using the Taqman ABI Prism 7700 Sequence Detector (PerkinElmer Applied ByoSystem, Foster City, CA). In patient 2, a 2 log-decrease of the P190(BCR/ABL) transcripts, as compared to diagnosis, was detected after consolidation with ASCT. Such level remained stable for the next 12 months during IFN-α maintenance and for the successive 21 months after IFN discontinuation. In a subsequent sample collected 6 month later (6 months before hematologic relapse), a significant increase of the hybrid mRNA level was detected. In patient 1, only 2 sequential samples taken during IFN maintenance were available. Hybrid mRNA molecules levels were 2 logs below the diagnostic level.

PCR-positivity in long-term HCR has been reported very recently in 2 of 5 BCR/ABL-positive ALL patients receiving IFN-α maintenance for longer than 2 years. The significance of persistent MRD in patients in both that study and our study is presently unclear. Although a beneficial effect of IFN maintenance was hypothesized by Visani et al, we remark that 1 of our 2 cases already had prolonged HCR prior to receiving IFN and remains in HCR 2 years after IFN discontinuation. Unfortunately, only a few Q-PCR determinations were made in our cases, and so we are unable to establish whether IFN really exerted a control on the leukemic clone. Further studies are needed in order to verify the real frequency of PCR-detectable MRD in long-term remission of BCR/ABL-positive ALL. The use of prospective Q-PCR evaluation in more patients with persistently detectable BCR/ABL transcripts should provide important information on the effect of IFN or newly developed agents (ie, tyrosine kinase inhibitors) in the control of the disease.

To the editor:

Lack of SH3 domain does not imply a more severe clinical course in Ph+ chronic myeloid leukemia patients

Philadelphia-positive (Ph+) hematological malignancies are characterized by different BCR-ABL rearrangements. Three breakpoint cluster regions on chromosome 22 have been described: major (M-BCR), involving BCR exons 13 or 14 (e13 and e14, or b2 and b3) and giving origin to protein P210; minor (m-BCR), if BCR exon 1 (e1) is fused with ABL and resulting in hybrid protein P190; and micro (µ-BCR), involving BCR exon 19 (e19) and producing the rare P230. On chromosome 9 the breakpoint portion is generally positioned upstream from ABL exon 2 (a2) and only rarely upstream from exon 3 (a3).

The vast majority of chronic myeloid leukemia (CML) patients express either b2/a2 or b3/a2 transcripts; only 4 patients with M-BCR lacking ABL exon 2 (ie, b2/a3 or b3/a3) have been described. Five more cases of pediatric acute lymphoblastic leukemia with similar or m-BCR (e1/a3) hybrid gene have also been reported.

We report the cases of 2 Ph+ CML patients who express the b3/a3 fusion transcript.

The first patient is a 69-year-old male who was diagnosed as having chronic-phase CML in April 1996: Hb 13.3 g/dL, platelet count 527 × 10^9/L, WBC count 18.0 × 10^9/L (82% neutrophils, 8% eosinophils, 4% basophils, 6% lymphocytes); bone marrow biopsy showed trilineal hyperplasia with eosinophilia. Serum chemistry was normal; epatosplenomegaly was absent. The karyotype analysis showed a three-way Philadelphia translocation 46, XY, t(4;9;22)(q31;q34;q11) in 10 mitoses, and normal pattern in 2 mitoses. The patient was initially observed for 9 months, after which, due to progressive leukocytosis and thrombocytosis, hydroxyurea therapy was started (initial dose 1 g/day, then 1.5 g/day); hematological remission was obtained, which lasted for 28 months. In May 1999 WBC and platelet counts rised again, and bone marrow biopsy was substantially stable; hydroxyurea was replaced by busulfan and 6-mercaptopurine. The patient is currently alive and well, in first chronic phase.

Reverse transcriptase polymerase chain reaction (RT-PCR) analysis using BCR exon 13 (b2) and ABL exon 3 (a3) primers was performed at diagnosis: the result was a fragment 174 bases shorter than b2/a2 and 100 bases shorter than b3/a2. This suggested a possible deletion of ABL exon 2, resulting in a b3/a3 junction, which was confirmed sequencing the target band.

