Genetic Aspects of Myelodysplastic/Myeloproliferative Neoplasms

Subjects: Oncology

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Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) are myeloid neoplasms characterized by the presentation of overlapping features from both myelodysplastic syndromes and myeloproliferative neoplasms. Although the classification of MDS/MPN relies largely on clinical features and peripheral blood and bone marrow morphology, studies have demonstrated that a large proportion of patients (~90%) with this disease harbor somatic mutations in a group of genes that are common across myeloid neoplasms. These mutations play a role in the clinical heterogeneity of these diseases and their clinical evolution.

Keywords: myelodysplastic/myeloproliferative neoplasms ; cytogenetics ; molecular landscape ; gene mutations

1. Introduction

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) constitute a heterogeneous group of clonal myeloid malignancies with clinical, laboratory, morphologic and genetic features that overlap with myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN). According to the 2017 World Health Organization (WHO) classification, this category currently includes four adult subtypes: chronic myelomonocytic leukemia (CMML), *BCR-ABL1*–negative atypical chronic myeloid leukemia (aCML), MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), MDS/MPN-unclassifiable (MDS/MPN-U), and one pediatric entity: juvenile myelomonocytic leukemia (JMML) [1].

MDS/MPN are usually characterized by a hypercellular bone marrow (BM) with increased proliferation in one or more of the myeloid lineages which is also accompanied by dysplastic features (as a result of increased programmed cell death). Simultaneously, cytopenia may also be present. The blast percentage in the BM and peripheral blood (PB) should be <20% [1].

Depending on the subtype, conventional cytogenetics allows the identification of chromosomal abnormalities in 10–50% of the cases, while around 90% of patients present with somatic mutations in myeloid-related genes [2][3][4].

2. Diagnostic Criteria of MDS/MPN

As previously mentioned, MDS/MPN represents a heterogeneous group of myeloid malignancies that share clinicopathological features with both MDS and MPN. According to the WHO criteria, diagnosis is primarily based on morphological and laboratory findings, as well as exclusion of specific genetic abnormalities [1].

The most common and most well characterized MDS/MPN subtype is CMML, which is characterized by sustained (≥3 months) PB monocytosis (≥1 × 10^9/L; monocytes ≥ 10%) and BM dysplasia [1]. Its incidence is estimated in four cases per 100,000 people per year [5]. Median age at diagnosis is 72 years and it is an infrequent disease in young adults [6][7]. Clinical course is highly variable, with a median overall survival (OS) that ranges between 12–24 months and 15–30% probability of progression to acute myeloid leukemia (AML) [6]. CMML was initially considered as an MDS subtype by the French–American–British (FAB) classification, which subdivided this entity based on leukocyte count into myelodysplastic (MD-CMML, <13 × 10^9/L) and myeloproliferative (MP-CMML, ≥13 × 10^9/L) variants [8]. In 2001, when the WHO assigned CMML to the overlap MDS/MPN group, two categories (CMML-1 and CMML-2) were distinguished according to BM or PB blast percentage [9]. In this case, the percentage of blasts represents the sum of monoblasts, promonocytes and myeloblasts. Both classifications hold prognostic value, since patients with MP-CMML or CMML-2 have shorter OS and a higher risk of AML transformation [10]. Years later, Schuler et al. proposed a refined categorization where CMML-1 subtype was divided into two groups [11]. Based on all these, current WHO classification recognizes three CMML categories: CMML-0 (<2% blasts in PB and <5% blasts in BM), CMML-1 (2% to 4% blasts in PB and/or 5% to 9% blasts in BM) and

CMML-2 (5% to 19% blasts in PB, 10% to 19% in BM, and/or when any Auer rods are present), but also recommends the separation of CMML into MD/MP-CMML, since this can guide the therapeutic approach [1].

Atypical CML is defined largely by morphologic criteria including leukocytosis, dysplastic neutrophils and their precursors. Cytogenetic and molecular studies should be negative for Philadelphia chromosome and *BCR-ABL* fusion gene [1]. The exact incidence of aCML is unknown, but it is estimated in <2 cases for every 100 cases of *BCR-ABL1*-positive CML [12]. Overall, aCML is generally associated with a very poor prognosis and a median OS of 10–20 months [4][13][14].

