Chapter 11
Atypical Chronic Myeloid Leukemia, *BCR/ABL1* Negative

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Introduction

Atypical chronic myeloid leukemia (aCML) is a neoplasm of hematopoietic stem cells characterized by overlapping myelodysplastic and myeloproliferative features at the time of diagnosis, and is hence classified under the myelodysplastic/myeloproliferative (MDS/MPN) disease category. The hallmark of this uncommon disorder is an overabundance of dysplastic mature granulocytic cells and their immature precursors, found in both the peripheral blood and bone marrow. To date, the specific causative molecular mechanisms underlying this enigmatic entity remain elusive, and there is no single genetic feature that defines the disease. Initially, studies investigating the biological underpinnings of aCML were limited by both technology and a lack of consensus criteria for diagnosing the disorder. The formalized diagnostic requirements for aCML, originally described in the 2001 World Health Organization (WHO) classification and later refined in 2008 and 2016, set the stage for an explosion of SNP array and next generation sequencing research in recent years. These efforts have identified numerous novel, recurrent somatic mutations seen in association with aCML as well as numerous other myeloid malignancies [1]. While none of these mutations is specific to aCML, alterations in certain genes, particularly *SETBP1* and *ETNK1*, appear to occur more frequently in aCML than in other myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN),

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and MDS/MPN. These findings are of critical importance due to their potential diagnostic, prognostic, and therapeutic implications for patients with aCML, which, at present, has few effective treatment options and a dismal overall prognosis.

Epidemiology

Atypical CML is one of the rarest myeloid neoplasms. For many years, it was postulated that there were only one to two cases of aCML (by definition t(9;22)*/BCR-ABL1*-negative) for every 100 cases of "typical" CML (by definition t(9;22)*/BCR-ABL1*-positive) [2]. A recent review of United States cancer registry data collected between 2001 and 2012 confirmed that aCML is indeed quite rare, with an incidence of 0.1 cases per one million person-years [3]. Atypical CML is somewhat more common in males, with an approximate male-to-female ratio of 1.5:1. Although its occurrence has been documented in patients of many ages, including rare cases in the pediatric population [4, 5], aCML is most commonly diagnosed in the sixth or seventh decades of life. Cases of aCML have been observed in individuals from many different racial/ethnic backgrounds, although recent data suggest that the disease is much more common in patients who identify as white than in patients who identify as Hispanic, black, or Asian/Pacific Islander [3].

Clinical Features

There is a paucity of published information regarding the clinical presentation of aCML. Based on the limited data available, the most common signs and symptoms of aCML are B-symptoms (fevers, night sweats, weight loss), occurring in 38% of patients [6], and hepato- or splenomegaly, sometimes with associated early satiety and abdominal pain, occurring in 44–75% of patients [2, 6, 7]. Patients with aCML can present with variable abnormalities in hemoglobin and platelet counts. Atypical CML case series have reported median hemoglobin concentrations ranging from 9.4 to 11.7 g/dL and median platelet counts ranging from 87 to 319 × 10^9 cells/L at the time of diagnosis [2, 5–9]. If cytopenias are severe enough, aCML patients may present with related clinical findings, including dyspnea on exertion and fatigue from anemia, as well as bleeding and bruising from thrombocytopenia. While aCML patients by definition produce an excessive number of leukocytes, they appear to have a predisposition to develop recurrent infections, presumably a consequence of the cells' qualitative dysfunction. A single case series showed that by the time patients were diagnosed with aCML, 30% had experienced infection, 38% had experienced hemorrhage, and 65% had developed a transfusion requirement [2].

Morphology and Immunophenotype

The diagnosis of aCML relies on a combination of morphologic features seen in the peripheral blood and bone marrow, which have been outlined by the WHO (see Table 11.1) [10, 11]. A summary of the findings that help distinguish aCML from chronic myelomonocytic leukemia (CMML) and chronic neutrophilic leukemia (CNL), two entities commonly in the differential diagnosis of aCML, is presented in Table 11.2.

Peripheral Blood Morphology

Examination of the peripheral blood smear can reveal many features required for the diagnosis of aCML. An essential peripheral blood finding is the presence of a persistent granulocytic leukocytosis in excess of 13×10^9 cells/L [12]. The median white blood cell count reported in aCML case series varies widely, ranging from 23.7 to 152×10^9 cells/L [2, 5–9, 12]. The leukocytosis of aCML is left-shifted, with immature precursors, including metamyelocytes, myelocytes, and promyelocytes, accounting for at least 10% of the total white blood cell differential count (Fig. 11.1). Dysgranulopoiesis should be present, although no numeric cutoff for percentage of dysplastic cells or rigorous dysplasia grading system have been formally established. Features of granulocytic dysplasia may include unusually small or large cell

Table 11.1 Summary of the 2016 WHO diagnostic criteria for atypical chronic myeloid leukemia

