indicative of lack of host (male) cells in female-to-male combinations, which is another surrogate marker, equivalent to elimination of BCR/ABL-positive host cells as well.

As mentioned by Kaeda et al, the graft-versus-leukemia (GVL) effect has the potential to achieve a cure in CML. The GVL effect is usually accompanied by graft-versus-host disease (GVHD). In this cohort of patients, full donor chimerism was achieved rapidly, without or with a short transition period of documented mixed chimerism, which probably had a major impact on the incidence of acute and chronic GVHD. In fact, as discussed in our manuscript, GVHD remains the single major obstacle of transplantation using NST for the treatment of CML, yet alloreactive donor lymphocytes increase the probability of elimination of the last tumor cell at the cost of acute and mostly chronic GVHD.

Finally, the most encouraging results of this study suggest that consistent and durable elimination of *BCR/ABL* transcripts may be accomplished in patients with CML who receive transplants in first chronic phase for up to 5 years. Therefore, even if the RT-PCR data were not too sensitive, due to the limitations pointed out by Kaeda et al, there seems to be no question that the GVL effects accomplished by NST were durable and clinically meaningful. Our conclusion is based on multiple analyses documenting durable 100% donor chimerism over a long period of time, as shown in our patients successfully treated with NST.

Taking into account the aforementioned considerations, assessing quantitative RT-PCR of *BCR-ABL* transcript as opposed to nonquantitative RT-PCR of *BCR-ABL* transcript appears to be of little practical value in assessing the long-term benefits of NST in clinical practice, unless one wishes to investigate the speed of full conversion of host to 100% donor chimerism, which was not the goal of our present report. Taken together, considering the consistently negative RT-PCR over a long period of time, including the surrogate marker, amelogenine gene PCR in some patients, we are convinced that NST is an effective, relatively safe, and potentially curative modality in CML.

Michael Y. Shapira, Reuven Or, Igor B. Resnick, Menachem Bitan, and Shimon Slavin

Correspondence: Michael Y. Shapira, Department of Bone Marrow Transplantation, Hadassah University Hospital, PO Box 12000, Jerusalem, Israel; e-mail: shapiram@hadassah.org.il

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To the editor:

Rapid identification of *CBFB-MYH11*–positive acute myeloid leukemia (AML) cases by one single *MYH11* real-time RT-PCR

The inv(16)(p13q22) rearrangement is present in approximately 10% of cases with de novo acute myeloid leukemia (AML) and results in a *CBFB-MYH11* gene fusion.[1] Patients with this fusion gene define a specific subgroup with a relatively good prognosis, and the accurate identification of *CBFB-MYH11*/inv(16)–positive cases is therefore essential. Recent studies have shown that *CBFB-MYH11* reverse transcriptase–polymerase chain reaction (RT-PCR)–positive cases can be missed by cytogenetic analysis.[2,3] RT-PCR may be an efficient method for identifying *CBFB-MYH11*–positive cases. To date, at least 12 different *CBFB-MYH11* fusion transcripts have been described that are caused by alternative splicing and variable breakpoints in both *CBFB* and *MYH11*.[1,4-8] This diversity complicates routine *CBFB-MYH11* RT-PCR diagnosis. Real-time quantitative PCR (qPCR) is currently being used for routine identification and quantification of many fusion genes and transcripts associated with hematologic malignancies. The amplicon size used in qPCR should be 300 bp or less. Because the distance between the smallest and longest *CBFB-MYH11* fusion transcript is more than 1200 bp the efficient detection of all fusion transcripts requires at least 4 different qPCRs.[8]

Because of the fusion to *CBFB*, the expression of the involved *MYH11* RNA sequences might be significantly altered compared with normal levels from the unrearranged alleles. This would allow for rapid identification of *CBFB-MYH11*–positive cases by quantifying *MYH11* mRNA expression. To test this hypothesis, we designed a *MYH11* qPCR downstream of all known *MYH11* fusion points. We determined the *MYH11* expression in 32 bone marrow and blood samples taken from cases with newly diagnosed AML. Of these samples, 11 were C*BFB-MYH11* positive as determined by cytogenetics and conventional RT-PCR.[8] Of the *CBFB-MYH11*–positive cases, 6 were positive for the most frequently occurring fusion transcript (type A), 2 were positive for transcript type D, 2 were positive for the longest transcript (type E), and one was positive for the smallest transcript (type S).[1,8] Within the group of *CBFB-MYH11*–positive cases the *MYH11* expression varied 7-fold. This is in line with an earlier observation that the *CBFB-MYH11* expression levels in a different group of 6 cases varied less than 5-fold at diagnosis.[9] A significantly higher *MYH11* expression was measured in all *CBFB-MYH11*–positive cases compared with negative cases ($P = .000\ 0046$, Mann-Whitney test, Figure 1). The median *MYH11* expression detected in *CBFB-MYH11*–positive cases was 298-fold higher compared with the negative cases. The smallest difference between the *CBFB-MYH11*–positive patient with the lowest *MYH11* expression and *CBFB-MYH11*–negative patient with the highest *MYH11* expression was 25-fold. In 2 *CBFB-MYH11*–positive cases where follow-up material was available, remission samples showed *MYH11* expression levels comparable to those observed in inv(16)-negative AML patients. Finally, we measured the *MYH11* expression in bone marrow and blood samples taken from cases with other hematologic malignancies ($n = 22$) and from healthy volunteers ($n = 2$) and observed, as in *CBFB-MYH11*–negative AML cases, a significant lower expression

