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CMML AND aCML: NOVEL PATHOGENETIC LESIONS

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Summary

Chronic myelomonocytic leukemia (CMML) and atypical chronic myeloid leukemia (aCML) are distinct, yet related, entities of myelodysplastic/myeloproliferative neoplasms (MDS/MPN) characterized by morphologic dysplasia with accumulation of monocytes or neutrophils, respectively. Our understanding of the molecular pathogenesis of CMML and aCML has advanced, mainly due to the application of novel technologies such as array-based karyotyping or next generation sequencing. In addition to previously known recurrent aberrations, somatic uniparental disomy affecting chromosomes 3, 4, 7, and 11 frequently occurs in CMML. Novel somatic mutations of genes, including those associated with proliferation signaling (*CBL*, *RAS*, *RUNX1*, *JAK2* (*V617F*)) and with modification of epigenetic status (*TET2*, *ASXL1*, *UTX*, *EZH2*) have been found. Various combinations of mutations suggest a multistep pathogenesis and may account for clinical heterogeneity. The prognostic and diagnostic significance of these molecular lesions, in particular their value as biomarkers of response or resistance to specific therapies, while uncertain now is likely to be clarified as large systematic studies come to completion.

Introduction

Chronic myelomonocytic leukemia (CMML) and atypical chronic myeloid leukemia (aCML) are distinct entities of overlap myelodysplastic/myeloproliferative neoplasms (MDS/MPN).¹ However, except for disease-specific lineage bias, there are several clinically shared characteristics including elevated leukocyte count, thrombocytopenia, and splenomegaly. Unlike CML, characterized by a *BCR-ABL1* fusion, the molecular pathogenesis of closely related CMML and aCML remains unclear and is likely heterogeneous.¹ A similar clinical phenotype in patients fulfilling the diagnostic criteria can be a result of diverse molecular events. While distinct proximal lesions may converge to affect a common molecular pathway, it is also possible that diverse pathogenetic pathways can result in common phenotypic features. Moreover, oncogenic driver mutations such as in *RUNX1* or *CBL-B* can coexist with mutation of genes involved in epigenetic regulation

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including *TET2*, *ASXL1* or *EZH2*. As a consequence, future therapies may need to target specific lesions present in molecularly defined subgroups of patients.

In this review, we will discuss the recent advances in the understanding of the molecular pathogenesis of CMML and aCML and the implications of these findings for clinical applications and future research directions.

Morphological diagnosis

Classically, CMML was defined based on morphologic criteria, but recent advances in the understanding of the molecular pathogenesis of CMML suggest that morphological diagnosis may be replaced or at least complemented by molecular markers.

Monocytosis is the hallmark of CMML; by definition, monocytes are $>1 \times 10^9/L$ and usually range from 2 to $5 \times 10^9/L$, but may exceed $80 \times 10^9/L$. Monocytes are almost always $>10\%$ of leukocytes, in contrast to aCML patients (where monocytes rarely exceed 10% of leukocytes). The monocytes can display a normal or abnormal morphology with granulation, unusual nuclear lobation or chromatin condensation patterns. Clearly, abnormal monocytes as well as immature monocytes with denser chromatin, nuclear convolutions and folds, and a more grayish cytoplasm may be present or even coexist. Blasts and promonocytes may also be seen but at $<20\%$ of total white cells. In some cases leukocyte counts may be normal or decreased, but in nearly one half of patients the leukocyte count is increased due to monocytosis or/and neutrophilia.¹ In fact, patients with significant neutrophilia otherwise consistent with the diagnosis of aCML but displaying monocytosis fulfilling diagnostic criteria would rather be classified as CMML. Of note is that many patients with MDS can at times show relative or even absolute monocytosis, but this observation does not suffice for the diagnosis of CMML due to the lack of chronic and consistent monocyte elevation.

