JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. Nat Genet. 2009;41(4):446-9.
6. Olcaydu D, Harutyunyan A, Jager R, Berg T, Gisslinger B, Pabinger I, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. Nat Genet. 2009;41(4):450-4.
7. Kilpivaara O, Mukherjee S, Schram AM, Wadleigh M, Mullally A, Ebert BL, et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2V617F-positive myeloproliferative neoplasms. Nat Genet. 2009;41(4):455-9.
8. Olcaydu D, Skoda RC, Looser R, Li S, Cazzola M, Pietra D, et al. The 'GGCC' haplotype of JAK2 confers susceptibility to JAK2 exon 12 mutation-positive polycythemia vera. Leukemia. 2009;23(10):1924-6.
9. Harrison CN, Campbell PJ, Buck G, Wheatley K, East CL, Bareford D, et al. Hydroxyurea Compared with Anagrelide in High-Risk Essential Thrombocythemia. N Engl J Med. 2005; 353(1):33-45.
10. Jones AV, Silver RT, Waghorn K, Curtis C, Kreil S, Zoi K, et al. Minimal molecular response in polycythemia vera patients treated with imatinib or interferon alpha. Blood. 2006;107(8):3339-41.
11. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447(7145): 661-78.

Hydroxyurea induced oscillations in twelve patients with polycythemia vera

Hydroxyurea is used in polycythemia vera for cytoreductive treatment. Some patients show marked fluctuations of platelet and leukocyte counts while taking hydroxyurea. We identified 12 patients in our patient cohort and performed a mathematical analysis which revealed oscillatory behavior with a typical period length of 27-30 days. During the presence of oscillations (25.5 years) no bleeding or thromboembolic episodes were observed.

Polycythemia vera is a chronic acquired stem cell disorder which affects primarily red blood cells and in many patients also granulocyte and platelet counts. Polycythemia vera is associated with an increased risk of thromboembolic and hemorrhagic complications.[1] Phlebotomy is the therapy of choice.[2] In refractory patients cytoreductive treatment with drugs such as hydroxyurea is advocated. Hydroxyurea primarily interferes with DNA synthesis by inhibiting ribonucleotide reductase in hematopoietic stem cells.[3] Recently, patients with polycythemia vera and hydroxyurea associated oscillations of the platelet and white blood cell count have been reported.[4-7]

We identified 12 patients (7 females and 5 males) who showed fluctuations of the platelet and white blood cell counts when treated with hydroxyurea. All patients were positive for the $JAK2^{V617F}$ mutation. Total observation time was about 76 patient years of which 29 years were under therapy with hydroxyurea (Table 1).

We performed a mathematical periodicity analysis by calculating the frequency spectrum with the Lomb periodogram[8] which was first used for the analysis of blood cell fluctuations by Bennett and Grunwald.[5] For the platelet count, all patients oscillated with a cycle length of 28.6 days (27-30). In contrast, only 5 patients showed oscillations of the white blood cell count with a cycle length of 27.8 days (26-29). Patients were observed for a

Table 1. Characteristics of 12 patients with HU induced oscillations: all patients were positive for the $JAK2^{V617F}$ mutation. Male patients: 5,6,8,9,10; female patients 1-4, 7, 11-12. Patients 1,3,4, 6, 10 took Litalir®, the others Syrea®. Mathematical analysis of the oscillations of the platelets and White blood cells (cycle length). Also shown are start, ending and duration of oscillations as well as duration of HU intake, dosage and total observation period.

Pt.	PLT cyle [days]	WBC cycle [days]	Oscillations started	Oscillations affected by	PLT nadir [x10⁹/L]	PLT zenith [x10⁹/L]	WBC nadir [x10⁹/L]	WBC zenith [x10⁹/L]	Duration of HU [months]	Duration of oscillation [months]	HU dose [g/day]	Total observation period [months]
1	28	26	After intake of HU with a 12 month delay	Anagrelide after a 9 month delay	21	1417	03.0	14.0	36	23	0.5-2	101
2	27	27	Immediately after intake of HU	Still oscillating	68	1690	06.9	33.3	40	40	1-2	48
3	29		Immediately after intake of HU	Anagrelide	71	1145	03.0	29.0	4	4	1-2	96
4	30	29	Immediately after intake of HU	Still oscillating	104	1420	01.9	06.9	120	120	1-2	125
5	30	29	Immediately after intake of HU	Busulphane	46	1122	04.5	38.2	10	10	0.5-3.5	120
6	30		Immediately after intake of HU	Ending HU therapy	116	598	10.5	21.7	15	15	1-2	96
7	27		Immediately after intake of HU	Still oscillating	136	1385	08.6	21.6	48	48	1-2	48
8	30		After splenectomy. Prior 6 years of HU without oscillations	Busulphane	27	4900	04.3	30.0	8	8	1-2	108
9	28		Immediately after intake of HU	An additional phlebotomy	189	1162	05.4	10.2	21	4	0.5-1	72
10	29	28	Immediately after intake of HU	Anagrelide	21	1495	04.3	12.4	21	21	1-2	48
11	28		Immediately after intake of HU	No obvious reason	140	530	06.5	09.4	18	6	1	18
12	27		Immediately after intake of HU	Anagrelide	116	989	05.5	16.4	7	7	0.5-1	36

