Hyperdiploidy is reported in 10% to 20% of childhood ALL, and seems to be associated with good prognosis; it is a rare finding in AML. No report on the association between hyperdiploidy and dysplasia is present in the literature; in our patient, hyperdiploidy and dysplasia were probably expressions of different phenomena. Dysplasia was probably a late effect of the previous chemotherapy: it was absent at diagnosis, and persisted at relapse even after disappearance of hyperdiploidy. The absence of BM blastosis at cytogenetic relapse is puzzling. We may surmise that in our patient the hyperdiploid clone was not the actual leukemic clone, but rather a preleukemic expansion of an (n-1) population from which leukemia emerged. Even if this is the case, the immunological control of the (n-1) clone may be of paramount importance in preventing leukemia relapse. Finally, it is possible that different forms of leukemia may have different sensitivity to an immunological treatment, depending essentially on cell-surface alterations that can be recognized by activated lymphocyte subsets. In this setting, a hyperdiploid clone might be a better target for an immunological attack, because cells may have an increased number of surface molecules. Thus, it may well be that immunologic treatment of a low-burden acute leukemia has selected indications, which are still to be defined.

Expression of AC133, a Novel Stem Cell Marker, on Human Leukemic Blasts Lacking CD34− Antigen and on a Human CD34+ Leukemic Cell Line: MUTZ-2

To the Editor:

A novel hematopoietic stem and progenitor cell marker, monoclonal antibody (MoAb) AC133, was recently published by Miraglia et al1 and Yin et al2 in the December 15, 1997 issue of Blood. AC133 recognizes only CD34 bright and CD38− subsets of human progenitor cells including colony-forming unit granulocyte macrophage (CFU-GM) needed for short-term engraftment and probably the severe combined immunodeficient (SCID) mouse repopulating cell.3 Investigators could detect AC133 in a majority of CD34+ cases of acute leukemia, suggesting that it may be an early marker for human progenitor cells. In contrast, they could not detect AC133 in the CD34− leukemic blasts of 1 patient with acute myelogenous leukemia (AML) or in any of 5 human AML cell lines, although expression was found in 3 nonhematopoietic cell lines of human origin.

We investigated several human cell lines by multiparameter immunophenotyping using AC133-PE (Miltenyi, Bergisch Gladbach, Germany) and CD34-Cy5, CD90-FTIC (Immunotech, Marseille, France). We found that a majority of cells of MUTZ-2 coexpressed CD34 and AC133 (Fig 1). Mutz-2 is a cell line derived from AML and displays an FAB:M2 morphology.4 This is the first known hematopoietic cell line expressing AC133, representing a possible additional tool for experiments concerning the function of the AC133 receptor.

We also characterized expression of CD34 and AC133 in bone marrow blasts from 10 patients with AML. None of the six patients with a pure CD34− blast population had detectable levels of AC133+ blasts. On the other hand, we saw a 53-year-old male AML patient with blasts of FAB:M4 morphology and a high percentage of bone marrow infiltration (>90% leukemic blasts) who had a mixed population of CD34− and CD34+ blasts (Fig 2). As expected, we found coexpression of CD34 and AC133 in 44% of the blast population. Only a very small population of leukemic blasts was AC133+ and CD34+. Interestingly, we could find a significant blast population (18%) in which expression of AC133 was not accompanied by expression of CD34 or CD90 (Thy-1). This first report of a CD34−CD90−AC133+ leukemic blast population could represent clonal diversity of the blast population. On the other hand, it may suggest a possible differentiation pathway, wherein CD34−CD90−AC133+ cells give rise to CD34+AC133+ cells. Alternatively, the CD34−AC133+ blasts could be progenitors of the CD34+AC133+ cells seen in this patient. Therefore, our finding of a CD34−AC133+ blast population raises questions about the significance of AC133 expression in AML. A more detailed investigation of the role of AC133 in AML differentiation pathways needs to be done.

REFERENCES
Fig 1. A MUTZ-2 cell sample was stained with CD34(PE-Cy5), AC133(PE) as well as isotype control antibodies conjugated with PE-Cy5 or PE. (Top left) The gate on forward versus side scatter (1) contained the leukemic blast cell population (10% probability dot plot). (Top right) Expression of CD34 and AC133 on gated (R1) cells is shown. (Bottom left) The pattern of IgG-PE-Cy5 and IgG-PE isotype MoAb expression on the R1 population.

Fig 2. A bone marrow sample (53-year-old male AML:M4) was stained with CD34(PE-Cy5), AC133(PE), CD90 (FITC) as well as isotype control antibodies conjugated with PE-Cy5, PE, FITC. (Top left) The gate on forward versus side scatter (1) contained the leukemic blast cell population (10% probability dot plot). (Top right) Expression of CD34 and AC133 on gated (R1) cells is shown. (Bottom right) Expression of CD90 in R2 (dotted line) versus R3. (Bottom left) The pattern of IgG-PE-Cy5 and IgG-PE isotype MoAb expression on the R1 population.
Deletions in the Long Arm of Chromosome 10 in Lymphomas With t(14;18): A Pathogenetic Role of the Tumor Suppressor Genes PTEN/MMAC1 and MXI1?