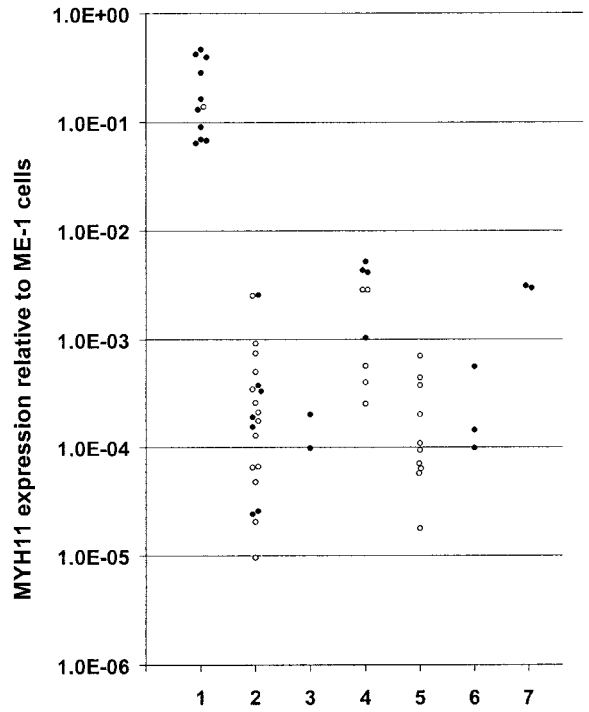

Figure 1. *MYH11* overexpression in *CBFB-MYH11*– positive cells. The expression in *CBFB-MYH11*–positive ME-1 cells was set at 1.0. From all cases informed consent was obtained in accordance with the Declaration of Helsinki. The numbers indicate the following: (1) newly diagnosed *CBFB-MYH11*–positive AML (n = 11); (2) newly diagnosed *CBFB-MYH11*–negative AML (n = 21); (3) *CBFB-MYH11*–positive cases in complete hematologic remission (n = 2); (4) de novo chronic myeloid leukemia (n = 9); (5) history of chronic myeloid leukemia (n = 10); (6) de novo acute lymphocytic leukemia (n = 3); and (7) healthy volunteers (n = 2). ○ and ● indicate blood and bone marrow samples, respectively. Primers and probes were developed downstream of all known *MYH11* fusion points using Primer Express version 1.5 (Applied Biosystems, Foster City, CA). Sequences (5′-3′) of *MYH11* forward and reverse primers and probe are respectively, AGTAGCCTGTCGGGAAGGAAC, GCCTGCTGTGTGGCTTTG, CACTCCAGGACGAGAAGCGCCG. The cDNA synthesis and input, cycling conditions, and *PBGD* expression measured for normalization were as described.[9] For quantification, serial log dilutions of cDNA in H_2O derived from the *CBFB-MYH11*–positive cell line ME-1 were used. Linear amplification extended down to a 4 log dilution.

compared with *CBFB-MYH11*–positive AML cases (Figure 1). We conclude that up-regulation of *MYH11* expression because of the fusion to *CBFB* can be used to rapidly identify *CBFB-MYH11*–positive cases in newly diagnosed AML.

Bert A. van der Reijden, Marion Massop, Evelyn Tönnissen, Louis van de Locht, Petra Muus, Theo de Witte, and Joop H. Jansen

Correspondence: Bert A. van der Reijden, Central Hematology Laboratory, University Medical Center Nijmegen, Geert Grooteplein zuid 8, PO Box 9101, 6500 HB, Nijmegen, The Netherlands; e-mail: b.vanderreijden@chl.umcn.nl

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To the editor:

No exon 4 polymorphism of cytochrome P450 CYP2C9 in Taiwanese

A recent article by Leung et al[1] analyzed the relationship between the genetic polymorphism in exon 4 of cytochrome P450 CYP2C9 and warfarin sensitivity in Chinese patients. They used polymerase chain reaction (PCR) and direct sequencing methods to analyze the genetic changes of the *CYP2C9* gene in 89 patients, and their results showed that 4 polymorphisms in exon 4 were found: heterozygosities for 608TTG>GTG (Leu208Val), 561CAG>CCG (Gln192Pro), 537CAT>CCT (His184Pro), and 527ATT>CTT (Ile181Leu) existed at frequencies 0.75, 0.20, 0.10, and 0.09, respectively. We used a similar approach to analyze the genetic polymorphisms in exon 4 of the *CYP2C9* gene in Taiwanese patients. The primers used to amplify the exon 4 were 5′-AATACAGTGTTTTATATCTAAAG-3′ (GenBank accession number L16879, nucleotide [nt] 1-23) and 5′-TAAGTGGTTTCTCAGGAAGC-3′ (nt 256 to 237) as forward and reverse primers, respectively. We were unable to find these 4 polymorphisms or other new polymorphisms in the exon area in 50 healthy people (Figure 1). In

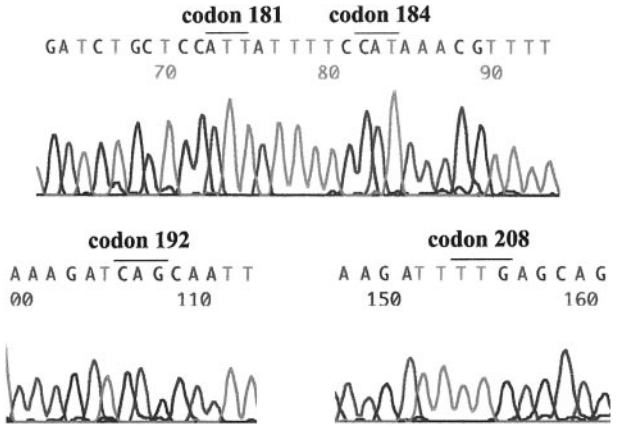

Figure 1. The representative case of direct sequencing analysis for exon 4 polymorphisms of *CYP2C9* gene. No polymorphism was found in exon 4 of 50 cases after sequencing analysis.