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To the editor:

Identification of monoclonal B-cell lymphocytosis among sibling transplant donors for chronic lymphocytic leukemia patients

In patients with chronic lymphocytic leukemia (CLL), a family history of hematologic malignancies is recorded in 12% of cases, half of the latter being CLL.[1] First-degree relatives of CLL patients have an 8-fold greater likelihood of harboring a CLL than members of the general population.[2] Monoclonal B cells with a CLL-like immunophenotype, defined as monoclonal B-cell lymphocytosis (MBL),[3] identifiable in approximately 3% to 5% of adults with normal blood counts,[4,5] can be found in 13% of first-degree apparently unaffected relatives of CLL patients.[6]

Since January 2005 we have routinely investigated all human leukocyte antigen (HLA)–identical siblings of CLL patients candidate to an allogeneic stem cell transplantation (SCT) for the presence of a MBL by 4-color flow cytometry and polymerase chain reaction (PCR) on peripheral blood (PB) cells, as previously described.[7] Thirteen HLA-matched siblings of 13 CLL patients have been so far evaluated: 9 males and 4 females, median age 52 years (range, 34-70 years). Three cases had a relative affected by a CLL, a chronic myeloid leukemia (CML), and a non-Hodgkin lymphoma, respectively. All donors showed PB counts within the normal range, with an overall lymphocyte count of 1.99×10^9/L (range, $1.06\text{-}3.06 \times 10^9$/L).

Of the 13 siblings analyzed, 2 were found to have a B-cell clone in the PB by flow cytometry and PCR, giving an overall incidence of MBL of 15.4%. Both were males (40 and 70 years of age). The family history of the first case was positive for CML. The physical examination was normal in both individuals. The B-cell clone accounted for 74×10^9 and 77×10^9 cells/L, respectively, with a λ light chain restriction and a VH4 family usage in both cases. There was no concordance in the VH family usage between donor and patient pairs. The 2 donors were considered ineligible for a stem cell donation.

Although our series is relatively small, it is the first prospective report which identified a MBL in 15.4% of HLA-identical siblings of CLL patients eligible for an allogeneic SCT, in line with the prevalence of MBL in apparently unaffected first-degree relatives of CLL patients.[6] At present, we know that people with MBL and a lymphocytosis greater than 4000/mm^3 develop a CLL requiring treatment at a rate of 1.1% per year.[5] Little is known about the evolution over the years of a MBL with a normal lymphocyte count. Because the incidence of MBL is 100-fold greater than the incidence of CLL, it can be assumed that in most people, MBL will not progress into a CLL.[8] Nevertheless, our decision to exclude the donors with MBL from the stem cell collection was determined by the risk of transferring clonogenic B cells, the risk of evolution from MBL to CLL in the context of posttransplantation immunodepression,[9-11] and the possible availability of alternate donors.

Because the age limits of recipients and of their related donors has increased with the advent of nonmyeloablative allogeneic SCT, the likelihood of transferring premalignant hemopoietic clones with the SCT process might be expected to increase. We recommend that flow cytometry analysis of PB be added to the eligibility screening of HLA-matched siblings of patients affected by CLL and candidate to an allogeneic SCT, as has been suggested by others.[12,13] This procedure is cost-effective, due to the relatively high incidence of positive cases, the noninvasive modality of the test, and the need to collect prospective data on the clinical impact of this relatively new issue.

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To the editor:

The role of CBFβ in AML1-ETO's activity

AML1-ETO is the chimeric protein generated as a result of the t(8;21) in acute myeloid leukemia. Understanding which of the proteins that interact with AML1-ETO are essential for its activity is vital for developing targeted small-molecule AML1-ETO inhibitors. Two papers recently assessed the importance of CBFβ, the non–DNA-binding subunit of the core binding factors, for AML1-ETO's activity.[1,2] In both studies, the authors introduced amino acid substitutions at the CBFβ-binding interface of the Runt domain of AML1-ETO, and assessed the effects of these mutations on AML1-ETO's ability to confer serial replating ability to primary mouse bone marrow cells. Our group (Roudaia et al)[1] and that of Kwok et al[2] reached opposite conclusions. Roudaia et al[1] reported that CBFβ is essential for AML1-ETO's activity, whereas Kwok et al[2] claimed it is dispensable.