MDS/MPN-RS-T was a provisional entity until the 2017 WHO classification update, and it is characterized by the presence of thrombocytosis (≥450 × 10^9/L), large atypical megakaryocytes, anemia and ring sideroblasts accounting for ≥15% of erythroblasts [1]. *SF3B1* mutation is reported in ~90% of patients [15]. In contrast to MDS-RS, the diagnosis of MDS/MPN-RS-T cannot be established if *SF3B1* mutation is accompanied by 5–<15% ring sideroblasts. It represents the subtype associated with the best prognosis among overlap syndromes, with a median OS of around 6 years [16].

MDS/MPN-U is the most heterogeneous and the least well-characterized entity, including patients that do not meet other MDS/MPN diagnostic criteria. Median OS is reported in 15–25 months and leukemia-free survival in 19 months [17][18][19]. Clinical characteristics and the natural history of patients with MDS/MPN-U are not well established, due to the heterogeneity of the patients, although poor prognosis among patients with MDS/MPN-U is reported in several studies [17][18][20].

Finally, JMML, the childhood counterpart of CMML, is a rare heterogeneous myeloid neoplasm that shares many clinical and molecular aspects of CMML, and is currently considered a bona fide RASopathy syndrome. It is the only pediatric-onset neoplasm within MDS/MPN and is characterized by excessive proliferation of granulocytic and monocytic lineages [21]. Age at diagnosis ranges from 1 month to early adolescence, but 75% of cases occur in children aged < 3 years [1][21]. Splenomegaly is present almost in all cases. The clinical course varies widely, thus, appropriate clinical management ranges from watchful observation to early allogenic hematopoietic stem cell transplantation (HSCT) [22].

3. Cytogenetic Abnormalities in MDS/MPN

In general, cytogenetic abnormalities and somatic copy number alterations (CNAs) are uncommon and unspecific across all MDS/MPN subtypes (Table 1, Figure 1A), considering that the same alterations are also found in other myeloid malignancies. In most cases, prognosis is not well defined for specific alterations.

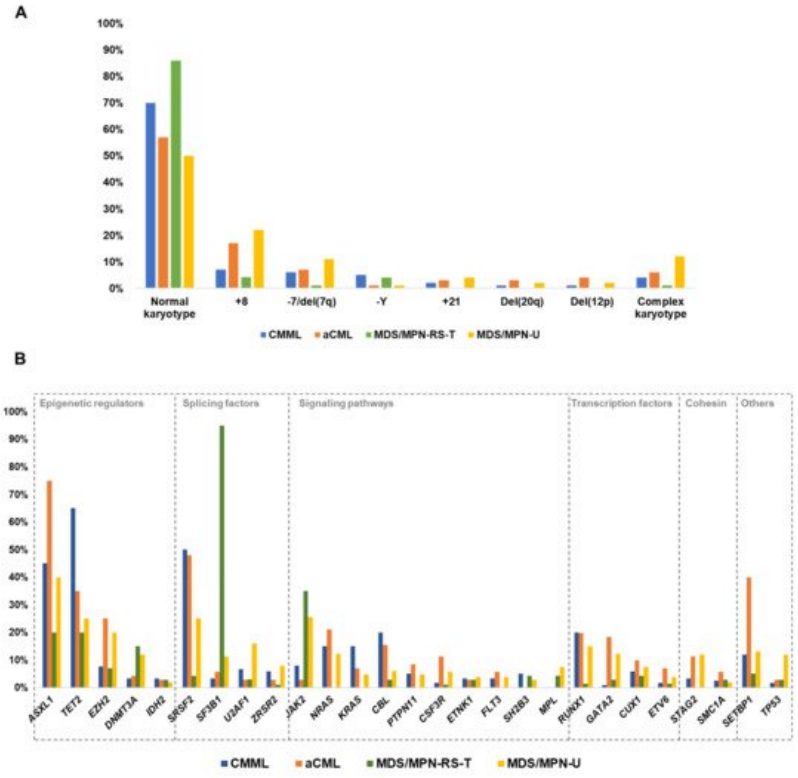

Figure 1. Genetic landscape of adult MDS/MPN. Frequency of recurrent cytogenetic alterations (**A**) and gene mutations (**B**) across the different subtypes of adult MDS/MPN. Based on data from Patnaik et al. [3], Palomo et al. [4], Breccia et al. [13], Jeromin et al. [15], DiNardo et al. [20], Such et al. [23], Tang et al. [24], Wassie et al. [25], Patnaik et al. [26] and Mangaonkar et al. [27]. Abbreviations: aCML: atypical chronic myeloid leukemia; CMML: chronic myelomonocytic

leukemia; MDS/MPN-RS-T: myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; MDS/MPN-U: myelodysplastic/myeloproliferative neoplasm unclassifiable.

