Chapter 10
Chronic Myeloproliferative Neoplasm, Rare Types

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Chronic Neutrophilic Leukemia

Introduction

Chronic neutrophilic leukemia (CNL) is a rare type of myeloproliferative neoplasm (MPN) characterized by sustained leukocytosis ($\geq 25 \times 10^9$/L) with neoplastic proliferation of neutrophilic granulocytes in blood and bone marrow. Since first described in 1920 by Tuohy, approximately 200 cases of CNL have been reported to date [1, 2]. The literature includes many case reports and a handful of small case series. The diagnostic criteria for CNL have only been defined more recently, and it is unclear from the literature how many are true cases of CNL. It is likely that less than 40% of reported cases meet the current WHO diagnostic criteria [3, 4].

In 2013, Maxon et al. reported high frequency of oncogenic mutations in colony stimulating factor 3 receptor (*CSF3R*) in CNL [5]. These findings were supported by another study that reported 100% frequency of *CSF3R* mutation in 12 patients with WHO-defined CNL [6]. *CSF3R* mutations have been incorporated in the diagnostic criteria in the 2016 WHO classification of CNL [7]. Table 10.1 lists the updated diagnostic criteria.

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Table 10.1 Diagnostic criteria for chronic neutrophilic leukemia [7]

1	Peripheral blood WBC ≥ 25 × 10^9/L Segmented neutrophils plus band forms ≥80% of WBC Neutrophil precursors (promyelocytes, myelocytes, and Metamyelocytes) <10% of WBC Myeloblasts rarely observed Monocytes <1 × 10^9/L No dysgranulopoiesis
2	Hypercellular bone marrow Neutrophil granulocytes increased in percentage and number Neutrophil maturation appears normal Myeloblasts <5% of nucleated cells
3	Not meeting WHO criteria for *BCR-ABL1*-positive CML, PV, ET, or PMF
4	No rearrangement of *PDGFRα, PDGFRβ, FGFR1,* or *PCM1-JAK2*
5	Presence of *CSF3R* T618I or other activating *CSF3R* mutation
or	In the absence of *CSF3R* mutation, persistent neutrophilia (at least 3 months), splenomegaly, and no identifiable cause of reactive neutrophilia including absence of a plasma cell neoplasm or, if present, demonstration of clonality of myeloid cells by cytogenetic or molecular studies

Clinical Features

With rare exceptions, CNL is primarily a disease of elderly with most patients presenting in their 60s. A review of 33 published cases of CNL reported a median age of 62.5 years and male to female ratio of 2:1 [8]. Many patients are asymptomatic at the time of diagnosis. In other patients, fatigue is the most common symptom [9]. A small number of patients presents with weight loss, night sweats, bone pain, gout, or pruritus [10]. The most common and consistent finding on physical examination is splenomegaly. Hepatomegaly can be seen, but lymphadenopathy is uncommon [10–12]. Some cases in the literature have reported increased incidence of hemorrhagic diathesis and mucosal bleeding [10, 11]. The bleeding tendency could result from thrombocytopenia and platelet dysfunction or from vascular wall infiltration by the neoplastic leukocytes [13, 14].

Morphology

Peripheral blood shows leukocytosis with neutrophilia. The white blood cell (WBC) count is moderately elevated with an average of 50 × 10^9/L. The diagnostic leukocyte count threshold in the 2008 WHO classification is ≥25 × 10^9/L. [2] In contrast to chronic myeloid leukemia (CML), leukocytosis in CNL consists of a proliferation of primarily mature neutrophilic granulocytes (Fig. 10.1). Segmented neutrophils and bands comprise >80% of the total WBCs [2]. Neutrophils frequently show toxic granulations and Döhle bodies, which suggest an activated state [15]. Features of dysplasia are usually absent. There are no monocytosis, basophilia, or

