increased in *BCR-ABL1* transcript levels and loss of MMR Disease progression to accelerated or blast phase

Modified from NCCN guidelines, CML, version 1.2019 [18]
MMR = major molecular response (≤ 0.1% *BCR-ABL1* IS)

Table 14.3 Therapeutic options based on the *BCR-ABL1* gene mutations

Mutation in *BCR-ABL1* gene	Treatment recommendation
Y253H, E255K/V, or F359V/C/I	Dasatinib
F317L/V/I/C, T315A, or V299L	Nilotinib
E255K/V, F317L/V/I/C, F359V/C/I, T315A, or Y253H	Bosutinib
T315I	Ponatinib, omacetaxine, or allogeneic HCT, or clinical trial

Adapted from NCCN guidelines, CML, version 1.2019 [18]
HCT, hemopoietic cell transplant

7. **What Are the Common Molecular Alterations in the *BCR-ABL1*-Negative Myeloproliferative Neoplasms?**
   - Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are collectively known as Philadelphia chromosome-negative myeloproliferative neoplasms. The identification of driver mutations in *JAK2*, *CALR*, and *MPL* provides a better understanding of the pathogenesis as well as therapeutic options.
   - PV is more homogenous in the molecular level, and most of the patients with PV (95–98%) harbor *JAK2* V617F mutation (exon 14), and the remainder of PV cases have small insertion or deletions in *JAK2* exon 12 [31–33].
   - ET and PMF have more heterogeneous molecular abnormalities with the most common mutation being *JAK2* V617F, followed by mutations in *CALR* (exon 9) and *MPL* (exon 10). These mutations are mutually exclusive to each other.
   - "Triple negative" refers to negative mutations in *JAK2*, *CALR*, and *MPL* genes. *CALR* mutations have been shown with favorable clinical courses, while "triple negative" is associated with a worse prognosis in PMF [34–36]. In the triple-negative MPNs, the mutations in *ASXL1*, *EZH2*, *TET2*, *IDH1*, *IDH2*, *SF3B1*, and *SRSF2* are also helpful in determining the clonal nature of the disease [37–40]. The comparison of these three Ph-negative MPNs is summarized in Table 14.4.
8. **Which Molecular Genetic Abnormality Is Frequently Associated with Chronic Neutrophilic Leukemia (CNL)?**
   - Chronic neutrophilic leukemia (CNL) is a rare, aggressive myeloproliferative neoplasm that is characterized with sustained neutrophilic leukocytosis (WBC ≥25 × 10^9/L with mature neutrophils ≥80% of WBC and immature neutrophilic precursors<10% of WBC; ≥ 3 months), bone marrow granulocytic hyperplasia, and frequent splenomegaly. The differential diagnosis between CNL and aCML is listed in Table 14.6 (question 16).

Table 14.4 Ph-negative myeloproliferative neoplasms

		PV	ET	PMF
Laboratory findings		↑Hb (>16.5 g/dL in men; >16 g/dL in women) ↑ Hct (>49% in men; >48% in women) ↑RBC mass (>25% of normal)	↑platelet (≥450 × 10^9/L)	Leukocytosis ≥11 × 10^9/L; anemia; leukoerythroblastosis
Morphological features		Panmyelosis, pleomorphic megakaryocytes	↑enlarged mature megakaryocytes with hyperlobated nuclei	Atypical myeloproliferation, atypical megakaryocytic hyperplasia, fibrosis of bone marrow
Molecular features	*JAK2* V617F	95–98%; *JAK2* exon 12 (2–5%)	50–60%	50–60%
	CALR		20–30%	20–30%
	MPL		3–5%	5–10%
	Triple neg.		10–16%	9–12%

PV, polycythemia vera; *ET*, essential thrombocythemia; *PMF*, primary myelofibrosis

- The colony-stimulating factor 3 receptor (*CSF3R*) mutations were identified in most of the patients with CNL [41–43]. The 2017 WHO diagnostic criteria endorsed the presence of *CSF3R* T618I or another activating *CSF3R* mutation as an important clonal marker for the diagnosis of CNL [44].
- There are two types of mutations in *CSF3R*: point mutations in the extracellular domain (exon 14)-activating JAK-STAT signaling pathway and less frequently, nonsense or frameshift mutations in the cytoplasmic tail (exon 17)-activating SRC tyrosine kinase [45]. In CNL, the most common mutation is the membrane proximal p.Thr618Ile (T618I) point mutation. Mutations in the cytoplasmic truncation mutations are often concurrent with T618I. The two distinct mutation regions and downstream kinases signaling pathways result in the different sensitivity to JAK2 inhibitors (e.g., ruxolitinib) and SRC inhibitors (dasatinib) [45, 46].

9. **How to Distinguish Chronic Neutrophilic Leukemia (CNL) from Chronic Myeloid Leukemia (CML)?**
   - A rare form of CML with p230 BCR-ABL protein may demonstrate prominent neutrophilic maturation and is called neutrophilic-CML (N-CML).
   - Both N-CML and CNL share morphological features such as prominent neutrophilic leukocytosis, minimal granulocytic precursors in the peripheral blood, and hypercellular bone marrow consisting of hyperplastic granulocytic cells.
   - The clinical course of the patients with N-CML is milder with a lower total WBC count, absent or minimal precursors in the peripheral smear, less severe anemia, less prominent splenomegaly, and blastic transformation which occurs much later [9, 43].
   - N-CML is invariably associated with a *BCR-ABL1* fusion and should be easily differentiated from CNL with proper molecular testing.
10. **What Molecular Changes Are Associated with Mastocytosis?**
    - Detection of the *KIT* D816V (exon 17)-activating mutation in the bone marrow, blood, or other extracutaneous organs is counted as a minor criterion for the diagnosis of cutaneous and systemic mastocytosis. *KIT* D816V can be detected in more than 80% of the patients. KIT D816V is considered as a major therapeutic target in advanced systemic mastocytosis (SM). Though D816V mutation is resistant to imatinib and masitinib, several drugs have been developed (e.g., nilotinib, dasatinib, and midostaurin) to target this mutation. Wild-type KIT and other mutations such as K509I or F522C are sensitive to imatinib [47, 48].
    - Besides D816V, other oncogenic variants of *KIT* in exons 8, 9, 10, and 11 have been detected. In advanced systemic mastocytosis (SM) and especially in patients with systemic mastocytosis with associated hematological neoplasm (SM-AHN), somatic mutations have been detected in *KIT* and its signaling pathways [49–51].
11. **What Is the Underlying Molecular Abnormality of *PDGFRA* Rearrangement?**
    - Myeloid/lymphoid neoplasms with eosinophilia involving recurrent genetic abnormalities of *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2* are a specific disease entity defined by the 2017 WHO [44]. Tyrosine kinase inhibitors have been proven successful for the treatment of *PDGFRA*-, *PDGFRB*-, and *PCM1-JAK2*-related diseases. However, *FGFR1*-associated neoplasms are resistant to imatinib therapy and associated with a poor prognosis [52, 53].
    - *FIP1L1-PDGFRA* fusion gene results from an 800-kb cryptic interstitial deletion that includes the cysteine-rich hydrophobic domain 2 (*CHIC2*) loci at 4q12. The deletion disrupts the *FIP1L1* and *PDGFRA* genes and fuses the 5′ part of *FIP1L1* to the 3′ part of *PDGFRA* [53, 54].
    - *FIP1L1-PDGFRA* fusion gene can only be detected by break-apart FISH or RT-PCR due to cryptic deletion [Fig. 14.1]. Since the *CHIC2* gene is in this deleted region, the test is also referred as "FISH for *CHIC2* deletion" [55, 56]. The *FIP1L1-PDGFRA* fusion has been identified in patients with increased eosinophilia associated with acute myeloid leukemia, mast cell neoplasms, and T-cell lymphoblastic lymphoma [57, 58].
12. **What Are the Common Underlying Genetic Abnormalities Involved in Chronic Myelomonocytic Leukemia (CMML)?**
    - Chronic myelomonocytic leukemia (CMML) is a clonal hematopoietic stem cell disorder with overlapping features of MDS and MPN and potential evolution to acute myeloid leukemia. It is characterized by the presence of sustained (>3 months) peripheral blood monocytosis ($\geq 1 \times 10^9$/L; monocytes ≥10% of white blood cells count) with or without dysplastic changes in the bone marrow [44]. The *BCR-ABL1* fusion and rearrangements of *PDGFRA*, *PDGFRB*, or *FGFR1* are absent.
    - Clonal cytogenetic abnormalities are seen in about 20–40% of patients. Most common abnormalities include trisomy 8, monosomy 7, del (7q), trisomy 21, and complex karyotypes [59, 60].
    - Recurrent somatic gene mutations have been identified in up to 90% of CMML cases. These gene muta-

