

Review of current classification, molecular alterations, and tyrosine kinase inhibitor therapies in myeloproliferative disorders with hypereosinophilia

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Abstract: Recent advances in our understanding of the molecular mechanisms underlying hypereosinophilia have led to the development of a ‘molecular’ classification of myeloproliferative disorders with eosinophilia. The revised 2008 World Health Organization classification of myeloid neoplasms included a new category called “myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*.” Despite the molecular heterogeneity of PDGFR (platelet-derived growth factor receptor) rearrangements, tyrosine kinase inhibitors at low dose induce rapid and complete hematological remission in the majority of these patients. Other kinase inhibitors are promising. Further discoveries of new molecular alterations will direct the development of new specific inhibitors. In this review, an update of the classifications of myeloproliferative disorders associated with hypereosinophilia is discussed together with open and controversial questions. Molecular mechanisms and promising results of tyrosine kinase inhibitor treatments are reviewed.

Keywords: hypereosinophilia, classification, myeloproliferative disorders, molecular alterations, tyrosine kinase inhibitor

Introduction to hypereosinophilia Eosinophil biology

The normal eosinophil count ranges between $0.05 \times 10^9/L$ and $0.5 \times 10^9/L$ in peripheral blood and between 1% and 6% in bone marrow aspiration. Eosinophils originate from CD34+ hematopoietic precursor cells under the control of transcription factors (eg, erythroid transcription factor [GATA-1], PU.1, CCAAT-enhancer-binding proteins [CEBPs], and signal transducer and activator of transcription 5A [STAT5]) and cytokines. ‘Eosinopoietic’ cytokines (mainly interleukin [IL]-5, granulocyte-macrophage colony-stimulating factor [GM-CSF], and IL-3) are produced by activated T lymphocytes, mast cells, and stromal cells, and promote proliferation, differentiation, and survival of normal and neoplastic eosinophils via specific cell surface receptors.^{1,2} Only IL-5 is specific for eosinophils, while IL-3 and GM-CSF stimulate other myeloid lineages. Under various conditions, eosinophils can invade tissues or organs. Eosinophils produce a number of active molecules in their granules, such as eosinophil peroxidase (EPX), eosinophil cationic protein, major basic protein (MBP), and various lipid mediators and several cytokines, including transforming growth factor beta (TGF- β). When eosinophils are activated by different stimuli for a long period of time, the release of eosinophil granule proteins can trigger local inflammation

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and alter the microenvironment, resulting in tissue fibrosis, thrombosis, and severe organ damage.

Definitions

Blood eosinophilia is usually divided into mild ($0.5\text{--}1.5 \times 10^9/\text{L}$), moderate or marked ($1.5\text{--}5.0 \times 10^9/\text{L}$), and severe or massive ($>5 \times 10^9/\text{L}$).⁴ Until recently, the definition of hypereosinophilic syndrome was based on the three criteria described by Chusid et al in 1975⁵: (1) a persistent absolute blood eosinophil count $>1.5 \times 10^9/\text{L}$ for more than 6 months (or death before 6 months associated with signs and symptoms of hypereosinophilic disease); (2) a lack of evidence of parasite, allergy or another known cause of eosinophilia; and (3) signs or symptoms of organ involvement, including hepatosplenomegaly, congestive heart failure, gastrointestinal dysfunction, diffuse or focal nervous system abnormalities, pulmonary fibrosis, fever, weight loss or anemia. A detailed description of organ damage induced by eosinophils was reviewed by Roufosse et al.⁶ A major issue is the lack of robust criteria to define hypereosinophilia–organ damage by radiological or histological examination of the affected tissues.⁸

In 2011, the Working Conference on Eosinophil Disorder and Syndromes (2011 Working Conference) updated the definition of eosinophilic disorders.⁷ The expert panel proposed that the term hypereosinophilia should be used for marked and persistent eosinophilia ($>1.5 \times 10^9/\text{L}$ in at least two measurements with a minimum interval of 4 weeks).⁷ Such a recommendation may be adapted to the urgent need of therapy in patients with hypereosinophilia-related end-organ damage.⁸ Tissue hypereosinophilia was defined by (1) the presence of more than 20% of eosinophils in bone marrow aspiration, (2) identification of tissue infiltration by eosinophils, or (3) identification of eosinophil granule proteins on biopsy material. However, objective criteria for tissue hypereosinophilia in extramedullary organs are not available. Immunohistochemical markers for eosinophils (eg, EPX, MBP) are not specific and there are no markers for immature or neoplastic eosinophils.⁸ Finally, the experts defined a new category of patients with eosinophil-related organ damage – eosinophil infiltrates with single-organ dysfunction.⁷

Classification of hypereosinophilia

The 2011 Working Conference's panel of experts determined a new classification of hypereosinophilia with four variants, as well as a classification of hypereosinophilic syndromes with three variants (Tables 1 and 2).⁷ Most hypereosinophilia are secondary or reactive; they are caused by allergic reaction (80% of the cases), helminth infections (8%), toxic or allergic drug reactions, atopic disorder, or other rare disorders.

