Chapter 12
Chronic Myelomonocytic Leukemia: Clinical and Pathologic Features

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Introduction

Chronic myelomonocytic leukemia (CMML) is a myeloid neoplasm characterized by a persistent absolute monocytosis, often with a background of dysplastic morphologic features. The diagnosis encompasses a heterogeneous group of cases with considerable variability in morphologic dysplasia, cytopenias, leukocytosis, and the presence or absence of organomegaly. As such, the diagnostic category has features of a myelodysplastic syndrome as well as a myeloproliferative neoplasm. It was originally categorized as a form of myelodysplastic syndrome (MDS) in the early French-American-British (FAB) classification [1]. But in later editions of the World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues, it was placed within the newly created category of "Myelodysplastic/Myeloproliferative Neoplasms" [2].

Clinical

The diagnosis of CMML requires a persistent peripheral blood (PB) monocytosis ($\geq 1 \times 10^9$ cells /L and ≥10% of the white blood cell differential count as defined in the 2016 WHO classification of hematopoietic neoplasms) with <20% blasts in the peripheral blood and bone marrow (BM) and the absence of a *BCR-ABL1* rearrangement [3]. There should be morphologic dysplasia; however, if dysplasia is minimal

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Table 12.1 Diagnostic criteria for CMML [2, 3]

Criteria
Persistent peripheral blood monocytosis $\geq 1 \times 10^9$/L & ≥10% of WBC
Absence of *BCR-ABL* rearrangement
No rearrangement of *PDGFRA, PDGFRB,* or *FGFR1* and no *PCM1-JAK2* fusion[a]
<20% blasts in peripheral blood and bone marrow[b]
Dysplasia in one or more myeloid lineages;
if dysplasia is minimal/absent then need:
Acquired clonal cytogenetic or molecular genetic present in hematopoietic cells or
Monocytosis has persisted ≥3 months and all other causes of monocytosis are excluded
CMML-0: <2% blasts in PB and <5% blasts in BM[c];
CMML-1: 2–4% blasts in PB and/or 5–9% blasts in the BM[c];
CMML-2: 5–19% blasts in PB, 10–19% blasts in the BM, and/or presence of any Auer rods[c]

[a]Should be excluded in cases with eosinophilia
[b]Blasts include myeloblasts, monoblasts, and promonocytes
[c]The PB or BM blast count that results in the highest category should be used

or absent, there should be an acquired clonal cytogenetic or molecular genetic abnormality or monocytosis lasting ≥3 months with exclusion of other causes of monocytosis [2]. Additionally, in cases with eosinophilia, rearrangements involving *PDGFRA*, *PDGFRB*, and *FGFR1*, as well as the *PCM1-JAK2* fusion, should be excluded [3]. If eosinophils are $>1.5 \times 10^9$ cells/L and there is no rearrangement with the aforementioned genes, the diagnosis is CMML with eosinophilia (see Table 12.1).

There is some evidence to support dividing CMML into "dysplastic" ($<13 \times 10^9$ WBC/L) and "proliferative" ($\geq 13 \times 10^9$ WBC/L) categories based on distinctive molecular and clinical features of each subset [4–10]. In addition, CMML is categorized by the percentage of blasts[1] present in the peripheral blood and bone marrow as CMML-0: <2% blasts in PB and <5% blasts in BM; CMML-1: 2–4% blasts in

[1] The blast count in either the PB or BM that results in the highest CMML category should be used.

PB and/or 5–9% blasts in the BM; and CMML-2: 5–19% blasts in PB, 10–19% blasts in the BM, and/or presence of any Auer rods [3].

In two large epidemiological studies in Europe and the United States, the incidence of CMML was 3–4.1/1,000,000 person-years [11, 12]. The median age at diagnosis was 76 years with a male predominance [12]. The overall survival at 5 years was 18% [11]. Patients have a spectrum of MDS to MPN-like presentations. The majority have elevated WBC counts, although some have normal or decreased counts. Symptoms include fatigue, weight loss, fever, night sweats, infections, and bleeding. Splenomegaly or hepatomegaly may occur, especially with the myeloproliferative subgroup. Typically, patients present with <5% circulating blasts and <10% BM blasts, corresponding to CMML-0/1 [2].