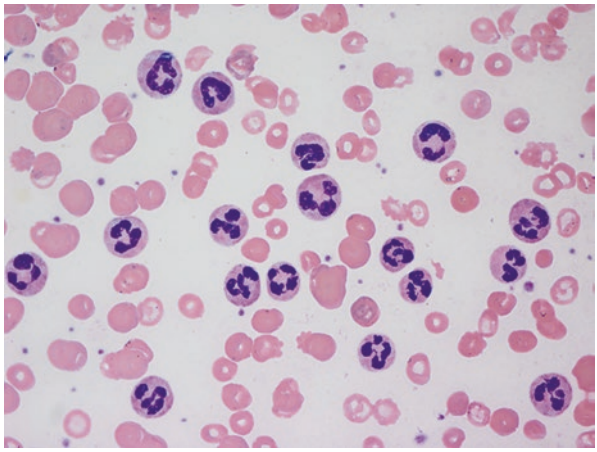

Fig. 10.1 Peripheral blood smear from a patient with CNL showing leukocytosis with neutrophilia without left shift (Wright-Giemsa stain, 500×)

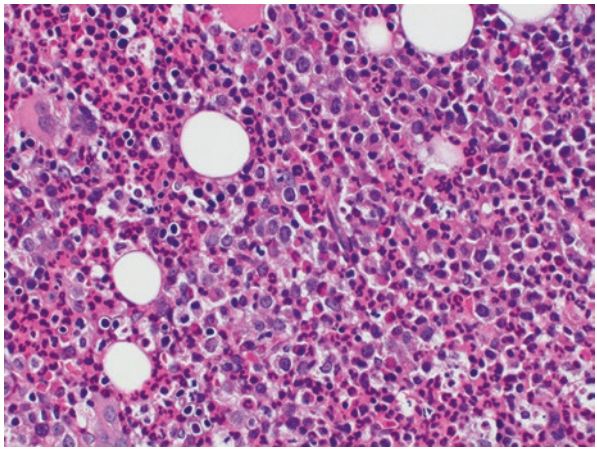

Fig. 10.2 Bone marrow trephine biopsy from a patient with CNL showing hypercellularity with marked myeloid hyperplasia. The erythropoiesis and megakaryocytes are normal in number and maturation (Hematoxylin & Eosin, 400×)

eosinophilia. Intermediate and early myeloid precursors and nucleated red blood cells are rare. In particular, circulating myeloblasts are almost never seen. Platelet count is usually normal except in late disease stages when mild thrombocytopenia can be seen with increasing splenomegaly and progression of disease.

The bone marrow is hypercellular with marked myeloid hyperplasia. Myeloid to erythroid ratio is often >20:1. There is an increase in myelocytes, metamyelocytes, and bands, but blasts or promyelocytes are not increased typically (Fig. 10.2). Erythroid precursors are relatively reduced and show normal maturation. In general, megakaryocytes are normal in number and morphology. Some cases may show mild megakaryocytic hyperplasia [2]. Significant dyspoiesis is not seen; if present, should prompt one to rule out atypical chronic myeloid leukemia (aCML). Reticulin fibrosis is not observed.

Rare cases of CNL have been reported to harbor clonal plasma cells in bone marrow. Bone marrow should be carefully examined for plasma cells, and appropriate

immunohistochemical stains should be performed while diagnosing CNL [16, 17]. In cases where clonal plasma cells are detected, the diagnosis of CNL should be supported by molecular or cytogenetic studies to prove clonality [2].

Splenomegaly is a consistent finding in CNL. Infiltration of spleen by CNL primarily involves red pulp cords and sinuses. The white pulp is relatively spared from leukemic infiltration. The red pulp is expanded and filled with segmented neutrophils and precursors. Rarely megakaryocytes and erythroid precursors can be seen along with neutrophils [11]. Similar to spleen, involvement of liver is seen as infiltration of the sinuses and portal areas by neutrophils [5, 6]. Lymph node involvement has been reported only rarely [12, 18].