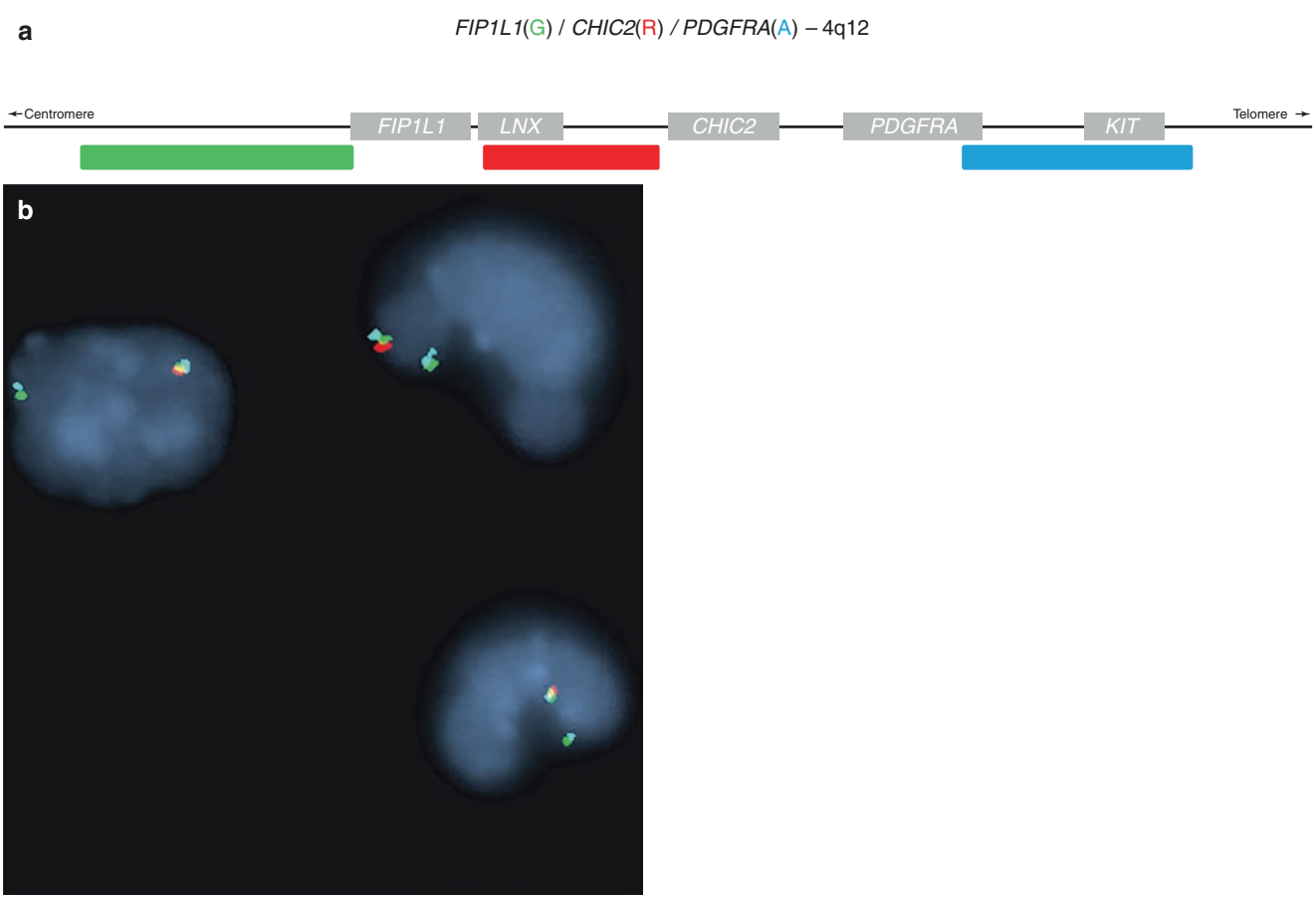

Fig. 14.1 (**a**) Schematic representation of the three probes for FIP1L1 (green), CHIC2 (red), and PDGFRA genes(aqua) that flank the 4q12 region. (**b**) The deletion of CHIC2 resulted in the fusion of 5′ of FIP1L1 to the 3′ part of PDGFRA. The absence of CHIC (red) signaling and the presence of the two flaking probes is indicative of the deletion of CHIC gene. (Credit for Jason Yuhas, Genomics Laboratory, Mayo Clinic, MN)

tions in CMML are divided into three groups: regulate cell signaling molecules (*KRAS*, *NRAS*, *CBL*, *PTPN11*, *FLT3*, *JAK2*), splicing factors (*SRSF2*, *SF3B1*, *ZRSF2*, *U2AF1*), and epigenetic control of transcription such as DNA methylation (*DNMT3A*, *IDH1*, *IDH2*, and *TET2*) and histone modification (*ASXL1*, *EZH2*, and *BCOR*) [61–71]. Of these, the most frequent mutations involve *TET2* (~60%), *SRSF2* (~50%), *ASXl1* (~40%), and the RAS signaling pathway (~30%). The triad of *TET2*, *SRSF2*, and *ASXL1* mutations is very specific for CMML [72, 73].

13. **What Is the Prognostic Significance of the Genetic Changes in Chronic Myelomonocytic Leukemia (CMML)?**
    - Karyotypic abnormalities occur in 20–30% of patients with CMML. The Spanish CMML-specific cytogenetic risk stratification (CPSS) system separates the patients into three prognostic groups: low risk (normal karyotype and isolated loss of Y chromosome), high risk (trisomy 8, chromosome 7 abnormalities and complex karyotype), and intermediate (all other karyotypic abnormalities). The 5-year overall survival (OS) was 35% for low-risk, 26% for intermediate, and 4% for high-risk groups [60, 74].
    - Mayo molecular model (MMM) that focused on the combination of hemoglobin, absolute monocyte, circulating immature myeloid cells and platelet values, and *ASXL1*. The univariate analysis showed a poor prognostic value of nonsense/frameshift *ASXL1* mutations [75, 76].
    - CMML-specific prognostic scoring system (CPSS) was updated to include molecular mutations in *RUNX1*, *NRAS*, *SETBP1*, and *ASXL1* in addition to the prior CPSS cytogenetic scores. The CPSS-Mol stratified CMML into four risk groups: low (0 risk factors), intermediate-1 (1 risk factor), intermediate-2 (2–3 risk factors), and high (≥4 risk factors) [77, 78]. *NPM1* mutation in CMML is rare and tends to be associated with normal cytogenetics, dysplastic CMML, *DNTM3A* mutations, and high risk of AML transformation [79, 80].