Morphology and Immunophenotype

In the blood (see Fig. 12.1), a monocytosis of ≥1 × 10^9cells/L and ≥10% of total WBCs is a requirement, in distinction from chronic myeloid leukemia, *BCR-ABL1* positive, which may have an absolute monocytosis, but it is typically <10% of all WBCs [2, 3]. The monocytes of CMML can have abnormal morphology with atypical granulation and nuclear lobation, or immature chromatin that is somewhat denser than that of promonocytes or monoblasts; overall however, the monocytes are usually mature and morphologically unremarkable [2]. Monoblasts are large with abundant gray-to-blue cytoplasm, possible pseudopod formation, and round nuclei with delicate chromatin and prominent nucleoli. Promonocytes also have abundant gray or blue cytoplasm and nuclei with finely reticulated chromatin, but the nuclei have delicate folds with or without a small nucleolus (see Fig. 12.2) [2]. Cytopenias are often present and there may be neutrophilia [2]. There is usually dysgranulopoiesis, which may manifest as nuclear hypolobation, abnormal nuclear lobation, or hypogranular granulocytes [2].

Of note, cases of MPN can be associated with monocytosis or can develop monocytosis during the course of the disease. In these rare situations, a previously documented history of MPN excludes CMML. Additionally, the presence of MPN features in the bone marrow and/or of MPN associated mutations (*JAK2*, *CALR* or *MPL*) tend to support MPN with monocytosis rather than CMML.

The bone marrow (see Fig. 12.1) is usually hypercellular, but it can be normocellular or hypocellular [2]. A granulocytic proliferation is often the most prominent finding and may obscure the monocytic proliferation [2]. Most cases have dysgranulopoiesis and dysmegakaryopoiesis, and many have dyserythropoiesis [2]. Reticulin fibrosis occurs in up to 30% of cases and 20% of cases have nodules of clonally related, neoplastic plasmacytoid dendritic cells [2]. In cases with splenomegaly, the red pulp is typically infiltrated by leukemic cells [2].

Phenotypically, the leukemic cells typically express the myeloid associated antigens CD33 and CD13 [2]. Monocytic antigens such as CD14, CD68, and CD64 are variably expressed [2]. Oftentimes, there is an aberrant immunophenotype on

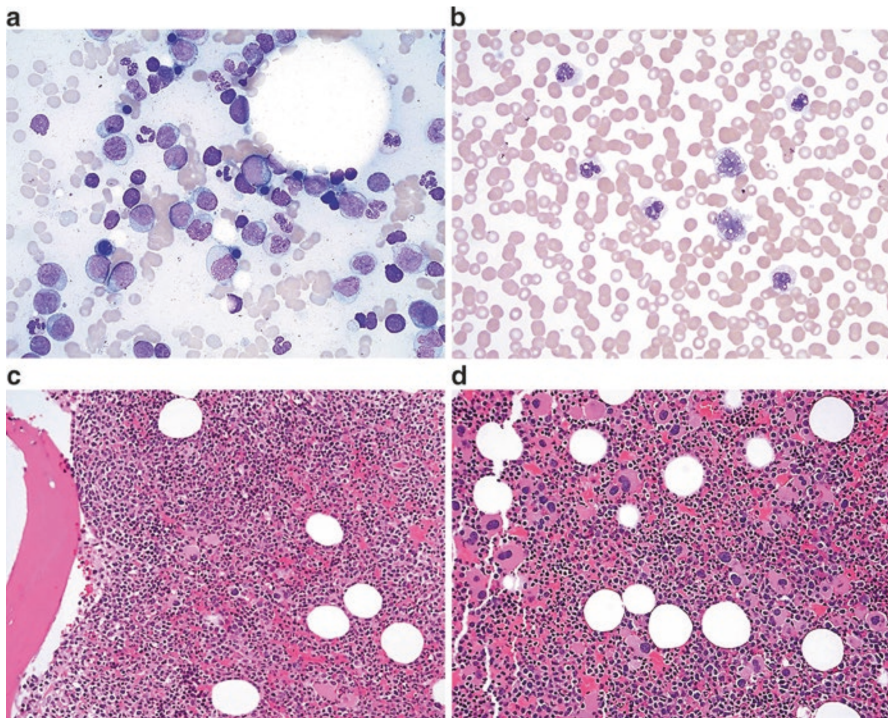

Fig. 12.1 (**a**) Bone marrow aspirate of CMML-2 (Wright stain, 500× original magnification). (**b**) Peripheral blood of CMML (Wright stain, 500× original magnification). (**c**) Bone marrow core biopsy (hematoxylin and eosin, 200× original magnification) with (**d**) numerous dysplastic megakaryocytes (hematoxylin and eosin, 400× original magnification) (Courtesy of Dr. H. Joyce Rogers, Pathology and Laboratory Medicine Institute, Cleveland Clinic, Cleveland, OH)

monocytes with ≥2 immunophenotypic aberrancies such as expression of CD56 or CD2, decreased expression of CD14 (possibly indicative of immaturity), and/or decreased expression of HLA-DR, CD13, CD15, CD64, and CD36 [2]. Monoblasts and promonocytes are typically negative for CD34. The monocytes are positive for lysozyme, nonspecific esterase, and are negative for naphthol-ASD-chloroacetate esterase [2].

