Diagnostic and therapeutic management of eosinophilia-associated chronic myeloproliferative disorders

Andreas Reiter, David Grimwade,° Nicholas C.P. Cross#*

**III. Medizinische Universitätsklinik, Medizinische Fakultät Mannheim der Universität Heidelberg, Mannheim, Germany; °Department of Medical and Molecular Genetics, Guy's, King's and St. Thomas' School of Medicine, Guy's Campus and Department of Haematology, University College London Hospitals, London, UK; #Wessex Regional Genetics Laboratory, University of Southampton, Salisbury District Hospital, Salisbury, U.K. E-mail: andreas.reiter@med3.ma.uni-heidelberg.de*

DOI: 10.3324/haematol.10328

Eosinophilia is commonly observed in a wide range of disparate non-clonal and clonal disorders.[1,2] In the majority of cases it is reactive, associated with atopic conditions, autoimmune disorders, infections or malignancies. In rare cases, a hematologic disorder underlies sustained eosinophilia which can be either non-clonal or clonal. The former (secondary eosinophilia) can be found in a variety of hematologic malignancies including Hodgkin's disease and lymphomas, predominantly of T-cell phenotype. Hypereosinophilic syndrome (HES) is diagnosed when the blood eosinophil count is persistently greater than 1500/μL for at least 6 months with no evidence of a reactive condition or clonality. HES is a potentially life-threatening condition associated with end-organ damage to heart, gastrointestinal tract, skin, joints or nervous system due to release of granular contents from infiltrating eosinophils. In the lymphocytic variant (L-HES), clonal T-lymphocytes induce non-clonal eosinophil proliferation through overproduction of eosinophilopoietic cytokines such as IL-3, IL-5 or GM-CSF. Clonal or primary eosinophilia is generally associated with chronic myeloproliferative disorders (Eos-MPD), including atypical chronic myeloid leukemia (aCML), myeloproliferative variant of HES (M-HES), chronic myelomonocytic leukemia (CMML), unclassifiable overlap syndromes of myelodysplastic syndrome/myeloproliferative disorders (MDS/MPD) and systemic mastocytosis (SM). Chronic eosinophilic leukemia (CEL) is diagnosed in the presence of increased numbers of blasts and/or proof of clonality through cytogenetic or molecular analyses.

Molecular pathogenesis

Acquired constitutive activation of protein tyrosine kinases is a central feature in the pathogenesis of chronic MPD. Activation occurs as a consequence of specific point mutations, e.g. *JAK2* V617F, or fusion genes, e.g. *BCR-ABL*, generated by chromosomal translocations, insertions or deletions. In Eos-MPD, cytogenetic analysis has identified four distinct recurrent breakpoint clusters that target the genes encoding platelet-derived growth factor receptor α (*PDGFRA*) at 4q12, platelet-derived growth factor receptor β (*PDGFRB*) at 5q31-33, fibroblast growth factor receptor 1 (*FGFR1*) at 8p11 and janus kinase 2 (JAK2) at 9p24.[2,3] To date, more than 35 different fusion genes have been identified in association with Eos-MPD (Figure 1), the most common of which is *FIP1L1-PDGFRA*, generated by a cytogenetically invisible 800kb interstitial deletion on chromosome 4q12.[4,5] However, this fusion is only seen in approximately 10% of cases with persistent unexplained eosinophilia, and most of the other fusions, all of which are associated with visible cytogenetic abnormalities, are considerably less common. Nevertheless, the finding of a chromosomal rearrangement is very helpful in indicating the likely presence of the underlying fusion gene and facilitating its identification. Thus, karyotype analysis is important for the initial assessment of patients with this spectrum of disorders.

In fusion proteins, the N-terminal part of a partner protein is fused to the C-terminal part of the tyrosine kinase, thus retaining the entire catalytic domain of the kinase. The vast majority of partners contain one or more dimerization domains that are required for the transforming activity of the fusion proteins. Homotypic interaction between specific domains of the partner protein leads to dimerization or oligomerization of the fusion protein mimicking the normal process of ligand-mediated dimerization and resulting in constitutive activation of the tyrosine kinase moiety.[6] Structurally and functionally, these fusion proteins are very similar to BCR-ABL in CML. Of note, FIP1L1 does not contain any self-association motifs and it was shown that the FIP1L1 moiety is dispensable for the transforming activity of the truncated PDGFRA protein.[7]