Peripheral blood:
Neutrophilic leukocytosis >13×10^9 cells/L
Myeloid precursors (promyelocytes, myelocytes, metamyelocytes) ≥10% of leukocytes
Dysgranulopoiesis
Monocytes <10% of leukocytes with no or minimal absolute monocytosis
Basophils usually <2% of leukocytes with no or minimal absolute basophilia
Blasts <20% of leukocytes
Bone marrow:
Hypercellular with granulocytic expansion
Dysgranulopoiesis with or without erythroid and megakaryocytic dysplasia
Blasts <20% of nucleated cells
Genetic:
No Philadelphia chromosome or *BCR-ABL1* fusion gene
No rearrangement of *PDGFRA, PDGFRB,* or *FGFR1*
No *PCM1-JAK2* fusion gene

Data from Swerdlow et al. 2008 and Arber et al. 2016 [10, 11]

Table 11.2 Comparative summary of 2016 WHO morphologic criteria for atypical chronic myeloid leukemia (aCML), chronic neutrophilic leukemia (CNL), and chronic myelomonocytic leukemia (CMML) in the peripheral blood (PB) and bone marrow (BM)

	aCML	CNL	CMML
WBC	>13 × 10^9 cells/L	≥25 × 10^9 cells/L	NA
% Immature granulocytes PB	>10%	<10%	NA
% Monocytes PB	<10%	NA	≥10%
Absolute monocytes PB	Normal to mildly increased	<1 × 10^9 cells/L	≥1 × 10^9 cells/L
% Blasts PB	<20%	Rarely observed	<20%
% Blasts BM	<20%	<5%	<20%
Granulocytic dysplasia	+	−	±
Other hematopoietic dysplasia	±	−	±

Adapted from Arber et al. (2016) [10]

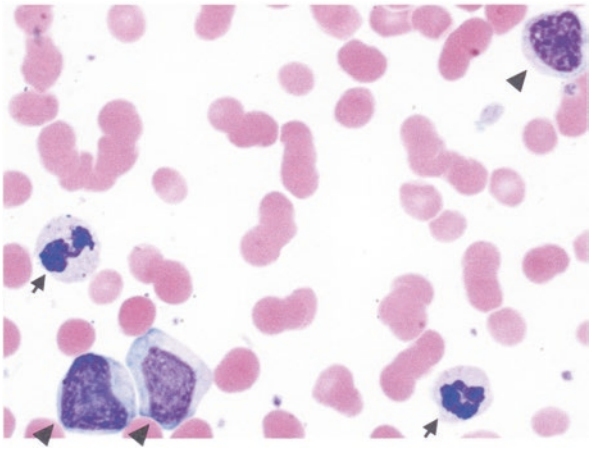

Fig. 11.1 High-power view of atypical CML peripheral blood smear with dysplastic neutrophils (*arrows*) and immature precursors (*arrowheads*). Wright-Giemsa, 100×

size, exaggerated chromatin clumping, abnormalities in nuclear segmentation (including hypolobation, pseudo Pelger-Huet cell formation, and irregular hypersegmentation), and abnormalities in granulation (including hypogranularity, agranularity, or enlarged, pseudo Chediak-Higashi granules) (Fig. 11.2) [11]. An accurate diagnosis of aCML hinges on the absence of certain additional findings. Basophils and monocytes should account for <2% and <10% of peripheral white blood cells, respectively. Blasts typically account for <5% of peripheral blood leukocytes and should never exceed 20% of the total.

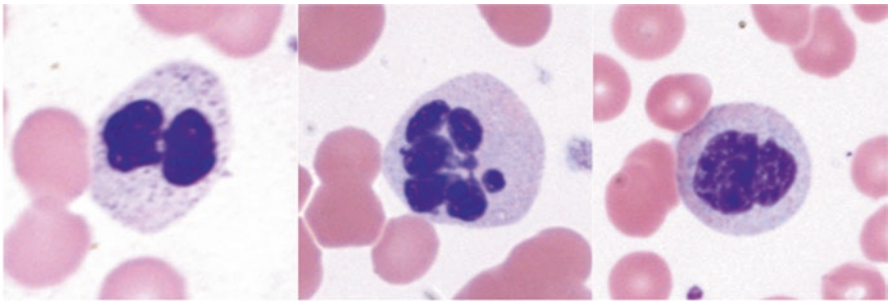

Fig. 11.2 High power view of dysplastic peripheral blood neutrophils in atypical CML. Pseudo Pelger Huet morphology (*left panel*); nuclear hyperlobation and cytoplasmic hypogranulation (*central panel*); and nuclear hypolobation and cytoplasmic hypogranulation (*right panel*). Wright-Giemsa, 100×