14. **In the 2017 WHO Classification, Chronic Myelomonocytic Leukemia (CMML) Is Further Categorized into "Proliferative CMML" and "Dysplastic CMML." What Are the Clinical and Molecular Genetic Differences Between These Two Types?**
    - CMML is heterogeneous with different clinical manifestations and underlying molecular changes. CMML is further divided into "dysplastic CMML (WBC < 13 × 10^9/L)" and "proliferative CMML" (WBC ≥ 13 × 10^9/L) [Table 14.5]. If myelodysplasia is absent or minimal, a diagnosis of CMML can still be made if clonal cytogenetic or molecular abnormalities are present [44, 61, 81, 82].
15. **Which Genetic Abnormalities Are Relatively Common in Atypical Chronic Myeloid Leukemia (aCML), *BCR-ABL1*-Negative?**
    - Atypical chronic myeloid leukemia (aCML) is a rare subtype of MDS/MPN. Patients tend to have severe anemia, thrombocytopenia, neutrophilic leukocytosis, granulocytic dysplasia, and splenomegaly. *BCR-ABL1* fusions as well as the rearrangements of *PDGFRA*, *PDGFRB*, or *FGFR1* are absent in aCML.
    - The most common cytogenetic abnormalities are gain of chromosome 8 and del(20q) [84, 85]. The other reported changes included −7/−7q and i17 (q); deletions of 5q, 13q, 17p, 12q, and 11q; t(6,8) (p23;q22); trisomy 14, 21, and 19; and complex karyotype [86]. However, none of these abnormalities is specific for aCML.
    - Currently, no specific molecular changes have been identified for aCML. Recurrent *SETBP1* mutations, which are encountered in 12–33% of aCML patients, are associated with worse prognosis than aCMLs with wild-type *SETBP1* [87–89]. However, *SETBP1* mutations have also been described in patients with CMML (15%) and JMML (<3%) [89–92].
    - Somatic missense mutations involving ETNK1 have been found in 8.8% of aCML cases [93]. Other somatic mutations involving *NRAS*, *KRAS*, *TET2*, *EZH2*, *JAK2*, *IDH2*, *CSF3R*, *SRSF2*, *RUNX1*, *CEBPA*, *ASXL1*, and *CBL* have also been detected in aCML, although at a much lower frequency [72, 86, 88, 94].
16. **What Are the Major Features to Differentiate Atypical Chronic Myeloid Leukemia (aCML), *BCR-ABL1*-Negative, from Chronic Neutrophilic Leukemia (CNL)?**

    There are strong morphological and clinical resemblances between aCML and CNL. Lack of specific molecular markers makes the diagnosis challenging in some cases. It is important to incorporate clinical presentations, morphology, and molecular markers for an accurate diagnosis [Table 14.6].
17. **Which Genes Are Most Commonly Mutated in Juvenile Myelomonocytic Leukemia (JMML)?**
    - Juvenile myelomonocytic leukemia (JMML) is a rare MDS/MPN disorder that occurs during infancy and early childhood, clinically characterized by the overproduction of myelomonocytic cells. It is associated

Table 14.5 Dysplastic CMML and proliferative CMML [44, 61, 72, 83]

	Dysplastic CMML	Proliferative CMML
Clinical features and prognosis	Easy bruising, recurrent infection, transfusion dependent	Fatigue, night sweats, organomegaly, worse prognosis than dysplastic CMML
Peripheral blood	WBC < 13 × 10^9/L Cytopenia	WBC ≥ 13 × 10^9/L Leukocytosis, monocytosis
Molecular	More mutations in splicing pathway (*SF3B1*, *SRSF2*, *ZRSR2*, and *U2AF35*)	More mutations in RAS-signaling pathway (*JAK2*, *NRAS*, *KRAS*, *CBL*, and *PTPN11*)

CMML, chronic myelomonocytic leukemia

Table 14.6 The WHO 2017 revised diagnostic criteria for CNL and aCML [44]

	aCML	CNL
Peripheral blood	WBC ≥ 13 × 10^9/L	WBC ≥ 25 × 10^9/L;
	↑ numbers of neutrophils	Segmented neutrophils plus bands ≥80% of WBC
	Neutrophil precursors ≥10% of WBC No/minimal absolute basophilia	Neutrophil precursors <10% of WBC Myeloblasts rare or absent
	No/minimal monocytosis[b]	
Bone marrow	Hypercellular BM, ↑neutrophils	Hypercellular BM, ↑neutrophils
	Dysgranulopoiesis; ± erythroid and megakaryocytic dysplasia	No dysgranulopoiesis
	Myeloblasts <20%	Myeloblasts<5%
Molecular Genetics	No specific alteration. See question 15	Presence of *CSF3R*T618I or other activating *CSF3R* mutation[a]
	Not meeting WHO criteria for *BCR-ABL1*+ CML, PMF, PV or ET[b] No rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1* or *PCM1-JAK2*[b]	

CML, atypical chronic myeloid leukemia, *BCR-ABL1*-negative; *CNL*, chronic neutrophilic leukemia

[a]For CNL, in the absence of a *CSF3R* mutation, alternative diagnostic criteria requires persistent neutrophilia (≥3 months) and no identifiable cause of reactive neutrophilia, including absence of a plasma cell neoplasm or if present, demonstration of clonality of myeloid cells by cytogenetic of molecular studies

[b]Common features present in both aCML and CNL

with a poor prognosis and shares some clinical and molecular features with CMML.

- The recurrent mutations in the RAS signaling pathway are the main driving events in JMML. About 90% of the patients harbor either a somatic or germline mutation in the genes of *PTPN11*, *NF1*, *NRAS*, *KRAS*, and *CBL*; these genetic mutations are largely mutually exclusive. Among these, the gain-of-function mutations in *PTPN11* are the most common molecular genetic changes (35%) in JMML [95–97]. A recent study with RNA-sequencing detected ALK/ROS1 tyrosine kinase fusion (18%) in JMML patients without RAS pathway mutations [98]. Germline mutations in *NF1* are present in 10% of children with JMML. In some cases, JMML may be the first sign of neurofibromatosis 1. The patients are mostly diagnosed after 5 years and have a higher blast count in BM and higher platelet count than the patients without NF1 [98, 99].
- Noonan syndrome is the most common RASopathy, involving germline mutations in *PTPN11* (~50%), *SOS1*, *RAF1*, *RIT1*, *KRAS*, or other genes of the RAS signaling pathway. Approximately 3% of neonates and infants with Noonan syndrome develop JMML [100].

18. **What Are the Common Molecular Genetic Abnormalities That Are Associated with Myelodysplastic/Myeloproliferative Neoplasm with Ring Sideroblasts and Thrombocytosis (MDS/MPN-RS-T)?**
    - MDS/MPN-RS-T is characterized by thrombocytosis (≥450 × 10^9/L), refractory anemia and dyserythropoiesis with ring sideroblasts (≥15% of erythroid precursors) in the bone marrow. Somatic mutations in the spliceosome gene SF3B1 are associated with ring sideroblasts and are highly associated (65–90%) with MDS/MPN-RS-T [101–103].
    - *SF3B1* mutations often coexist with *JAK2* V617F (~50%) and less commonly *CALR* (0–3%) or *MPL* (1–3%) in MDS/MPN-RS-T [104–106]. *SF3B1* mutations confer increased risk of thrombosis in patients with MDS/MPN-RS-T [107]. Mutations of *TET2*, *ASXL1*, *SETBP1*, and *DNMT3A* were also detected in several cohort studies [105].
    - The prognosis of MDS/PMN-RS-T is better than that of MDS-RS but inferior to that of ET. The patients with *SF3B1* mutations had fewer cytopenias and longer event-free survival than those with wild-type [102]. The presence of a *SF3B1* mutation is an independent predictor for a favorable clinical outcome, while *ASXL1* or *SETBP1* mutations are associated with poor prognosis [101, 105].

19. **What Are the Disease-Defining Chromosomal Abnormalities in Myelodysplastic Syndrome (MDS)?**
    - Chromosomal abnormalities can be detected by cytogenetics in 50% of de novo MDS cases [110]. Of these, the most common abnormalities are monosomy 5/del(5q), trisomy 8, and monosomy 7/del(7q) [111]. Balanced chromosomal translocations are relatively rare (<2–3%) in MDS and are also important to help the diagnosis of MDS with equivocal morphological dysplastic features [112, 113]. The recurrent chromosomal abnormalities are summarized in Table 14.7. Presence of one of these chromosomal abnormalities is presumptive evidence of MDS in patients with otherwise unexplained refractory cytopenia and no morphologic evidence of dysplasia [45]. Complex chromosomal abnormalities are defined as multiple (≥3) chromosomal abnormalities and often associated with TP53 mutation and a poor clinical course [114].
    - It should be noted that certain cytogenetic alterations have been found in the normal elderly population, such as -Y. Without definitive morphological evidence, these cytogenetic changes are insufficient to establish a diagnosis of MDS.