Although considerable progress has been achieved in our understanding of the pathogenesis of Eos-MPD, the majority of cases have a normal karyotype and are negative for the *FIP1L1-PDGFRA* fusion gene. Of note, point mutations known to be associated with classical MPDs, e.g. *JAK2* V617F in polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) or *KIT* D816V in SM are very uncommon in Eos-MPD.[8] However, the clinical phenotype of *FIP1L1-PDGFRA* negative cases is frequently indistinguishable from those in whom the fusion is present, suggesting the likely presence of as yet uncharacterized activating mutations or rearrangements in genes encoding tyrosine kinases or unknown molecular mechanisms which remain to be identified. Because the successful treatment of HES with imatinib facilitated the identification of the *FIP1L1-PDGFRA* fusion gene, it is possible that a similar strategy through the use of imatinib or alternative inhibitors may lead to the identification of these unknown molecular abnormalities.

Similar to CML it is likely that fusion gene associated MPD are stem cell disorders, and this notion is supported by the fact that *FIP1L1-PDGFRA* positive T-cells have been described in cases with an associated lymphoma.[9] It is unclear why *BCR-ABL* induces expansion of the granulo-

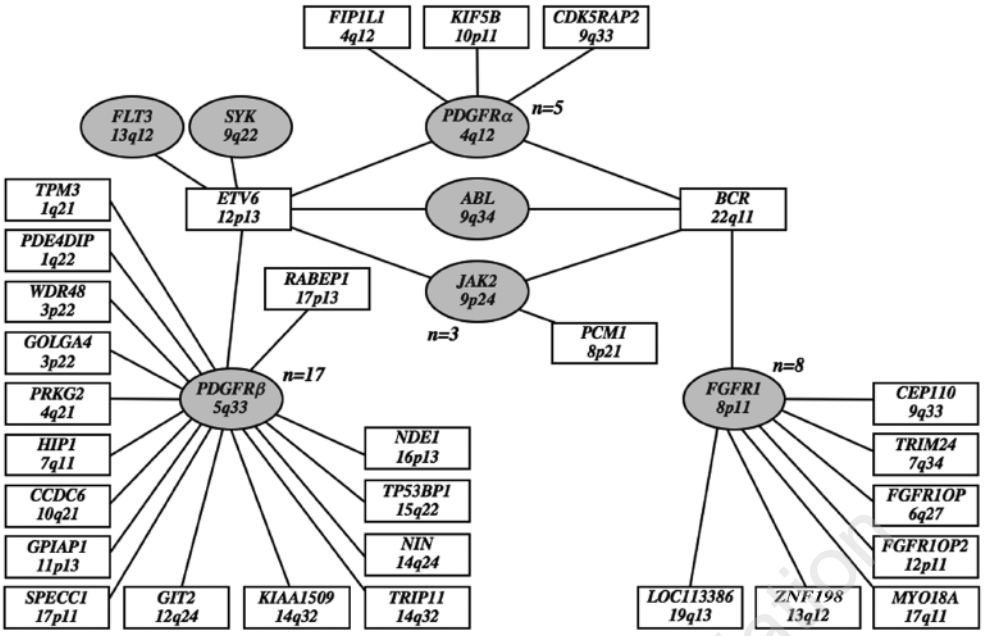

Figure 1. Network of tyrosine kinase fusion genes in eosinophilia-associated chronic myeloproliferative disorders.

cytic lineage whereas *FIP1L1-PDGFRA* predominantly affects eosinophils, but clear differences in the penetration of different cell compartments by these two fusions has been described. For example the great majority of CFU-GM are *BCR-ABL* positive in CML patients whereas only a small minority are positive for *FIP1L1-PDGFRA* in CEL associated with this abnormality, suggesting that *FIP1L1-PDGFRA* does not confer a proliferative advantage to this compartment.[10]

Diagnostic procedures

A careful diagnostic work-up is needed for the correct classification of HES/CEL following exclusion of reactive eosinophilia. In addition to regular quantitative measurements of the peripheral blood, smears should be screened for monocytosis, dysplastic features, indicators of myelofibrosis (e.g. dacrocytes, erythroid and myeloid precursors) and blasts. A lymphoproliferative HES (L-HES) is suggested by the identification of T-cells with unusual phenotype, e.g. $CD3^-/CD4^+$ or less frequently $CD3^+/CD4^-/CD8^-$, and/or proof of T-cell clonality by T-cell receptor gene rearrangement analysis. Skin involvement, hypergammaglobulinemia and increased levels of IL-5 and IgE in the absence of allergies are indicative of L-HES whereas elevated levels of vitamin B12 and tryptase are suggestive of M-HES.