Cytogenetics and Molecular Findings

Many case reports and a few small series have supported the clonal nature of CNL based on X-inactivation partners and karyotypic abnormalities [19]. However, in majority of the cases, cytogenetic studies showed normal karyotype [2, 3]. One review series reported cytogenetic abnormalities in 37% cases of CNL [3]. The reported abnormalities included trisomy 8, trisomy 21, deletion 11q, and deletion 20q [20–22]. The most frequent cytogenetic abnormality was deletion 20q. 20q deletions are not specific for CNL and have been reported in other myeloproliferative neoplasms (MPNs). It is possible that the reported cytogenetic abnormalities are secondary events in pathogenesis and represent cytogenetic evolution [23].

The major breakthrough in the pathogenesis of CNL came in 2013 when mutations in the gene coding for colony stimulating factor 3 receptor (*CSF3R*) were reported in 16 of 27 patients (59%) with CNL or *BCR-ABL1*-negative atypical CML. The reported mutation frequencies were 89% in CNL cases (8/9) and 40% in aCML cases (8/18) [5]. These findings were supported by another study that reported 100% frequency of *CSF3R* mutation in 12 patients with WHO-defined CNL [6].

The *CSF3R* gene maps on chromosome 1p34.3 and encodes the transmembrane receptor for granulocyte colony stimulating factor (G-CSF; CSF3). It is known to play an important role in proliferation and differentiation of granulocytes. Two types of mutations are found in *CSF3R*: the majority occurs in the extracellular domain (membrane proximal point mutations) and a small number occurs in the cytoplasmic portion of the receptor (nonsense or frameshift mutations) leading to truncation of the cytoplasmic domain. The most common membrane proximal mutations include T618I and T615A. These mutations result in ligand-independent activation of *CSF3R* that initiates downstream signaling through JAK2 [5]. The point mutation is usually present in isolation or can be seen along with compound frameshift or nonsense mutations. *CSF3R* mutations have not been reported in patients with reactive neutrophilia [5, 6]. *CSF3R* T618I or other activating mutations in *CSF3R* are part of the diagnostic criteria in revised 2016 WHO classification of hematolymphoid neoplasms [7].

Since the initial discovery of the *JAK2* V617F mutation, a few cases of CNL with this mutation have been published [24, 25]. Despite being rare, when detected,

JAK2 V617F mutation indicates the clonal nature of the disease. Other mutations that have been reported to occur in CNL include *SETBP1*, calreticulin (*CALR*), and *ASXL1* mutations [26–28]. In one study, *SETBP1* mutations were seen along with *CSF3R* T618I mutations in 33% patients [26]. A recent study has reported *SETBP1* and *ASXL1* mutations in 14 cases of CNL with mutated *CSF3R*. Eight cases (57%) showed *SETBP1* mutation whereas five (38%) cases showed *ASXL1* and/or *SETBP1* mutations. The presence of coexisting *SETBP1* and *CSF3R* mutations may indicate a worse prognosis [26].

Clinical Course and Disease Progression

The clinical course of the disease is variable. The survival time ranges from 6 months to more than 20 years [9, 22]. Unlike CML, there are no established criteria for progression. The disease progression is characterized by progressive neutrophilia, worsening splenomegaly, resistance to previously effective therapy, anemia, and thrombocytopenia. Transformation to acute myeloid leukemia has been reported in 10–15% of cases [22]. A review of 40 cases of CNL reported median overall survival of 23.5 months and median time to progression to acute myeloid leukemia (AML) of 21 months [28]. The most frequently reported causes of death were intracranial hemorrhage, progressive disease, and treatment-related toxicity from chemotherapy or transplantation [21]. A recent study evaluated role of various factors for prognostication including age, LDH levels, splenomegaly, hemoglobin level, thrombocytopenia, total bilirubin levels, *SETBP1* mutation, *ASXL1* mutation, and "T618I versus other *CSF3R* mutation" in a group of 14 cases of *CSF3R*-mutated CNL. On a multivariate analysis, only *ASXL1* mutation and thrombocytopenia were found to be independently predictive of short survival. The median survival in this group was 23.2 months [27]. A trend of short survival has been reported in patients with coexisting *CSF3R* and *SETBP1* mutations [5]. A case of CNL has been reported with coexistent *CSF3R* and *SETBP1* mutations that showed in vitro lack of response to JAK inhibitor [24]. A study reported transformation of two *SETBP1*-mutated cases of CNL to acute myeloid leukemia. The same study also reported evolution of CNL to chronic myelomonocytic leukemia (CMML) in patients with the presence of *ASXL1* mutation and lack of *SETBP1* mutation [27]. These co-operative mutations likely play an important role in disease transformation.