Table 1 Classification of hypereosinophilia

Proposed terminology	Pathogenesis/definition
Hereditary (familial) hypereosinophilia	Pathogenesis unknown; familial eosinophilia.
Hypereosinophilia of undetermined significance	No underlying cause of hypereosinophilia, no family history. No symptom of hypereosinophilia.
Primary (clonal/neoplastic) hypereosinophilia	Underlying stem cell, myeloid or eosinophilic neoplasm (WHO criteria).
Secondary (reactive) hypereosinophilia	Underlying condition/disease in which eosinophils are non-clonal cells. Hypereosinophilia is triggered by cytokines.

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Abbreviation: WHO, World Health Organization.

Secondary hypereosinophilia are polyclonal processes mediated by 'eosinopoietic' cytokines that promote proliferation of eosinophils and their precursors. Overproduction of IL-5 by a subtype of CD4 T helper cell (Th2) can be documented in many cases (eg, in allergic and parasitic disorders). However, the classification of hypereosinophilia is more complex.⁹ Hypereosinophilia can be reactive in hematopoietic neoplasms, such as in Hodgkin's lymphoma, T-cell lymphoma, B-lymphoblastic leukemia/lymphoma, or T-lymphoblastic leukemia with molecular alteration such as t(5;14)(q35;q32) that activates the IL-3 gene.⁷ In these patients, eosinophils are non-neoplastic cells. The lymphoid variant of hypereosinophilic syndrome is a special subgroup of reactive hypereosinophilic syndrome caused by the non-malignant expansion of clonal Th-2 lymphocytes with an aberrant

Table 2 Classification of syndromes and conditions accompanied by hypereosinophilia

Proposed terminology	Pathogenesis/definition
Idiopathic hypereosinophilic syndrome	No underlying cause of hypereosinophilia.
Primary (clonal/neoplastic) hypereosinophilic syndrome	Underlying stem cell, myeloid or eosinophilic neoplasm (WHO criteria).
Secondary (reactive) hypereosinophilic syndrome	Underlying condition/disease in which eosinophils are non-clonal cells. Hypereosinophilia is triggered by cytokines. Subvariant: lymphoid variant hypereosinophilic syndrome (clonal T-cells identified as the only potential cause).

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Abbreviation: WHO, World Health Organization.

immunophenotype (mainly CD3⁻, CD4⁺) producing IL-5.¹⁰ Eosinophils are not in the malignant clone but their number increases reactively in response to eosinopoietic cytokines produced by clonal, aberrant T lymphocytes. This variant has to be differentiated from hematopoietic stem cell disorders in which both the eosinophils and the lymphocytes belong to the neoplastic clone by molecular and cytogenetic studies. Classification of clonal (neoplastic) hypereosinophilia was recently revised by the 2008 World Health Organization (WHO) classification of myeloid neoplasms.¹¹

Myeloproliferative disorders with eosinophilia

Diagnostics and classification

In patients with myeloid or stem cell-derived neoplasms, eosinophils usually belong to the malignant clone, although this is difficult to establish in routine tests. Both clonal and non-clonal eosinophils can coexist. There is no robust immunophenotypic marker or combination of markers to detect immature or neoplastic eosinophils available.⁸ Molecular markers associated with cytogenetic abnormalities are highly indicative of clonal hypereosinophilia in myeloid neoplasms and stem cell neoplasms with eosinophilia. The most common fusion genes involve *PDGFRA* (platelet-derived growth factor receptor, alpha polypeptide), *PDGFRB* (*PDGFR*, beta polypeptide), *FGFR1* (fibroblast growth factor receptor 1), *ABL1* (*c-abl* oncogene 1, non-receptor tyrosine kinase), and *JAK2* (*Janus kinase 2*).¹² The recurrent molecular abnormalities reported in more than five patients are listed in Table 3.^{8,12-14} Many of them can be detected by conventional karyotyping. *CHIC2* (cysteine-rich hydrophobic domain 2) deletion associated with the *FIP1L1* (*FIP1 like 1 [S. cerevisiae]*)-*PDGFRA* fusion gene is only found using fluorescent in situ hybridization (FISH). In all cases, polymerase chain reaction (PCR) allows the confirmation of molecular alterations, but this is usually not necessary.

The revised 2008 WHO classification of myeloid neoplasms introduced molecular markers as disease-related criteria. Two different categories of myeloid neoplasms with eosinophilia are proposed by the 2008 WHO classification: (1) “chronic eosinophilic leukemia [CEL], not otherwise specified [CEL-NOS];”¹¹ and (2) “the myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1*.”¹¹ The WHO classification specifies that all patients with *PDGFRA*, *PDGFRB* or *FGFR1* abnormalities need further diagnostic evaluation to get a final diagnosis of myeloproliferative neoplasms (MPN) or another malignancy.¹¹ The 2011 Working Conference’s panel of experts agreed

Table 3 Recurrent molecular abnormalities detected in myeloproliferative neoplasms with eosinophilia