Screening for the cytogenetically invisible *FIP1L1-PDGFRA* fusion gene from peripheral blood cells should be performed at an early stage. Two different screening methods have been used, namely reverse transcriptase polymerase chain reaction (RT-PCR) and fluorescence *in situ* hybridization (FISH), testing for deletion of the *CHIC2* locus as a surrogate marker as this gene lies between *FIP1L1* and *PDGFRA* on 4q12.[4,11,12] The relative frequency of the *FIP1L1-PDGFRA* fusion has varied markedly in reported series, ranging between 3 and 56%.[4,11-16] This may reflect differing levels of stringency in the diagnosis of HES and the study of highly selected series, but may also be compounded by difficulties in establishing a molecular diagnosis. The latter relates to the considerable heterogeneity in breakpoints within the *FIP1L1* locus, variable mechanisms leading to formation of an in-frame fusion product involving use of cryptic splice sites, in addition to the marked alternative splicing between *FIP1L1* exons.[4] *FIP1L1-PDGFRA* transcripts can be difficult to detect by single-step RT-PCR and nested PCR is required for reliable identification of the fusion. This is likely to be accounted for by the relatively low level of expression of the fusion gene in some cases, as revealed by real-time quantitative PCR (RQ-PCR).[16] Moreover, FISH has shown that the proportion of cells harbouring the fusion varies considerably between cases and in some cases with clearly documented *FIP1L1-PDGFRA* fusion by RT-PCR, the proportion of cells with loss of *CHIC2* signal is no greater than background for the methods used. Overall it appears that a combination of RT-PCR and other approaches such as FISH provide the best way to pick up all positive cases. If cases are detected by FISH then the precise breakpoint should also be determined by RT-PCR and sequencing to enable the monitoring of molecular response to imatinib using specific RQ-PCR assays.[16] Bone marrow examinations should include cytomorphology and histology with reticulin staining and immunohistochemistry for mast cells (tryptase, CD117, CD25, CD2). Diagnostic work-up should also include cytogenetic analysis: rearrangements of 4q12 (*PDGFRA*) and 5q31-33 (*PDGFRB*) are suggestive of underlying fusion genes associated with imatinib sensitivity, whereas

involvement of 8p11 (*FGFR1*), 9p24 (*JAK2*) or 13q12 (*FLT3*) predict a poor clinical response to this agent.[2,3] However cytogenetic analysis alone is insufficient and RT-PCR should be performed to confirm the presence of a suspected fusion. RACE-PCR and long-distance inverse PCR[17] can be used to identify fusions formed by novel cytogenetic abnormalities if one partner is a known gene.

Clinical presentation

Eos-MPD with PDGFRA or PDGFRB fusion genes

The dominant clinical feature of cases with *PDGFRA* or *PDGFRB* fusion genes, besides eosinophilia of the peripheral blood and marrow is the male predominance which exceeds a 9:1 male/female ratio.[2,3] The reasons for this are unknown and it can only be speculated about possible secondary genetic changes or other gender-related differences. Additional important clinical features are the frequent presence of marrow fibrosis and of loose aggregates of mast cells identified by immunostaining with tryptase and CD117, particularly in *FIP1L1-PDGFRA* positive disease.[11,13] This is not exclusively seen with *FIP1L1-PDGFRA*, however and similar features have also been seen in patients with rearrangements of *PDGFRB* and *JAK2*.[17,18] Useful markers indicating an underlying *FIP1L1-PDGFRA* fusion are elevated levels of serum tryptase and vitamin B12.[13] In addition to eosinophilia, patients with a rearrangement of the *PDGFRB* gene can present with a variable degree of monocytosis and thus have features that are generally suggestive of both aCML and CMML.[19-21] Evolution to secondary AML occurs in some cases after a highly variable interval,[9] but the probability of transformation seems to be lower than that seen in *BCR-ABL* positive CML.

