Molecular basis of juvenile myelomonocytic leukemia

Andrica C.H. de Vries, C. Michael Zwaan, and Marry M. van den Heuvel-Eibrink

Department of Pediatric Oncology/Hematology, Erasmus University Medical Center, Sophia Children's Hospital, Rotterdam, The Netherlands
E-mail: m.vandenheuvel@erasmusmc.nl. *doi:10.3324/haematol.2009.016865*

Juvenile myelomonocytic leukemia (JMML) is classified as a combined myeloproliferative/ myelodysplastic disease by the World Health Organization and accounts for less than 3% of all childhood hematologic malignancies.[1,2] Children typically present at young age (median age at diagnosis: two years) with hepatosplenomegaly, monocytosis, anemia, thrombocytopenia and elevated HbF (Figure 1).[3,4] Morphology of the peripheral blood smear is important for the diagnosis, and shows low blast percentages and myeloid precursors. A bone marrow aspiration is necessary to exclude acute myelomonocytic leukemia, AML M4. In JMML, absence of the Philadelphia chromosome (*BCR/ABL* fusion gene) is one of the mandatory diagnostic criteria.[3,4] Cytogenetic analysis reveals monosomy 7 in 25% of the cases, random aberrations in the karyotype in 10%, whereas in 65% of the cases a normal karyotype is found.[5] Figure 1 shows the currently used diagnostic criteria.[3,4]

Molecular aberrations

RAS signaling pathway

In vitro GM-CSF hypersensitivity has been a hallmark of JMML for the past two decades.[6] This GM-CSF hypersensitivity results from continuous activation of the GM-CSF-receptor-RAS-RAF-MEK-ERK signal transduction pathway. Currently, molecular aberrations, which will be discussed below, have become more important than the hypersensitivity assay for diagnostic classification (Figure 1).[3,4]

Proteins encoded by the genes of the RAS family play a role in the transduction of extracellular signals to the nucleus and control proliferation and differentiation of many cell types (Figure 2). RAS proteins are signaling molecules, which regulate cellular processes by switching between an active [guanosine triphosphate (GTP) bound RAS] and inactive [guanosine diphosphate (GDP) RAS] form. The active GTP RAS activates the RAF kinase, resulting in a downstream proliferative effect. The amount of GTP-RAS is regulated by guanine nucleotide exchange factors (GNEFs), and GTPase activating proteins (GAPs). GNEFs are necessary for the conversion of GDP-RAS into GTP-RAS and can be stimulated by upstream proteins like Shp1 and Son of Sevenless (SOS). GAPs, like neurofibromin, are responsible for the termination of the RAS signaling by RAS-GTP conversion in RAS-GDP.[7] Somatic mutations in this pathway are frequently found in JMML, are mutually exclusive and result in continuous activation and cell proliferation. Analysis of genetic syndromes related to germline aberrations of the RAS pathway, like neurofibromatosis type I and Noonan syndrome, have increased the knowledge of the pathogenesis of JMML. In reverse, the discovery of somatic JMML related

