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.

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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

be widely used as an *MDS cell line*, and I recently received several manuscripts for review in which generalizations about MDS pathobiology were made on the basis of *in vitro* experiments performed in P39/Tsugane cells. In order to determine whether an aliquot of unique P39/Tsugane cells exists and to confirm the findings of the JCBR, DSMZ, and Sanger Institute, I obtained P39/Tsugane cells from two laboratory groups with publications that made use of these cells. (These laboratories graciously supplied the cells despite knowing that I intended to evaluate them for cross-contamination with HL-60 cells.)

In a laboratory area that had never worked with HL-60 cells, I grew the putative P39/Tsugane cells to confluence in RPMI 1640 media supplemented with 10% fetal calf serum, and compared them to HL-60 cells from the NCI (HL-60 cells courtesy of Scott. H. Kaufmann, MD PhD, Mayo Clinic, Rochester, MA, USA). Methods of comparison included G-banded karyotyping supplemented by fluorescent *in situ* hybridization (FISH, courtesy of Rhett Ketterling, MD, Cytogenetics Laboratory, Mayo Clinic) and a 12-marker DNA Variable Nucleotide Tandem Repeat (VNTR) panel used for forensic work (courtesy of W. Edward Highsmith, PhD, Division of Laboratory Genetics, Mayo Clinic).

Both of the P39/Tsugane aliquots tested exhibited karyotyping/FISH and VNTR results identical to the HL-60 cells. The published karyotype of P39/Tsugane cells is "45,XY,+del(6)(q15),9q+, t(14;16)-(q24;q21),-16,-17".

In contrast, among the aliquots I examined, there were no metaphases with this karyotype, FISH results for chromosome 17p deletion were normal, and the karyotype was instead female as for HL-60 cells (other investigators who are working with P39/Tsugane cells and wish to easily determine whether their aliquot is actually HL-60 cells might begin by assessing for presence of a Y chromosome by FISH, since P39/Tsugane cells were obtained from a man and HL-60 cells from a woman).

While it is possible that an aliquot of a unique P39/Tsugane cell line exists, and the published results might still retain some relevance even though the experiments were actually performed in HL-60 cells, MDS investigators should be aware that the P39/Tsugane cells available from major cell banks and in widespread use in research laboratories are contaminated by HL-60 cells. Drexler recently reviewed all putative MDS cell lines and found only 3 of them (MDS92, M-TAT, and TER-3) that had both been established in the MDS phase (i.e. rather than after AML transformation, as for P39/Tsugane) and might be valid, although more analysis at the genome and proteome levels is required.[4]

Obtaining a suitable cell line that mimics characteristics of MDS and is suitable for mechanistic biological analysis remains a research priority.

P39/Tsugane cells cannot provide any special insight into MDS and should not be used for this purpose.

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Autoimmune hemolytic anemia in patients with chronic lymphocytic leukemia is associated with IgVH status

Recent investigations in chronic lymphocytic leukemia (CLL) have correlated the mutational status of immunoglobulin heavy-chain variable-region (*IgVH*) gene to the development of secondary immune thrombocytopenia, the risk being higher for unmutated cases.[1,2] A similar association between autoimmune hemolytic anemia (AHA) and IgVH gene status has not yet been defined. Therefore, whereas it is well known that the prevalence of autoimmune hemolytic anemia is highest in the more advanced stages of the disease,[3] and may depend on the type of treatment administered,[4,5] the impact of this autoimmune complication on the outcome of patients with chronic lymphocytic leukemia remains controversial.[4-7]

To address this, we searched our database of 473 chronic lymphocytic leukemia patients consecutively referred to our Department from 1st January 2000 to 1st January 2009. We identified 35 chronic lymphocytic leukemia patients (7%) who developed overt autoimmune hemolytic anemia. All patients met the chronic lymphocytic leukemia diagnostic criteria of the National Cancer Institute. Autoimmune hemolytic anemia was defined with standard criteria: a fall in hemoglobin level of at least 2 g/dL, associated with a positive direct antiglobulin test and/or increased reticulocyte count, and a rise in indirect bilirubin with no other causes of anemia identified.

As part of the diagnostic work-up, IgVH DNA sequence analysis was performed on peripheral blood or bone marrow specimens of all the 238 consecutive chronic lymphocytic leukemia patients diagnosed at our Institution since June 2004. Mutational status was obtained retrospectively, using the samples collected at diagnosis, in 12 of the 35 cases of chronic lymphocytic leukemia with autoimmune hemolytic anemia occurring before June 2004. Sequences were aligned to IMGT and V-BASE directories and analyzed using DNAPLOT and IMGT/VQUEST software. Those differing more than 2% from the corresponding germ-line gene were considered mutated.

Median follow-up from diagnosis of chronic lymphocytic leukemia was 66 months (range 6-118). All patients with autoimmune hemolytic anemia except 2 presented with RAI stage 0 or 1, and developed autoimmune hemolytic anemia in a median time of 48 months (range 0-102), all requiring specific therapy for the anemia. In