Table 1. Cytogenetic abnormalities in MDS/MPN.

MDS/MPN	Abnormal Karyotypes (%)	Common Abnormalities (Frequency %)
CMML	30%	+8: 6–7% −Y: 4–6% −7/del(7q): 2–9% +21: 1–2% CK: 3–6% Deletions of 20q (1–2%) and 12p (1%)
aCML	43%	+8: 17% −7/del(7q): 6–8% CK: 4–8%
MDS/MPN-RS-T	10–17%	+8: 4% −Y: 4% CK: 0–4%
MDS/MPN-U	50%	+8: 14–25% −7/del(7q): 11% CK: 12%
JMML	19–35%	−7: 9–25% Others (del(7q), +8): 10%

Abbreviations: aCML: atypical chronic myeloid leukemia; CMML: chronic myelomonocytic leukemia; CK: complex karyotype; JMML: juvenile myelomonocytic leukemia; MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; MDS/MPN-RS-T: myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; MDS/MPN-U: myelodysplastic/myeloproliferative neoplasm unclassifiable.

The majority of CMML patients have a normal karyotype; however, around 25–30% present with clonal cytogenetic abnormalities. Common alterations include trisomy 8 (+8), loss of Y chromosome (−Y), abnormalities of chromosome 7 (chr7), trisomy 21 (+21) and complex karyotypes (≥3 cytogenetic abnormalities; CK) [23]. Trisomy 8 is commonly found in isolation and is detected in 6–7% of CMML patients [23][24]. Chr7 abnormalities, which mainly constitute monosomy 7 (−7) and 7q deletion (del(7q)), are reported in 2–9% of CMML cases [23][24]. These abnormalities are also present at different frequencies in other myeloid neoplasms such as MDS, MPN and AML. Finally, −Y is reported in 4–6% of CMML patients [23][24][25], although its impact is a matter of debate because, even when it has been described in several neoplasms, it is also found in healthy elderly men [28][29]. To date, three different CMML-specific cytogenetic risk classification systems have been proposed, which stratify patients in groups that differ in their OS and risk of AML progression [23][24][25] (Table 2). According to these, normal karyotypes and isolated −Y are associated with favorable outcomes. In contrast, chr7 abnormalities, CK and monosomal karyotypes (defined by the presence of two monosomies or one monosomy + ≥1 structural abnormality) are associated with a poor outcome, while the prognostic impact of +8 remains controversial.

Table 2. Clinical relevance of cytogenetic abnormalities and gene mutations in MDS/MPN.

MDS/MPN Subtype	Diagnosis	Prognosis
CMML	-WHO [1]: presence of mutations in genes often associated with CMML (*TET2, SRSF2, ASXL1, SETBP1*) in the proper clinical contest can be used to support diagnosis -Associated with the following gene mutation combinations: *TET2-SRSF2,* biallelic *TET2*, *SRSF2-RUNX1* [2][4][30]	Cytogenetics -Three cytogenetic stratification systems have been proposed [23][24][25] -Recurrent findings: • Low risk karyotypes: normal karyotype, isolated loss of Y • High risk karyotypes: chr7 abnormalities, complex karyotype, monosomal karyotype Gene mutations: -Unfavorable outcome: mutations in *ASXL1*, *RUNX1, NRAS* and *SETBP1* [2][30][31] -Favorable outcome: *TET2* mutations, especially in the absence of *ASXL1* mutations ($TET2^{MUT}/ASXL1^{WT}$). These patients also show better response to HMA [32][33][34]. Prognostic stratification: -GFM Model [2], stratification in 3 risk groups based on: Age > 65 years; Hb < 10 g/dL in females and <11 g/dL in males; WBC > 15 × 10^9/L; Platelet count < 100 × 10^9/L; *ASXL1* mutations -Mayo Molecular Model (MMM) [31], stratification in 4 risk groups based on: Hb < 10 g/dL; AMC > 10 × 10^9/L; Platelet count < 100 × 10^9/L; Presence of circulating IMCs; *ASXL1* mutations -CPSS-Mol [30], stratification in 4 risk groups based on: WBC ≥ 13 × 10^9; BM blasts ≥ 5%; RBC transfusion dependency; Genetic risk group (includes CMML-specific cytogenetic risk stratification [23] and mutations in *ASXL1, RUNX1, NRAS* and *SETBP1*).
aCML	-Associated with the following gene mutation combinations: *ASXL1/SETBP1, SETBP1/SRSF2, ASXL1/EZH2, RUNX1/EZH2* [3][4][35]	Unfavorable outcome: mutations in *TET2, RUNX1, NRAS* and *CUX1* [3][4] Prognostic stratification: Mayo Prognostic Model for aCML [3], stratification in 2 risk groups based on: Age > 67 years; Hb < 10 g/dL; *TET2* mutations