Bone Marrow Morphology

An aCML bone marrow biopsy should be hypercellular, with mature granulocytic cells and precursors comprising the majority of the cellularity (Fig. 11.3). Typically, the myeloid to erythroid ratio is in excess of 10:1 [11]. The overall number of megakaryocytes can be decreased, normal, or increased [11]. Similar to the peripheral blood, dysplasia should be present in the granulocytic lineage (Fig. 11.4). Median frequencies of erythroid dysplasia seen in aCML have varied substantially, ranging from 12 to 91% [2, 5, 7, 8]. Dysmegakaryopoiesis is slightly more common, with median reported frequencies ranging from 44 to 90% [2, 5, 7, 8]. The dysplastic megakaryocytes seen in aCML may have features typical of MDS, such as small cell size, abnormal nuclear lobation, or nuclear hypolobation; features typical of MPN, such as large cell size, nuclear hypersegmentation, and clustering; or features that fall somewhere in between (Fig. 11.5) [7]. A single, recent study found that among 61 patients with aCML, 54% had MDS-like megakaryocytes, 26% had MPN-like megakaryocytes, and 8% had mixed MDS/MPN-like megakaryocytes [7]. Reticulin fibrosis is uncommon in aCML, but may be seen in occasional cases, with a frequency of 18% reported in one case series [2]. The bone marrow blast count is usually <5% [2, 5, 7] and must be <20%. In addition, blasts should not form visible sheets or clusters [11].

Immunophenotype and Cytochemistry

There are no specific data regarding the immunophenotype of aCML. However, immunophenotyping of peripheral blood and bone marrow for CD14, CD68R, and/or CD163 may facilitate monocyte quantification in cases where both aCML and CMML are in the differential diagnosis [11]. It should be noted that immunophenotyping,

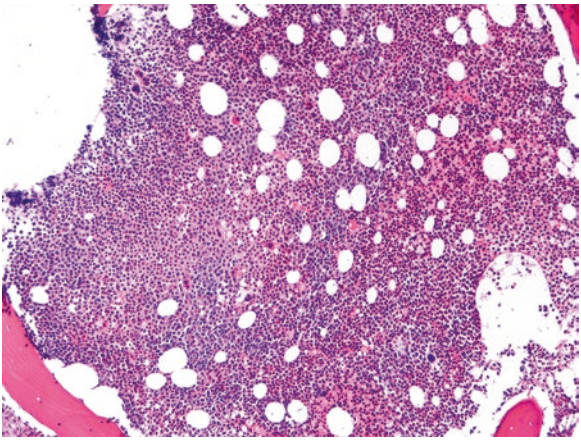

Fig. 11.3 Low power view of a bone marrow core biopsy of atypical CML. The biopsy is notable for hypercellularity and a markedly increased myeloid to erythroid ratio. Hematoxylin and eosin 10×

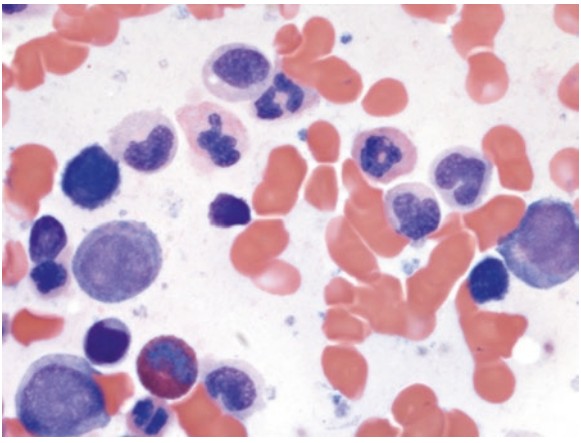

Fig. 11.4 High-power view of atypical CML bone marrow aspirate smear with dysplastic granulocytic cells. Wright-Giemsa, 100×

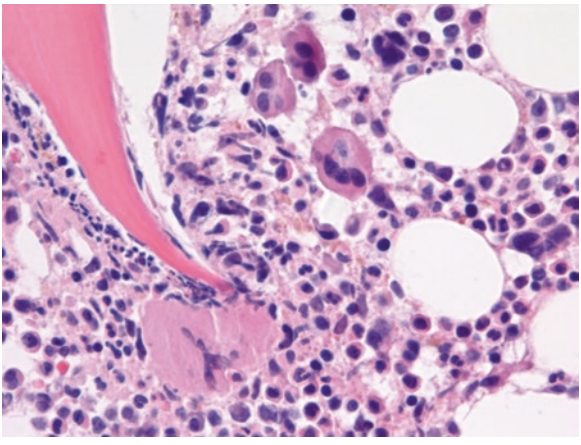

Fig. 11.5 High power view of bone marrow core biopsy of atypical CML showing abnormal megakaryocytes with both myelodysplastic and myeloproliferative features. Hematoxylin and eosin 40×

even in this limited capacity, is not without challenges. Flow cytometric and immunohistochemical evaluation of monocytes may be impacted by alterations of antigen expression, including CD14, in immature and neoplastic populations [13, 14]. While such aberrancies may be helpful in the qualitative identification of abnormal monocytic populations, they can also limit precise enumeration by both flow cytometry and immunohistochemistry. In addition, it has been shown that staining of bone marrow core biopsies for CD68R and CD163 could not reliably distinguish between aCML and CMML in one small study [15]. For these reasons, cytochemical staining for nonspecific esterase is still advocated in addition to flow cytometry and immunohistochemistry as the most reliable means of identifying neoplastic monocytic populations [11, 13, 16].