Fusion gene/mutation (molecular)	Cytogenetic abnormality
<i>PDGFRA</i>	
<i>FIP1L1</i> - <i>PDGFRA</i>	del(4q12)
<i>PDGFRB</i>	
<i>ETV6</i> - <i>PDGFRB</i>	t(5;12)(q33;p13)
<i>CCDC6</i> - <i>PDGFRB</i>	t(5;10)(q33;q21)
<i>FGFR1</i>	
<i>ZMYM2</i> - <i>FGFR1</i>	t(8;13)(p11;q12)
<i>FGFR1</i> <i>OP2</i> - <i>FGFR1</i>	ins(12;8)(p11;p1p22)
<i>CEP110</i> - <i>FGFR1</i>	t(8;9)(p12;q33)
<i>JAK2</i>	
<i>PCM1</i> - <i>JAK2</i>	t(8;9)(p21;p24)
<i>ETV6</i> - <i>JAK2</i>	t(9;12)(p24;p13)
Others	
<i>CBFB</i> - <i>MYH11</i>	inv(16)
<i>BCR</i> - <i>ABL1</i>	t(9;22)(q34;q11)
Point mutations	
<i>KIT</i> D816V	–
<i>JAK2</i> V617F	–

Note: For an exhaustive list of fusions, see supplemental tables in Medves and Demoulin.¹²

Abbreviations: *PDGFR*, platelet-derived growth factor receptor; *PDGFRA*, *PDGFR* alpha polypeptide; *PDGFRB*, *PDGFR* beta polypeptide; *FGFR1*, Fibroblast growth factor receptor 1; *FIP1L1*, *FIP1* like 1 (*S. cerevisiae*); *ETV6*, ets variant 6; *CCDC6*, coiled-coil domain containing 6; *ZMYM2*, zinc finger, MYM-type 2; *CEP110*, centriolar coiled coil protein 110 kDa; *JAK2*, Janus kinase 2; *PCM1*, pericentriolar material 1; *CBFB*, core-binding factor, beta subunit; *BCR*, breakpoint cluster region; *ABL1*, *c-abl* oncogene 1, non-receptor tyrosine kinase.

with the cytogenetic and molecular WHO classification but underlined two weaknesses – the lack of histologic subclassification and the absence of subgroups with more than one driver mutation.^{7,8} The second mutation can be produced by a subclone, or two separate neoplasms can coexist. Furthermore, eosinophilia is of prognostic significance and each subtype diverges regarding disease biology, prognosis, and response to kinase inhibitors. The *KIT* D816V mutation is associated with clonal hypereosinophilia in advanced systemic mastocytosis (SM) but not in indolent SM. The high level of serum tryptase (>100 ng/mL) can reveal an indolent SM in cases of MPN-eo or CEL. The WHO classification does not include lymphocytic and familial categories.

A transient solution was proposed by the expert’s panel in the 2011 Working Conference. Minimal diagnostic criteria for CEL and acute eosinophilic leukemia (AEL) were established (Table 4). The molecular and cytogenetic defects in the 2008 WHO classification were detailed and a provisional histopathologic classification was proposed (Table 4).⁷ The cytohistomorphological criteria remain the primary criteria and the molecular and cytogenetic markers will be minor diagnostic criteria. For myeloid neoplasms with hypereosinophilia where criteria for CEL or AEL are not fulfilled,

Table 4 Hematopoietic neoplasms with hypereosinophilia: comparison between WHO-based definitions and a provisional working definition for morphologic disease variants

Molecular classification and recurrent molecular/chromosome defects

1. Myeloid, lymphoid, and hematopoietic stem cell neoplasm with hypereosinophilia and a recurrent somatic gene defect
 - A. *PDGFRA*-rearranged neoplasms
 - B. *PDGFRB*-rearranged neoplasms
 - C. *FGFR1*-rearranged neoplasms
 - D. Other defects: *JAK2* fusion gene, *FLT3* fusion gene
2. Eosinophilic leukemia without a recurrent somatic gene defect (listed above)
 - A. No gene defect and no chromosome defect detectable
 - B. With a nonspecific chromosome/gene abnormality
3. WHO-defined myeloid neoplasm with hypereosinophilia (MPN-eo)
 - A. Ph+ chronic myeloid leukemia (CML-eo)
 - B. *JAK2* V617F myeloproliferative neoplasms (MPN-eo)
 - C. *KIT* D816V+ systemic mastocytosis (SM-eo)
 - D. *CBFB*-fusion gene-related acute myeloid leukemia (AML-eo)
 - E. Myelodysplastic syndromes with hypereosinophilia (MDS-eo)
 - F. Other WHO-defined myeloid neoplasms with hypereosinophilia

Histopathologic classification

- A. Acute eosinophilic leukemia: hypereosinophilia and eosinophils $\geq 30\%$ and myeloblasts $\geq 20\%$
- B. Chronic eosinophilic leukemia: hypereosinophilia and eosinophils $\geq 30\%$ and myeloblasts $< 20\%$ and no underlying stem cell, myeloid or lymphoid neoplasm found
- C. Other myeloid neoplasm or stem cell neoplasm with hypereosinophilia – WHO classification criteria and hypereosinophilia but eosinophils $< 30\%$

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Abbreviations: WHO, World Health Organization; *PDGFR*, platelet-derived growth factor receptor; *PDGFRA*, *PDGFR alpha polypeptide*; *PDGFRB*, *PDGFR beta polypeptide*; *FGFR1*, *Fibroblast growth factor receptor 1*; MPN, myeloproliferative neoplasms; CML, chronic myeloid leukemia; SM, systemic mastocytosis; AML, acute myeloid leukemias; MDS, myelodysplastic syndromes; *JAK*, Janus kinase; *CBFB*, *core-binding factor, beta subunit*; *FLT3*, *fms-related tyrosine kinase 3*.

the WHO criteria will be followed and the appendix ‘-eo’ will be added in the final diagnosis.