MDS/MPN Subtype	Diagnosis	Prognosis
MDS/MPN-RS-T	-WHO [1]: presence of a *SF3B1* mutation. -Associated with the following gene mutation combinations: *SF3B1*, either alone or in combination with *DNMT3A* or *JAK2*, or *DNMT3A/JAK2* [4][26][36]	Unfavorable outcome: -Presence of altered karyotype [4][26] -Mutations in *ASXL1, SETBP1, EZH2* [4][26] Prognostic stratification: Mayo Prognostic Model for MDS/MPN-RS-T [26], stratification in 3 risk groups based on: Hb < 10 g/dL; Abnormal karyotype; mutations in *ASXL1* or *SETBP1*
MDS/MPN-U	-	Unfavorable outcome: -Presence of chr7 abnormalities and complex karyotypes [19] -Mutations in *ASXL1, CBL, CEBPA, EZH2, STAG2, TP53* [4][27][37] Prognostic stratification: -Genomics-based stratification system (Figure 4), classification in 5 subtypes with prognostic relevance based on mutational profile [4]
JMML	-WHO [1]: presence of (1 finding sufficient): • Somatic mutation: *PTPN11, KRAS, NRAS* • Clinical diagnosis of *NF1* or *NF1* mutation • Germline *CBL* mutation CBL LOH	Prognostic stratification: According to the methylation level, three groups that correlate molecular features and clinical outcome have been proposed [38]: • High: characterized by somatic *PTPN11* mutations and poor clinical outcome • Intermediate: enriched in somatic *KRAS* mutations and monosomy 7 • Low: characterized by somatic *NRAS* and *CBL* mutations and a favorable prognosis

Abbreviations: aCML: atypical chronic myeloid leukemia; AMC: absolute monocyte count; chr: chromosome; CMML: chronic myelomonocytic leukemia; CPSS-Mol: molecular CMML-specific prognostic scoring system; GFM: Groupe Francophone des Myelodysplasies; Hb: Hemoglobin; HMA: hypomethylating agents; HSCT: hematopoietic stem cell transplantation; IMCs: immature myeloid cells; JMML: juvenile myelomonocytic leukemia; LOH: loss of heterozygosity; MDS/MPN-RS-T: myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; MDS/MPN-U: myelodysplastic/myeloproliferative neoplasm unclassifiable; RBC: red blood cells; WBC: white blood cell count; WHO: World Health Organization.

In the case of aCML, few patient cohorts with cytogenetic data have been described until now [3][4][13][24], with the largest series consisting of 65 and 71 patients, respectively [4][24]. According to these two studies, approximately 43% of patients present with cytogenetic abnormalities, +8 and chr7 alterations being the most common, with reported frequencies of approximately 17% and 7%, respectively. CK are seen in 4–8% of the cases. In contrast, only 10–17% of MDS/MPN-RS-T patients have an abnormal karyotype. Commonly detected alterations include +8 and -Y, while other chromosomal abnormalities, as well as CK, are rare (0–4%) [4][15][26]. Among all MDS/MPN overlap syndromes, MDS/MPN-U is the subtype with the highest frequency of chromosome instability, with near 50% of altered karyotypes. Trisomy 8 (mostly found as a sole abnormality) is the most frequent alteration (15–25%), followed by chr7 alterations (12%) and CK (12%) [4]

[20][27]. Other less common abnormalities include del(12p), +9 and del(20q) [20][27]. Overall, the presence of cytogenetic abnormalities is generally associated with an inferior OS in all adult MDS/MPN subtypes, except aCML [4]. This impact seems to be especially strong in MDS/MPN-RS-T, where abnormal karyotypes are rare but, if detected, confer a very poor outcome [4][26] (Table 2).