Eos-MPD with an aggressive clinical course

The term '8p11 myeloproliferative syndrome (EMS)' has been suggested for the distinctive disease associated with 8p11 translocations and underlying rearrangement of *FGFR1*.[22] The majority of patients present with typical features of MDS/MPD-like disease. Marked eosinophilia in the peripheral blood and/or bone marrow is usually present but not mandatory for diagnosis. EMS can also resemble CMML, but the distinguishing feature of this condition is the strikingly high incidence of co-existing non-Hodgkin's lymphoma and lymphoblastic leukemias that may be either of B or, more commonly, T cell phenotype[22,23] particularly in patients with the t(8;13)(p11;q12) and a *ZNF198-FGFR1* fusion gene. In many cases lymphadenopathy is present at diagnosis, whereas in others it appears during the course of the disease. EMS is typically an aggressive disease and rapidly transforms to acute leukemia, usually of myeloid phenotype, within 1 or 2 years of diagnosis. However, we are aware of occasional anecdotal reports of patients who have lived much longer than this despite minimal treatment (e.g. hydroxyurea). Since its first description in 2005,[18] the clinical course of *PCM1-JAK2* positive disorders resulting from a t(8;9)(p21;p24) has been reported in 15 patients.[24-26] The most striking clinical feature, similar to *PDGFR*-related disorders, is a marked male predominance. In the absence of blasts, eosinophilia, splenomegaly and marrow fibrosis are common features of a chronic phase CML-like disease. A substantial proportion of patients present with significantly increased numbers of blasts leading to a diagnosis of *de novo* or secondary AML similar to blast crisis in CML following chronic-phase disease. Moreover, in common with *BCR-ABL*, the fusion gene has also been identified in acute lymphoblastic leukemia.[18]

Treatment

Following the success of imatinib in *BCR-ABL* positive CML and its capacity to occupy the ATP binding sites of several other tyrosine kinases such as ARG (ABL2), KIT, PDGFRA, PDGFRB or FMS, there has been considerable interest in extending its clinical use to diseases in which other activated tyrosine kinases are implicated. In this issue Baccarani *et al.*[27] present the results of a phase-II-trial in which patients with *FIP1L1-PDGFRA*-positive CEL or HES without a known molecular target were treated with imatinib at a target dose of 400 mg/day. This is the largest such study reported to date and has the advantage of relatively long follow-up. Based on the high rates of rapid complete hematologic remissions previously reported by other groups,[27] it is not surprising that all *FIP1L1-PDGFRA* positive patients achieved complete hematologic remissions after a median of 2 months. In addition, all patients achieved complete molecular remission, as determined by nested RT-PCR, after a median of 6 months, consistent with the relatively rapid kinetics of disease response demonstrated using relatively sensitive quantitative PCR approaches.[16] Moreover, in accordance with other recently presented data,[16] discontinuation of imatinib in three patients led to rapid molecular relapse, with all of them achieving a second complete molecular remission following reinstatement of imatinib.

After a median observation time of 25 months, all patients are still on imatinib and in sustained complete molecular remission with the majority of patients treated with 100-200 mg/day. Other studies have shown that sustained use of even lower doses of imatinib are sufficient to induce and maintain durable complete hematologic and molecular remissions at low toxicity in *FIP1L1-PDGFRA* positive disease.[28,29] Treatment with imatinib as monotherapy or as maintenance after intensive chemotherapy was also highly effective in *FIP1L1-PDGFRA* positive secondary AML with seven of seven patients disease-free and in complete hematologic and molecular remission after a median time of 20 months (range, 9-36) on imatinib.[9] These clinical findings correlate with the exquisite *in vitro* sensitivity of the FIP1L1-PDGFRA fusion to imatinib as compared to *BCR-ABL*, for which the kinetics of disease response are much slower and molecular remissions are infrequently observed. However these very encouraging findings have to be set against the fact that for many cases

the sensitivity with which *FIP1L1-PDGFRA* can be detected is relatively low.[16]

Occurrence of severe left ventricular dysfunction within the first days of treatment with imatinib was described in some cases of HES.[30] The cardiac symptoms could be reversed promptly by systemic steroid treatment. Patients at risk should therefore be closely monitored for relevant symptoms, levels of troponin I and by echocardiography. Short-term treatment with steroids before and during the first 2 weeks of imatinib therapy should therefore be considered in patients at risk. Thus far, amongst patients maintained on imatinib, relapse has only been reported in two patients, both of whom had the appearance of a T674I mutation in PDGFRA which was shown to confer resistance to imatinib.[4,31] This mutation is analogous to the T315I mutation in the ABL kinase in imatinib-resistant CML. It was shown recently that the T674I mutant is *in vitro* effectively inhibited by PKC412, sorafenib and nilotinib.[32-34] Excellent clinical responses to imatinib have also been reported in cases with other *PDGFRA*[2,3] and *PDGFRB* fusion genes.[17,35] The largest series demonstrated rapid normalization of blood counts in 11 of 12 patients with complete resolution of cytogenetic abnormalities and decrease or disappearance of fusion transcripts as measured by RT-PCR in ten patients after a median of 47 months (range, 0.1-60 months) treatment with imatinib.[35] In addition, updates were sought from eight further patients previously described in the literature; prompt responses were described in seven and persist in six. In general, *PDGFRB*-rearranged patients have been treated with 400 mg/day imatinib although responses to lower doses have been seen.