Distinct driving molecular alterations

Almost half of the patients with criteria for hypereosinophilic syndrome in two different studies published in the early 1980s exhibited features of myeloproliferative disorder (eg, bone marrow hypercellularity, cell lineage abnormalities, myelofibrosis, splenomegaly, vitamin B12 level elevation).^{15,16} Data suggested that these patients had an aggressive disease with a poor prognosis and did not respond to treatment with steroids.¹⁷

Eosinophilia associated with myeloid leukemia

Clonal eosinophilia can represent an expanded population derived from the malignant clone. The most frequent example

is the subgroup of core-binding factor acute myeloid leukemias (AML). The WHO classification listed a category of AML with recurrent genetic abnormalities including two subgroups: AML with inversion of chromosome 16 (*inv*[16]) (*p13.1q22*) or translocation (*t*(16;16)(*p13.1;q22*) (*CBFB-MYH11*AML); and AML with *t*(8;21)(*q22;q22*) (*RUNX1-RUNX1T1*).¹¹ Several morphological features of eosinophils are characteristic of these AML subtypes.⁹ An increased number of abnormal eosinophils with characteristic large, basophilic, and dense granules are typically found in bone marrow aspiration of AML patients with *inv*(16). There is no obvious arrest in maturation. The eosinophils derive from the leukemic clone and possess the *inv*(16) rearrangement.^{18,19} *CBFB* at 16q22 encodes the β -subunit of core-binding factor (CBF), whereas *MYH11* at 16q13 encodes the smooth muscle myosin heavy chain (SMMHC).^{19,20} The fusion oncoprotein impairs hematopoietic differentiation but is not sufficient to induce AML. Cooperating mutations in *RAS* or receptor tyrosine kinase (RTK) (such as *FLT3* ITD or *KIT* mutation) that confer a proliferative and/or survival advantage were found in 70% of the AML patients with *inv*(16).¹⁹ One-third of AML patients with *t*(8;21) have increased eosinophil precursors and blood eosinophilia. In these cases, *t*(8;21) is detected in eosinophils that are part of the malignant clone.^{21,22} *RUNX1* (also known as *AML1*) at 21q22.12 encodes a CBF subunit and the *RUNX1-RUNX1T1* fusion disrupts the CBF function, leading to the transcriptional repression of *RUNX1* target genes.²³

In rare cases, clonal eosinophilia can be associated with chronic myeloid leukemia (CML), chronic myelomonocytic leukemia, myelodysplastic syndromes (MDS), or other MPNs, MDS/MPN overlap disorders, and a subset of patients with SM.

Eosinophilia with myeloid neoplasms and *PDGFRA* abnormalities

The identification of the *FIP1L1-PDGFR*A rearrangement led to remarkable advances in the understanding and treatment of clonal myeloproliferative eosinophilias.²⁴ The disease was named as CEL or myeloproliferative hypereosinophilic syndrome and is now recognized as a subgroup of myeloid neoplasm in the 2008 WHO classification.¹¹ The overwhelming majority of patients with *PDGFRA*-associated myeloid neoplasms are male. Bone marrow biopsy shows a characteristically hypercellular marrow with increased eosinophils and precursors; eosinophil maturation is typically normal.²⁵ In peripheral blood however, eosinophils may exhibit a wide spectrum of

morphological abnormalities, including hyposegmented or hypersegmented nuclei with cytoplasmic vacuoles and small and sparse granules with clear areas of cytoplasm.^{9,11,25} These alterations are not entirely specific. In many cases, a pronounced mastocytosis is present in scattered or loose non-cohesive aggregates.²⁵

The *FIP1L1-PDGFR*A fusion gene is the most frequently recurrent aberration, detected in 5%–15% of all cases with clonal hypereosinophilia. The fusion gene was detected in eosinophils, neutrophils, mast cells, monocytes, and T-cells or B-cells in some patients, suggesting that the rearrangement arises in a pluripotent hematopoietic progenitor cell.²⁶ The fusion transcript results from an 800-kilobase internal deletion on band 4q12 containing the gene *CHIC2*.^{24,27} The deletion is cryptic – these patients have a normal karyotype. The deletion results in a fusion of the 5' end of *FIP1L1* and the 3' end of *PDGFR*A.²⁴ The breakpoints are variables in both genes but the fusions are always in frame.²⁴ The breakpoints of *FIP1L1* are extended on a region of 40 kb. The role of *FIP1L1* in clonal eosinophilia is unknown. *FIP1L1* encodes for a protein involved in messenger RNA processing. Breakpoints in *PDGFR*A occur in a small region that always involves exon 12.^{24,27} *PDGFR*A encodes an RTK, platelet-derived growth factor receptor α . The deletion removes the autoinhibitory *PDGFR*A juxtamembrane domain and leads to the constitutive activation of the tyrosine kinase activity.²⁸ In addition, the fusion protein is resistant to degradation, in contrast to wild-type receptors.²⁹