Cytogenetics and Molecular

Approximately 30% of CMML cases have cytogenetic abnormalities [13, 14]. Among the most common findings are +8, −Y, del(20q), +21, der(3q), and chromosome 7 abnormalities including −7 and del(7q) [14, 15]. Karyotypes having multiple abnormalities are also common [13–15]. Isolated abnormalities of chromosome 5 (−5/del(5q)) are relatively rare [13–15]. Single nucleotide polymorphism (SNP)

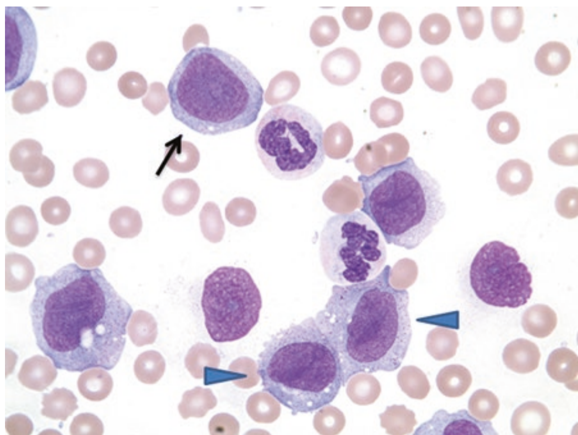

Fig. 12.2 Peripheral blood of CMML highlighting a monoblast (*arrow*) and promonocytes (*arrowhead*) (Wright stain, 1000× original magnification)

arrays have shown increased numbers of chromosomal alterations than appreciated by karyotype analysis alone including frequent copy neutral loss of heterozygosity (LOH) [16, 17].

Over 90% of CMML cases have identifiable gene mutations [18, 19] (see Table 12.2). Molecular genetic or cytogenetic abnormalities can be used as evidence of clonality in cases without significant morphologic dysplasia, especially when involving gene mutations commonly associated with CMML. Many of these genetic mutations can be broadly divided into 3 pathways: epigenetic regulation/histone modification, spliceosome machinery, and cell signaling/transcription factors.

It is important to bear in mind that many of the mutations found in CMML (such as *TET2, ASXL1, SRSF2, CBL*) can also be found in isolation in hematologically normal appearing patients or patients with cytopenia(s) who do not otherwise meet criteria for a myelodysplastic syndrome (MDS) or CMML [20, 21]. Thus, the presence of one of these mutations should be carefully considered in the context of the duration of the monocytosis, exclusion of other reactive causes of monocytosis, and of the presence of other clinical data such as cytopenias or splenomegaly that might support CMML.

In CMML patients, the most commonly identified mutations in genes encoding proteins involved in epigenetic regulation/histone modification involve *TET2, DNMT3A, IDH2, ASXL1, EZH2,* and *UTX* (see Table 12.2) [19, 22, 23]. Of these, *TET2* and *ASXL1* are the most frequent and most important. *TET2* catalyzes the hydroxylation of methylated DNA and as a result, somatic mutations in *TET2* are believed to lead to epigenetic dysregulation [24] . *TET2* mutations are found in 46–58% of CMML cases [19, 22] and *TET2* deletions have been detected in 7% CMML cases, with a higher incidence of cryptic deletions noted than in acute myeloid leukemia (AML) or MDS [25]. Mutations of *IDH1, IDH2* and *TET2* tend to be mutually exclusive since they are functionally redundant [22, 26] . Mutated *IDH1/IDH2* results in abnormal production of the metabolite 2-hydroxyglutarate(2-HG) [24], which inhibits multiple enzymes including *TET2,* leading to hypermethylation [26]. *ASXL1* is thought to affect histone modification through effects on the

Table 12.2 Mutational profile of CMML [19, 22]