Results are different for imatinib-treated HES/CEL patients without known chromosomal or molecular aberrations. Baccarani *et al.*[27] performed a careful diagnostic work-up with conventional cytogenetic analysis, FISH and RT-PCR to detect the presence of the most common fusion genes known to be associated with Eos-MPD in 36 patients. Partial and complete hematological remissions were only seen in five of 36 (14%) patients and importantly these responses were only transient (1-15 months). The reasons for the discrepancy between early studies with higher response rates[27] and this study are unclear but it is suggested that an unknown proportion of patients in previous reports might have carried imatinib-sensitive fusion genes or observation times were too short. Baccarani *et al.* therefore suggest, that imatinib should not be routinely used in HES/CEL without known molecular aberrations.

Eos-MPD with involvement of *FGFR1* and *JAK2* are frequently associated with an aggressive clinical course and poor prognosis with a median survival time of less than 2 years.[18,23] Both these kinases are unaffected by imatinib and due to the current lack of effective alternative tyrosine kinase inhibitors and poor prognosis, early hematopoietic stem cell transplantation (HSCT) should be considered for eligible patients with a suitable donor.[18,23] A number of other compounds have been developed, e.g. SU5404, SU6668 and PD173074 that also function as ATP binding site blockers and which possess anti-FGFR activity, suggesting that it may be possible to develop targeted therapeutic approaches with clinical activity in this subset of disorders. These compounds are inactive against BCR-ABL but can specifically inhibit the growth of *ZNF198-FGFR1* and *BCR-FGFR1* transformed cells.[36] PKC412 had some activity in a patient with *ZNF198-FGFR1* positive EMS in advanced phase[37] but we are unaware of any patient who has achieved a significant chromosomal response after treatment with this compound. Several JAK2 inhibitors are also in development that may be of use for the treatment of cases with *PCM1-JAK2* but thus far there are no data to support this contention.

Concluding remarks

The identification of more than 35 different fusion genes as the consequence of various chromosomal abnormalities in Eos-MPD has highlighted the fundamental role of constitutively activated tyrosine kinases in the pathogenesis of these disorders. Imatinib is the treatment of choice for all Eos-MPD which are associated with a kinase-activating fusion gene involving *PDGFRA* or *PDGFRB*. In contrast to *BCR-ABL*-positive CML, complete molecular response rates seem to be higher and potentially more durable with low rates of acquired resistance due to mutations within the ATP binding domain.[27] While there is a paucity of long-term follow-up data, studies to date indicate that imatinib therapy may need to be continued to maintain remission since patients taken off the drug are likely to develop rapid disease recurrence.[16,27] While the quiescent stem cell population in CML has been shown to be resistant to imatinib,[38] the stem cell pool in Eos-MPD remains poorly characterized and it is possible that in at least some of these disorders imatinib may effectively target this population.

Addressing these issues experimentally would represent a considerable challenge given the relative rarity of these conditions. However, circumstantial evidence could be gained through studies in which imatinib is given for different time-frames following achievement of molecular remission, to establish whether molecular relapse invariably follows drug cessation. Such studies would be most reliably performed using RQ-PCR assays which enable accurate measurement of kinetics of disease response and identify patients with molecularly persistent disease or molecular relapse. These strategies have proven to be highly effective in CML in which kinetics of response to imatinib has been shown to be predictive of outcome, with poor molecular response or rising fusion transcript levels frequently indicating the presence of subclones harboring resistance mutations.[39,40]

The small minority of patients with *FGFR1* or *JAK2* fusion genes are resistant to imatinib. The *in vitro* and *in vivo* activity of several promising new tyrosine kinase inhibitors is currently being explored. Until they are available in clinical practice, allogeneic HSCT should be considered at an early stage for eligible patients with an HLA-

matched donor who present with an aggressive clinical course. Once a wider range of inhibitors are clinically available it seems likely that MRD monitoring using RQ-PCR will become increasingly useful to identify primary resistance or emergence of resistant subclones, prompting mutation screening for resistance mutations as a guide to the most appropriate treatment approach.

A key challenge remains the characterization of molecular aberrations underlying the majority of Eos-MPD in patients in whom the pathogenesis is completely unknown. The activity of imatinib and other inhibitors might prove helpful serving as a guide for the underlying molecular lesion, as was a factor in the identification of the *FIP1L1-PDGFRA* fusion gene in CEL. The presence of the *JAK2* V617F mutation in patients with PV, ET or PMF and the *KIT* D816V mutation in about 80-90% of patients with SM has highlighted the potential benefit of high-throughput screening for single point mutations in important regulatory domains of all known kinases[41] and ultimately a similar approach may be necessary to find other abnormalities in Eos-MPD. However this will not identify cryptic rearrangements and other approaches such as expression arrays, single nucleotide polymorphism analysis and array comparative genomic hybridization will be required to identify novel molecular aberrations in Eos-MPD.