*FIP1L1-PDGFR*A is present in the cell line EOL-1, derived from a patient with AEL.^{30,31} Several studies have aimed to reproduce the disease in mice and hematopoietic stem/progenitor cell models. The activated fusion protein was shown to impose eosinophil-lineage commitment in murine hematopoietic progenitor/stem cells in vitro.³² However, in human hematopoietic progenitor cells, *FIP1L1-PDGFR*A induced colony formation in the absence of cytokines but did not only favor eosinophil development.³³ We recently transduced in vitro human CD34+ cord blood hematopoietic progenitor cells with *FIP1L1-PDGFR*A and showed that the fusion oncogene can induce cell proliferation in the absence of cytokine and eosinophilia with IL-3 and IL-5.³⁴ Interestingly, we found that *FIP1L1-PDGFR*A activated the transcription factors STAT (eg, STAT5) and nuclear factor (NF)- κ B.³⁴ The fusion oncoprotein seems to be a major player in the development of eosinophilia. We cannot rule out that secondary mutations may contribute to the physiopathology of the disease, but until now they were not found. Other myeloid cytogenetic alterations, such as loss of the Y chromosome,

trisomy 8, trisomy 15, del(6q), del(20q), and i(17q) have been rarely reported in patients with eosinophil neoplasms, supporting the clonal nature of hypereosinophilia.³⁵

However, 65%–80% of cases of eosinophilia associated with myeloid neoplasms remain without known underlying genetic aberration. A few case reports described isolated patients with other fusion products of *PDGFR*A resulting from chromosomal translocations. These rare patients are sensitive to imatinib. Erben et al developed a quantitative reverse transcriptase PCR to detect overexpression of the 3'-regions of *PDGFR*A or *PDGFR*B as a possible indicator of an underlying fusion.³⁶ Sequencing of 87 *FIP1L1-PDGFR*A-negative hypereosinophilic syndrome patients showed several *PDGFR*A point mutations (R481G, L507P, I562M, H570R, H650Q, N659S, L705P, R748G, and Y849S).³⁷ Four of these in vitro mutations induced growth factor-independent cell proliferation and constitutive phosphorylation of *PDGFR*A and STAT5.³⁷ Mice injected with *PDGFR*A-mutant cells were treated with oral imatinib. The drug significantly decreased leukemic growth and prolonged survival.³⁷ Whether patients carrying such mutations can be successfully treated with imatinib remains to be tested.

Eosinophilia with myeloid neoplasms and *PDGFR*B or *FGFR*1 abnormalities

The 2008 WHO classification regrouped under one entity “myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFR*A, *PDGFR*B, or *FGFR*1.”¹¹ Both *PDGFR*B at 5q33 and *FGFR*1 at 8p11 encode RTK and the mechanisms are similar to *PDGFR*A. *PDGFR*B fusion gene is an uncommon cause of clonal eosinophilia reported in only isolated individuals. The most common translocation t(5;12)(q33;p13) involving *ETV6* is found in patients with chronic myelomonocytic leukemia.³⁸ The extracellular ligand-binding domain of *PDGFR*B is replaced by the pointed domain of *ETV6*, which is required for oligomerization and activation of the kinase domain. Remarkably, the fusion protein also retains the *PDGFR*B transmembrane domain but is localized in the cytosol. We showed that this hydrophobic domain plays an essential role in the fusion active conformation.³⁹ As in the case of *FIP1L1-PDGFR*A, studies have tried to reproduce the disease in mice and hematopoietic stem/progenitor cell models. *ETV6-PDGFR*B, in the absence of growth factors, stimulates the proliferation of Ba/F3 cell and in vivo promotes hematopoietic cell proliferation in mouse transplantation models, leading to a myeloproliferative disease, but without eosinophilia.⁴⁰ We transduced human CD34+ cord blood hematopoietic stem cells with *ETV6-PDGFR*B

and showed an increase in proliferation and eosinophil differentiation with eosinopoietic cytokines.³⁴ NF- κ B seems to be an important mediator of the effects of ETV6-PDGFRB on hematopoietic cell growth and differentiation.³⁴

FGFR1 fusion genes are also uncommon. Patients present with eosinophilia and hypercellular bone marrow with variable increase in eosinophils. The cell of origin is believed to be a progenitor cell or a T-cell precursor with potential for myeloid differentiation. The biopsy shows T-cell lymphoblastic leukemia/lymphoma or mixed myeloid/T-cell lineage. The course of the disease is usually aggressive.⁴¹ The translocation results in a chimeric protein with constitutive activation of FGFR1. The most common translocation is t(8;13)(p11;q12) involving *ZNF198* at 13q12. This disease is also known as 8p11 myeloproliferative syndrome or stem cell leukemia/lymphoma.

CEL-NOS

The definition of CEL-NOS in the 2008 WHO classification is based on clonal peripheral blood hypereosinophilia in the absence of diagnostic features associated with another myeloproliferative disorder or AML. The malignant nature of the disease should be confirmed by the presence of a clonal genetic abnormality or blast cells. Genetic alterations may include trisomy(8) but not breakpoint cluster region (BCR)-ABL fusion gene, inv(16) or rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*. In the absence of genetic alteration, the percentage of blast cells should be more than 2% in the peripheral blood or more than 5% in bone marrow, but should not reach the threshold of 20% associated with AML.¹¹ Cases of CEL-NOS are extremely rare.