20. **What Are the Typical Clinicopathological Findings in Patients with Myelodysplastic Syndrome (MDS) with Isolated del(5q)?**
    - MDS with isolated del(5q) is defined by the presence of cytogenetic abnormality involving an interstitial deletion of the long arm of chromosome 5 (5q) with or without one additional cytogenetic abnormality (except del(7q) or monosomy 7) and in the absence of increased blasts [110, 111]. There are two common deleted regions (CDR): one is the region flanking 5q32–33.1 which is with 5q-syndrome that confers a good clinical course; the other one is 5q31.2 which is more common in patients with high-risk MDS and therapy-related myeloid neoplasms [112–114]. In the 2017 WHO classification, isolated del(5q) is the only cytogenetic abnormality to define a specific MDS subtype.
    - MDS with isolated del(5q) is one of the most common cytogenetic changes in patients with MDS (10–15%), and mostly affect elderly women and typically present macrocytic anemia, thrombocytosis, neutropenia, and hypolobated small megakaryocytes in the bone marrow [115].
    - Patients with MDS with isolated del(5q) generally have a favorable prognosis with a median survival and a low risk of transformation to AML [116, 117]. Gain of an additional clonal aberration with monosomy 7 or del(7q) was shown to confer a poor prog-

Table 14.7 The cytogenetic scoring system in MDS, revised [44, 108, 109]

Chromosomal abnormalities		Prognosis	Frequency (%)
Unbalanced	de1(11q)	Very good	3
	Loss Y[b]	Very good	5
	del(5q)[a]	Good	10
	del(12p)	Good	3
	del(20q)[b]	Good	5–8
	Double, including del(5q)	Good	
	Trisomy 8[b]	Intermediate	10
	Isochromosome 17 or t(17p)	Intermediate	3–5
	Trisomy 19	Intermediate	
	Any other single- or double-independent abnormalities	Intermediate	
	inv(3), t(3q), or del(3q)	Poor	
	Monosomy 7[c]	Poor	[c]
	Double including loss of 7 or del(7)	Poor	
	Complex karyotype (>3)	Very poor	
	Loss of chromosome 13 or del(13q)	Favorable	3
	del(9q)		1–2
	Idic(X)(q13)		1–2
Balanced	t(3;21)(q26.2;q22.1)		
	t(1;3)(p36.3;q21.3)		1
	t(2;11)(p21;q23.3)		1
	Inv(3)(q21.3q26.2)		1
	t(6;9)(p23.3;q34.1)		1
	t(11;16)(q23.3;p13.3)		

[a]Isolated del(5q)/del(5q) plus one other abnormality (with the exception of monosomy7/del(7q))
[b]Without definitive morphological evidence, trisomy8, -Y, and del(20q) cannot be used for establishing diagnosis of MDS
[c]The combined frequency for monosomy7 and del(7q) is 10%

nosis [118]. TP53 mutation has been found to correlate with a significantly worse outcome, which is helpful to further refine the prognostic risk [119, 120]. *ASXL1* mutation is associated with a higher risk of AML transformation [111].

- Lenalidomide, an immunomodulatory drug with efficacy in multiple myeloma (MM), is the standard therapy for the patients with MDS with isolated del(5q). However *TP53* mutation has been shown to confer lenalidomide resistance [115].

21. **What Are the Common Molecular Abnormalities Associated with MDS?**
    - Gene mutations in MDS can occur with or without chromosomal abnormalities and have been shown to have prognostic and therapeutic significance. The common driver genes mutated in MDS with mutational frequency and respective common associations are summarized in Table 14.8.
    - The driver genes are classified into several functional pathways including DNA methylation, RNA spliceosome machinery, histone modification, transcription, signal transduction, DNA repair, and cohesion complexes [121].
    - The most frequently mutated genes are *SF3B1*, *TET2*, *SRSF2*, and *ASXL1* (>10%), followed by *DNMT3A* and *RUNX1* (5–10%) [122]. Most of these mutations are associated with functional loss, instead of activating mutations. The wide spectrum of the mutations contributes to the different clinical courses in the patients with MDS.
    - Keep in mind that germline mutations in *DDX41*, *RUNX1*, *GATA2*, and *TP53* may also occur, and it is crucial to screen the family members when bone marrow transplant is the treatment of choice.
22. **How to Differentiate Idiopathic Cytopenia of Undetermined Significance (ICUS), Clonal Hematopoiesis of Indeterminate Potential (CHIP), Clonal Cytopenia of Undetermined Significance (CCUS), and Myelodysplastic Syndrome from Each Other?**
    - ICUS, CHIP, and CCUS are considered precursor conditions that can progress to MDS, AML, or other hematologic malignancies [Table 14.9]. It is important to make accurate diagnosis for monitoring cytopenia and clinical follow-up.
    - ICUS is defined as persistent cytopenia (≥6 months) in one or more lineages and absence of fulfillment of the diagnostic criteria for MDS. CCUS is defined as persistent cytopenia (≥4 months) in one or more lineages as well as at least one somatic mutation in

Table 14.8 Common recurrent mutations in MDS

Mutated genes		Prognostic impact	Frequency (%)	Associations with
RNA splicing	*SF3B1*	Favorable	20–30	Ring sideroblasts; *DNMT3A* mutation
	SRSF2	Adverse	10–20	*RUNX1*, *TET2*, *IDH1* mutations *RUNX1* overexpression
	U2AF1	Unknown	5–10	*ASXL1*, *IDH2* mutations
	ZRSR2	Unknown	<5	*TET2* mutations
DNA methylation	*TET2*	Unknown	20–30	60% CMML
	DNMT3A	Unfavorable	5–10	*IDH2* mutations
	IDH1/2	Unfavorable	5	*DNMT3A*, *ASXL1*, and *SRSF* mutations
Histone modification	*EZH2*	Unfavorable	5	*U2AF1* mutations
	ASXL1	Unfavorable	15–20	
Transcription	*RUNX1*	Unfavorable	5–10	t-MN, *SRSF2* mutations, −7/del(7)
	NRAS	Unfavorable	5	−7/del(7)
	ETV6	Unfavorable	<5	
	SETBP1	Unfavorable	2–5	del(7q) and *ASXL1* mutations
	BCOR	Unfavorable	5	
	GATA2	Unfavorable	Rare	
Signaling	*CBL*	Unfavorable	5	
	JAK2		5%	In 50% of cases of MDS/MPN-RS-T
	FLT3	Unfavorable	<5	
	KIT		Rare	
DNA repair	*TP53*	Unfavorable	5–10	Poor prognosis; rarely a/w spliceosome mutations
Cohesion complex	*STAG2*	Unfavorable	5–7	Rarely identified as a founding clone
	SMC3		<3	
	RAD21		<3	

Modified [109, 121, 123]

Table 14.9 Comparison of ICUS, CHIP, CCUS, and MDS

	ICUS	CHIP	CCUS	MDS
Dysplasia	–	–	–	+
Cytopenia	+	–	+	+
Clonality	–	+	+	+

ICUS, idiopathic cytopenia of undetermined significance; *CHIP*, clonal hematopoiesis of indeterminate potential (CHIP); *CCUS*, clonal cytopenia of undetermined significance; *MDS*, myelodysplastic syndrome

MDS-associated genes (allele burden ≥2% in bone marrow or peripheral blood).

- CHIP is defined as the absence of persistent cytopenia but presents ≥1 somatic mutation in MDS-associated genes (≥2% variant allele frequency) [124, 125].

Case Presentation

Case 1

Learning Objectives

To become familiar with the underlying cytogenetic abnormalities for CML accelerated and blast phase.

Case History

A 30-year-old male with a history of CML (Philadelphia chromosome-positive, p210) presented with fever and diarrhea with CBC: Hb 10.9 g/dL; RBC 3.79 × 10^{12}/L; MCV 89.7 fL; RDW 20.8%; WBC 11.0 × 10^{9}/L; and platelet 341 × 10^{9}/L. Peripheral blood smear was reviewed as no cytological abnormalities with no blasts identified.