Acknowledgements

The authors gratefully acknowledge support by the Eurropean LeukemiaNet.

References

1. Tefferi A, Patnaik MM, Pardanani A. Eosinophilia: secondary, clonal and idiopathic. Br J Haematol 2006;133:468-92.
2. Gotlib J, Cross NC, Gilliland DG. Eosinophilic disorders: molecular pathogenesis, new classification, and modern therapy. Best Pract Res Clin Haematol 2006;19:535-69.
3. Reiter A, Walz C, Cross NCP. Tyrosine kinases as therapeutic targets in BCR-ABL negative chronic myeloproliferative disorders. Current Drug Targets 2007;8:205-16.
4. Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med 2003; 348:1201-14.
5. Griffin JH, Leung J, Bruner RJ, Caligiuri MA, Briesewitz R. Discovery of a fusion kinase in EOL-1 cells and idiopathic hypereosinophilic syndrome. Proc Natl Acad Sci USA 2003; 100:7830-5.
6. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. N Engl J Med 2005; 353:172-87.
7. Stover EH, Chen J, Folens C, Lee BH, Mentens N, Marynen P, et al. Activation of FIP1L1-PDGFRalpha requires disruption of the juxtamembrane domain of PDGFRα and is FIP1L1-independent. Proc Natl Acad Sci USA 2006;103:8078-83.
8. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood 2005; 106:2162-8.
9. Metzgeroth G, Walz C, Score J, Siebert R, Schnittger S, Haferlach C, et al. Recurrent finding of the FIP1L1-PDGFRA fusion gene in eosinophilia-associated acute myeloid leukemia and lymphoblastic T-cell lymphoma. Leukemia 2007;21:1183-8.
10. Crescenzi B, Chase A, Starza RL, Beacci D, Rosti V, Galli A, et al. FIP1L1-PDGFRA in chronic eosinophilic leukemia and BCR-ABL1 in chronic myeloid leukemia affect different leukemic cells. Leukemia 2007;21:397-402.
11. Pardanani A, Ketterling RP, Brockman SR, Flynn HC, Paternoster SF, Shearer BM, et al. CHIC2 deletion, a surrogate for FIP1L1-PDGFRA fusion, occurs in systemic mastocytosis associated with eosinophilia and predicts response to imatinib therapy. Blood 2003;102:3093-6.
12. Pardanani A, Brockman SR, Paternoster SF, Flynn HC, Ketterling RP, Lasho TL, et al. FIP1L1-PDGFRA fusion: prevalence and clinicopathologic correlates in 89 consecutive patients with moderate to severe eosinophilia. Blood 2004; 104:3038-45.
13. Klion AD, Noel P, Akin C, Law MA, Gilliland DG, Cools J, et al. Elevated serum tryptase levels identify a subset of patients with a myeloproliferative variant of idiopathic hypereosinophilic syndrome associated with tissue fibrosis, poor prognosis, and imatinib responsiveness. Blood 2003; 101:4660-6.
14. Vandenberghe P, Wlodarska I, Michaux L, Zachee P, Boogaerts M, Vanstraelen D, et al. Clinical and molecular features of FIP1L1-PDFGRA (+) chronic eosinophilic leukemias. Leukemia 2004;18:734-42.
15. Roche-Lestienne C, Lepers S, Soenen-Cornu V, Kahn JE, Lai JL, Hachulla E, et al. Molecular characterization of the idiopathic hypereosinophilic syndrome (HES) in 35 French patients with normal conventional cytogenetics. Leukemia 2005;19:792-8.
16. Jovanovic JV, Score J, Waghorn K, Cilloni D, Gottardi E, Metzgeroth G, et al. Low-dose imatinib mesylate leads to rapid induction of major molecular responses and achievement of complete molecular remission in FIP1L1-PDGFRA positive chronic eosinophilic leukemia. Blood 2007;109-4635-41.
17. Walz C, Metzgeroth G, Haferlach C, Schmitt-Graff A, Fabarius A, Haferlach T, et al. Characterization of three new imatinib-responsive fusion genes in chronic myeloproliferative disorders generated by disruption of the platelet-derived growth factor receptor beta gene. Haematologica 2007; 91: 163-9.
18. Reiter A, Walz C, Watmore A, Schoch C, Blau I, Schlegelberger B, et al. The t(8;9)(p22;p24) is a recurrent abnormality in chronic and acute leukemia that fuses PCM1 to JAK2. Cancer Res 2005;65:2662-7.
19. Apperley JF, Gardembas M, Melo JV, Russell-Jones R, Bain BJ, Baxter J, et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N Engl J Med 2002;347:481-7.
20. Steer EJ, Cross NC. Myeloproliferative disorders with translocations of chromosome 5q31-35: role of the platelet-derived growth factor receptor Beta. Acta Haematol 2002;107:113-22.
21. Baxter EJ, Kulkarni S, Vizmanos JL, Jaju R, Martinelli G, Testoni N, et al. Novel translocations that disrupt the platelet-derived growth factor receptor beta (PDGFRB) gene in BCR-ABL-negative chronic myeloproliferative disorders. Br J Haematol 2003;120:251-6.
22. Macdonald D, Aguiar RC, Mason PJ, Goldman JM, Cross NC. A new myeloproliferative disorder associated with chromosomal translocations involving 8p11: a review. Leukemia 1995;9:1628-30.
23. Macdonald D, Reiter A, Cross NCP. The 8p11 myeloproliferative syndrome: a distinct clinical entity caused by constitutive activation of FGFR1. Acta Haematol 2002;107:101-7.
24. Bousquet M, Quelen C, De M, V, Duchayne E, Roquefeuil B, Delsol G, et al. The t(8;9)(p22;p24) translocation in atypical chronic myeloid leukaemia yields a new PCM1-JAK2 fusion gene. Oncogene 2005;24:7248-52.
25. Murati A, Gelsi-Boyer V, Adelaide J, Perot C, Talmant P, Giraudier S, et al. PCM1-JAK2 fusion in myeloproliferative disorders and acute erythroid leukemia with t(8;9) translocation. Leukemia 2005;19:1692-6.
26. Adelaide J, Perot C, Gelsi-Boyer V, Pautas C, Murati A, Copie-Bergman C, et al. A t(8;9) translocation with PCM1-JAK2 fusion in a patient with T-cell lymphoma. Leukemia 2006; 20:536-7.
27. Baccarani M, Cilloni D, Rondoni M, Ottaviani F, Messa F, Merante S, et al. The efficacy of imatinib mesylate in patients with FIP1L1-PDGFRα-positive hypereosinophilic syndrome. Results of a multicenter study. Haematologica 2007;92:1173-9.
28. Pardanani A, Ketterling RP, Li CY, Patnaik MM, Wolanskyj AP, Elliott MA, et al. FIP1L1-PDGFRA in eosinophilic disorders: Prevalence in routine clinical practice, long-term experience with imatinib therapy, and a critical review of the literature. Leuk Res 2006;30:965-70.