There are other immunohistochemical findings in bone marrow core biopsies that can serve as a useful alternative to counting monocytes when attempting to distinguish aCML from CMML. For example, immunohistochemical staining for CD123 can be used to identify plasmacytoid dendritic cell nodules on bone marrow core biopsies, which are a specific (although somewhat insensitive) finding that favors a diagnosis of CMML [15].

Cytogenetics and Molecular Findings

Cytogenetics

There is no single cytogenetic feature that can confirm or establish a diagnosis of aCML. Although the Philadelphia chromosome is by definition absent in all cases of aCML, other nonspecific karyotypic abnormalities are quite common, having been reported in 20–88% of patients carrying the diagnosis [2, 5–8]. Studies have shown that there are some recurrent cytogenetic findings in aCML patients, many of which are also observed in MDS and CMML [11]. The most common aberrations in aCML, observed across six case series, are trisomy 8 and deletions of the long arm of chromosome 20 [2, 5–8, 12]. The next most common cytogenetic abnormalities noted in those studies were alterations in chromosome 7 (either monosomy or deletion of the long arm) and the presence of isochromosome 17q. Additional abnormalities of chromosomes 5, 11, 12, 13, 14, 17, 19, 21, and X were identified at lower frequencies. While complex karyotypes and multiple-anomaly karyotypes are relatively frequent findings in aCML, balanced translocations are quite rare.

Molecular

Scientific understanding of aCML's molecular underpinnings remains in its infancy. Until recently, aCML's only defining molecular features were the absence of key genetic findings diagnostic of other similar neoplasms, specifically the *BCR-ABL1* fusion seen in CML and the *PDGFRA*, *PDGFRB*, *FGFR1*, and, most recently,

PCM1-JAK2 fusions seen in myeloid and lymphoid neoplasms associated with eosinophilia [10]. Over the past several years, aCML has transitioned from a disease defined by the mutations it lacks into a disease with a few signature molecular alterations. Recent genetic sequencing studies have revealed that two genes, *SETBP1* and *ETNK1*, are recurrently mutated in aCML. Because mutations in these genes are considered relatively specific for aCML, they will be the primary focus of this section. There are, however, multiple additional, nonspecific genetic alterations seen in aCML which recur across the spectrum of myeloid neoplasms; these will be summarized here and discussed elsewhere. The relative frequencies of the various mutations seen in aCML and other closely related disorders are summarized in Table 11.3.

Table 11.3 Frequencies of gene mutations as seen in atypical chronic myeloid leukemia (aCML) and other morphologically similar diagnostic entities, including chronic myelomonocytic leukemia (CMML), myelodysplastic/myeloproliferative neoplasm – unclassifiable (MDS/MPN-U), and chronic neutrophilic leukemia (CNL)

	aCML	CMML	MDS/MPN-U	CNL
SETBP1	24–33%	4–15%	10%	14–55%[a]
ETNK1	9%	3–14%	0%	0%
CSF3R	<10%[b]	0–1%	0%	43–100%
Cell Signaling				
N/KRAS	0–40%	4–57%	10–14%	NR
CBL	7–12%	10–21%	>10%	0%
JAK2	0–7%	0–13%	0–19%	0%
FLT3	0–7%	0–3%	3%	NR
CALR	0–4%	3%	0%	0–8%
MPL	0–2%	<1%	0%	0%
Transcription Regulation				
CEBPA	5–12%	4–20%	0–4%	NR
RUNX1	2%	9–37%	14%	NR
RNA Splicing				
SRSF2	40%	36–51%	NR	21%
U2AF1	13%	5–15%	NR	NR
Epigenetic Regulation				
ASXL1	20–66%	27–49%	NR	57%
TET2	25–41%	36–61%	29–30%	29%
EZH2	13–20%	6–13%	10%	NR
IDH1/2	0–5%	1–10%	0–10%	NR

NR not reported

Sequencing methodologies and extent of gene analyzed were heterogeneous across different studies. The categories denoted with a "/" (*N/KRAS* and *IDH1/2*) each feature two closely related genes which were sometimes reported individually and sometimes reported as an aggregate

[a]Percentages are from small studies and may not reflect the true mutational frequency in the designated patient population

[b]Average percentage across majority of available studies, although initial research reported mutational frequencies of up to 44%

SETBP1

The gene SET binding protein 1 (*SETBP1)* is located on chromosome 18q21.1, encodes a protein of the same name which contains 1596 amino acids (NCBI reference sequence NP_056374.2), and is predominantly located in the nucleus [17]. The structure of the SETBP1 protein has not been fully elucidated, but its known structural components are depicted in Fig. 11.6. Although its biological function is still under investigation, SETBP1 has been proposed to influence cell proliferation by inhibiting the known tumor suppressor phosphatase 2A (PP2A) via interactions with its substrate SET [17–20], by regulating the expression of cell differentiation homeobox genes, homeobox A9 (*Hoxa9)* and homeobox A10 (*Hoxa10)* [21], and, possibly, by modulating Ski/Ski homodimer and/or Ski/SnoN heterodimer formation via its SKI homologous domain [17].