Bone Marrow, Biopsy, and Aspirate

- Bone marrow core biopsy with hypercellular bone marrow with abnormal interstitial infiltration of immature cells [Fig. 14.2a].
- Bone marrow aspirate smear showed the immature cells have high N/C ratio, scant basophilic cytoplasm, fine chromatin, and conspicuous nucleoli, consistent with blast cells [Fig. 14.2b].

Differential Diagnosis

- CML chronic phase
- CML blast phase
- CML accelerated phase

Ancillary Studies

- IHC demonstrated that the blasts are mostly negative for CD34 and positive for TdT. On the flow cytometry analysis, the blasts are positive for CD19 and CD10 but are negative for CD20 and MPO.

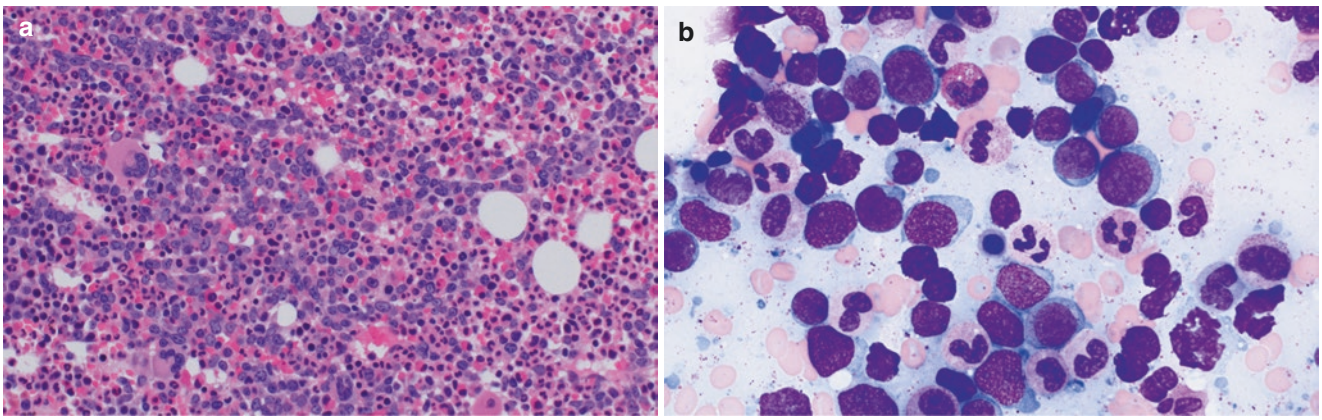

Fig. 14.2 (**a**) Bone marrow biopsy (40×). (**b**) Bone marrow aspirate smear (100×)

- Flow cytometry on the bone marrow showed 22% blasts with B-ALL phenotype that are positive for CD34 (partial), CD19, CD10 (partial), CD45 (dim), CD13 (partial), CD33 (dim), HLA-DR, CD38, and CD9 (partial) and negative for CD3, CD15, CD16, CD117, CD2, CD7, CD56, CD36, CD64, CD20, and MPO.
- Cytogenetic: 46,XY,add(5)(q13),t(9;22)(q34;q11.2)[4]/46,Y,t(X;5)(p10;p10),add(5)(q13),t(9;22)(q34;q11.2),der(17)t(5;17)(q13;q21) [4]/46,XY,t(2;21)(p13;q22),add(5)(q13),t(9;22)(q34;q11.2) [1]/46,XY [10]. Of the 20 metaphases, 10 were normal, and 10 had a t(9,22)(q34;q11.2) and additional abnormalities.
- FISH analysis: 1.8% of nuclei had *BCR-ABL1* fusion.
- Molecular studies: *BCR-ABL1* RT-qPCR result of 1.47 (IS).

Final Diagnosis

Chronic myeloid leukemia, *BCR-ABL1*-positive, blast phase (22% B-lymphoblasts)

Take Home Messages

1. CML can progress into blast phase with increased blast, ≥ 20% in the blood or bone marrow, or the presence of extramedullary blast proliferation.
2. Progression is often associated with additional cytogenetic changes including a second Ph chromosome, trisomy 8, trisomy 19, and isochromosome 17q.
3. The blast lineage can be myeloid (70–80%) or lymphoid (20–30%). The prognosis of the blast phase is poor.

Case 2

Learning Objectives

To become familiar with the molecular basis of essential thrombocythemia.

Case History

A 46-year-old male presented with thrombocytosis for a few years. He denies headaches, dizziness, blurred vision, fevers, chills, weight loss, or night sweat. *JAK2* mutation analysis from outside the hospital showed negative for V617F. Current CBC: Hb 15.4 g/dL; RBC 4.70 × 10^{12}/L; MCV 94.5 fL; RDW 12.9%; WBC 6.6 × 10^{9}/L; platelet 1151 × 10^{9}/L.

Bone Marrow, Biopsy and Aspirate

- Normocellular bone marrow with trilineage hematopoietic maturation and increased number of large megakaryocytes dispersed throughout. No significant increase in erythropoiesis or granulopoiesis [Fig. 14.3a].
- The megakaryocytes are presented with abundant mature cytoplasm and hypersegmented (staghorn-like) nuclei. Some form loose clusters [Fig. 14.3b].

Differential Diagnosis

- Reactive thrombocytosis
- Essential thrombocythemia
- Primary myelofibrosis
- Polycythemia vera

Ancillary Studies

- Iron stain, bone marrow aspirate: increased storage iron. Sideroblasts present. No ring sideroblasts seen
- Reticulin stain, bone marrow biopsy: no increase in reticulin fibrosis, grade 0 of 3
- Cytogenetics (bone marrow): 46, XY [20]
- Molecular analysis (*JAK2*, *CALR*, and *MPL* mutation): positive for *CALR* mutation [Fig. 14.3c]. Negative for *JAK2* V617F and *MPL* mutation