29. Helbig G, Stella-Holowiecka B, Grosicki S, Bober G, Krawczyk M, Wojnar J, et al. The results of imatinib therapy for patients with primary eosinophilic disorders. Eur J Haematol 2006;76:535-6.
30. Pitini V, Arrigo C, Azzarello D, La Gattuta G, Amata C, Righi M, et al. Serum concentration of cardiac troponin T in patients with hypereosinophilic syndrome treated with imatinib is predictive of adverse outcomes. Blood 2003;102:3456-7.
31. von Bubnoff N, Sandherr M, Schlimok G, Andreesen R, Peschel C, Duyster J. Myeloid blast crisis evolving during imatinib treatment of an FIP1L1-PDGFR α-positive chronic myeloproliferative disease with prominent eosinophilia. Leukemia 2005;19:286-7.
32. Cools J, Stover EH, Boulton CL, Gotlib J, Legare RD, Amaral SM, et al. PKC412 overcomes resistance to imatinib in a murine model of FIP1L1-PDGFR α-induced myeloproliferative disease. Cancer Cell 2003;3:459-69.
33. Lierman E, Folens C, Stover EH, Mentens N, Van MH, Scheers W, et al. Sorafenib is a potent inhibitor of FIP1L1-PDGFRα and the imatinib-resistant FIP1L1-PDGFRalpha T674I mutant. Blood 2006;108:1374-6.
34. von Bubnoff N, Gorantla SP, Thone S, Peschel C, Duyster J. The FIP1L1-PDGFRA T674I mutation can be inhibited by the tyrosine kinase inhibitor AMN107 (nilotinib). Blood 2006; 107:4970-1.
35. David M, Cross NC, Burgstaller S, Chase A, Curtis C, Dang R, et al. Durable responses to imatinib in patients with PDGFRB fusion gene-positive and BCR-ABL-negative chronic myeloproliferative disorders. Blood 2007;109:61-4.
36. Demiroglu A, Steer EJ, Heath C, Taylor K, Bentley M, Allen SL, et al. The t(8;22) in chronic myeloid leukemia fuses BCR to FGFR1: transforming activity and specific inhibition of FGFR1 fusion proteins. Blood 2001;98:3778-83.
37. Chen J, DeAngelo DJ, Kutok JL, Williams IR, Lee BH, Wadleigh M, et al. PKC412 inhibits the zinc finger 198-fibroblast growth factor receptor 1 fusion tyrosine kinase and is active in treatment of stem cell myeloproliferative disorder. Proc Natl Acad Sci USA 2004;101:14479-84.
38. Graham SM, Jorgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 2002;99:319-25.
39. Hughes T, Branford S. Molecular monitoring of BCR-ABL as a guide to clinical management in chronic myeloid leukaemia. Blood Rev 2006;20:29-41.
40. Hochhaus A, Erben P, Ernst T, Mueller MC. Resistance to targeted therapy in chronic myelogenous leukemia. Semin Hematol 2007;44:S15-S24.
41. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, et al. Patterns of somatic mutation in human cancer genomes. Nature 2007;446:153-8.