SETBP1 became relevant to aCML when multiple publications showed evidence of recurrent *SETBP1* mutations in up to 33% of patients with the disease [22–25]. Mutations in *SETBP1* were also identified in patients with CMML and myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U), but they occurred at lower rates than in aCML, at frequencies of 4–15% [23–29] and 10% [25], respectively. A handful of *SETBP1* mutations were also identified in 1 of 4 [25], 2 of 14 [23], 5 of 13 [30], 5 of 9 [31], and 4 of 12 [29] patients with CNL, although the small number of cases examined in these studies makes it difficult to know the true mutation frequency in CNL. While the initial study found no evidence of *SETBP1* mutations in hundreds of other hematologic and nonhematologic malignancies [25], subsequent studies showed that they can be seen with some frequency in secondary AML (2–17%) and less commonly in cases of MPN (0–3%), MDS (2–4%), and primary AML (0–< 1%) [24, 26, 28, 32].

The initial study reporting *SETBP1* alterations in aCML patients showed that the *SETBP1* mutations were all heterozygous missense mutations occurring almost exclusively in the protein's SKI-homologous region [25]. Six of the mutations seen in the

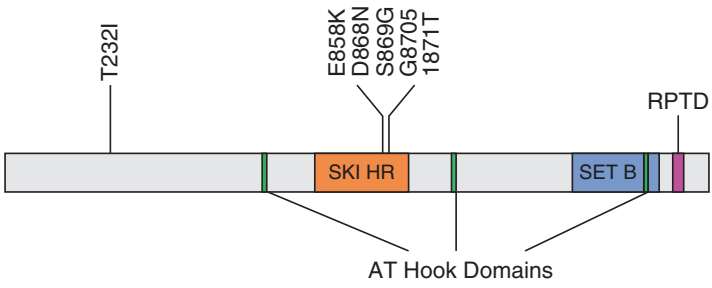

Fig. 11.6 Schematic representation of SETBP1 protein. The primary protein isoform consists of 1596 amino acids and contains three AT hook domains (*green*), a SKI homologous domain (SKI HR, *orange*), SET binding domain (SET B, *blue*), and repeat domain (RPTD, *pink*). The mutations specifically associated with aCML are listed, and those confirmed to be somatic are in bold (Adapted from Piazza 2012 [33])

aCML patients were confirmed to be somatically acquired by tandem analysis of constitutive DNA [25]. The mutational hotspot included 16 amino acid residues in positions 858–874 [24, 25]. Comparative RNA analysis between *SETBP1* wild type and *SETBP1* mutated aCML cases revealed 14 differentially expressed genes belonging to the group transcriptionally controlled by TGF-β, a finding which was highly statistically significant [25]. Because of the known associations between SKI and TGF-β signaling [33], these findings suggested one potential mechanism by which mutations in *SETBP1*'s SKI homologous domain could induce oncogenesis in aCML. It was also noted that a portion of the *SETBP1* mutational hotspot (amino acids 868–873) encodes a virtually perfect binding site for β-TrCP1, the substrate recognition subunit of the protein-degrading E3 ubiquitin ligase [25]. Additional experiments not only confirmed that the mutated versions of SETBP1 seen in aCML are resistant to degradation, but also demonstrated that cells expressing mutant *SETBP1* have significantly lower levels of PP2A activity and significantly increased proliferation rates compared to wild type controls [25], suggesting a second mechanism by which mutations in *SETBP1* could induce oncogenesis in aCML.

There are only limited data connecting *SETBP1* mutations to clinical features, cytogenetic findings, and other mutations specifically in aCML. The seminal study of *SETBP1* mutations in aCML revealed that the only clinical variable significantly associated with *SETBP1* mutations was a higher white blood cell count at diagnosis, with a median of 81×10^9 cells/L in the *SETBP1* mutated group compared to a median of 38.5×10^9 cells/L in those with wild type *SETBP1* [25]. One subsequent study found that aCML patients with *SETBP1* mutations had significantly higher hemoglobin concentrations than those with wild type *SETBP1* [23], a finding which was not seen in the initial study [25]. There is currently no aCML-specific information regarding associations between *SETBP1* mutations and cytogenetic alterations, although studies of other myeloid neoplasms have shown significant correlations between *SETBP1* mutations and several cytogenetic anomalies that are frequently seen in aCML, including i(17)(q10), monosomy 7, and del(7q) [24, 28] . Several studies of patients with aCML have noted preliminary associations between *SETBP1* mutations and alterations in other genes that are commonly mutated across the broad spectrum of myeloid neoplasms. The only statistically significant association observed has been between *SETBP1* mutations and *SRSF2* mutations [23]. Multiple other genes have been reportedly mutated in tandem with *SETBP1* in aCML, including *CBL* [24, 25], *ASXL1* [22, 24, 25], *EZH2* [25], *N/KRAS* [22, 25], *TET2* [25], *ETNK1* [22], and *CSF3R* [31]. These findings, however, were either not statistically significant or were only anecdotal. Additional, larger studies will be needed to firmly establish these associations and to expand our understanding of how *SETBP1* mutations interact with other concurrent molecular alterations to produce the unique aCML disease phenotype.