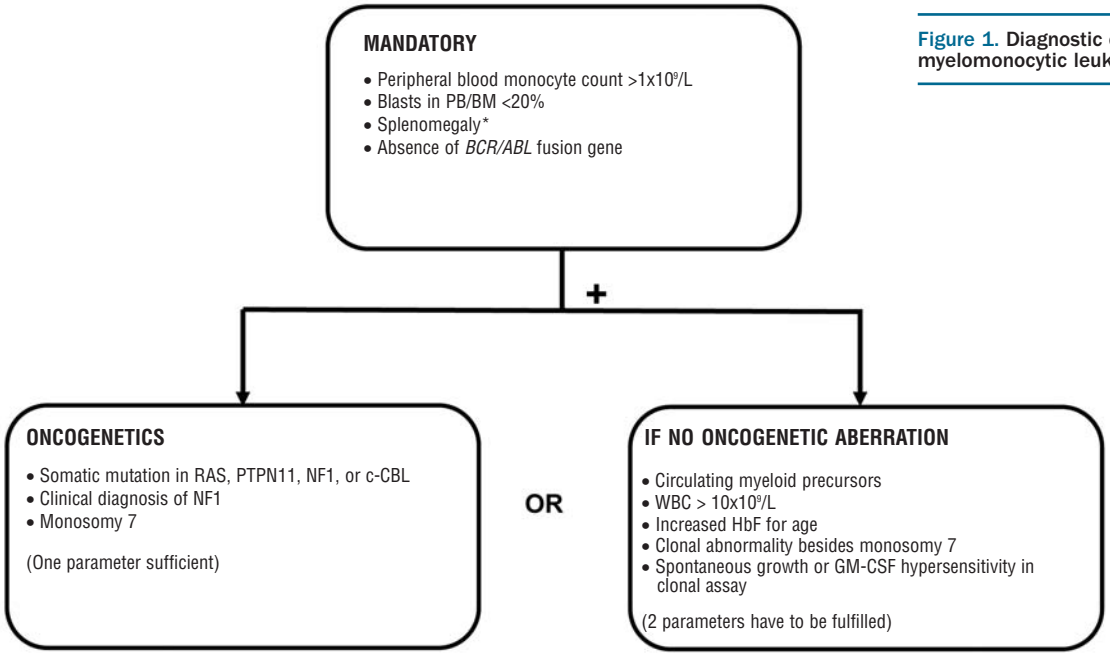

Figure 1. Diagnostic criteria of juvenile myelomonocytic leukemia.

*PB: peripheral blood; BM: bone marrow; NF1: neurofibromatosis type 1; WBC: white blood cell count; HbF: fetal hemoglobin; GM-CSF: granulocyte macrophage- colony stimulating factor; * Splenomegaly is present at first presentation in 95% of cases. The majority of the patients are under than 13 years of age.*

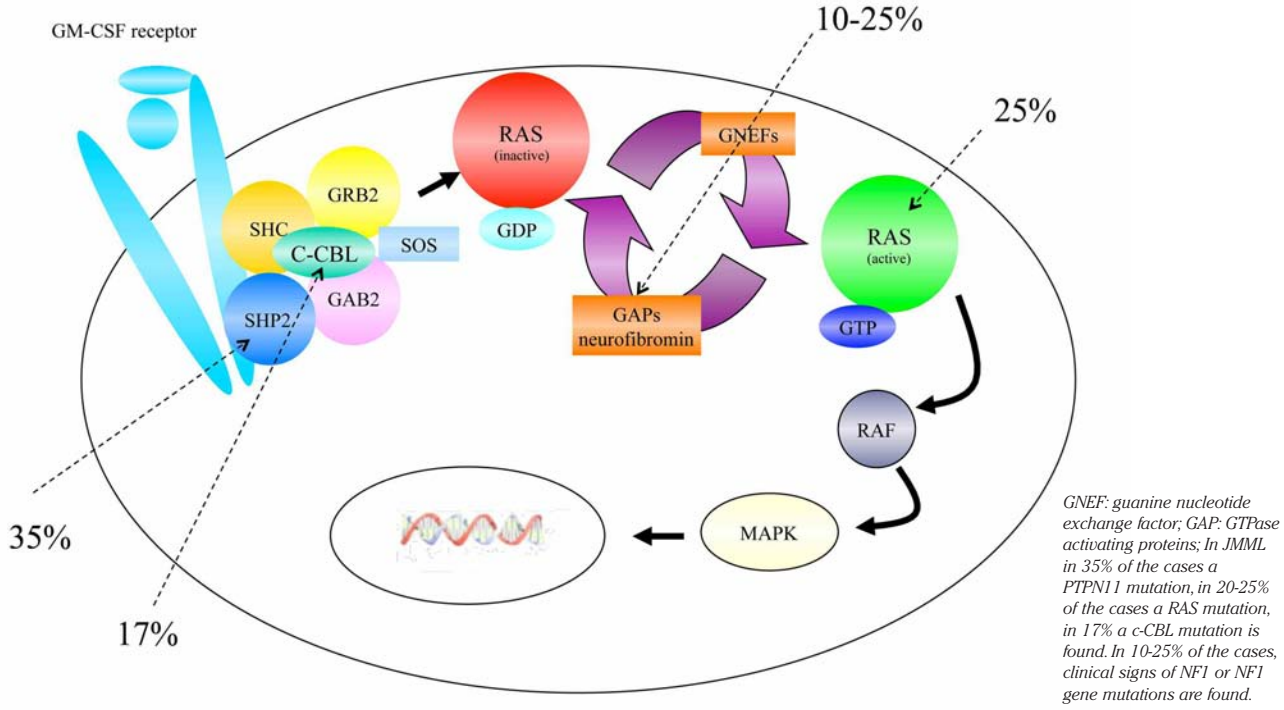

Figure 2. RAS-RAF-MEK-ERK signaling pathway.