Conclusion

CNL is a rare myeloprolife*βα*rative neoplasm that is characterized by persistent neutrophilic leukocytosis in peripheral blood and bone marrow and by frequent hepatosplenomegaly. Oncogenic mutations in *CSF3R* appear to be specific driver events in CNL. The role of additional subclonal mutations such as *CALR, JAK2,*

SETP1, and *ASXL1* is being evaluated. *SETBP1* and *ASXL1* are emerging as new prognostic indicators while awaiting more conclusive studies. Before testing for *CSF3R* mutations, one should always keep in mind that CNL is a very rare myeloid neoplasm. Although molecular testing of *CSF3R* mutations will quickly become available in diagnostic laboratories, other common causes of neutrophilia must be ruled out before considering the confirmative molecular test.

Chronic Eosinophilic Leukemia, Not Otherwise Specified

Introduction

Chronic eosinophilic leukemia, not otherwise specified (CEL-NOS) is defined by 2008 WHO classification as clonal eosinophil disorder with persistent increase of eosinophils in blood, bone marrow, and peripheral tissues. The diagnosis requires a blood eosinophil count of $>1.5 \times 10^9$/L plus at least one of the following two criteria: increase of blasts in blood and/or bone marrow (>2% in blood, >5% in marrow) and evidence of clonality in eosinophils. Cases with greater than 20% blasts in blood or bone marrow are excluded from CEL and are diagnosed as acute myeloid leukemia. Evidence of clonality is demonstrated by cytogenetics or pathological mutations [29]. There is no major update in the 2016 WHO classification on CEL [7]. Table 10.2 lists the 2008 WHO diagnostic criteria of CEL.

CEL is a rare disease. There are no epidemiological reports on the incidence. CEL mainly occurs in adult male with a peak incidence of fourth decade. Clinical symptoms are associated with eosinophil-mediated organ damage, such as cardiomyopathy, pneumonitis, dermatitis, neuropathy, and gastrointestinal (GI) inflammation. The patients typically present with nonspecific constitutional symptoms including fever, malaise, cough, angioedema, pruritus, muscle pain, and diarrhea. A small subset of patients presents with symptoms of cardiac damage with restrictive cardiomyopathy and congestive heart failure, and valve damage. Dislodge of cardiac thrombi may result in emboli of end organs. CNS and peripheral neuropathies are also frequent. Extensive bone marrow infiltration of eosinophils often results in

Table 10.2 Diagnostic criteria of chronic eosinophilic leukemia, not otherwise specified [29]

1	Eosinophil count $\geq 1.5 \times 10^9$/L
2	No Ph chromosome or *BCR-ABL1* or other myeloproliferative neoplasms (PV, ET, PMF) or MDS/MPN (CMML or aCML)
3	No rearrangement of *PDGFRα, PDGFRβ, FGFR1, or PCM1-JAK2*
4	No inv.(16)(p13q22) or t(16;16)(p13;q22) or other feature of diagnostic of AML
5	Blast count in peripheral blood or bone marrow <20%
6	There is a clonal cytogenetic or molecular genetic abnormality, or blasts >2% in peripheral blood or >5% in bone marrow