Blast cells plus promonocytes usually account for fewer than 5% of blood leukocytes and fewer than 10% of the nucleated marrow cells. A higher number of blasts (plus promonocytes) may identify patients who have a rapid transformation to acute leukemia. Thus, the 2008 revision of the World Health Organization (WHO) classification system recommended that CMML be further divided into two subcategories, depending on the number of blasts (plus promonocytes) found in blood and marrow: CMML-1, with blast $<5\%$ in blood and $<10\%$ in the bone marrow, and CMML-2, with blasts (including promonocytes) 5-19% in blood or 10-19% in the marrow, or with Auer rods irrespective of the blast plus promonocytes count.¹ In contrast to WHO, the French-American-British (FAB) system has recommended a division of CMML in two classes upon leukocyte count: leukocytosis $<13 \times 10^9/L$ defines CMML with myelodysplastic form (MD-CMML) and leukocytosis $>13 \times 10^9/L$ defines CMML with myeloproliferative form (MP-CMML).²

aCML is a leukemic disorder with dysplastic as well as proliferative features at the time of initial diagnosis. It is characterized principally by involvement of the neutrophil lineage with leukocytosis resulting from an increase of morphologically dysplastic neutrophils and their precursors. However, multilineage dysplasia is common and reflects the stem cell origin of aCML. The neoplastic cells do not have a *BCR-ABL1* fusion gene. As mentioned before, the presence of monocytosis assigns the patient to a diagnosis of CMML.

Primary monocytic acute myeloid leukemia (AML; FAB M4 and M5) and secondary AML (sAML) transformed from CMML should be added to the list of differential diagnosis when CMML or aCML is suspected. By definition of WHO 2008, blast and monocyte percentage ($>20\%$) defines the AML diagnosis. Juvenile myelomonocytic leukemia (JMML) has a very similar clinical phenotype with increasing of abnormal monocytes, but exclusively occurs in

young children. The spectrum of molecular lesions is overlapping but there are also mutations which are exclusive to either CMML or JMML.

Recurrent Cytogenetic abnormalities

Balanced abnormalities

A distinctive type of CMML occurs in association with the rearrangement of *PDGFRB* at 5q31-33. There are several translocations, including t(5;12)(q31-33;p12) with formation of an *ETV6-PDGFRB* fusion gene. The hematological features are most often those of CMML (usually with eosinophilia), but some patients have been characterized as aCML. These patients with *PDGFRB* rearrangement are sensitive to tyrosine kinase inhibitors such as imatinib,³ so confirmation of *PDGFR* rearrangement by FISH or another methodology in patients with CMML features is important for clinical decision making. According to the updated WHO classification (2008),¹ the *ETV6-PDGFRB* fusion gene is included in the criteria of a novel entity, namely “myeloid and lymphoid neoplasms with eosinophilia”, defined abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*.

Unbalanced abnormalities

Until recently, conventional metaphase cytogenetics and gene mutational analyses were applied to routine diagnosis of CMML; clonal cytogenetic abnormalities are found 20-40% of cases but are not pathognomonic. A close pathological relationship to MDS is indicated by an overlapping spectrum of chromosomal defects. In CMML, the most common abnormalities are trisomy 8 and monosomy 7/deletion 7q.¹ In comparison to CMML, aCML patients have a higher frequency of cytogenetic abnormalities (80%), including trisomy 8, trisomy 13, isochromosome 17q and deletion 20q.^{1,4-6}

Recently, more sophisticated methodologies, such as array-based comparative genomic hybridization (CGH-A) or single nucleotide polymorphism arrays (SNP-A) have been utilized to investigate pathogenetic lesions in hematological malignancies. Both technologies can detect microdeletions and microduplications that are usually missed by conventional metaphase analysis. Copy number abnormalities were found by SNP-A analysis in 49% of 63 CMML patients, in particular recurrent losses of 7q22.1 (n=3) but also 4q24 (n=1) and 11q23.3 (n=1).⁷ Additionally, and perhaps more importantly, SNP-A can detect loss of heterozygosity (LOH) due to acquired uniparental disomy (UPD), which is a commonly observed abnormality in hematological malignancies including MDS/MPN.⁸ Mapping recurrent areas of LOH may aid in the identification of genes harboring mutations, as demonstrated for UPD9p and *JAK2* (*V617F*) mutations in polycythemia vera (PV).⁹ In CMML, genome-wide SNP-A analysis revealed that UPD is frequent on chromosomes 1p, 4q, 7q, and 11q. Interestingly, UPD11q is also frequently detected in aCML.⁹ This observation indicates that pathogenic gene mutations in a homozygous form may differ between CMML/aCML and MPN.