RAS-pathway aberrations has resulted in the discovery of germline RAS-pathway related mutations in Noonan like syndromes such as Leopard syndrome, Costello syndrome and Cardio-Facio-Cutaneous syndrome.[8,9]

NF1 mutations and clinical signs of neurofibromatosis type 1

Niemeyer *et al.* reported that 11% of the JMML patients have clinical signs of neurofibromatosis type 1.[5] Thereafter, Side *et al.* found *NF1* gene mutations in 15% of the JMML patients without clinical signs of NF1 (Figure 2).[10] The *NF1* gene is a tumor suppressor gene encoding for neurofibromin, and is a GTPase activating protein hydrolysing GTP-RAS into GDP-RAS.[10] JMML cells from children with NF1 showed a reduced neurofibromin activity, resulting in elevated GTP-RAS expression.[11] In 2007, Flotho *et al.* described somatic loss of heterozygosity (LOH) in 4 out of 5 JMML cases with NF1. In the leukemic cells the wild-type *NF1* gene was replaced by a second copy of the NF1 mutant 17q arm, resulting from uniparental disomy.[12] In the current issue of this journal, Steinemann *et al.*[13] describe bi-allelic *NF1* gene inactivation in all cases in a larger cohort of JMML patients with NF1. Again, this was either caused by LOH (n=10), or by somatic mutations of the *NF1* gene (n=5). This indicates that although NF1 predisposes for JMML and other leukemias, second events are necessary to abolish the complete function of the *NF1* gene in the development of malignancies. Recently, comparable bi-allelic mutations in the *NF1* gene were also found in non-syndromic AML and T-ALL patients, which underscores the fact that bi-allelic inactivation of the *NF1* gene is an important mechanism in hematologic malignancies, but also that it is not JMML-specific.[14]

RAS gene mutations

Mutations in the *RAS* genes are found in 20-30% of all human tumors. In 25% of all JMML, activating point mutations are found in codon 12, 13 and 61 of NRAS and *KRAS* resulting in a continuous activation of the *RAS* pathway (Figure 2).[15]

PTPN11 mutations

PTPN11 is a gene encoding for the non-receptor protein tyrosine phosphatase SHP-2. Mutations in the *PTPN11* gene cause a gain of function of the SHP2 protein. This results in activation of the GNEFs and in this way to a continuous activation of RAS. Tartaglia *et al.* observed somatic mutations in exon 3 and 13 of the *PTPN11* gene in 35% of the JMML patients without Noonan syndrome (Figure 2).[16]

Germline mutations in the *PTPN11* gene have been described in 50% of the Noonan syndrome cases. These mutations differ from the somatic mutations found in JMML.[17] Noonan syndrome is characterized by developmental disorders, short stature, facial dysmorphia, skeletal anomalies and heart defects. Children with Noonan syndrome are at increased risk for developing JMML. However, in Noonan syndrome, JMML seems to behave differently from sporadic JMML as it occurs at a very young age (infancy) and tends to regress spontaneously.[18-20] Therefore, recognising Noonan syndrome in a JMML patient is important in order to identify those patients who might benefit from a watch and wait policy.[17,18]

c-CBL mutations

Recently Loh *et al.* identified c-CBL mutations in 17% of the JMML patients, lacking RAS, PTPN11 or NF1 abnormalities (Figure 2). c-CBL is an E3 ubiquitin ligase, responsible for the intracellular transport and degradation of a large number of tyrosine kinase receptors, but also has important adaptor functions. One of the proteins regulated by c-CBL is Grb2. This adaptor molecule binds to c-CBL and in this way binding of c-CBL to SOS is prevented. Mutations in *c-CBL* have been shown to result in a continuous activation of RAS.[21,22]

Other genes of the RAS pathway

So up till now, in about 80-85% of the JMML cases a somatic mutation in the RAS pathway is found, indicating that hyperactivation of the RAS pathway plays a central role in the pathogenesis of JMML. Therefore, other genes involved in the RAS-RAF-MEK-ERK pathway have been investigated. Analysis of *SHC1*, *GRB2*, *GAB1*, *SOS1*, *BRAF* and *MEK 1* and *MEK 2* genes revealed no mutations.[23,24] We and others showed that, upstream, *FLT3* mutations are rare and we found no constitutively activated *FLT3*.[25,26]