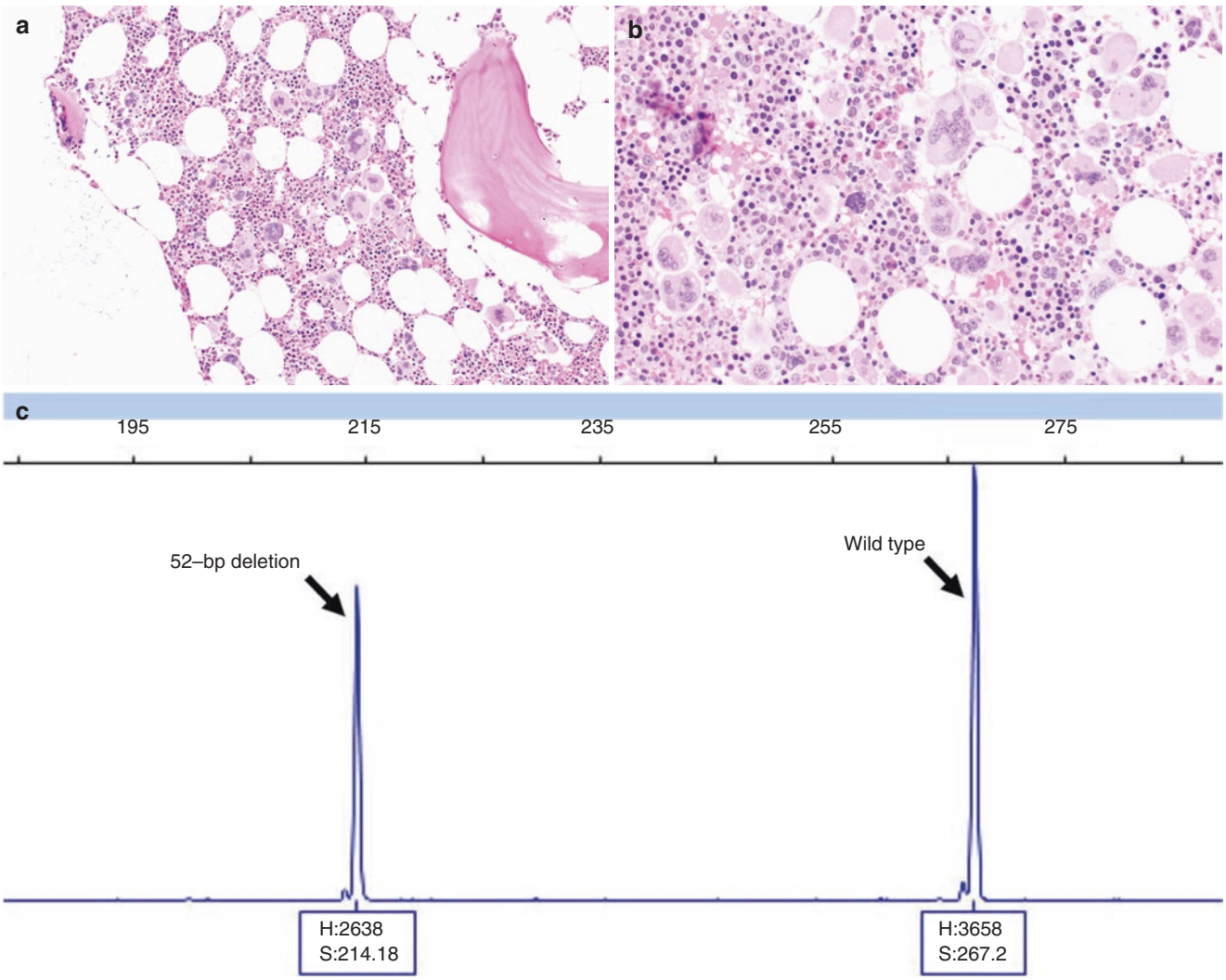

Fig. 14.3 (**a**) Bone marrow biopsy (20×). (**b**) Bone marrow biopsy (40×). (**c**) CALR mutation result

Final Diagnosis

Essential Thrombocythemia

Take Home Messages

1. ET is characterized with an elevated platelet count (≥ 450 × 10^9/L) on routine CBC and presence of a clonal marker. In most cases (>95%), mutations of one of the three genes (*JAK2*, *CALR*, and *MPL*) can be detected, and they are mutually exclusive.
2. The majority of *CALR* mutational changes are insertion or deletion in exon 9 resulting in truncated protein. The 52-bp deletion (type 1) and the 5-bp insertion (type 2) are the most frequent *CALR* mutations.
3. Triple-negative ETs have a better prognosis, whereas triple-negative PMFs are associated with a worse survival rate.
4. Morphology is important to distinguish ET from prefibrotic/early primary myelofibrosis (PMF), which may also present as thrombocytosis [Table 14.4]. The patients with ET have a low risk of progression to acute leukemia and superior overall survival than those with pre-PMF.

Case 3

Learning Objectives

To become familiar with the diagnostic criteria for systemic mastocytosis.

Case History

A 56-year-old female with a history of urticaria pigmentosa presented with persistent diarrhea. Image studies showed mild hepatomegaly, significant splenomegaly, and mild thickening of the stomach walls. The biopsy of the colon demonstrated mast cell infiltration. The tryptase level was

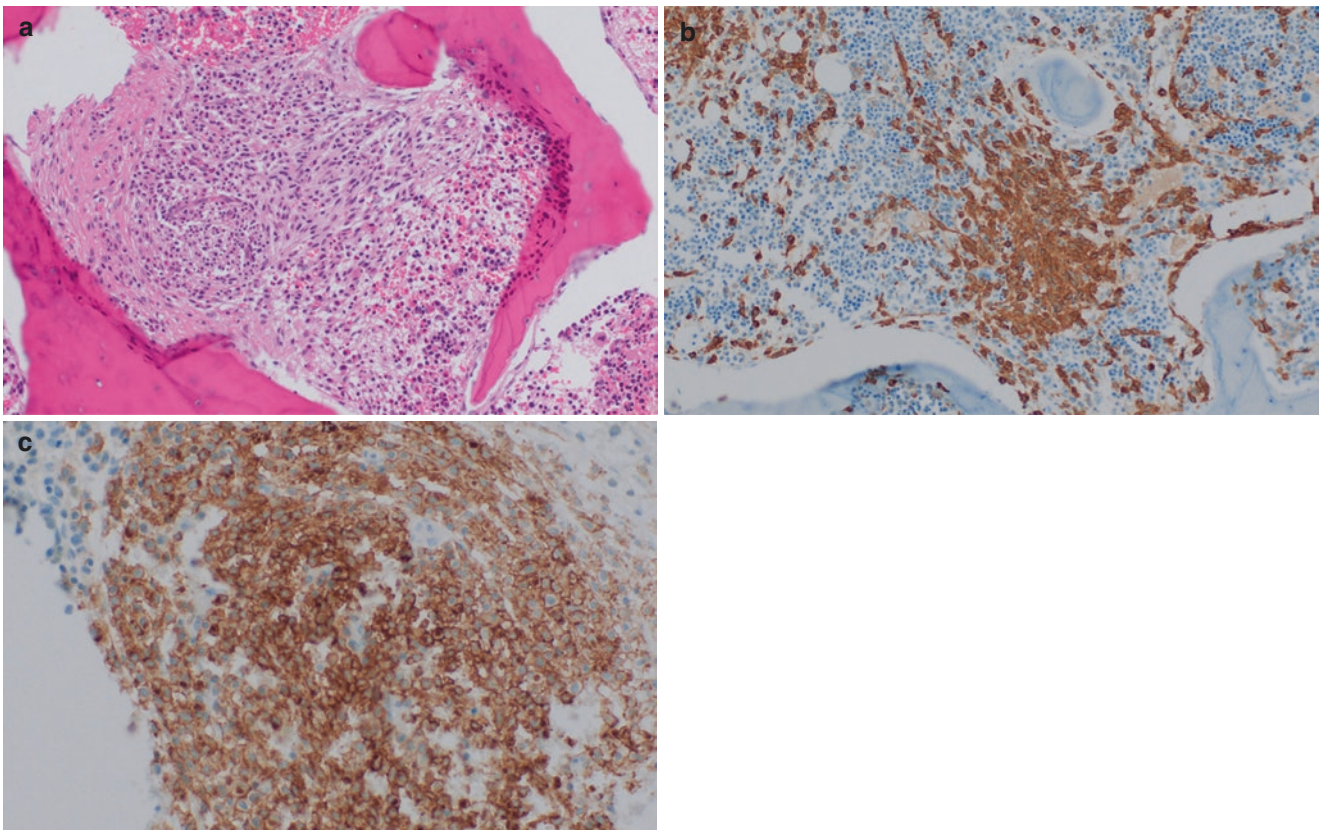

Fig. 14.4 (**a**) Bone marrow biopsy (20×). (**b** and **c**) Immunohistochemical stains for CD117 and CD25, respectively

498 ng/ml. Current CBC: Hb 8.2 g/dL; RBC 2.6 × 10^{12}/L; MCV 91.7 fL; RDW 16.2%; WBC 2.7 × 10^{9}/L (lymphocytes 1%, monocytes 9%, eosinophil 17%); platelet 23 × 10^{9}/L.

Bone Marrow, Biopsy, and Aspirate (BM-184793)

- Abnormal paratrabecular infiltration of dense spindle-shaped cells with increased eosinophils [Fig. 14.4a]

Differential Diagnosis

1. Reactive mastocytosis
2. Systemic mastocytosis

Ancillary Studies

- On immunohistochemistry stain, the neoplastic cells are strongly positive for CD117 [Fig. 14.4b] and CD25 [Fig. 14.4c].
- Cytogenetics (bone marrow): 46,XX [20].
- Molecular analysis (bone marrow): positive for KIT p. Asp816Val.

Final Diagnosis

Systemic mastocytosis

Take Home Messages

1. The diagnosis for systemic mastocytosis requires one major criterion and at least one minor criterion; or ≥ 3 minor criteria are met.
2. The major criterion is the presence of multifocal clusters of mast cells (≥15 mast cells in aggregates) in the bone marrow and/or extramedullary site(s).
3. The minor criteria include atypical morphology of mast cells (≥25% of mast cells), activating mutation at codon 816 of *KIT*, abnormal expression CD25, with or without CD2, and serum total tryptase >20 ng/ml.