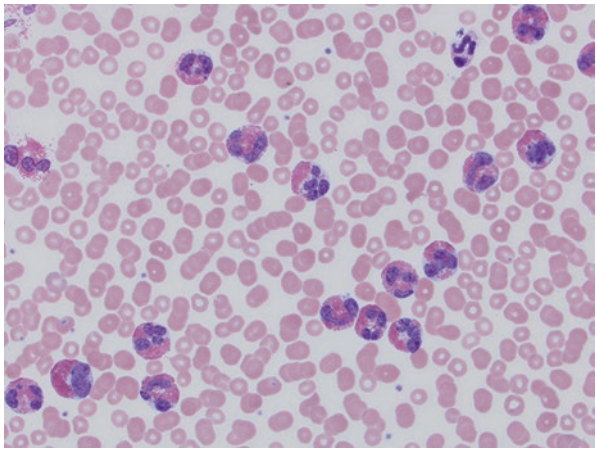

Fig. 10.3 Peripheral blood smear from a patient with CEL showing numerous eosinophils. The eosinophils are mature in morphology and show no cytological atypia (Wright-Giemsa stain, 500×) (Courtesy of Dr. Zenggang Pan)

anemia and thrombocytopenia. Liver and spleen involvement is present in 30–50% patients. Some patients may be asymptomatic, and the diagnoses are made by incidental finding [29, 30].

Morphology

Peripheral blood eosinophilia is a consistent finding, usually greater than 1.5×10^9/L. The eosinophils are predominantly segmented forms, and immature eosinophils are infrequent. Dysplasia such as hypersegmented nuclei, hypogranular cytoplasm, cytoplasmic vacuoles, and enlarged nuclear size are often present in eosinophils (Fig. 10.3). However, these features are not specific for leukemic eosinophils as similar morphology can present in reactive eosinophilia. Neutrophilia may be present but dysplastic neutrophils are absent. Monocytosis is usually absent.

The bone marrow is hypercellular, and the dominant feature is eosinophilic infiltrate. Maturation of eosinophils is usually orderly although left shift is not uncommon. Increase of blasts may be seen, and their presence supports the diagnosis of CEL. If blasts are greater than 20%, then a diagnosis of acute myeloid leukemia with associated eosinophilia should be entertained. Neutrophilic granulocytes, erythrocytes, and megakaryocytes are usually normal in morphology and maturation (Fig. 10.4).

Cases having similar clinical presentation of CEL but lack clonal cytogenetic changes or increase of blasts are classified as idiopathic hypereosinophilic syndrome (HES). The 2008 WHO classification defines HES as persistent eosinophilia $>1.5 \times 10^9$/L for greater than 6 months with no demonstrable clonal cytogenetic changes or increase of blasts. In addition, the diagnosis requires demonstration of organ damage such as cardiomyopathy, pulmonary infiltrate, and renal disease [29]. It is now widely accepted that HES represents a heterogeneous group that includes

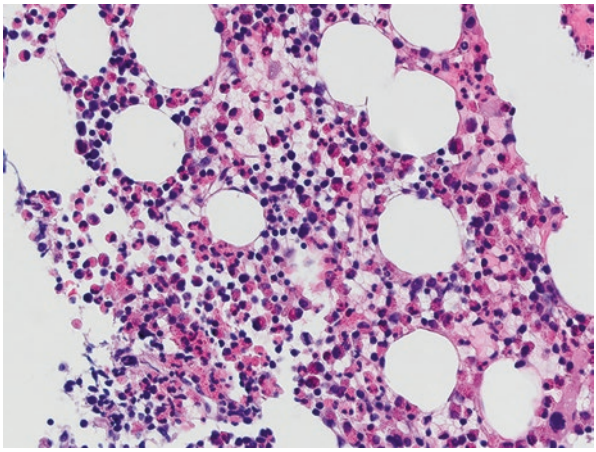

Fig. 10.4 Bone marrow trephine biopsy from a patient with CEL showing hypercellularity with increase of eosinophils and precursors (Hematoxylin & Eosin, 400×) (Courtesy of Dr. Zenggang Pan)

patients of CEL and reactive eosinophilia. The CEL patients have mutations or cryptic chromosome abnormalities that are not detected by the currently available technology. The remaining patients have sustained eosinophilia caused by reactive conditions that are cytokine- driven due to hyperproliferation of T cells or other cytokine producing cells.