SM with eosinophilia

The current WHO definition of SM requires the presence of either one major and one minor criterion or three minor criteria.⁴² The major criterion is multifocal dense infiltrate of mast cells in bone marrow or another extracutaneous organ. The minor criteria are (1) >25% of mast cells in bone marrow or non-cutaneous tissue biopsy sections with spindle-shaped or atypical morphology; (2) mast cells in the bone marrow, blood, or involved tissue expressing CD25 and/or CD2; (3) detection of a codon 816 c-kit point mutation in blood, bone marrow, or involved tissue; and (4) serum tryptase levels persistently elevated at greater than 20 ng/mL. SM are usually separated into disease variants based on the mast cell burden, involvement of non-mast cell lineages, and disease aggressiveness.⁴²

Bone marrow examinations are hypercellular with focal dense, paratrabecular aggregates of atypical spindle-shaped

mast cells and increased number of eosinophils and lymphocytes.^{16,42,43} Myelofibrosis and osteolytic or osteosclerotic changes are common in advanced disease. Peripheral blood eosinophilia is found in >50% of patients with D816V KIT-positive mast cell leukemia. The mutation can be detected in both eosinophils and CD34+ hematopoietic stem cells in 30% of patients.⁴⁴

Treatments of clonal eosinophilia: the tyrosine kinase inhibitor (TKI) area

In contrast with classifications, treatment decisions should be based on symptoms and on molecular defects rather than histomorphological criteria alone. This can be difficult as patients with starkly different underlying diseases can present with identical clinical manifestations. A second obstacle is that only one large multicenter retrospective study is available, in addition to small cases series.^{45,46} Treatment of hypereosinophilic syndrome aims to limit organ damage by controlling the eosinophil count. Standard treatments included prednisone, hydroxyurea, and interferon alfa. In 2002, the TKI imatinib revolutionized the treatment and prognosis of patients with hypereosinophilic syndrome and PDGFR alterations. Imatinib is effective in patients with *ABL1*, *PDGFRA* or *PDGFRB* fusion genes as well as with some *KIT* mutations, but not in neoplasms with other kinase mutations such as the *FGFR1* fusion gene.

First generation of TKIs: imatinib

The first case of imatinib treatment of hypereosinophilic syndrome was reported in 2001.⁴⁷ The hypothesis was based on the efficacy of imatinib in chronic myeloid leukemia patients and the probable common pathogenesis. After 4 days of imatinib at 100 mg, a complete hematological response was observed and peripheral eosinophils disappeared at day 35. Subsequently, Gleich et al treated five patients suffering from hypereosinophilic syndrome of unknown origin with 100 mg of imatinib mesylate daily.⁴⁸ Four of the patients with normal serum IL-5 showed a complete hematological response. In 2003, Cools et al treated eleven patients with hypereosinophilic syndrome.²⁴ Nine of them had a response to imatinib lasting more than 3 months with an eosinophil count that returned to normal. Cools et al discovered the fusion oncoprotein FIP1L1-PDGFRB in five of the patients. Relapse in one patient was associated with the detection of the T674I mutation in *PDGFRA* that confers resistance to imatinib.²⁴ All published case reports of imatinib treatment of patients with *FIP1L1-PDGFRB* or *PDGFRB* rearrangements are listed in Table 5. Nearly all patients with *FIP1L1-PDGFRB* can be

Table 5 Published reports of imatinib in hypereosinophilic syndrome with PDGFR alteration

Author, year	Number of patients treated with imatinib	Responses	FIP1L1-PDGFR α	PDGFR β alteration
Schaller et al ⁴⁷	1	1 CR	NA	
Gleich et al ⁴⁸	5	4 CR	NA	
Ault et al ⁸⁷	1	1 CR	NA	
Pardanani et al ⁸⁸	7	3 CR, 1 PR	NA	
Cortes et al ⁸⁹	9	4 CR	NA	
Cools et al ²⁴	11	9 CR	5	
Pardanani et al ⁹⁰	5	3 CR	3	
Klion et al ⁹¹	7	7 CR	7	
Vandenbergh et al ⁹²	4	4 CR	4	
Pardanani et al ⁹³	26	12 CR	8	
Roche-Lestienne et al ⁹⁴	9	7 CR	6	
La Starza et al ⁹⁵	12	9 CR	7	
Jovanovic et al ⁵⁰	11	11 CR	11	
Baccarani et al ⁶⁰	63	27 CR	32	
Helbig et al ⁴⁹	24	13 CR	14	
Metzgeroth et al ⁹⁶	31	22 CR	16	
Helbig et al ⁹⁷	22	22 CR	22	
Helbig et al ⁹⁸	8	4 CR	0	
Arefi et al ⁹⁹	19	15 CR	8	
				PDGFRβ alteration
Apperley et al ¹⁰⁰	4	4 CR	4	
David et al ¹⁰¹	12	10 CR	12	
Arefi et al ⁹⁹	8	7 CR	7	
		1 NA		
Metzgeroth et al ⁹⁶	5	3 CR	5	

