CORRESPONDENCE

Detection of AML1/ETO Fusion Transcripts in Patients With t(8;21) Acute Myeloid Leukemia After Allogeneic Bone Marrow Transplantation or Peripheral Blood Progenitor Cell Transplantation

To the Editor:

We have evaluated the occurrence of the AML1/ETO fusion transcripts by reverse transcription-polymerase chain reaction (RT-PCR) analysis[1] in 7 patients with t(8;21)(q22;q22) acute myeloid leukemia (AML) who underwent allogeneic bone marrow transplantation (BMT; n = 6) or peripheral blood progenitor cell transplantation (PBPCT; n = 1). We found here that the AML1/ETO fusion transcripts were detectable in only 2 of 7 patients in the long-term follow-up posttransplant (12 and 112 months post-BMT). One of these two patients who were tested positive for the AML1/ETO fusion transcript at 1 month and at 12 months posttransplant remained also to be mixed chimeric by variable number of tandem repeats PCR (VNTR-PCR) analysis at 12 months post-BMT, but relapsed 5 months later and subsequently died. However, another patient who was AML1/ETO positive up to 112 months posttransplant remained in complete remission during the whole observation period. This patient had a complete chimerism status by VNTR-PCR at 3 months and at 48 months post-BMT.

Three of five patients who were initially AML1/ETO positive in the RT-PCR assay converted 6, 9, and 30 months posttransplant, respectively, and showed at least two consecutive negative PCR assays for the AML1/ETO fusion transcript. All patients who achieved a molecular remission by the AML1/ETO PCR assay remained in stable cytogentic remission.

Studies about the presence of the AML1/ETO fusion transcripts after allogeneic BMT or PBPCT are rare and results are discussed controversely.[1-5] Recently, Jurlander et al[2] reported that AML1/ETO fusion transcripts in patients treated with allogeneic BMT for t(8;21) leukemia were still persistent post-BMT in all 9 of 9 evaluable patients.[1] According to these investigators, persistence of the leukemic clone after BMT suggests that allogeneic BMT, like conventional chemotherapy and autologous BMT, is usually not sufficient to eliminate expression of AML1/ETO transcripts.[2] Contrary to Jurlander et al,[2] we showed here, using a similar sensitive method, that allogeneic transplantation leads to sustained suppression or elimination of the leukemic clone in most of the studied patients due to a combination of pretransplantation marrow-ablative conditioning regimen and allogeneic immune reaction (graft-versus-leukemia effect). Our results are supported by a study of Miyamoto et al,[3] who reported that the AML1/ETO fusion transcript could not be detected in 4 patients who had been in maintaining remission for more than 30 months after allogeneic BMT. In three other studies,[4-6] a total of 5 patients were analyzed by RT-PCR for the AML1/ETO fusion transcripts post-BMT. In 3 of 5 patients the AML1/ETO fusion transcript was consistently detected in the long-term follow-up, whereas 2 patients achieved a molecular remission in these studies.

These data may suggest that patients who achieved a molecular remission after allogeneic transplantation might have a better prognosis with respect to leukemic relapse than their counterparts with a persisting positive AML1/ETO PCR test. However, the detection of the AML1/ETO fusion transcript seems to have not that high of a risk of leukemic relapse after allogeneic BMT as, for example, the detection of BCR/ABL transcripts.

In absence of quantitative PCR assays, chimerism analysis using sensitive VNTR-PCR techniques might help to increase the possible predictive value of a positive AML1/ETO PCR assay in regards to leukemic relapse posttransplant. By having a lower sensitivity usually by a factor of 10^1 to 10^2, chimerism analysis by VNTR-PCR may detect a larger number of cells of host-type hematopoiesis. For example, it has thus been shown that, in patients with chronic myeloid leukemia after BMT, a mixed chimerism is associated with a higher incidence of MRD and risk of leukemic relapse.[7,8]

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Response

To our knowledge there are now reports on a total of 23 patients who have been studied for expression of the AML1/ETO transcript after allogeneic BMT.[1-7] Of these patients, 15 have been found to have persistent expression, 7 were or became negative, and 1 was not evaluable. The discrepancy most likely reflects the lack of consensus on when to score a given sample as negative. Our data suggested that the sensitivity of each assay, the amount of starting material, or the source of the material (ie, BM *v* blood) might all contribute to some of the negative results obtained.[7] For a patient to be considered negative at a given timepoint in our study, samples had to (1) be amplified in three independent experiments using 2.0 μg of total cellular RNA per reaction, (2) be succesfully amplified for the β-actin in each reaction; (3) be performed simultaneously with an RT-PCR showing a sensitivity for detection of the AML1/ETO transcript of $\geq 1 \times 10^5$ in all three reactions, and (4) assays had to be performed on blood and BM. Taken together, these data suggest that, in the majority of patients, persistent expression of the AML1/ETO is compatible with continued clinical remission and, with the reported follow-up times of up to 10 years, even cure. Recently, similar results, although not after allogeneic BMT, were reported in childhood ALL.[8]

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Which Are the Nonerythroid Cells That Constitutively Express the Duffy Antigen?

To the Editor:

The Duffy blood group antigen has generated great interest because it is the receptor for the human malarial parasite *Plasmodium vivax*, simian malarial parasite *Plasmodium knowlesi*, and a new class of chemokine receptor for several proinflammatory cytokines.[1-4] The finding that nonerythroid organs produce Duffy mRNA motivated the identification of cells that constitutively produce the Duffy protein (gp-Fy).[5] Immunohistochemical studies were performed by Hadley et al,[6] Peiper et al,[7] and by Chaudhuri et al.[8] Hadley et al[6] and Peiper et al[7] used only monoclonal antibody anti-Fy6. Chaudhuri et al[8] used anti-Fy6 and rabbit polyclonal antibody 6615. The latter is a Duffy-specific antibody reacting with the sugar moiety of gp-Fy.[8] According to Hadley et al[6] and Peiper et al,[7] endothelial cells of postcapillary venules of all organs and Purkinje cells of the cerebellum are the only nonerythroid cells that constitutively express gp-Fy. Chaudhuri et al[8] identified the same cells; however, their studies showed gp-Fy in other cell types. Thus, in kidney, the endothelium of glomeruli, peritubular capillaries, vasa recta, and the principal cells (epithelial) of collecting ducts showed expression of gp-Fy. Duffy protein was also noticed in the endothelial cells of large venules and epithelial cells (type-I) of pulmonary alveoli. In thyroid, only the endothelial cells of capillaries produced gp-Fy. In spleen, in addition to the endothelial cells of capillaries and sinusoids, which is consistent with the observations of Peiper et al,[7] endothelial cells of high endothelial venule (HEV) also produced abundant gp-Fy according to Chaudhuri et al.[8] Furthermore, ultrastructural studies performed with antibody 6615 showed that apical and basolateral plasma membrane domains, including caveolae, contained gp-Fy. This indicates that the Duffy antigen is not limited to the membrane domain lining the vessels.

Hadley and Peiper[9] challenged these findings in a recently published and well-documented review article. They disputed the specificity of rabbit polyclonal antibody 6615. However, Chaudhuri et al[8] unequivo-