In some HES patients, increase of CD4+ T cells and clonal T-cell gene rearrangement can be demonstrated in bone marrow and/or blood. These cases are known as "lymphoid variant of HES" (L-HES). In order to make a diagnosis of L-HES, the cases must meet the diagnostic criteria of HES plus additional evidence of increased CD3-CD4+ T cells. Clonal T-cell receptor gamma gene rearrangement can be detected in the majority, if not all, patients. Expansion of CD3-CD4+ T cells (also known as Th2 T cells) results in overproduction of interleukin 5 (IL-5) which in turn drives proliferation of eosinophils. Clinical manifestations related to IL-5 include atopic skin disorders (up to 80%), diarrhea and abdominal pain (~20%), eosinophilic pneumonia (~20%), and rheumatologic arthralgia, arthritis, and synovitis (~30%). The CD3-CD4+ Th2 T cells may have variant expression of other T cell markers such as CD7-, CD25+, and TCRαβ-. Clonal TCR gamma gene rearrangement can be detected in up to 75% of patients. The majority of the patients have no evidence of overt T-cell lymphoma. In rare patients, concurrent T-cell lymphoma is present suggesting that the abnormal T cells are lymphoma cells [31, 32].

Cytogenetics and Molecular Findings

Various chromosome abnormalities have been described in CEL and in other eosinophil-associated neoplasms. Cases with recurrent translocations involving tyrosine kinase genes *PDGFRα*, *PDGFRβ*, *FGFR1*, and *PCM1-JAK2* are categorized separately by WHO classification to emphasize the potential therapeutic

options of tyrosine kinase inhibitors for these patients. These cases are excluded from CEL. Chronic myeloid leukemia with *BCR-ABL1*, often accompanied by eosinophilia, can occasionally mimic CEL. The presence of *BCR-ABL1* excludes CEL [29].

After the aforementioned entities are excluded, the neoplastic nature of CEL must be proved by the presence of clonality or increase of blasts. Clonality can be demonstrated by chromosome abnormalities, pathogenic mutations, or by an alternative method such as X chromosome inactivation (Humara). One must aware that eosinophilia is not uncommon in other bone marrow neoplasms, in which the eosinophils can be either a part of neoplastic components (e.g., acute myeloid leukemia with inversion 16; systemic mastocytosis) or a reactive response to neoplastic stimuli (e.g., certain peripheral T-cell lymphomas). In these situations, the diagnoses are based on their primary malignancies. Due to these reasons, the presence of another neoplastic process must be first ruled out before a diagnosis of CEL can be made.

The majority of CEL is diagnosed based on the presence of clonal cytogenetic alteration. The chromosomal changes range from single karyotype abnormality to complex karyotypes. These changes encompass a wide variety with no specific recurrent chromosome types. Myelodysplastic syndrome (MDS)-associated chromosome changes such as trisomy 8 and deletion 5q are also seen as isolated abnormalities in CEL [33, 34]. Isochromosome 17p, most frequently found in accelerated and blast phases of chronic myeloid leukemia, has been reported as sole abnormality in CEL [35, 36]. Approximately 25% CEL have a complex karyotype [37].