JAK-STAT pathway

It has been suggested that JMML is the juvenile counterpart of chronic myelomonocytic leukemia (CMML) which mainly occurs in adults, as also in CMML GM-CSF hypersensitivity is found. However, *JAK2* mutations (V617F) which are found in in 3-13% of all CMML cases are a very rare event in JMML, underscoring the differences between JMML and CMML.[26,27] Using flow cytometry, Kotecha *et al.* found hyperphosphorylation of pSTAT5 in response to subsaturating concentrations of GM-CSF. This supports the hypothesis that the JAK2-STAT5 pathway plays a collaborating role in the pathogenesis of JMML but that JAK-STAT5 activation mainly seems to occur by upstream of activation of the RAS in the aberrant response of JMML cells to GM-CSF.[21,27]

PTEN

Although all above mentioned studies have shown the role of hyperactivation of the RAS pathway in JMML, only few data are available documenting the status of the other components downstream of RAS, like P13K and MAPK. One of the other regulators of the P13K pathway is *PTEN*, a tumor suppressor gene which antagonizes the function of *P13K* and subsequently of *AKT*, and which is involved in cell growth, proliferation, apoptosis and differentiation. Liu *et al.* found PTEN protein deficiency in 67% of the JMML patients and hypothesized that this deficiency might lead to insufficient negative growth signals to counter the hyperactive RAS pathway.[28]

Epigenetics

It has been suggested that PTEN protein deficiency can be caused by hypermethylation of the promoter region of *PTEN*.[28] Apart from hypermethylation of *PTEN*, it was recently shown that other epigenetic changes might play a role in the pathogenesis of JMML as illustrated by Furlan *et al.* They reported the first JMML patient, also characterized by monosomy 7, treated with a DNA hypomethylating agent. The patient revealed an excellent clinical and molecular-genetic response, as illustrated by the disappearance of the monosomy 7 and hypomethylation of the promoter region of the *CALCA* gene after treatment.[29] In addition, other JMML studies showed mild hypermethylation of specific genes, like *p15*, *p16* and *RASSF1A*.[30,31] These studies are important as they may point towards new, less toxic treatment approaches which are already available for use in the near future for at least subsets of JMML patients.

Treatment and prognosis

To date, the only curative treatment option for JMML is stem cell transplantation (SCT). Intensive chemotherapy does not represent a curative strategy, and splenectomy before transplantation has never been shown to be of benefit.[32] The median survival time without SCT is about one year.[5] In the treatment protocol of the European Working Group on Childhood MDS (EWOG MDS), the 5-year event free survival after transplantation was in the 50% range and the cumulative relapse rate was 35%, with a therapy related mortality rate of 15%.[32] Results of a study performed by the Children's Oncology Group including a farnesyltransferase in the pre-transplantation window are underway.[33] So far, no further data on the effect of treatment with RAS pathway inhibitors are available. As indicated above, hypomethylating agents may be a promising treatment option for the future.

Stem cell transplantation in this relatively young age category of children with JMML harbors the risk of serious toxicity, not only during the procedure but also in later life. Hence, there is a need for unravelling the biology of JMML in order to identify potential drug targets.

Conclusion

JMML is a rare hematologic malignancy in childhood. Increased knowledge of the molecular background has enhanced diagnostic classification, as in more than 80% of the cases mutations in RAS pathway related genes can be found. *NF1* is one of the genes which plays an important role in the pathogenesis of JMML. The paper of Steinemann *et al.* in the current issue[13] of Haematologica underscores the fact that in NF1, for developing JMML, bi-allelic inactivation is necessary by either mutations or LOH as a second event to abolish the complete function of the *NF1* gene.

Andrica de Vries is a pediatric oncologist at the Erasmus MC, Sophia Children's Hospital Rotterdam, The Netherlands, and PhD student of childhood MDS and JMML. Michael Zwaan is an Associate Professor of pediatric oncology/hematology at the Erasmus MC. Marry van den Heuvel-Eibrink is Associate Professor in pediatric oncology/hematology at the Erasmus MC and Chair of the Scientific Board of the European Working Group of MDS/JMML in Childhood (EWOG-MDS).

We would like to acknowledge the collaboration within the European Working Group for MDS (EWOG).

No potential conflicts of interests relevant to this article were reported.