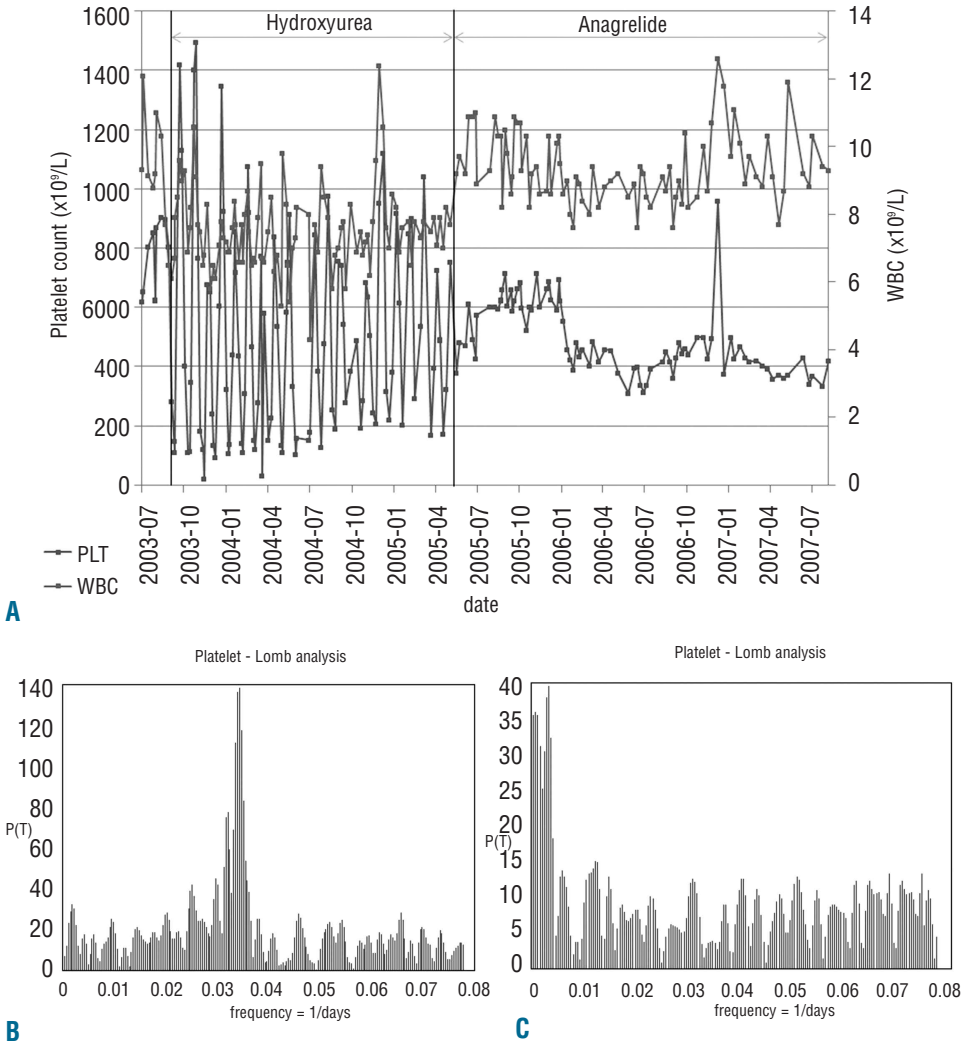

Figure 1. (A) Development of platelet and WBC count during therapy with hydroxyurea and after switch to anagrelide in a 60-year old man (Patient 10 in Table 1). (B) Verification of platelet count under HU therapy with the Lomb periodogram: a significant peak is present. (C) Verification of platelet count under anagrelide therapy with the Lomb periodogram: no significant peak is present.

median of 84 months (18-125).