Gene	No. of tested Samples	Number mutated	% range	overall %
ASXL1	487	207	40–46.9	42.5
TET2	437	231	45.7–58	52.9
SRSF2	395	194	46–53.1	49.1
RUNX1	438	64	14.3–15	14.6
NRAS	438	50	11–12	11.4
CBL	439	52	10–14.3	11.8
JAK2	438	28	4–8	6.4
KRAS	263	20	8	7.6
ZRSF2	364	24	5.1–8	6.6
IDH2	404	21	4.5–6	5.2
SF3B1	395	23	5.7–6	5.8
U2AF1	395	25	5–8	6.3
EZH2	348	10	1.1–5	2.9
FLT3	439	9	0.57–3	2.1
DNMT3A	402	14	2–5.1	3.5
CEBPA	175	11	6.3	6.3
SETBP1	370	45	6.2–18.9	12.2
PTPN11	175	8	4.5	4.5
SH2B3	175	8	4.5	4.5
TP53	377	11	1–5.1	2.9
BCOR[a]	54	4	7.4	7.4
STAG2[a]	88	9	10.2	10.2
IDH1	404	4	<1–1.7	1

These data taken from separate studies [40, 42]

polycomb group repressive complex proteins (PRC1/2) [27]. The most common mutations associated with *ASXL1* are c.1934dupG;p.Gly646TrpfsX12 and 1900_1922_ del [28]. *ASXL1* mutations are associated with a higher WBC count, lower hemoglobin, extramedullary disease and an abnormal karyotype [15, 22]. *EZH2* is a component of the PRC2 complex [29] and *UTX* is a lysine specific demethylase with effects on histone H3K27 [23].

The most commonly identified spliceosome mutations involve the following genes: *SF3B1, SRSF2, U2AF1*, and *ZRSR2* [19, 22]. Mutations within this category affect the machinery involved in pre-mRNA splicing [30, 31] and tend to be exclusive of each other in cases of CMML [22, 30, 32]. Of these genes, mutations of *SRSF2* are by and far the most common. Mutations of *SRSF2* tend to result in alterations at the 95th amino acid residue, normally occupied by proline and are associated with a normal karyotype [15, 30] . Mutations of *SF3B1* have an association with der(3q) and, as with cases of MDS, tend to be associated with ring sideroblasts [15, 30]. Recurrent *SF3B1* mutations include K700E, H662Q, and K666N [30, 32]. *U2AF1* encodes a small nuclear RNA auxiliary factor. Mutations in this gene (most commonly S34F, Q157, [30] are associated with a normal karyotype, but can also

be seen with a monosomal karyotype (defined as having loss of two chromosomes or loss of one chromosome plus another structural alteration) [15].

There are several recurring mutations in genes involved in signaling/tyrosine kinase pathways including *JAK2*, *RAS* (*KRAS* + *NRAS*), *CBL*, *PTPN11*, and *BRAF* [22, 33–35] . The *RAS* gene family is composed of multiple isoforms, including *KRAS* and *NRAS*, which have GTPase activity and are involved in cell signaling pathways [36]. *BRAF* is a kinase intimately involved with *RAS* signaling and is an important activator of the *MEK/ERK* pathway [37]. *JAK2* is a tyrosine kinase involved with cell signaling and proliferation via the *STAT* pathway [38]. *JAK2* mutated CMML tends to share some morphologic features with JAK2+ myeloproliferative neoplasms such as mild/moderate reticulin fibrosis, erythroid and megakaryocytic hyperplasia, occasional megakaryocytic clustering and atypia, and dilated sinusoids [39]. Overall, these morphologic features appear to be less developed than in a pure MPN. Additional factors that would lend support to a diagnosis of JAK2+ CMML would include a lack of a history of MPN or lack of cell counts consistent with an MPN, and finding morphologic features of dysplasia. *CBL* regulates receptor tyrosine kinase activity by ubiquitination [34]. It is associated with *TET2* mutations and monosomy 7 and tends to associate with wild type *JAK2* and *KRAS/NRAS* [34].

Other reported common mutations found in CMML are of *RUNX1, SETBP1, STAG2*, and *BCOR* [19, 22, 33, 40–43]. The cohesin complex is a multimer composed of four subunits, including *STAG2*, thought to be involved in cohesion of sister chromatids during cell division, postreplicative DNA repair, and regulation of gene expression [40]. Among myeloid neoplasms, *STAG2* mutations are often found with other mutations such as *TET2, ASXL1*, and *EZH2* [40]. *RUNX1* encodes the alpha subunit of the core-binding factor and is essential for hematopoiesis/differentiation and helps regulate expression of GCSF and MPO [44]. *SETBP1* is a binding partner for *SET* protein, a protein which has downstream effects on transcription and nucleosome assembly [45, 46]. *SETBP1* mutations have an association with mutations of *ASXL1* or spliceosome machinery and often occur with a normal karyotype [47].