Somatic gene mutations

In CMML patients, various somatic mutations can be found, including those associated with proliferation signaling (*CBL*, *RAS*, *RUNX1*, *JAK2* (*V617F*)), and with modification of epigenetic status (*TET2*, *ASXL1*, *DNMT3A*) (summarized in Table 2). However, CMML patients are rarely found to have mutations in *PTPN11*,¹⁰ which is seen in significant proportion of patients with JMML.¹¹ In contrast, mutations in *CBL-B*,^{12,13} *RAS*,¹⁴⁻¹⁶ and *ASXL1*^{17,18} are shared by CMML and JMML.

CBL

In various myeloid neoplasms including CMML, RING finger domain (RFD) mutations were found in *CBL-B*, which codes for an E3 ubiquitin ligase involved in degradation of activated receptor tyrosine kinases, which results in the augmentation of proliferative signals mediated by various growth factor receptors.^{8,19,20} Mutations of *CBL* are frequently associated with UPD11q, which results in a homozygous configuration and the absence of RFD function in clonal cells. Recently, molecular analyses of large CMML cohorts revealed the precise percentage (10-19%) of CMML patients with somatic *CBL* mutation.^{7,21} In an aCML cohort of 152 cases, somatic *CBL* mutations were also identified in 12 (8%), including cases with UPD11q.²²

RAS

Mutations in the *RAS* family of oncogenes have been associated with multiple types of solid tumors as well as hematopoietic neoplasms. A proportion of cases of CMML and aCML also exhibit *NRAS* or *KRAS* mutations.^{23,24} Based on recent reports of large CMML cohorts with comprehensive gene mutational analyses, the proportion of *RAS* mutations is around 11–27 %.^{7,21,25} Ricci et al. reported that MP-CMML patients have higher frequency of *RAS* mutations compared with MD-CMML, and clinical evolution was associated with expansion of a clone mutated in *RAS*.²⁶

RUNX

RUNX1 is essential for normal hematopoiesis. Kuo *et al.* found *RUNX1* mutations in 30 of 81 patients (37%) with CMML.²⁷ They reported that there was no difference in overall survival between patients with and without *RUNX1* mutations, but a trend towards a higher risk of AML progression was observed in mutation-positive patients (16/30 vs 17/51, $p=0.102$), especially in patients with C-terminal mutations ($p=0.023$).

JAK2 (V617F.)

JAK2 is a tyrosine kinase involved in the transduction of cellular growth stimuli. A somatic activating mutation, *JAK2 (V617F)*, was described in a majority of patients with PV and in approximately half those with essential thrombocythemia (ET) and myelofibrosis (MF).^{28,29} *JAK2 (V617F)* was also identified in a proportion of CMML patients (2-10 %),^{7,30-32} and was more recently reported to be associated with the myeloproliferative form (MP-CMML) rather than with myelodysplastic form (MD-CMML).²⁶

TET2

TET2 on chromosome 4q24 was initially identified as candidate tumor suppressor gene by SNP-A analysis.³³⁻³⁶ Sequencing studies revealed *TET2* mutations in 10-25% of patients with sAML, MDS and MPN, while the frequency appears to be higher in CMML. *TET2* mutations can occur in heterozygous or hemizygous forms, as well as in homozygous forms specifically in the context of UPD4q24.³³⁻⁴⁰ Recently, studies of large cohort identified mutations of *TET2* in approximately 50% of patients with CMML (Table 2).^{7,21,41} These findings suggested a specific association of the *TET2* gene with regulation of the monocytic lineage. *TET* family proteins are involved in the conversion of methylcytosine to hydroxymethylcytosine. It is thereby possible that TET proteins regulate the maintenance of methylation-based silencing or prevent aberrant hypermethylation.^{40,42,43} The prognostic impact is being explored, but findings to date have been contradictory. Kohlmann et al. reported *TET2* mutated CMML patients have significantly better survival²¹ while Kosmider *et al.* described a significant adverse effect of the *TET2* mutations on survival in CMML-1 patients.⁴⁴