To the Editor:

The translocation t(14;18)(q32;q21), characteristic for follicular lymphomas and a subset of diffuse large cell lymphomas, juxtaposes the bcl-2 proto-oncogene with the Ig heavy-chain (IgH) gene. This results in deregulation of the bcl-2 gene expression and elevation of BCL-2 protein, which protects the cells against induction of programmed cell death and, thus, confers a survival advantage leading to immortalization of t(14;18)-carrying cells. The acquisition of additional chromosomal aberrations is necessary for the malignant transformation and clonal progression of BCL-2 overexpressing lymphocytes. These secondary changes can be observed by karyotyping in more than 90% of lymphomas with t(14;18).1,2

By karyotyping a series of 201 lymphomas at the Department of Human Genetics in Kiel, we detected deletions in the long arm of chromosome 10 in 6 of 57 (11%) B-cell lymphomas with a t(14;18), but in only 1 of 144 (1%) lymphomas without a t(14;18). Thus, loss in 10q seems to be a characteristic secondary abnormality for t(14;18)-positive lymphomas (P < .001). The frequency of del(10q) in t(14;18)-positive lymphomas observed in our series are in line with other cytogenetic studies reporting abnormalities of the long arm of chromosome 10 in 7 of 66 (11%) and 9 of 75 (12%) follicular lymphomas.3

The common region of cytogenetic loss in our series encompassed the chromosome band 10q24, suggesting the existence of a tumor suppressor gene in 10q involved in the pathogenesis of follicular lymphomas. Thus, the common region of molecular cytogenetic loss comprises a region proximal to the MXII gene, but encompassing the PTEN/MMAC1 locus.

As to the consistent loss of one allele of the PTEN/MMAC1 gene in the lymphomas with 10q-deletions according to our FISH study we further investigated the role of this gene by complete sequencing of both strands of all nine exons as described by Steck et al4 with slight modifications. For these analyses, genomic DNA from primary lymphoma tissue was available for six patients shown to contain loss of one PTEN/MMAC1 allele by FISH. Additionally, a further t(14;18)-positive primary lymphoma not evaluable by FISH but containing a karyotypically detectable deletion in 10q and the cell line Karpas 422 were included. Except for well-known polymorphisms, no alterations of the PTEN/MMAC1 gene were detected in any of the primary lymphomas. Nevertheless, the cell line Karpas 422 was found to contain a splice acceptor site mutation (A → C) at position −2 of exon 3 of the PTEN/MMAC1 gene (Fig 1B), which was confirmed by sequencing a second sublineage of this cell line. Remarkably, the same mutation has been recently described in a primary glioblastoma.5 To determine the pathogenetic relevance of this alteration, reverse transcriptase-polymerase chain reaction (RT-PCR) analysis was performed on Karpas 422 with primers surrounding exon 3.6 This failed to amplify the expected germline 397-bp fragment but showed a shorter PCR product, which, by sequencing, was shown to be a splice variant caused by skipping of the 45-bp sized exon 3 (Fig 1C). Thus, the intact PTEN/MMAC1 transcript is completely absent in Karpas 422 due to deletion of the first and mutation of the second allele. The truncated transcript lacks part of conserved protein domain with high homology to tensin and auxillin and thus can be assumed to lack activity.7

In summary, our data provide evidence that deletions in the long arm of chromosome 10 are recurrent secondary changes in t(14;18) lymphomas. As to our FISH analyses, the distal border of the interstitial deletions in most cases is proximal to the MXII gene, rendering this gene as well as the candidate gene DMBT1, which is localized more distally in 10q25.3-26.1,13 highly unlikely as tumor suppressors in germinal center lymphomas. We detected consistent loss of the PTEN/MMAC1 gene by FISH in the cases investigated. Nevertheless, a mutation leading to inactivation of the second allele has been detected only in the cell line Karpas 422 but not in primary lymphomas. Inactivating mutations have also been described in a series of other cell lines derived from hematological neoplasms.12,14,15 In accordance with our results, alterations of the PTEN/MMAC1 have been reported by two groups to occur only in a small minority of unselected primary lymphoid malignancies. Grundbæk et al14 reported mutations of the PTEN/MMAC1 in 2 of 170 and Sakai et al15 in 1 of 42 primary lymphoid malignancies. Considering the scarcity of PTEN/MMAC1 mutations in primary lymphoid malignancies, the existence of a thus far unknown tumor suppressor gene in 10q involved in the pathogenesis of at least germinal center lymphomas might be assumed.
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