Although they can be found in multiple different myeloid neoplasms, and while they are not evident in the majority of aCMLs, the current data suggest that *SETBP1* mutations are one of the most promising molecular markers in the diagnosis and pathogenesis of aCML. Preliminary research has suggested multiple potential mechanisms by which mutations in *SETBP1* can induce neoplastic transformation

in hematopoietic cells. More investigation is needed to further our understanding of the precise molecular mechanisms by which *SETBP1* mutations contribute specifically to the development of aCML.

ETNK1

The gene ethanolamine kinase 1 (*ETNK1*, also known as *EKI1*) is located on chromosome 12p12.1 and encodes a 452 amino acid protein called ETNK1 [34]. Little is known about the structure of the protein, which is depicted in Fig. 11.7. ETNK1 is responsible for facilitating the ATP-dependent phosphorylation of ethanolamine (Etn) to produce phosphoethanolamine (P-Etn). The conversion of Etn to P-Etn is the first step in a biochemical chain of events known as the CDP-ethanolamine pathway (also known as the Kennedy pathway), which ultimately results in the production of phosphatidylethanolamine (PE) [35]. PE is the second most abundant phospholipid in mammalian cells, and is involved in many essential cellular processes, including cell division and membrane protein orientation [35]. These functions are, of course, quite nonspecific. Although mechanisms linking them to neoplasia could be postulated, further research is needed to link PE and ETNK1 to oncogenesis at the molecular level.

The only study examining *ETNK1* mutations in aCML performed whole exome and transcriptome sequencing on 15 patients with aCML, and identified two somatic, heterozygous missense mutations in *ETNK1* that altered two adjacent amino acid residues, H243Y and N244S [22]. In that same study, targeted sequencing of numerous additional clonal hematologic disorders revealed *ETNK1* mutations in 4 of 53 additional cases of aCML, bringing the overall *ETNK1* mutation frequency in aCML to 9% (6 of 68 cases). Although *ETNK1* mutations were also identified in 2 of 77 (3%) cases of CMML, they were not seen in MDS/MPN-U ($n = 10$), CNL ($n = 1$), other hematologic neoplasms, solid tumors, cancer cell lines, or healthy controls [22]. The additional six mutations were all heterozygous N244S mutations, bringing the overall mutation counts to one H243Y variant and seven N244S variants [22]. Review of the ETNK1 structure revealed that the mutations were clustering in the region encoding the protein's highly conserved kinase domain [22]. Follow up experiments on seven aCML primary samples indicated that *ETNK1*

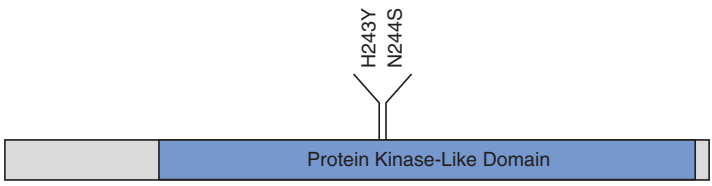

Fig. 11.7 Schematic representation of ETNK1 protein. The primary protein isoform consists of 452 amino acids, with a protein kinase-like domain spanning amino acids 100–444 (*blue*). The mutations specifically associated with aCML are listed (Adapted from Gambacorti-Passerini 2015 and Lasho 2015 [30, 42])

mutations significantly lowered intracellular levels of P-Etn relative to wild type controls, and that transduction of myeloid cell lines with wild type and mutant forms of *ETNK1* produced similar results [22]. These findings suggest that mutations in *ETNK1* interfere with the function of the ETNK1 enzyme, but until more research is performed, it remains unknown how diminished ETNK1 activity contributes to oncogenesis.

At present, there are no data available regarding the clinical characteristics and cytogenetic findings associated with *ETNK1* mutations in aCML or in any other hematologic neoplasm. Very limited data suggest that concurrent *ETNK1* and *SETBP1* mutations may be seen in aCML [22], although how these mutations may interact and contribute to oncogenesis has not been explored. Another important consideration is that, although the breakthrough study suggested that *ETNK1* mutations were highly specific for aCML, an additional study reported *ETNK1* mutations in 4 of 29 (14%) cases of CMML, which would make the mutational frequency in CMML higher than that reported in aCML [36]. In addition, *ETNK1* mutations were seen in 5 of 82 (6%) cases of systemic mastocytosis (SM) and in 1 of 137 (<1%) cases of "idiopathic hypereosinophilia" [36]. All of these findings suggest that alterations in *ETNK1* may not be as unique to aCML as previously thought. Larger-scale studies are needed to clarify the relative incidences of *ETNK1* mutations in aCML, CMML, SM, and "idiopathic hypereosinophilia" so that we may better understand their utility in classifying these related disorders.