ASXL1

ASXL1, located on chromosome 20q11.1, belongs to the polycomb gene family. *ASXL1* is reported to cooperate with heterochromatin proteins and modulate lysine-specific demethylase 1 activity, leading to a change in histone H3 methylation and thereby retinoic acid receptor repression.⁴⁵ *ASXL1* is mutated in substantial proportion of CMML patients (27-49%),^{7,25,46}. Gelsi-Boyer et al. reported that *ASXL1* mutations are associated with progression to acute phase and lower overall survival.²⁵

UTX and EZH2

UTX encodes a histone H3 lysine 27 (H3K27) demethylase, which removes the same histone modification that is introduced by the H3K27 methyltransferase *EZH2*,⁴⁷ suggesting that alterations of histone H3K27 trimethyl levels, as a consequence of either *EZH2* or *UTX* loss, may contribute to the pathogenesis of malignant evolution. In CMML and aCML patients, *EZH2* mutations were initially found by gene mapping using SNP-A (shared regions of UPD and microdeletion on 7q) and/or next generation sequencing.^{38,39} In a larger cohort of patients with CMML (N=63), the frequency of *EZH2* mutations was 6%.⁷ Jankowska *et al.* reported that *UTX* mutations were found in patients with advanced disease, in contrast to *EZH2* mutations were mainly in low risk CMML.⁷ Interestingly, it is likely that mutations in *UTX* and *EZH2* are mutually exclusive, i.e., a patient with mutations in both genes has not been found.

IDH family

Somatic mutations of *IDH1* and *IDH2* were initially described in central nervous system tumors.^{48,49} *IDH* family mutations are gain of function mutations that lead to the production of 2-hydroxyglutarate; consequently, heterozygous mutations may be sufficient to facilitate malignant progression.^{50,51} These genes are also mutated in primary AML^{52,53} and in sAML evolved from MPN, but not in chronic-phase MPN.⁵⁴ These findings suggest that *IDH* family genes play a secondary role in the acquisition of a more aggressive phenotype in MPN. We and others have detected canonical *IDH* mutations in 5-10% of patients with CMML, both in MP-CMML and MD-CMML.^{7,25}

Other gene mutations and combinations of mutations

PTPN11 mutations are rarely found in adult CMML; Loh et al. reported that only 1 of 84 patients with CMML possessed mutated *PTPN11*,¹⁰ in contrast to JMML. Mutations in *NPM1*⁵⁵, *FLT3*²⁵, *CEBPA*⁵⁶ are also reported to be rare events in CMML patients.

Genes mutated in CMML can be divided into two groups; genes associated with proliferating signaling (*CBL*, *RAS*, *RUNX1*, *JAK2*), and with modification of epigenetic status (*TET2*, *ASXL1*, *UTX*, and *EZH2*). Most of the gene mutations in CMML are not mutually exclusive; i.e., several gene mutations can occur in a single patient. Jankowska *et al.* reported that 38% CMML patient have ≥ 2 known gene mutations, and those patients prognosis was significantly inferior to patients with single gene mutation.⁷ However, still significant proportion of CMML patients (20-30%)^{7,21} do not display mutations in any of the known genes despite enthusiastic gene mutational surveys.