References

1. Hasle H, Wadsworth LD, Massing BG, McBride M, Schultz KR. A population-based study of childhood myelodysplastic syndrome in

British Columbia, Canada. Br J Haematol. 1999;106(4):1027-32.
2. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. World Health Organization classification of tumours: Pathology and genetics of Tumours of Haematopoietic and Lymphoid tissues: IARC Press, Lyon, 2001.
3. EWOG-MDS. Clinical Trial Protocol EWOG-MDS 2006. Prospective non-randomized multi-center study for epidemiology and characterization of Myelodysplastic Syndromes (MDS) and Juvenile Myelomonocytic Leukemia (JMML) in childhood. 2007.
4. Chan RJ, Cooper T, Kratz CP, Weiss B, Loh ML. Juvenile myelomonocytic leukemia: a report from the 2nd International JMML Symposium. Leuk Res. 2009;33(3):355-62.
5. Niemeyer CM, Arico M, Basso G, Biondi A, Cantu Rajnoldi A, Creutzig U, et al. Chronic myelomonocytic leukemia in childhood: a retrospective analysis of 110 cases. European Working Group on Myelodysplastic Syndromes in Childhood (EWOG-MDS). Blood. 1997;89(10):3534-43.
6. Emanuel PD, Bates LJ, Castleberry RP, Gualtieri RJ, Zuckerman KS. Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. Blood. 1991;77(5):925-9.
7. Emanuel PD. RAS pathway mutations in juvenile myelomonocytic leukemia. Acta Haematol. 2008;119(4):207-11.
8. Kratz CP, Schubbert S, Bollag G, Niemeyer CM, Shannon KM, Zenker M. Germline mutations in components of the Ras signaling pathway in Noonan syndrome and related disorders. Cell Cycle. 2006;5(15):1607-11.
9. Syndromes run together in the RAS pathway. Nat Genet. 2006; 38:267.
10. Side LE, Emanuel PD, Taylor B, Franklin J, Thompson P, Castleberry RP, et al. Mutations of the NF1 gene in children with juvenile myelomonocytic leukemia without clinical evidence of neurofibromatosis, type 1. Blood. 1998;92(1):267-72.
11. Bollag G, Clapp DW, Shih S, Adler F, Zhang YY, Thompson P, et al. Loss of NF1 results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. Nat Genet. 1996;12(2):144-8.
12. Flotho C, Steinemann D, Mullighan CG, Neale G, Mayer K, Kratz CP, et al. Genome-wide single-nucleotide polymorphism analysis in juvenile myelomonocytic leukemia identifies uniparental disomy surrounding the NF1 locus in cases associated with neurofibromatosis but not in cases with mutant RAS or PTPN11. Oncogene. 2007; 26(39):5816-21.
13. Steinemann D, Arning L, Praulich I, Stuhrmann M, Hasle H, Stary J, et al. Mitotic recombination and compound-heterozygous mutations are predominant NF1-inactivating mechanisms in children with juvenile myelomonocytic leukemia (JMML) and neurofibromatosis type 1. Haematologica. 2010;95(1):320-3.
14. Balgobind BV, Van Vlierberghe P, van den Ouweland AM, Beverloo HB, Terlouw-Kromosoeto JN, van Wering ER, et al. Leukemia-associated NF1 inactivation in patients with pediatric T-ALL and AML lacking evidence for neurofibromatosis. Blood. 2008;11188):4322-8.
15. Flotho C, Valcamonica S, Mach-Pascual S, Schmahl G, Corral L, Ritterbach J, et al. RAS mutations and clonality analysis in children with juvenile myelomonocytic leukemia (JMML). Leukemia. 1999; 13(1):32-7.
16. Tartaglia M, Niemeyer CM, Fragale A, Song X, Buechner J, Jung A, et al. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet. 2003;34(2):148-50.
17. Kratz CP, Niemeyer CM, Castleberry RP, Cetin M, Bergsträsser E, Emanuel PD, et al. The mutational spectrum of PTPN11 in juvenile myelomonocytic leukemia and Noonan syndrome/myeloproliferative disease. Blood. 2005;106(6):2183-5.
18. Bader-Meunier B, Tchernia G, Miélot F, Fontaine JL, Thomas C, Lyonnet S, et al. Occurrence of myeloproliferative disorder in patients with Noonan syndrome. J Pediatr. 1997;130(6):885-9.
19. Fukuda M, Horibe K, Miyajima Y, Matsumoto K, Nagashima M. Spontaneous remission of juvenile chronic myelomonocytic leukemia in an infant with Noonan syndrome. J Pediatr Hematol Oncol. 1997;19(2):177-9.