CSF3R

CSF3R (granulocyte colony-stimulating factor 3 receptor, also known as *GCSFR*) encodes the trans-membrane cell surface receptor for granulocyte-colony stimulating factor (G-CSF, also known as CSF3), which has long been known to promote the proliferation and differentiation of granulocytic cells via its interaction with CSF3R [37]. Given the essential role that *CSF3R* plays in granulocytic cell growth and maturation, it is not surprising that *CSF3R* mutations would be seen in neoplasms characterized by aberrations in the myeloid lineage, including aCML and CNL. Although initially reported in 40–44% of patients with aCML [31, 38], *CSF3R* mutations are now considered rare in aCML, occurring in fewer than 10% of cases [7, 22, 23, 29]. The *CSF3R* mutations seen in aCML generally fall into one of two main categories: either missense mutations occurring predominantly in the membrane proximal domain or truncating mutations occurring in the cytoplasmic domain [38]. Occasionally, both mutation types occur simultaneously [38]. Follow-up experiments have demonstrated that both mutation subtypes can induce cell line transformation in vitro [38]. Although *CSF3R* mutations are occasionally identified in aCML, they are substantially more common in CNL, occurring in 43–4100% of cases [23, 30, 31, 38, 29]. Consequently, the 2016 WHO classification of myeloid neoplasms lists *CSF3R* mutations among the diagnostic criteria for CNL and notes that the detection of *CSF3R* mutations in potential cases of aCML should prompt efforts to exclude an alternative diagnosis of CNL or other myeloid neoplasm [10].

Other Molecular Findings in aCML

Many of the other mutations seen in aCML are not disease specific, but rather, are seen across a wide spectrum of myeloid neoplasms, including CMML, MDS/MPN-U, and CNL. These mutations, summarized in Table 11.3, occur in genes involved with cell signaling, such as *N/KRAS* [1, 7, 8, 22, 25, 39–41], *CBL* [1, 23–25, 41–43], *JAK2* [1, 7, 23, 29–31, 39, 41, 42, 44], *FLT3* [1, 7, 39, 45], *CALR* [7, 23, 30, 46], and *MPL* [7, 23], transcription regulation, such as *CEBPA* [1, 7, 25] and *RUNX1* [1, 25, 41], RNA-splicing, such as *SRSF2* [1, 23] and *U2AF1* [1, 22], and epigenetic regulation, such as *ASXL1* [1, 22–25, 30, 41], *TET2* [1, 23–25, 41, 42], *EZH2* [1, 22, 25, 41, 47], and *IDH1/2* [1, 7, 25, 41]. The mechanisms by which these various genes promote cellular proliferation and oncogenesis are quite diverse and beyond the scope of this chapter. Because none of these mutated genes is particularly unique to aCML, finding alterations in them is of little value diagnostically. However, mutations in these genes should not be overlooked, as they may have prognostic and therapeutic implications for aCML patients, which will be discussed in the next section. In addition, observations regarding which mutations tend to co-occur and which ones are mutually exclusive will hopefully shed light on the molecular pathogenesis of aCML and other myeloid neoplasms.

Therapy and Prognosis

Presently, aCML has a fairly dismal prognosis, with a median survival ranging from 12.4 to 36 months across several case series [2, 5–8]. The most robust risk factor associated with shorter overall survival in aCML is leukocytosis in excess of 50×10^9 cells/L, documented in two separate case series [2, 7]. A few other features, including older age (>65 years) [2], female sex [2], and higher numbers of circulating immature precursors [7], have also been associated with shorter overall survival, but these were noted only in single case series.

The only molecular marker that has been associated with prognosis specifically in aCML is the presence of mutated *SETBP1,* however, the studies examining this relationship have shown inconclusive results [24, 25]. While one study showed that *SETBP1* mutations were significantly associated with worse prognosis in aCML, with a median survival of 22 months compared to 77 months in nonmutated patients (median follow up not specified) [25], a second study found that aCML patients with *SETBP1* mutations had an overall survival of 32.9 months, compared to 15.6 months in nonmutated aCML patients (median follow up 17.1 months) [24]; importantly, the latter finding did not reach statistical significance. The fact that there have been significant associations between *SETBP1* mutations and other adverse prognostic factors such as increased white blood cell count [25], supports the notion that *SETBP1* mutations are markers of poor prognosis in aCML, although more studies are needed to confirm this finding.

In addition to having a poor overall prognosis, patients with aCML have a substantial risk of transforming into acute leukemia, with an absolute progression risk of 13–40% [2, 6, 7] and a median time to leukemic transformation of 11.2–18 months [2, 7]. Many clinical and laboratory parameters have been associated with the risk of transformation in aCML, including transfusional requirement, palpable hepatosplenomegaly, higher white blood cell count, higher percentage circulating myeloid precursors, percentage monocytes of 3–8% with an absolute monocyte count $<1 \times 10^9$ cells/L, >5% bone marrow blasts, marked dyserythropoiesis, and karyotypic changes [2, 7].