Prognostic schemes

Clinical characteristics including cytogenetics

In general, the diagnosis of CMML carries a poor prognosis, with a median survival of 12 months. CMML without monocytosis has been included in International Prognostic Scoring System (IPSS) criteria for MDS; consequently, in such cases prognosis can be estimated

based on the blast count, number of cytopenias and cytogenetic grouping (low, intermediate and high), thus prognosis for a major fraction of CMML cases can be assigned⁵⁷. In a cohort of 213 CMML patients, univariate analysis identified various factors, including abnormal cytogenetics by conventional methods, associated with shorter survival. Abnormal cytogenetics was not included in the multivariable analysis, which identified only 4 independent variables: hemoglobin level below 120 g/L (12 g/dL), presence of circulating immature myeloid cells, absolute lymphocyte count above $2.5 \times 10^9/L$, and marrow blasts 10% or more⁵⁸. These factors were used to generate the MD Anderson prognostic score (MDAPS), which identified 4 subgroups of patients with median survival of 24, 15, 8, and 5 months for low, intermediate-1, intermediate-2, and high risk groups, respectively.

Gene mutations

As described above, some gene mutations are reported to be associated with a specific clinical phenotype and survival outcomes. However, various somatically mutated genes could concurrently exist in the same patient,^{7,21} which makes it difficult to calculate the impact of each gene mutation. Recently, *RAS* mutations have been reported to be associated with disease progression, fitted to a multistep gene mutation disease model.²⁶ Jankowska *et al.* reported that patients who accumulated more mutations had inferior outcomes compared to those with single mutations.⁷ A revised clinical prognostic system with gene mutations can be established by future studies utilizing large cohorts with extensive longitudinal clinical findings and comprehensive gene mutational studies.

Conclusion

CMML and aCML are distinct clinical entities of MDS/MPN and share several molecular pathogenetic pathways, such as *PDGFRB* rearrangement and homozygous *CBL* mutations. Recent technologies, including genome-wide SNP-A karyotyping and next generation sequencing, have revealed that a substantial number of CMML patients have mutations of epigenetic regulatory genes (*ASXL1*, *TET2*, *EZH2*, and *UTX*), which could modify chromatin condensation and subsequently affect gene transcription. These findings have led to a paradigm shift in our comprehension of the pathogenesis of these disorders. However, although a number of molecular lesions have been identified specifically in these myeloproliferative disorders, to date the prognostic impact or therapeutic implications of these mutations has not been fully clarified. In the future, more comprehensive surveys in larger patient cohorts may allow us to understand the biological importance and/or clinical impact of these novel findings.

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Table 1**Differential diagnosis of monocytosis**

	CMML	aCML	JMML
Absolute monocyte count in PB	$> 1 \times 10^9/L$	-	$> 1 \times 10^9/L$
% of monocyte in PB	usually $\geq 10\%$	$< 10\%$	usually $\geq 10\%$
% of blast in PB/BM	$< 20\%$	$< 20\%$	$< 20\%$
Patient age	Elderly	Elderly	Young Children

Secondary AML derived from CMML and some primary forms of AML with monocytoid features [for example, AML with MLL translocation such as t(9;11)] have morphologic and phenotypic similarity.

CMML, chronic myelomonocytic leukemia; aCML, atypical chronic myeloid leukemia; AML, acute myeloid leukemia; JMML, juvenile myelomonocytic leukemia; PB, peripheral blood; BM, bone marrow.

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Table 2

Gene mutational status in CMML patients (cohort with >50 patients).

Number (n) of patients	Mutated genes								References	
	<i>CBL</i>	<i>RAS</i>	<i>RUNX1</i>	<i>JAK2</i> (V617F)	<i>TET2</i>	<i>ASXL1</i>	<i>UTX</i>	<i>EZH2</i>		<i>IDH1/2</i>
63	14%	11%	-	2%	48%	27%	9%	6%	5%	(7)
81	19%	27%	9%	10%	51%	-	-	-	-	(21)
53	10%	13%	21%	-	36%	49%	-	-	10%	(25)
88	-	-	-	-	50%	-	-	-	-	(44)
69	-	-	-	-	42%	-	-	-	-	(41)
81	-	-	37%	-	-	-	-	-	-	(27)
78	-	-	-	10%	-	-	-	-	-	(30)
52	-	-	-	13%	-	-	-	-	-	(31)