Case 4

Learning Objectives

To become familiar with the diagnostic criteria for chronic myelomonocytic leukemia.

Case History

A 64-year-old male was referred with progressive drop in hemoglobin, white blood cell count, and platelet count over several years. He also complained of intermittent left-sided abdominal pain and low back pain. He denied any recurrent

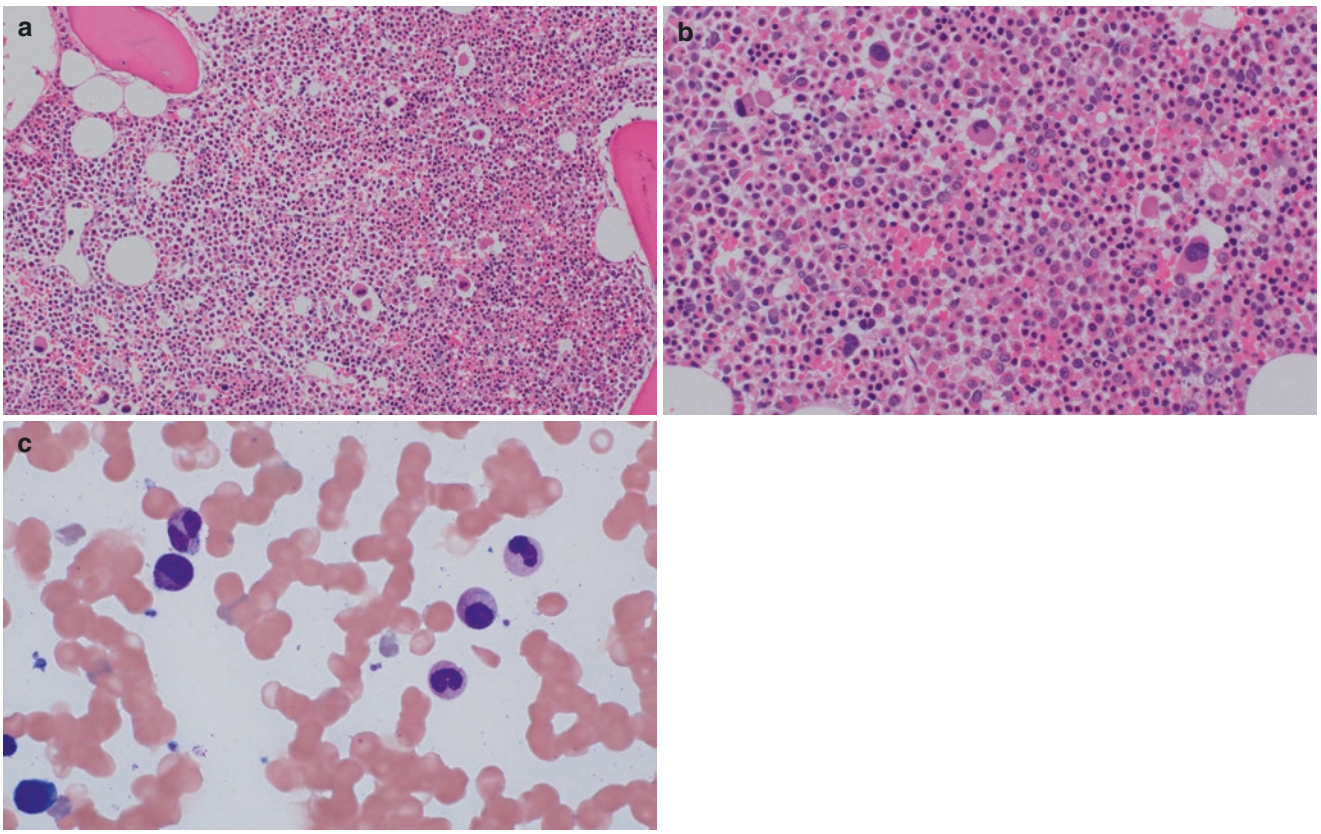

Fig. 14.5 (**a**) Bone marrow biopsy (20×). (**b**) Bone marrow biopsy (40×). (**c**) Bone marrow aspirate smear (100×)

infection or bleeding tendencies. Imaging showed mild splenomegaly. Current CBC: Hb 8.1 g/dL; RBC 2 × 10^{12}/L; MCV 90 fL; RDW 14%; WBC 3.2 × 10^{9}/L; platelet 106 × 10^{9}/L. White blood cell differential showed absolute monocytosis with monocytes 36%.

Bone Marrow, Biopsy, and Aspirate

- Hypercellular marrow with granulocytic and megakaryocytic proliferation. Frequent small forms dysplastic megakaryocytes with monolobated and/or hyperchromatic nuclei are noted [Fig. 14.5a and b].
- Marrow aspirate smear demonstrated the dysgranulopoiesis with hypolobated/pseudo-Pelger-Huet and hypogranular forms. Blasts are minimally increased (4%) [Fig. 14.5c].

Differential Diagnosis

1. Atypical chronic myeloid leukemia, *BCR-ABL1*-negative
2. Chronic myelomonocytic leukemia
3. Myelodysplastic syndrome with multilineage dysplasia
4. Reactive monocytosis

Ancillary Studies

Iron stains (bone marrow aspirate): normal stainable storage iron. Sideroblasts present. Rare ring sideroblasts are not seen.

Cytogenetics (bone marrow): 46, XY [20].

Molecular analysis: pathogenic mutations detected as follows:

1. *TET2*: c.538C > T; p.Gln180* (6%)
   c.774dup; p.Glu259* (14%)
   c.2524dup; p.Ser842Phefs*4 (6%)
   c.4546C > T; p.Arg1516* (7%)
2. *ZRSR2*: c.122-1G > A; p.? (89%)

Final Diagnosis

Chronic myelomonocytic leukemia-0 (CMML-0)

Take Home Messages

1. CMML is characterized by persistent monocytosis (≥1 × 10^{9}/L and ≥ 10% of WBC) in the peripheral blood as well as dysplastic changes in the bone marrow. Cytopenias and splenomegaly are common. It is divided

into proliferative type (WBC ≥ 13×10^9/L) and dysplastic type (<13×10^9/L).
2. The most common molecular mutations in CMML are *TET2*, *SRSF2*, *ASXL1*, and *SETBP1*.
3. The mutation profile of this case provides clonal evidence; although not entirely specific, is extremely helpful for the definitive diagnosis.

Case 5

Learning Objectives

To become familiar with the clinical presentation and diagnostic criteria for MDS with isolated del(5q) (correlation and comparison with Case 6).

Case History

A 80-year-old female presented with fatigue, and she denies any recent weight loss, night sweats, or fever. No history of pulmonary or cardiovascular diseases. No exposure to chemotherapy, radiation, or mutagens and normal levels of folate, vitB12, copper, iron/ferritin, TSH, and LDH. Current CBC: Hb 7.2 g/dL; RBC 1.8×10^{12}/L; MCV 122.7 fL; RDW17%; WBC 2.5×10^9/L; PLT 159×10^9/L.

Bone Marrow, Biopsy, and Aspirate

- Many small and monolobated forms distributed in loose clusters [Fig. 14.6a]
- Bone marrow smears of the same case [Fig. 14.6b]

Differential Diagnosis

1. Myelodysplastic syndrome with isolated del(5q)
2. Myelodysplastic syndrome with single lineage dysplasia

Ancillary Studies

Iron stain (bone marrow aspirate): normal storage iron, sideroblasts are present, no ring sideroblasts.