Cytogenetic studies of JMML show a normal karyotype in approximately 65–80% of cases [21][39][40]. Monosomy 7 is the most frequent alteration, reported in 9–25% of the cases. Other aberrations (such as del(7q) and +8) are reported in 10% of cases [21][39]. It is to note that −7 is most often seen in *KRAS*-mutated cases [22].

4. Other Chromosomal Abnormalities

As previously mentioned, the karyotype is often normal across all MDS/MPN subtypes [1]. Studies using single nucleotide polymorphism arrays (SNP-A) or other techniques that allow the detection of cryptic CNAs and copy number neutral loss of heterozygosity (LOH) are limited. To date, most of these studies report MDS/MPN cases (mainly CMML) within heterogeneous cohorts including other myeloid malignancies [41][42][43]. Overall, SNP-A allows the detection of chromosomal alterations in 75% of MDS/MPN patients compared to 30–40% by conventional cytogenetics [41][42][43][44][45][46].

Two SNP-A studies performed in large cohorts of CMML patients with normal karyotype reported 40–65% of abnormalities (CNAs + LOH) in these patients [45][46]. According to these studies, CNAs are detected in one third of patients but are highly heterogeneous, with very few recurrent alterations, including gains in 21q22 and losses in 4q24 and 12p13.2. In contrast, large interstitial LOH regions are detected in 25–35% of cases, recurrently affect 4q, 7q and 11q, and are often accompanied by the presence of homozygous mutations in *TET2*, *EZH2* and *CBL*, respectively. Prognostic impact of these abnormalities remains unclear.

Besides CMML, LOH were also reported in 38% MDS/MPN-U, especially in 11q23.3 were *CBL* gene is located [44]. Similarly, Jankowska et al. described that LOH of chromosome 4q (where *TET2* gene is located) was frequent in CMML and in secondary AML arising from these cases; however, it was absent in refractory anemia with ring sideroblasts and thrombocytosis (current MDS/MPN-RS-T) and aCML patients [47].

Overall, very few cryptic alterations are seen in patients with MDS/MPN, either by SNP-A or sequencing techniques, and these are highly heterogeneous and not specific to any of the subtypes [4]. Thus, even when chromosomal microarray testing is included as a suggested test by the European LeukaemiaNet 2013 and by the Spanish Group of MDS for the diagnosis of primary MDS, it has not been recommended for clinical work-up of myeloid malignancies by the WHO 2017, nor by the NCCN 2017 guidelines [48].

5. Functional Pathways Affected in MDS/MPN

The development of next generation sequencing (NGS) techniques has helped to define the molecular landscape of MDS/MPN. More than 90% of these patients harbor somatic mutations in a group of genes that is common across the spectrum of myeloid neoplasms [4]. Pediatric entity JMML, considered a RASopathy, is primarily characterized by germline and somatic mutations in genes involved in the RAS pathway [38]. In contrast, the spectrum of gene mutations in adult MDS/MPN is much more heterogeneous, with driver genes affecting specific cellular processes that can be categorized according to their function. Mutations recurrently affect epigenetic regulators, splicing factors, genes involved in signaling pathways, transcription factors and cohesin complex components [2][3][4][26][37] (Figure 1B and Figure 2). The acquisition of mutations in these patients occurs in a multi-step manner, as reported in both myeloid and lymphoid neoplasms [49]. Many cases probably arise from previous asymptomatic clonal hematopoiesis, and thus founder driver mutations are frequently found in epigenetic regulators and splicing factors. Secondary acquired driver mutations commonly affect transcription factors and signal transduction genes, which sometimes drive disease progression to AML, along with cell-intrinsic and -extrinsic factors. However, gene mutation frequencies and clonal evolution patterns differ among the four adult MDS/MPN subtypes [4] (Figure 1B and Figure 3).

