To the editor:

Monitoring CML after nonmyeloablative transplantations: how negative is negative?

It is widely accepted that allogeneic stem cell transplantation can cure selected patients with chronic myeloid leukemia (CML), and that this result is due, in part, to the graft-versus-leukemia effect mediated by donor-derived T cells. The evidence for a cure is based to a large degree on long survival without detectable evidence of residual leukemia as determined by a very sensitive reverse transcriptase–polymerase chain reaction (RT-PCR) for *BCR-ABL* transcripts.[1] The Hadasseh group has now reported impressive results with the use of nonmyeloablative stem cell transplantation with 21 of 24 survivors at a median follow-up of 42 months.[2] These survivors all apparently had 100% donor hematopoietic cells and were all negative by RT-PCR for *BCR-ABL*. We believe these molecular data may need more detailed consideration.

It is now conventional in specialist centers to serially monitor *BCR-ABL* transcript numbers by quantitative PCR in CML patients after initially successful treatment with imatinib or after allogeneic stem cell transplantation.[3] The assay is usually performed by reverse transcribing mRNA to cDNA using random hexamers followed by a 2-step competitive nested-primer PCR[4] or by Taqman or Lightcycler real-time PCR to amplify *BCR-ABL* transcripts.[5,6] The concomitant quantitative assay of a suitable "housekeeping" gene, such as *ABL*, *G-6PD*, β_2-microglobulin, or possibly *BCR*, is mandatory to exclude false-negative results.[3] These techniques typically detect *BCR-ABL* transcripts with a maximum sensitivity of about 1 in 10,[5] and the number of control gene transcripts indicates the sensitivity with which *BCR-ABL* can be excluded for each "negative" sample. The PCR assay employed in Jerusalem involves reverse transcription using gene-specific primers followed by nested 2-step PCR amplification.[2,7] No quantitation for either *BCR-ABL* or a control gene was reported and therefore the true sensitivity of the assay for each sample is uncertain. This is essential information for comparing results of nonmyeloablative and conventional transplantations. It thus seems to us of great importance that the Jerusalem group should report the results of serial follow-up of the surviving patients using a technique that includes assessment of the sensitivity of the assay. Such data could greatly strengthen the claim that their survival and disease-free survival curves are superimposable. Their report also serves indirectly to highlight the urgent need for validation and standardization of RT-PCR techniques employed in the different laboratories internationally.

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References

1. Mughal T, Yong A, Szydlo RM, et al. Molecular studies in patients with chronic myeloid leukaemia in remission 5 years after allogeneic stem cell transplant define the risk of subsequent relapse. Brit J Haematol. 2001;115: 569-574.
2. Or R, Shapira MY, Resnick I, et al. Nonmyeloablative allogeneic stem cell transplantation for the treatment of chronic myeloid leukemia in first chronic phase. Blood. 2003;101:441-445.
3. Kaeda J, Chase A, Goldman JM. Cytogenetic and molecular monitoring of residual disease in chronic myeloid leukaemia. Acta Haematologica. 2002;107: 64-75.
4. Cross NCP, Lin F, Chase A, Bungey J, Hughes TP, Goldman JM. Competitive PCR to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. Blood. 1993;82:1929-1936.
5. Branford S, Hughes T, Ruszki Z. Monitoring chronic myeloid leukaemia therapy by real time quantitative PCR in blood is a reliable alternative to bone marrow cytogenetics. Brit J Haematol. 1999;107:587-599.
6. Wang L, Pearson K, Pilliteri L, Ferguson J, Clark R. Serial monitoring of BCR-ABL by peripheral blood real time polymerase chain reaction predicts the marrow cytogenetic response to imatinib mesylate in chronic myeloid leukaemia. Brit J Haematol. 2002;118:771-777.
7. Radich JP, Gehly G, Gooley T, et al. Polymerase chain reaction detection of the BCR-ABL fusion transcript after allogeneic marrow transplantation for chronic myeloid leukemia: Results and implications in 346 patients. Blood. 1995;85:2632-2638.

Response:

Quantitative RT-PCR after NST for CML

In response to our paper published recently in *Blood*,[1] Kaeda et al claim that only quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) of *BCR-ABL* transcript rather than nonquantitative RT-PCR assay is essential for confirming the quality of remission in chronic myeloid leukemia (CML) patients after nonmyeloablative stem cell transplantation (NST), in comparison with remission accomplished by conventional myeloablative conditioning.

The data presented in our paper were based on the combination of variable number of tandem repeat (recently switched to short tandem repeats) assay in sex-matched donor-recipient combinations and the amelogenine gene PCR assay for sex-mismatched donor/recipient pairs, as well as cytogenetic analysis of bone marrow aspirates as a disease-specific marker (Philadelphia chromosome). The amelogenine gene PCR used for posttransplant follow-up of chimerism was informative in 5 of 9 cases from 9 of 24 sex-mismatched patients reported in this study. It should be noted that in our hands, the amelogenine gene assay is a most sensitive assay, and a positive signal may be obtained using DNA samples containing as little as one in 10^5 to 10^6 male cell in male/female mixtures.[2] Therefore, a negative amelogenine gene PCR, as reported in our study in 5 of 9 informative cases, also is

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.

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References

1. Or R, Shapira MY, Resnick I, Amar A, Ackerstein A, Samuel S, et al. Non-myeloablative allogeneic stem cell transplantation for the treatment of chronic myeloid leukemia in first chronic phase. Blood. 2003;101:441-445.
2. Pugatsch T, Oppenheim A, Slavin S. Improved single-step PCR assay for sex identification post-allogeneic sex-mismatched BMT. Bone Marrow Transplant. 1996;17:273-275.

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