Abbreviations: PDGFR, platelet-derived growth factor receptor; PDGFR α , PDGFR alpha polypeptide; PDGFR β , PDGFR beta polypeptide; CR, complete response; PR, partial response; NA, not applicable; FIP1L1, FIP1 like 1 (*S. cerevisiae*).

managed with low-dose imatinib (100 mg daily to as low as 100 mg weekly).^{49,50} Rapid institution of therapy is important to avoid irreversible complications. The response is usually very rapid; the majority of the patients experienced clinical and hematological responses within the first week of therapy and resolution of bone marrow alterations within the first month.⁴⁶ Resistances were very rare and occurred within the first year of diagnosis. In contrast with *BCR-ABL* domain mutations, which are a common problem in the treatment of CML, only seven cases of acquired resistances due to a point mutation in the *PDGFR α* kinase domain have been reported so far, with a median time of 5 months of imatinib therapy.^{24,27,51–56}

The T674I mutation within the kinase domain of *FIP1L1-PDGFR α* (adenosine-5'-triphosphate [ATP]-binding region) seems to be the most frequent mutation that appears under imatinib treatment and that causes resistance through steric hindrance mechanisms. The isoleucine (Ile) to threonine (Thr) substitution prevents the deep penetration of imatinib

into the ATP-binding pocket. The critical hydrogen bond between Thr and imatinib is lost and imatinib-binding is destabilized in the kinase domain.⁵⁷ Another patient with resistance to imatinib had two mutations, S601P and L629P in *FIP1L1-PDGFR α* .⁵⁴ S601P is located within the nucleotide binding loop and the new conformation of *PDGFR α* destabilizes the inactive conformation of the kinase domain that is necessary for the binding of imatinib or sorafenib.⁵⁸ Von Bubnoff et al⁵⁹ identified 27 different *FIP1L1-PDGFR α* kinase domain mutations, including 25 novel variants which attenuated the imatinib, nilotinib or sorafenib response but did not confer complete inhibitor resistance. It seems that a small number of residues are critical to the interference with binding and inhibition done by *PDGFR* kinase inhibitors. Of note, in vitro and in vivo findings suggest that imatinib may be effective in patients with activating *PDGFR α* point mutations.³⁷ Imatinib does not seem to be curative in patients with *FIP1L1-PDGFR α* as the fusion transcript became rapidly detectable after stopping imatinib.^{60,61} Reinitiation of imatinib led to molecular remission. When resistance occurs or side effects do not allow for use of imatinib, another tyrosine kinase inhibitor may be effective. Allogeneic stem cell transplantation was also successfully used in *FIP1L1-PDGFR α* -positive patients but has to be restricted for patients unresponsive or intolerant to TKIs.⁶²

Second generation of TKIs: nilotinib and dasatinib

Nilotinib can be efficient on the *FIP1L1-PDGFR α* fusion gene. In vitro, in the EOL-1 cell line, nilotinib was as potent as imatinib in inducing apoptosis and inhibiting proliferation.⁶³ Both drugs inhibit the phosphorylation of the *PDGFR α* tyrosine kinase. In a xenograft model of CEL, complete remission was obtained after 1 week of therapy with both imatinib and nilotinib.⁶⁴ Treatment of two patients resistant to imatinib with nilotinib was successful.^{55,65} Another patient intolerant to imatinib responded to nilotinib and dasatinib.^{66,67} The sensitivity of the T674I mutation to second generation TKIs has been a matter of debate. Von Bubnoff et al reported that nilotinib suppresses the growth of Ba/F3 cells transfected with the T674I *FIP1L1-PDGFR α* mutant.⁶⁸ However, Stover et al reported that nilotinib could not overcome the imatinib resistance conferred by the point mutation T674I in *FIP1L1-PDGFR α* in the same cellular model, even at high concentrations.⁶⁹ Metzgeroth et al reported a patient with T674I mutation that was insensitive to both nilotinib and sorafenib.⁵¹ Dasatinib is a dual SRC/ABL1 inhibitor that also inhibits *PDGFR α* and *FIP1L1-PDGFR α* fusion but has no effect on imatinib-resistant *FIP1L1-PDGFR α* T674I and D842V mutants.⁵⁴

Third generation of TKIs: ponatinib

Ponatinib has a potent activity towards BCR-ABL1, as well as numerous imatinib-resistant *BCR-ABL1* kinase domain mutants, including the T315I mutation.⁷⁰ This third generation TKI was also efficient against the FIP1L1-PDGFR and FGFR1OP2 (FGFR1 oncogene partner 2)-FGFR1 fusion proteins, as shown in the leukemic EOL and KG1 cell lines.⁷¹ Ponatinib reduces proliferation, induces apoptosis, and reduces phosphorylation of the FGFR1OP2-FGFR1 fusion protein and substrates in KG1a cell lines.⁷² Importantly, both FIP1L1-PDGFR T674I and FIP1L1-PDGFR-D842V mutant kinase were also sensitive to ponatinib.^{71,73} Ponatinib in vitro can also strongly inhibit CUX1 (cut-like homeobox 1)-FGFR1 fusion.⁷³ Ren et al recently confirmed that ponatinib can not only inhibit phosphoactivation of six different FGFR1 fusion kinases and their downstream effectors but also inhibit cell growth and clonogenicity of the CD34-positive cells transformed by FGFR1 fusion kinases.⁷⁴ Taken together, these preclinical data point to ponatinib as a very promising therapy for eosinophilic neoplasms associated with RTK mutations. Clinical trials have not yet been reported for this indication.