Large-scale sequencing has been recently applied to CEL and HES, and recurrent mutations were found in both categories. Pathological mutations were detected in up to 50% of CEL and 30% of HES. The most frequent mutations were found in *ASXL1* (43%), *TET2* (36%), *EZH2* (29%), and genes involved in DNA methylation and chromatin modification. Other frequently mutated genes included *SETBP1*, *CBL*, *NOTCH1*, and spliceosome genes [37]. Contradictory results were reported on *KIT* mutations in CEL and HES. Some studies reported high frequency of *KIT* mutations, whereas others found no *KIT* mutations [37, 38]. It is possible that the cases with eosinophilia and *KIT* D816V mutations are not true CEL but rather systemic mastocytosis with eosinophilia. Rare cases with *JAK2* mutations were reported in CEL/HES. Likewise, it is uncertain whether these cases are CEL or other MPN with increased eosinophils [39, 40]. *NRAS* mutations were reported in rare cases [41].

Methylation studies have identified considerable differences in methylation patterns between HES and reactive eosinophilia. HES patients have shown frequent hypomethylation signature, whereas patients with reactive eosinophilia are constitutively hypermethylated. These methylation alterations were seen in a 128 relevant gene signatures, with the highest numbers of methylation abnormalities seen in *Mir886*, *GSTM5*, *TNXB*, *ZADH2*, *LGR6*, *HLA-C*, *HLA-DRB1*, *S100A13*, and *HIVEP3*. These genes involve various functional pathways in tumorigenesis such as cancer, cell death and survival, hematologic diseases, and inflammatory response [42].

In female patients, clonality can be assessed by X-inactivation analysis of human androgen receptor gene (HUMARA). This assay detects polymorphisms of trinucleotide repeats adjacent to differential methylation sites in human androgen receptor genes in X chromosome. Due to high levels of polymorphism, amplification of this region using methylation-specific polymerase chain reaction (PCR) can determine tumor of paternal or maternal origin (monoclonal) or both (polyclonal). There are several limitations in this method. (1) The method can be only used in females. (2) The method is less informative in hematologic malignancies as compared with solid tumor due to highly skewed X-inactivation in hematologic cells. (3) The result is affected by reactive cell components within the tumor. (4) The assay requires normal control sample from the same patient. Due to these limitations, HUMARA assay has lost favor in assessing clonality in hematologic malignancies [43].

Prognosis and Therapy

CEL is a clinically aggressive disease with poor prognosis. A recent study showed median survival of 22 months and acute transformation developed in half of the patients within 3 years. Median survival from acute transformation to death was 2 months [44]. Hydroxyurea, combination chemotherapy, and stem cell transplantation are used to treat aggressive CEL with variable outcomes. Rare patients that have *KIT* M541 L mutations have shown good response to Imatinib [45]. Corticosteroid is effective in control of organ damage and is the first-line treatment for HES. The asymptomatic patients are usually managed by "watch and wait" approach with close follow-up [46].

Conclusions

Although CEL and HES are rare clinical syndromes, it is important to distinguish them from reactive eosinophilia due to their severe clinical consequences. Recent studies have shown that recurrent oncogenic mutations are present in a subset of CEL and HES. Higher frequencies of mutations are found in genes involving DNA methylation and chromatin modification. The finding of similar pathological mutations in CEL and HES suggests that a subset of HES is more closely resemble CEL. These patients are older in age, more likely to have abnormal eosinophil morphology, and have shorter overall survival. On the other hand, the HES mutation-negative patients are younger in age and are more frequently associated with symptoms of eosinophil activation such as GI, pulmonary, skin, and rheumatoid manifestations. These findings suggest that the subset of mutation-positive HES may represent clonal neoplastic process similar to CEL. Due to the limited targets in most of the currently performed next generation sequencing panels, mutations involving targets outside of the panels are not investigated. Large-scale sequencing

such as whole-exon sequencing may help us to further understand the pathogenesis and to better classify CEL and HES. It is anticipated that as the new pathological mutations been discovered in HES, some patients who are currently been classified as HES will be reclassified as CEL.

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