From a molecular perspective, the myelodysplastic type of CMML(MD-CMML) is associated with mutations of spliceosome proteins such as SRSF2, SF3B1, ZRSR2 and U2AF35 and epigenetic regulators of DNA methylation such as TET2 and IDH1/2 [10]. The myeloproliferative type of CMML (MP-CMML) is associated with mutations of ASXL1 and signal pathway mutations such as CBL, FLT3, JAK2 and KRAS/NRAS, in addition to the mutations involving spliceosome machinery and regulators of DNA methylation [8, 10, 39]. Even in cases originally diagnosed as MD-CMML, the identification of signal pathway mutations (RAS) at the time of diagnosis or during the disease course has been associated with progression to MP-CMML [8]. Investigation of the mutational hierarchy of CMML [18] indicates that mutations affecting epigenetic regulators (such as TET2 and ASXL1) and spliceosome mutations are often associated with early neoplastic clones whereas signal pathway mutations tend to be later mutational hits.

Prognosis and Therapy

Although there have been several large studies that have examined various clinical and pathologic markers for prognostic utility regarding CMML, there is no universally accepted prognostic model for CMML. Factors that have been found to have some prognostic significance at one time or another in multivariate analysis include increased age, high WBC count, increased bone marrow blasts, cytogenetic risk stratification, circulating immature myeloid cells, thrombocytopenia, anemia, and the presence of frameshift or nonsense *ASXL1* mutations [6, 22, 28, 47–50].

Three prognostic models with external validation include the CPSS (CMML-specific prognostic scoring system), the GFM (Group Francophone des Myélodysplasies), and the Mayo Model (see Table 12.3) [22, 28, 49]. The CPSS model identified 4 factors for stratifying overall survival (OS) and (acute) leukemia free survival (LFS) risk: French-American-British (FAB) classification, WHO classification, the CMML-specific cytogenetic risk stratification, and blood transfusion dependency. The CMML-specific cytogenetic risk stratification considered a normal karyotype and –Y as low risk; complex cytogenetics (≥3 chromosomal abnormalities), chromosome 7 abnormalities, and trisomy 8 as poor risk; and other chromosomal abnormalities as intermediate risk [14]. All positive risk factors were assigned a value of 1, except for high risk cytogenetics, which is assigned a value of 2. A total score was obtained from the sum of the individual scores and is placed into 1 of 4 categories: Low risk, Intermediate-1, Intermediate-2, and high risk. The GFM model used five risk factors for OS and LFS: age > 65 years, WBC $>15 \times 10^9$/L, anemia, platelets $<100 \times 10^9$/L, and presence of a nonsense or frameshift *ASXL1* mutation. Positive risk factors were assigned a value of 1, 2, or 3 and summed for a total score that was placed in one of three categories: low, intermediate, and high risk. The Mayo Model identified four significant prognostic variables for OS and LFS: absolute monocyte count ($>10 \times 10^9$/L), circulating immature mononuclear cells (defined as any of myeloblasts, promyelocytes, myelocytes, metamyelocytes), anemia (<10 g/dL), and thrombocytopenia ($<100 \times 10^9$/L). Three prognostic categories were created from this: low risk (0 risk factors), intermediate risk (1 risk factor), and high risk (≥2 risk factors).

Although mutations of various genes (*RUNX1, TET2, NRAS, CBL, SETBP1, SRSF2*) have been associated with differences in overall survival (OS) or leukemia-free survival (LFS), the data are inconsistent and further validations are necessary before drawing conclusions [19, 22, 30, 33, 34, 41, 43, 47, 51, 52].

Cytogenetic risk stratifications have also yielded variable results with regard to prognosis, but some common themes have emerged. These common themes include complex karyotypes are associated with a worse prognosis and a normal karyotype or –Y is associated with a better OS [14, 15]. When applied to multiple prognostic models in multivariate analysis, the Mayo-French cytogenetic model retained independent prognostic significance [15]. This model effectively predicted leukemic transformation and stratifies cases into one of three risk groups—high risk: complex karyotype or monosomal (defined as having at least one autosomal monosomy and

Table 12.3 Comparison of three CMML prognostic models with external validation [22, 28, 49]