Other kinase inhibitors

Sorafenib is a biaryl urea compound with multikinase inhibitory activity.⁷⁵ Sorafenib seems to be an in vitro potent inhibitor for hematological malignancies with *FIP1L1-PDGFR* and *FIP1L1-PDGFR* T674I mutant.⁷⁶ Lierman et al described a *FIP1L1-PDGFR* T674I patient that responded to sorafenib.⁷⁶ However, the clinical response was short because of the emergence of another D842V mutation. This mutation is highly resistant to sorafenib, imatinib, and dasatinib.⁵⁵ Sorafenib also failed to block S601P-mutated *FIP1L1-PDGFR*.⁵⁸ Structural modeling revealed that the newly identified S601P mutated form of *PDGFRA* destabilizes the inactive conformation of the kinase domain that is necessary to bind imatinib as well as sorafenib.⁵⁸

Other small molecules have been tested against imatinib-resistant *FIP1L1-PDGFR* T674I. PKC412 (midostaurin) is an inhibitor of the protein kinase C family of enzymes.⁷⁷ PKC412 was shown to inhibit FIP1L1-PDGFR and its T674I mutant in transformed Ba/F3 cells (as in murine models) but not the D842V mutant.^{55,78} The novel tyrosine kinase inhibitor EXEL-0862 seems to have an inhibitory activity towards *FIP1L1-PDGFR* and even towards the *FIP1L1-PDGFR* T674I mutant.^{75,79} Finally, triptolide, a transcription inhibitor, also seems to shut down the expression of *FIP1L1-PDGFR*, even with the T674I mutation.⁸⁰

Patients with *PDGFRB* rearrangement are usually sensitive to imatinib. The imatinib-resistant mutant *TEL-PDGFRB* T681I was sensitive in vitro and in vivo to nilotinib.⁶⁹ In contrast, patients with JAK2 or FGFR1 abnormalities are less likely to respond to imatinib. Ponatinib showed promising results on FGFR1 fusion kinases. Another interesting drug is TKI258 (dovitinib), which is a RTK inhibitor that increases apoptosis of Ba/F3 cells transformed by *ZNF198-FGFR1* or *FGFR1OP2-FGFR1*-positive KG168.⁸¹

Moreover, imatinib can be efficient in 14%–60% of patients with *FIP1L1-PDGFR*-negative hypereosinophilic syndrome. Glucocorticosteroid is the first-line therapy in this group of patients. Eosinophils possess receptors for glucocorticoids which inhibit eosinophil growth and function. The number of glucocorticosteroid receptors detectable in eosinophils correlates with the responses of these cells to glucocorticosteroids.^{82,83} Via an anti-inflammatory effect, glucocorticosteroids inhibit cytokine-induced expression of adhesion molecules on eosinophils and endothelial cells, and thus eosinophil adhesion and transendothelial migration. If glucocorticosteroid treatment fails, a 1 month dose of standard imatinib (400 mg daily) can be tried.^{46,84} If the patient responds, they probably suffer from a myeloid neoplasm characterized by an unknown mutation sensitive to imatinib.

Is hypereosinophilic syndrome a receptor-tyrosine kinase disease? Remarkably, when a mutated gene is found in patients with a hypereosinophilic syndrome, it is in most cases an RTK such as *PDGFRA*, *PDGFRB* or *FGFR1*, or less frequently *KIT* or *FLT3*.¹⁴ Conversely, mutations and fusions of PDGF receptors have not been associated with other hematological diseases, except in a few isolated case reports, such as the KANK1-PDGFRB that we have described in a thrombocytopenia patient.⁸⁵ Non-receptor type tyrosine kinases, such as JAK2 and ABL1, may be associated with hypereosinophilia, but only in rare cases. The reason why these receptors are specifically associated with hypereosinophilia remains unclear. PDGF receptors do not seem to play a major role in normal eosinophil development and may not even be consistently expressed in these cells.^{34,86} We speculate that these receptors may activate a unique set of transcription factors, such as STAT5 and NF- κ B, which drive eosinophil-differentiation from multipotent progenitors. Our data suggest new opportunities for the treatment of resistant patients.

Conclusion

Myeloproliferative neoplasms associated with eosinophilia regroup a heterogeneous population of patients with different molecular alterations. The discovery of rearrangements of

PDGFRA, *PDGFRB*, and *FGFR1* allow for a new molecular classification of these patients. The pathogenesis of PDGFR rearrangement and eosinophilia is still not completely understood. The exquisite response of patients with *PDGFRA* or *PDGFRB* rearrangement to imatinib underscores the importance of identifying the underlying molecular alteration. Future challenges remain in testing inhibitors targeting *FGFR1* or *JAK2* fusion genes or other therapeutic strategies targeting signaling pathways or mechanisms of protein stabilization and degradation.

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Disclosure

The authors report no conflicts of interest.

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