Roudaia et al[1] compared the effect of a single T161A mutation, which decreases the affinity of CBFβ for the Runt domain of AML1-ETO by 63-fold, with a combined Y113A/T161A mutation that decreases binding by 430 fold, on AML1-ETO's activity (Figure 1A). The side chains of both T161 and

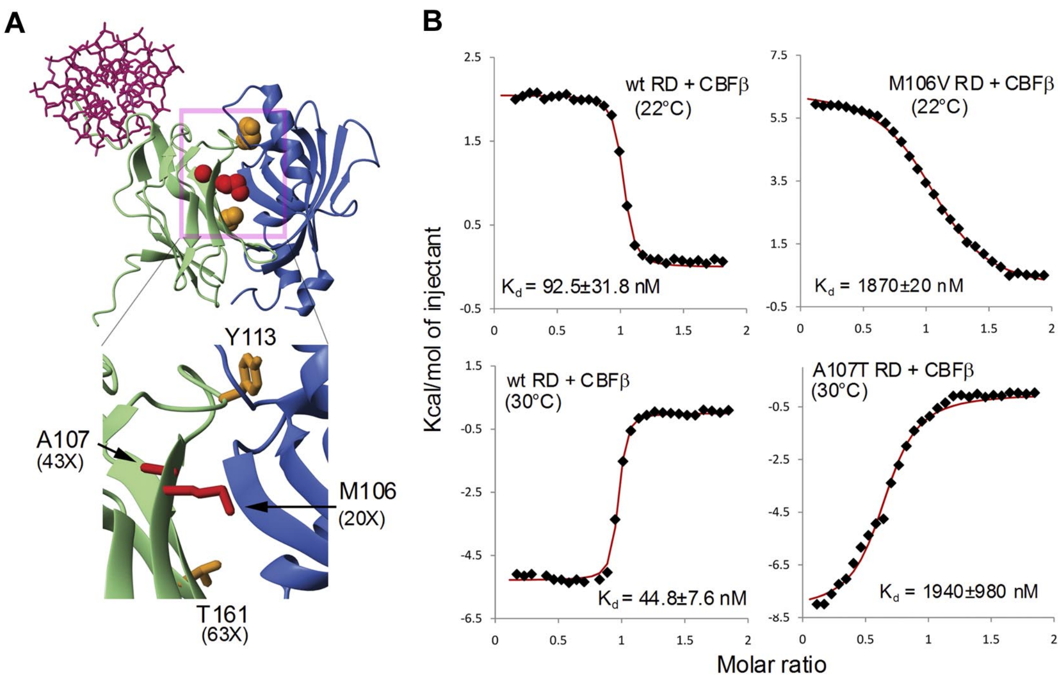

Figure 1. Location of mutations and results of binding measurements. (A) Structure of the CBFβ–Runt domain–DNA complex (PDB code 1h9d) with Runt domain amino acids mutated in the Roudaia et al (orange) and Kwok et al (red) studies indicated.[1,2] CBFβ is indicated in blue; Runt domain in green; and DNA in pink. (B) Results of isothermal titration calorimetry (ITC) measurements of the binding of CBFβ to wild-type Runt domain, M106V Runt domain, and A107T Runt domain. Measurements for the M106V mutant were carried out at 22°C, as done in the Roudaia et al study. Measurements for the A107T mutant were carried out at 30°C as a result of very low signals at 22°C for this mutant, precluding an accurate measurement. The wild-type Runt domain has also been measured at this temperature to provide an accurate comparison between the 2. The enthalpy of the reaction changes from exothermic to endothermic upon going from 22°C to 30°C, resulting in the mirror image appearance of the data for the wild-type Runt domain at the 2 different temperatures.