The second patient is a 51-year-old male with a diagnosis of chronic-phase CML in July 1989: Hb 15.1 g/dL, platelet count 566 × 10^9/L, WBC count 19.9 × 10^9/L (circulating metamyelocytes); karyotype analysis showed a classic Philadelphia translocation 46, XY, t(9;22)(q34;q11), whereas molecular studies were not performed. After 3 months interferon (IFN) therapy was started, obtaining in 11 months a major cytogenetic response (70% Ph− metaphases). IFN was withdrawn, and autologous bone marrow...
transplantation (ABMT) was performed. Two months later karyotype analysis showed 70% Ph1 cells, and IFN was resumed at 9 MU/day; 16 months later (April 1992) major cytogenetic response (73% Ph1) was achieved. The status was maintained (last karyotype analysis performed in January 2000), with a complete cytogenetic response (0% Ph1 metaphases) since January 1997. The daily IFN dose was progressively reduced, down to the current dose of 3 MU/week.

Evaluation of BCR-ABL transcript was firstly performed in June 1996 (83 months after diagnosis), in major cytogenetic response: following the same technique described above, presence of a b3/a3 junction was revealed. Three more samples, collected between January 1997 (first evidence of complete cytogenetic response) and June 1999, were negative for BCR-ABL rearrangement, both at first and second step of the “nested” RT-PCR. The last sample (January 2000) is weakly positive, indicating the recurrence of the same b3/a3 transcript.

ABL exon 2 encodes for part of the SH3 domain, which is generally considered to play a negative regulatory role for the tyrosine kinase activity (SH1 region). But recent data suggest that, although “in vitro” SH3 is not required in the BCR-ABL protein for growth factor–independent proliferation, inhibition of differentiation, and protection from apoptosis, “in vivo” lack of SH3 reduces the leukemogenic potential. In addition, proliferation rate and binding capability to extracellular matrices is lower in SH3-deleted BCR-ABL expressing hematopoietic cells than in those with wild-type BCR-ABL.6

The long-lasting and quite indolent disease history of these 2 patients, supported by the evidence of a complete cytogenetic and molecular response obtained with IFN therapy in patient 2, are in agreement with the data of this study and do not confirm the hypothesis of a possible correlation between the lack of ABL exon 2 in BCR-ABL rearrangement and a more aggressive form of CML, as described by others.2,3,5,7

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**References**


To the editor:

**Levels of circulating CD19+ cells in patients with multiple myeloma**

Clonotypic B cells at different stages of differentiation are present in patients with multiple myeloma (MM). But the nature and frequency of the less mature clonotypic B cells expressing CD19 is a standing controversy in myeloma research.1,2

Currently, the numbers of CD19+ cells are determined by flow cytometry using monoclonal antibodies (MoAbs) specific for the CD19 surface molecule. It has been suggested that the controversy concerning the levels of CD19+ cells in the peripheral blood (PB) of MM patients stems from the use of different CD19 MoAbs that recognize different epitopes with a heterogeneous pattern for clonal and polyclonal B-cells.2,3 In addition, it has been reported that the CD19 epitope defined by the Leu-12 MoAb (Becton Dickinson Immunocytometry Systems, San Jose, CA), not normally recognized on these clonal cells, is exposed when they are treated with neuraminidase.3

The frequency of CD19+ cells in MM patients have become clinically important because clonal CD19+ cells may contain the drug-resistant compartment in myeloma,4 and the CD19+ cell levels at diagnosis have been shown to be a prognostic factor.5 We therefore investigated whether the CD19 MoAbs B4 (Coulter Clone, Miami, FL), Leu-12, and FMC63 (Serotech, Oxford, England) recognized different epitopes and whether these epitopes displayed a different pattern on B cells from healthy donors when compared to MM patients.

In brief, 3 different CD19 double stainings were used. The 2 MoAbs were added together; cells were incubated for 30 minutes with MoAb-1, followed by the addition of MoAb-2; and cells were incubated for 30 minutes with MoAb-2, followed by the addition of MoAb-1. With each experiment, single staining of the actual MoAbs and an isotype-matched control were performed. All combinations of MoAbs including the FITC and the PE-conjugated MoAbs were tested in this setting. For all combinations maximum fluorescence intensity was obtained for both CD19 MoAbs in at