In oscillations, two mathematical parameters can be different: length of period and amplitude. Whereas the length of period (28 days) for platelet counts and white blood cell counts (27.8 days) was very similar, the amplitude was quite different. Platelets oscillated between a minimum platelet count of 21×10^9/ L and a maximum platelet count of $4{,}900\times10^9$/L. White blood cell count oscillated between 1.9×10^9/L and 38.2×10^9/L. Moreover, platelet and white blood cell counts varied in amplitude within each patient.

Daily hydroxyurea dosage varied from 0.5 to 3.5 g/day. Median duration of intake of hydroxyurea was 19.5 months (4–120). Hydroxyurea interferes with DNA synthesis by inhibiting ribonucleotide reductase in hematopoietic stem cells,[3] so that many cells reach mitosis at the same time causing partial synchronization. This synchronization increases the chances for oscillatory behavior. In Germany, there are 2 different hydroxyurea formulations available (Syrea® and Litalir®) which both caused oscillatory behavior which indicates that potential differences in pharmacokinetics are not relevant.

Hydroxyurea triggers these oscillations since in the large majority (10 out of 12 patients) the oscillations started immediately after the intake of this drug. In addition, 7 of 9 patients showed no more oscillations after discontinuation of hydroxyurea. However, there are some exceptions: in one patient platelet and white blood cell counts started to oscillate after twelve months of hydroxyurea intake. Another patient took hydroxyurea for six years without any oscillations. He developed oscillations only after splenectomy. This may indicate that a splenic dysfunction contributes to oscillations in non-splenectomized patients. In most of the patients, discontinuation of hydroxyurea leads to disappearance of the oscillations which did not reappear after switch of treatment to anagrelide (4 patients) or busulphan (2 patients) (Figure 1). In 2 patients, the oscillations disappeared slowly under therapy with hydroxyurea. Time to complete disappearance of oscillations may require several months in some individuals. This can be concluded from our observations in one patient: the oscillations persisted after a switch to anagrelide albeit with smaller amplitude and were no longer detectable nine months later. Three patients are still oscillating under hydroxyurea therapy.

When oscillations are observed under hydroxyurea therapy several strategies to reduce them can be used. In some patients oscillations are spontaneously diminished when the hydroxyurea dosage is kept constant. Another option is to discontinue hydroxyurea and replace it by

phlebotomies, aspirin or anagrelide. In contrast to hydroxyurea, anagrelide has no effect on hematopoetic stem cells. But the latter strategy is not working in all patients.

Is a change of treatment in polycythemia vera patients with oscillations necessary and how dangerous are these oscillations at all? The two main concerns are thromboembolic and bleeding complications. We have not observed any bleeding or thrombotic event during the time of the oscillations (25.5 patient years). In contrast to our observations other authors have reported clinical complications.[5,6]

Oscillations have been observed in platelet and white blood cell counts but not in erythrocytes. One possible explanation is the different life span of platelets, white blood cells, and erythrocytes. Platelets and white blood cells regenerate faster and are more rapidly degraded which makes oscillations more pronounced.

Oscillations under hydroxyurea therapy are a rare phenomenon since they can only be detected when patients donate blood samples at time intervals which are different from the typical oscillation period of 28 days. Chances of detecting oscillations are higher when patients are subjected to blood cell analyses at irregular time intervals. Since this practice is often used the number of patients with oscillations is probably more frequent than usually assumed. Therefore no statement can be made concerning the incidence of these hydroxyurea induced oscillations.

Since oscillations also occur in healthy individuals, even hematopoiesis itself is considered to be an oscillatory system, hydroxyurea seems to amplify preexisting conditions in sensitive individuals.[9,10] The underlying molecular mechanism remains unknown. To address the issue of the clinical relevance of hydroxyurea induced oscillations, it can only be speculated whether these oscillations are harmful or not. We did not see any bleeding or thrombotic events in our patients which underscores the present concept that thrombocytosis and leukocytosis are not the only cause of thrombotic events but hematocrit is the highest risk factor in patients with polycythemia vera. It is also unclear why such platelet oscillations have not been observed in other bcr/abl negative chronic myeloproliferative neoplasms such as primary thrombocythemia and primary myelofibrosis.