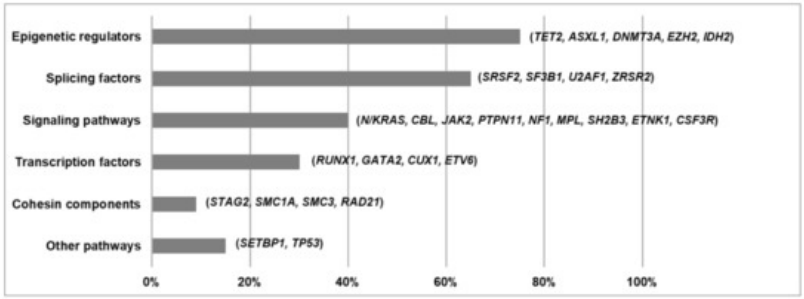

Figure 2. Functional pathways affected in MDS/MPN. Frequency of mutations affecting each pathway/functional category in MDS/MPN overlap syndromes. Based on data from Itzykson et al. [2], Patnaik et al. [3], Palomo et al. [4], Patnaik et al. [26] and Bose et al. [37].

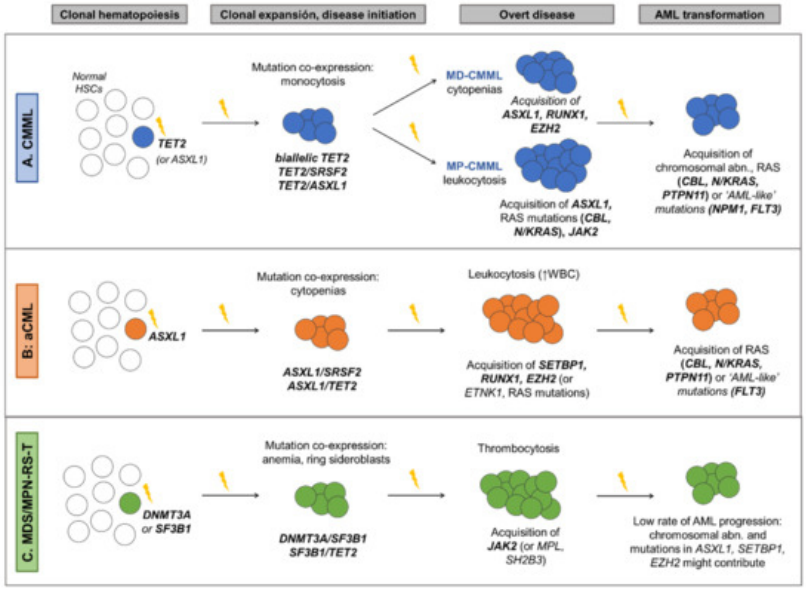

Figure 3. Clonal evolution model in adult MDS/MPN. The figure depicts the clonal evolution patterns most frequently observed in CMML (**A**), aCML (**B**) and MDS/MPN-RS-T (**C**). MDS/MPN arise from asymptomatic clonal hematopoiesis. Over time there is clonal expansion that leads to MDS/MPN phenotype and overt disease that, in some cases, eventually progresses to AML. This process takes place through the acquisition of molecular hits (chromosomal abnormalities and gene mutations) that confer to the neoplastic clone a selective advantage. The type of mutations and the order in which they are acquired shapes the disease phenotype and influences the clinical outcome. Based on data from Itzykson et al. [2], Palomo et al. [4], Elena et al. [30], Patnaik et al. [31], Coltro et al. [32], Palomo et al. [33], Meggendorfer et al. [35], Patnaik et al. [26], Steensma et al. [49], Itzykson et al. [50], Ricci et al. [51], Mason et al. [52], Awada et al. [53] and Patel et al. [54]. Abbreviations: aCML: atypical chronic myeloid leukemia; AML: acute myeloid leukemia; CMML: chronic myelomonocytic leukemia; HSCs: hematopoietic stem cells; MD-CMML: myelodysplastic CMML; MDS/MPN-RS-T: myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; MDS/MPN-U: myelodysplastic/myeloproliferative neoplasm unclassifiable; MP-CMML: myeloproliferative CMML; WBC: white blood cell count.

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