20. Silvio F, Carlo L, Elena B, Nicoletta B, Daniela F, Roberto M. Transient abnormal myelopoiesis in Noonan syndrome. J Pediatr Hematol Oncol. 2002;24(9):763-4.
21. Loh ML, Sakai DS, Flotho C, Kang M, Fliegauf M, Archambeault S, et al. Mutations in CBL occur frequently in juvenile myelomonocytic leukemia. Blood. 2009;114(9):1859-63.
22. Schmidt MH, Dikic I. The Cbl interactome and its functions. Nat Rev Mol Cell Biol. 2005;6(12):907-18.
23. de Vries AC, Stam RW, Kratz CP, Zenker M, Niemeyer CM, van den Heuvel-Eibrink MM. Mutation analysis of the BRAF oncogene in juvenile myelomonocytic leukemia. Haematologica. 2007;92(11): 1574-5.
24. Kratz CP, Niemeyer CM, Thomas C, Bauhuber S, Matejas V, Bergstrasser E, et al. Mutation analysis of Son of Sevenless in juvenile myelomonocytic leukemia. Leukemia. 2007;21:1108-9.
25. de Vries AC, Stam RW, Schneider P, Niemeyer CM, van Wering ER, Haas OA, et al. Role of mutation independent constitutive activation of FLT3 in juvenile myelomonocytic leukemia. Haematologica. 2007;92(11):1557-60.
26. Gratias EJ, Liu YL, Meleth S, Castleberry RP, Emanuel PD. Activating FLT3 mutations are rare in children with juvenile myelomonocytic leukemia. Pediatr Blood Cancer. 2005;44(2):142-6.
27. Kotecha N, Flores NJ, Irish JM, Simonds EF, Sakai DS, Archambeault S, et al. Single-cell profiling identifies aberrant STAT5 activation in myeloid malignancies with specific clinical and biologic correlates. Cancer Cell. 2008;14(4):335-43.
28. Liu YL, Castleberry RP, Emanuel PD. PTEN deficiency is a common defect in juvenile myelomonocytic leukemia. Leukemia Research. 2009;33(5):671-7.
29. Furlan I, Batz C, Flotho C, Mohr B, Lübbert M, Suttorp M, et al. Intriguing response to azacitidine in a patient with juvenile myelomonocytic leukemia and monosomy 7. Blood. 2009;113(12): 2867-8.
30. Hasegawa D, Manabe A, Kubota T, Kawasaki H, Hirose I, Ohtsuka Y, et al. Methylation status of the p15 and p16 genes in paediatric myelodysplastic syndrome and juvenile myelomonocytic leukaemia. Br J Haematol. 2005;128(6):805-12.
31. Johan MF, Bowen DT, Frew ME, Goodeve AC, Reilly JT. Aberrant methylation of the negative regulators RASSFIA, SHP-1 and SOCS-1 in myelodysplastic syndromes and acute myeloid leukaemia. Br J Haematol. 2005;129(1):60-5.
32. Locatelli F, Nollke P, Zecca M, Korthof E, Lanino E, Peters C, et al. Hematopoietic stem cell transplantation (HSCT) in children with juvenile myelomonocytic leukemia (JMML): results of the EWOG-MDS/EBMT trial. Blood. 2005;105(1):410-9.
33. EWOG-MDS. EWOG-MDS RC 06, TCRVbeta repertoire analysis and PNH clones in children with Refractory Cytopenia (RC). An open non-randomised multi-center prospective study. 2006.

High hematocrit as a risk factor for venous thrombosis. Cause or innocent bystander?

Anja J.M. Schreijer,[1] Pieter H. Reitsma,[2] and Suzanne C. Cannegieter[1]

[1]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden; [2]Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, the Netherlands
E-mail: p.h.reitsma@lumc.nl. *doi:10.3324/haematol.2009.017285*

In the lay press it is frequently stated that long haul air travel causes venous thrombosis through dehydration in the airplane, leading to hyperviscosity of the blood, which in its turn favors thrombosis. The common advice that is given to prevent thrombosis after air travel is, therefore, 'to drink ample amounts of fluids' (http://www.britishairways.com/travel/healthmedcond/public/en_gb#DVT). This concept of thick, slowly flowing blood is apparently intuitively appealing as a cause of thrombosis. It would also fit the classical triad of Virchow, who postulated that thrombosis is due to the occurrence of stasis, to disturbances of the composition