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.

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 MYH11.4-8 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 CBFB-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
To the editor:

No exon 4 polymorphism of cytochrome P450 CYP2C9 in Taiwanese

A recent article by Leung et al observed 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 polymorphisms in exon 4 of cytochrome P450 CYP2C9 and concluded 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.

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References


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.