Inherited thrombocytopenias

Alan T. Nurden, Paquita Nurden

Centre de Référence des Pathologies Plaquettaires, Plateforme Technologique et d'Innovation Biomédicale, Hôpital Xavier Arnozan, Pessac, France. E-mail: alan.nurden@cnrshl.u-bordeaux2.fr.

DOI: 10.3324/haematol.11256

Bleeding syndromes that arise through an inherited defect of platelet production constitute a heterogeneous group of rare platelet disorders of growing importance.[1,2] Some, including the Bernard-Soulier syndrome (BSS) and Wiskott-Aldrich syndrome (WAS), associate a low circulating platelet count with a deficiency in a known functional protein (Table 1). In others, platelet dysfunction has not been shown and the genetic cause lies in the inability of megakaryocytes (MK) to mature and to produce platelets in sufficient numbers. In congenital amegakaryocytic thrombocytopenia, there is an increased tendency towards the development of leukemia, while in others such as the Jacobsen syndrome, the defects extend outside megakaryocytopoiesis and interfere with the development and/or functioning of major organs. In many of these rare diseases, the low platelet count is accompanied by changes in platelet morphology including the presence of enlarged or giant platelets. The elucidation of the genetic basis of familial thrombocytopenias is providing basic knowledge of how MK develop from the pluripotent hematopoietic stem cell (HSC) under the influence of thrombopoietin (TPO) and other cytokines. This short review will mainly deal with the biology and genetics of inherited thrombocytopenias.

Mediterranean macrothrombocytopenia

Over 30 years ago, a series of 145 subjects from Italy and the Balkan peninsula were reported to have what was termed Mediterranean macrothrombocytopenia.[3] The diagnostic criteria included a moderately low platelet count (70,000-150,000/μL), increased mean platelet volume and mild bleeding. These patients mostly had autosomal dominant inheritance. A series of unrelated Italian families was subsequently studied by linkage analysis and mutation screening.[4] In six of them, a heterozygous A156V missense substitution was identified in GPIBα while in eight of ten patients GPIb-IX density on platelets was at levels reduced to those of BSS heterozygotes. This is somewhat enigmatic, for BSS is classically a disorder with autosomal recessive inheritance and an increased percentage of large platelets in obligate carriers is not an absolute rule.[5] It is possible that another as yet unidentified factor contributes to the Mediterranean macrothrombocytopenia phenotype.

DiGeorge or velocardiofacial syndrome

Although this disorder can show autosomal recessive inheritance, in most patients it is acquired. The phenotype is linked to a monoallelic chromosome 22q11.2 microdeletion. Phenotypic features include conotruncal cardiac abnormalities, learning disabilities, velopharyngeal insufficiency, immunodeficiency, facial dysmorphism and thymic hypoplasia. Studies on mutant mice suggest that a haploinsufficiency of a single gene, *TBX1* (encoding a T-box containing transcription factor), largely accounts for the phenotype.[6] Surveys of patients with DiGeorge syndrome suggest that mild thrombocytopenia and platelets of increased size affect about 20% of patients.[7] Adjacent to *TBX1* is the *GPIBB* gene, and its deletion can give rise to BSS when accompanied by a pathological mutation on the second allele.[8] Defining the factors that give rise to giant platelets