On immunohistochemical stain, CD61 highlights many small hypolobated/monolobated megakaryocytes [Fig. 14.6c]

Cytogenetic (bone marrow): 46,XX,del(5)(q15q33)[18]/46,XX [1]

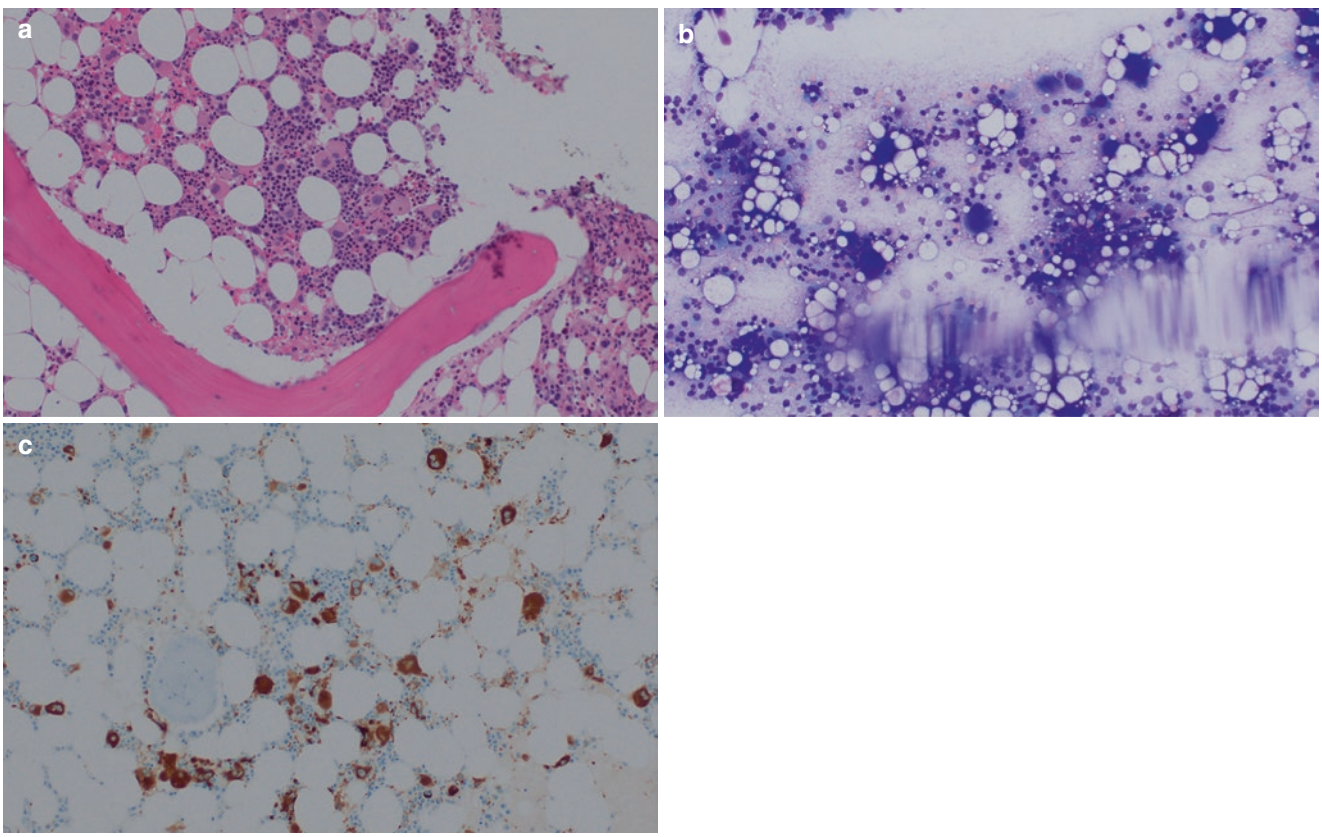

Fig. 14.6 (**a**) Bone marrow biopsy (20×). (**b**) Bone marrow smear (100×). (**c**) Immunohistochemical stain for CD61

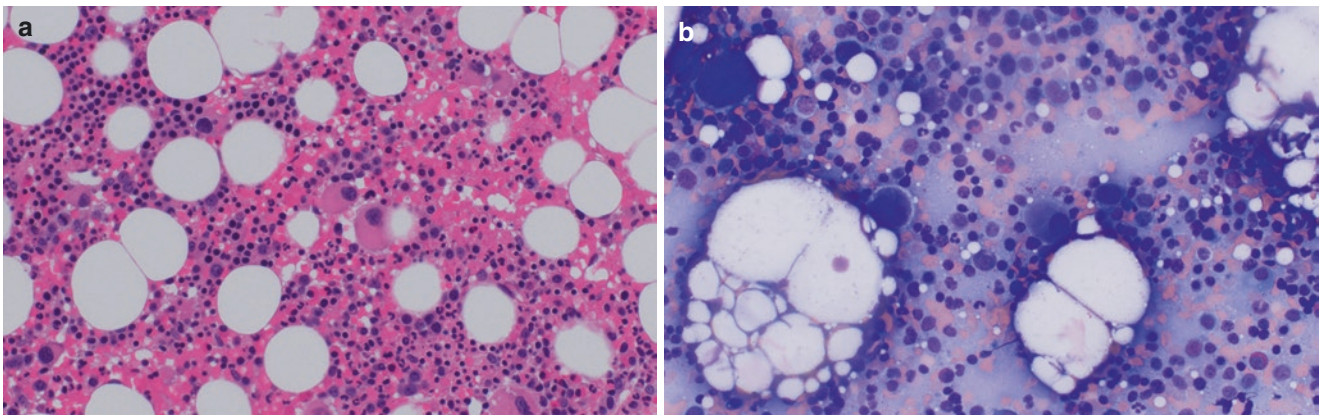

Fig. 14.7 (**a**) Bone marrow biopsy (40×). (**b**) Bone marrow smear (100×)

Molecular analysis (bone marrow): negative for *TP53* gene mutation

Final Diagnosis
Myelodysplastic syndrome with isolated del(5q).

Take Home Messages

1. MDS syndrome with isolated del(5q) is characterized by macrocytic anemia with or without other cytopenia and/or thrombocytosis with female predominance.
2. Bone marrow shows increased megakaryocytes with non-lobated and hypolobated nuclei. Blast count<5%. Of note, similar findings can be seen in MDS with inv(3).
3. Can present with one additional cytogenetic abnormality, other than monosomy 7 or del(7q).
4. *TP53* mutation is associated with increased risk of leukemia and poor survival.

Case 6

Learning Objectives
To become familiar with the classification for MDS.

Case History
An 80-year-old male presented with fatigue for several months. He has been followed up for several years for a history of mild asymptomatic splenomegaly without any demonstrable underlying hematological disorder. He denies having recurrent infections, fevers, chills, nausea, vomiting, diarrhea, overt bleeding, skin changes, lymphadenopathy, unintentional weight loss, and drenching night sweats. Current CBC: Hb 8.4 g/dL; RBC 2.2×10^{12}/L; MCV 110.4 fL; RDW 18.8%; WBC 5×10^{9}/L; platelet 166×10^{9}/L.

Bone Marrow, Biopsy, and Aspirate

- Both erythroid and myeloid lineages show full range of maturation with normal morphology. Blasts are not increased.
- Abnormal megakaryocytes with many monolobated forms [Fig. 14.7a and b].
- Iron stain shows storage iron present without ring sideroblasts.

Differential Diagnosis

1. Myelodysplastic syndrome with single lineage dysplasia
2. Myelodysplastic syndrome with isolated del(5q)

Ancillary Studies
Iron stains (bone marrow aspirate): normal stainable storage iron. Sideroblasts present. Rare ring sideroblasts seen (1% of erythroid precursors)

Cytogenetics (bone marrow): 46,XY,del(5)(q13q33)[1]/46,idem,del(7)(q22q34)[7]/46, XY [11]

Molecular analysis: *TP53* Arg175Cys, a variant of uncertain clinical significance

Final Diagnosis
Myelodysplastic syndrome with single lineage dysplasia (MDS-SLD).

Take Home Messages

1. The diagnostic criteria for MDS-SLD include single cytopenia or bicytopenia and ≥ 10% dysplastic cells in one cell line and blasts <5%. The diagnosis requires correlation with clinical and other laboratory tests to exclude nutrition, toxic metals, medications, and other factors that can also cause dysplastic changes.

2. Patients with MDS with isolated del(5q) have a relatively better prognosis and reduced risk of progression to AML. Chromosomal 7 abnormalities are associated with worse prognosis and reduced overall survival. MDS with isolated del(5q) as well as del7q/monosomy 7 should be best diagnosed as MDS-SLD.

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