There is no current gold standard for managing patients with aCML. Many different treatment modalities are utilized in aCML, including hydroxyurea, immunomodulators, hypomethylating agents, histone deacetylase inhibitors, traditional chemotherapy, and hematopoietic stem cell transplantation (HSCT) [2, 5–7]. While there are rare case series demonstrating these therapies can be beneficial in aCML [6, 48], the data regarding the efficacy of these treatments and their impact on patient survival are quite limited. The most evidence-based therapeutic strategy in aCML is HSCT, and even this approach is only supported by a few small studies, which followed an aggregate total of 18 patients over median follow up periods of 22–97.6 months [49–51]. Across these three studies, two patients relapsed, six patients died, nine developed acute graft versus host disease, and 15 developed chronic graft versus host disease. These aggregate data seem to suggest that HSCT is a viable treatment option for aCML, but the morbidity and mortality associated with the procedure highlights a need for additional therapeutic options.

The advent of molecular diagnostics in aCML has introduced the possibility of targeted, lower-toxicity treatment options for patients with certain disease-associated mutations. At present, most of the data regarding these treatments are limited to single case reports. For example, one group showed that transformed cells expressing an oncogenic *SPTBN1-FLT3* fusion protein derived from an aCML patient exhibited dose-dependent growth inhibition by three different FLT3 inhibitors [45]. Another study showed that an aCML patient with a heterozygous *NRAS* mutation had improvements in cell counts, normalization of liver and kidney function tests, and increased energy levels after receiving off-label trametinib, an FDA-approved MEK1/2 inhibitor [52]. There is a somewhat more substantial body of evidence supporting mutation-targeted treatment in aCML patients harboring *CSF3R* mutations. Although *CSF3R* mutations are not as prevalent in aCML as initially posited, studies have suggested that drugs targeting downstream effectors of CSF3R may be helpful therapeutic options for patients expressing mutant forms of the protein. In vitro experiments with *CSF3R*-mutated patient samples, cell lines, and murine bone marrow cells have demonstrated that cells with membrane proximal mutations like T618I are sensitive to JAK inhibitors and that cells with cytoplasmic tail truncating mutations like S783fs are sensitive to the SRC (and ABL1) inhibitor dasatinib [38]. There are multiple case reports showing that patients and mice with *CSF3R* T618I-mutated neoplasms respond to ruxolitinib therapy [4, 38, 53, 54]. The magnitude of response to treatment has varied from case to case, ranging from isolated reduction in white blood cell count [4] to complete normalization of peripheral blood counts with fewer

circulating granulocytes, improved neutrophil granulation, improvements in bone marrow morphology, reduction in splenic volume, and improvement in symptom scores [53]. Interestingly, a single aCML patient with concurrent *CSF3R* T618I and *SETBP1* G870S mutations did not respond to ruxolitinib therapy, suggesting that *SETBP1* mutations may modify *CSF3R* T618I mutations' responsiveness to therapy by an as-of-yet unknown mechanism [55].

In addition to guiding therapeutic decision making in aCML, *CSF3R* mutations may also have a role in monitoring therapeutic efficacy. *CSF3R* T618I mutation levels were found to correlate with the presence of disease in two aCML HSCT patients, suggesting this molecular marker may be useful in monitoring disease burden post-transplantation [56]. Although a single case report showed that clinical responses to ruxolitinib therapy are not necessarily accompanied by reductions in *CSF3R* T618I allele frequency [53], it will be worthwhile to further assess the utility of *CSF3R* allele frequency as a marker for response to targeted therapies. While these reports offer hope for the future of therapeutics in aCML, it is important to note that these findings are only anecdotal. Many more studies will be required to optimize therapeutic approaches and significantly alter patient prognosis.

The lack of consensus regarding the optimal management of aCML patients is multifactorial, and generating informative clinical trials will be challenging for several reasons. First, correctly identifying cases of aCML can be difficult, as the current diagnostic criteria were only recently established, and even these criteria may be open to subjective interpretation. In addition, aCML is a rare disease, making it hard to generate large clinical trials comparing efficacies of different treatment modalities. Finally, there are no formally accepted guidelines for evaluating response to treatment or for assessing disease progression in aCML, which hampers accurate assessment of therapeutic efficacy in research studies. Although such metrics are established in MDS [57] and subtypes of MPN [58], they are suboptimal for use in MDS/MPN because those criteria do not account for the simultaneous myelodysplastic and myeloproliferative features seen in this unique disease category. These important issues were discussed in a recent commentary where the authors proposed sets of criteria specifically designed to measure treatment response and disease progression in patients with MDS/MPN [59]. These recommendations are an excellent step toward standardizing aCML research, but they will require further refinement and thorough validation before they can be adopted in clinical practice.

Conclusion

Atypical CML is an uncommon myeloid neoplasm with overlapping myelodysplastic/myeloproliferative features and a poor prognosis. Although aCML is currently best defined by morphologic criteria, advances in the field of genetics have identified *SETBP1* and *ETNK1* as relatively disease-specific molecular markers. Many other mutations have also been reported in aCML. Although these mutations are not unique to aCML and are therefore of limited use diagnostically, they may carry

increasing significance as the arsenal of targeted therapeutic options expands. At present, more research is needed to further our understanding of the molecular mechanisms driving the development of aCML and to identify treatment regimens supported by robust, well-designed clinical trials.

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