Prognostic model	Risk factors	Prognostic score	Low risk category survival (months)	Intermediate risk category survival (months)		High risk category survival (months)	Risk of acute leukemic transformation
				1	2		
CPSS	• CMML FAB type (WBC <13 × 10^9=0 pt; >13 × 10^9 = 1 pt) • CMML WHO type (CMML-1= 0 pt, CMML-2=1 pt) • CMML-specific cytogenetics (low risk=0, intermediate = 1, high=2) • RBC transfusion dependence (1 pt)	Low risk: 0 pt Intermediate risk 1: 1 pt Intermediate risk 2: 2–3 pts High risk: 4–5 pts	72	31	13	5	% AML transformed at 5 years: 13, 29, 60 and 73%, respectively
GFM	• Age >65 years (2 pt) • WBC >15 × 10^9/L (3 pt) • Anemia (2 pt) females Hb<10 g/dL males Hb<11 g/dL • Platelets <100 × 10^9/L (2 pt) • ASXL1 mutation (nonsense or frameshift) (2 pt)	Low risk: 0–4 pts Intermediate risk: 5–7 pts High risk: 8–12 pts	Not reached	385		14.4	AML free survival: 56.0, 274, 9.2 months, respectively

(continued)

Table 12.3 (continued)

Prognostic model	Risk factors	Prognostic score	Low risk category survival (months)	Intermediate risk category survival (months)		High risk category survival (months)	Risk of acute leukemic transformation
				1	2		
Mayo Model	• Absolute monocyte count >10 × 10^9/L • Circulating immature mononuclear cells (myeloblasts, promyelocytes, myelocytes, metamyelocytes) • Hemoglobin <10 g/dL • Platelet count <100 × 10^9/L	Low risk: 0 risk factor Intermediate risk: 1 risk factor High risk: ≥2 risk factors	32	18.5		10	Relative risk for AML transformation: 4.9 for high risk; 2.6 for intermediate risk

one more structural abnormality or having at least two autosomal monosomies) karyotype; low risk: normal karyotype, −Y, order(3q); or intermediate risk: all others. Another study [53] examining the prognostic impact of cytogenetic abnormalities acquired during the course of CMML disease showed they were associated with an overall decrease in LFS by multivariate analysis. Acquisition of a complex karyotype was associated with leukemia progression, but del(20q) was associated with stable disease [53].

Therapy for CMML has largely been drawn from treatments for myelodysplastic syndrome and myeloproliferative neoplasms. Supportive care including erythropoietic stimulating agents and transfusions are typically utilized for significant anemia [54]. Hypomethylating agents, including 5-azacitidine and decitabine, have been approved by the United States Food and Drug Administration for treatment of CMML. In several studies, these two drugs collectively have shown overall response rates ranging from 25–69% and median OS from 12–37 months [55–61]. Proliferative phase CMML is typically treated with hydroxyurea. In a randomized control trial comparing hydroxyurea with etoposide, hydroxyurea was associated with better treatment response (60% versus 36%) and OS (20 months versus 9 months) than etoposide [62]. Even so, treatment outcomes with hypomethylating agents and hydroxyurea are still relatively poor and there is a strong need for more effective therapies. Allogeneic hematopoietic stem cell transplant (HSCT) is the only known cure; however, due to the advanced age and/or comorbidities often associated with CMML patients, this option is often not available [63].

Although *TET2* mutations have been associated with response to hypomethylating agents in MDS patients, there are no consistent molecular mutation predictors of response to hypomethylating agents in CMML patients [58, 64–66], and analysis of predictive models of methylation patterns have so far yielded mixed results [66]. Numerous investigational therapies such as the *JAK2* inhibitor ruxolitinib and *RAS* pathway inhibitors (farnesyltransferase inhibitors) have been evaluated in patients with CMML with variable but limited responses [67–69].

Conclusion

CMML is a myeloid neoplasm with overlapping features of a myelodysplastic and myeloproliferative neoplasm. The diagnosis requires a combination of a persistent absolute monocytosis (>1 × 10^9 cells/L) and either a background of morphologic dysplasia, clonal cytogenetic/molecular genetic abnormalities or persistence of monocytosis for ≥3 months with exclusion of other causes of monocytosis. There are no disease-defining cytogenetic or molecular genetic abnormalities. However, the presence of a cytogenetic or molecular abnormality may help to make a diagnosis of CMML in the appropriate clinical context. The independent prognostic and therapeutic value of molecular mutations is currently limited. Yet, as our knowledge of the mutational landscape is expanded and refined, and newer therapies become

available, our understanding of the molecular basis of CMML may yield additional insight into the treatment potential of CMML.

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