Jan Tauscher, Fabian Siegel, and Petro E. Petrides

Hematology Oncology Center and Ludwig-Maximilians-University Medical School Munich, Germany

Correspondence: Petro E. Petrides, Hematology Oncology Center and Ludwig-Maximilians-University Medical School Munich, Germany, Zweibrueckenstrasse 2, 80331 Munich, Germany. Phone: international +49.89.229009. Fax: international +49.89.229448. E-mail: petrides@onkologiemuenchen.de

Key words: polycythemia vera, hydroxyurea, anagrelide, platelet oscillations, WBC oscillations, cyclic hematopoiesis, spectral analysis, periodicity, Fourier analysis, Lomb analysis, JAK2V617F mutation.

Citation: Tauscher J, Siegel F, and Petrides PE. Hydroxyurea induced oscillations in twelve patients with polycythemia vera. Haematologica 2010; 95:1227-1229. doi:10.3324/haematol.2010.022178

References

1. Finazzi G, Barbui T. Evidence and expertise in the management of polycythemia vera and essential thrombocythemia. Leukemia. 2008;22(8):1494-502.
2. Spivak J. Polycythemia vera: myths, mechanisms, and management. Blood. 2002;100(13):4272-90.
3. Yarbro J. Mechanism of action of hydroxyurea. Semin Oncol. 1992;19(3 Suppl 9):1-10.
4. Burthem J, Chaudhry MS. Hydroxycarbamide associated platelet count oscillations in a patient with polycythaemia vera. A case report and review of the literature. Platelets. 2008;19(3):234-5.
5. Bennett M, Grunwald AJ. Hydroxyurea and periodicity in myeloproliferative disease. Eur J Haematol. 2001;66(5): 317-23.
6. Steensma DP, Harrison CN, Tefferi A. Hydroxyurea-associated platelet count oscillations in polycythemia vera: A report of four new cases and a review. Leukemia. 2001; 42(6):1243-53.
7. Tefferi A, Elliott MA, Kao PC, Yoon S, El-Hemaidi I, Pearson TC. Hydroxyurea-induced marked oscillations of platelet counts in patients with polycythemia vera. Blood. 2000;96(4):1582-4.
8. von Schulthess GK, Gessner U. Oscillating platelet counts in healthy individuals: experimental investigation and quantitative evaluation of thrombocytopoietic feedback control. Scand J Haematol. 1986;36(5):473-9.
9. Morley A, King-Smith EA, Stohlman F. Oscillatory nature of hemopoiesis. Hemopoietic Cellular Proliferation. In: Stohlman, F Jr. (Ed.). New York Grune and Stratton, Inc. (1970), 3-14.

P39/Tsugane cells are a false cell line contaminated with HL-60 cells and are not suitable for mechanistic studies in myelodysplastic syndromes

The P39/Tsugane myelomonocytoid cell line was generated in 1983 by Nagai and colleagues from a 69-year old man with chronic myelomonocytic leukemia (CMML) that had progressed to acute myeloid leukemia (AML), French-American-British classification subtype M2.[1] This cell line was deposited in the Japanese Cell Repository Bank (JCRB, now known as the Japanese Collection of Research Bioresources), in July 1986, where its unique identifier is JCRB0092.

After DNA fingerprinting technology became widely available in the 1990s, the *Deutsche Sammlung von Mikroorganismen und Zellkulturen* (DSMZ) and the JCRB applied fingerprinting techniques to repository cell lines, and both cell banks found that their P39/Tsugane cells share genetic identity with HL-60 cells, which were derived at the National Cancer Institute (NCI) from a woman with suspected acute promyelocytic leukemia (later found to be AML FAB M2), and first reported in 1976.[2]

The JCRB now lists P39/Tsugane as a misidentified or false cell line (*http://cellbank.nibio.go.jp/cellbank_e.html*), stating that evidence of cross-contamination with HL-60 cells was "found by DNA fingerprinting first, later confirmed by STR-PCR [short tandem repeat-polymerase chain reaction]."

Likewise, using Affymetrix 10K Single Nucleotide Polymorphism (SNP) arrays as part of the Cancer Genome Project, the Wellcome Trust Sanger Institute in the United Kingdom observed 97% genetic identity between P39/Tsugane cells and HL-60 cells from the NCI60 cell line set (*http://www.sanger.ac.uk/genetics/CGP/Genotyping/nci60.shtml*). As Hans Drexler of the DSMZ has observed, "a distressingly large percentage of purported MDS cell lines had been cross-contaminated", and the list of cell lines recognized as false or contaminated by the DSMZ includes P39/Tsugane cells.[3,4]

Despite these findings, P39/Tsugane cells continue to