Mll Cleavage Occurs in Approximately 5% of De Novo Acute Myeloid Leukemia, Including in Patients Analyzed Before Treatment Induction

To the Editor:

Aplan et al recently reported a site-specific DNA cleavage induced by topoisomerase II (Topo II) inhibitors within the Mll gene breakpoint cluster region on chromosome 11q23. The finding was initially identified by Southern blot analysis of circulating blasts taken from a case of T-cell acute lymphoblastic leukemia (T-ALL) 16 hours after the induction of multiagent chemotherapy, including doxorubicin, a known inhibitor of Topo II. The investigators subsequently reproduced the same pattern of DNA fragmentation by in vitro culture of malignant cell lines with doxorubicin and etoposide, showing that cleavage was not restricted to the T-lymphoid lineage because it was observed in B-lymphoid, myeloid, and nonhematopoietic malignant cell lines and even in the peripheral blood lymphocytes of normal individuals. They suggested that Topo II inhibitor induced Mll cleavage correlated with sensitivity to epidophyllotoxins and that lymphoid lines may be more sensitive to such cleavage, because it was observed in 4 of 6 malignant T-

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cell lines and 3 of 4 B-cell lines, but in only 1 of 6 myeloid cell lines and 1 of 6 solid tumor cell lines. We show here that an identical MLL DNA cleavage occurs relatively frequently in de novo AML and that it is not restricted to cases analyzed after induction with Topo II inhibitors.

Having demonstrated that MLL rearrangements occur frequently in de novo acute myeloid leukemia (AML) of diverse French-American-British (FAB) subtypes, we have instituted routine screening for MLL rearrangement for patients included in the EORTC/GIMEMA AML 10 trial (No. 06931) by Southern blot hybridization of BamHI and HindIII-digested DNA with an MLL cDNA probe, B859, encompassing exons 5 through 13 (kindly provided by Giuseppe Cimino, La Sapienza, Rome, Italy). Briefly, this trial includes patients 15 to 60 years of age with untreated, newly diagnosed AML of all FAB subtypes other than M3. Seventy clinical centers from 9 countries submit patients to AML 10, but the current molecular analysis is restricted to data from the French centers, which represent approximately 10% of patients. Parallel molecular analyses are performed on Dutch, Belgian, and Italian patients. Patient recruitment for the French centers started in January 1994 and prospective molecular screening began in January 1996. All patients with cryopreserved material obtained between January 1994 and January 1996 were studied retrospectively. MLL status was assessed by Southern blotting in 62 of 96 (65%) patients from the French centers included in the AML 10 protocol between January 1994 and April 1996. Whereas 9 of 62 (15%) of patients were shown to have major MLL rearrangements, a further 3 (5%) patients showed minor, biallelic rearranged bands with both enzymes, with identically sized BamHI fragments to those identified by Aplian et al.1 The intensity of the rearranged bands corresponded to approximately 5% to 10% of the DNA (Fig 1). Further analysis of EcoRI and Bgl II digested DNA (Fig 1) from 2 of these 3 cases with the B859 cDNA and an intron 9 genomic probe, 1,3 showed that the MLL breakpoint localized to the 2-kb EcoRI-Bgl II genomic fragment containing exons 9 and 10 of the breakpoint cluster region, with the size of the rearranged fragments identified (Table 1) corresponding exactly to the breakpoint putatively localized to a Topo II consensus recognition sequence 1,470 bp 5' of the BamHI site at the 3' extremity of the breakpoint cluster region.1 Both rearranged bands were of equivalent intensity in patients no. 593 and 722 (≈10%; Fig 1 and data not shown), whereas the 5' fragment was relatively weak in Bgl II digests from patient no. 975, with the 3' fragment more clearly visible when probed with B859 (Fig 1). The amount of cleaved DNA was lower in this patient and no clear rearranged band could be discerned with the 300-bp genomic 19 probe. BamHI analysis of patient no. 593 showed 2 further minor rearrangements of approximately 4.3 and 4 kb (Fig 1), which are compatible with a second cleavage site, but these bands were not detected with other digests and may therefore represent star activity. The possible presence of a minor translocation leading to transcription of one of the common AML fusion transcripts was analyzed in patient no. 593, but RT-PCR analysis failed to show MLL-AF6, MLL-AF9, MLL-ENL, MLL-ELL, or MLL duplication (data not shown).

In contrast to the T-ALL reported by Aplian et al,1 samples from all 3 patients were taken before the induction of chemotherapy. Clinical and biologic details of the patients are shown in Table 1. All 3 were men and presented with monocytic M4/S AML, in which karyotypic 11q23 abnormalities are found most frequently.4 Questioning did not show any prior malignancy or chemotherapy and there was no apparent exposure to toxic agents. Cytogenetic analysis was normal in 2 patients and the third demonstrated an isolated trisomy 8. The only finding of possible relevance was the presentation white blood cell (WBC) count, because both patients with easily detectable MLL cleavage had marked leukocytosis at diagnosis. The original case described by Aplian et al also showed a high presentation blast count, as is commonly found in T-ALL.

We therefore show that 5% of patients with AML demonstrate MLL cleavage in a minor proportion of blasts tested before the induction of chemotherapy. This observation has obvious significance for the interpretation of Southern blots in routine molecular diagnostic screening, when it is conceivable that a proportion of apparent MLL rearrangements may in fact be MLL cleavage. It is also interesting with regard to the known capacity of Topo II inhibitors to induce secondary leukemias with MLL rearrangement, as discussed by Aplian et al.1 It would obviously be interesting to determine whether MLL cleavage is more common in relapsed AML. Our data also suggest that MLL cleavage may occur preferentially in cases with a rapid cell doubling time. This intrinsic propensity may be further accentuated by treatment with Topo II inhibitors.

Routine molecular screening of AML at diagnosis, as undertaken in the EORTC/GIMEMA AML 10 trial, will allow us to determine whether MLL cleavage has prognostic significance, particularly with regard to secondary malignancies, provided that this particular Southern blot profile is recognized.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>FAB</th>
<th>WBC</th>
<th>% Age Blasts</th>
<th>Cytogenetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>593</td>
<td>35/M</td>
<td>M5</td>
<td>104</td>
<td>96</td>
<td>46,XY [25]</td>
</tr>
<tr>
<td>722</td>
<td>38/M</td>
<td>M4</td>
<td>160</td>
<td>80</td>
<td>46,XY[11]/47,XY, +8 [39]</td>
</tr>
<tr>
<td>975</td>
<td>55/M</td>
<td>M4</td>
<td>23.5</td>
<td>42</td>
<td>46,XY [20]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Restriction Enzyme</th>
<th>Genomic Fragments (kb)</th>
<th>Topo II Inhibitor (kb)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BamHI</td>
<td>8.3</td>
<td>6.7, 1.5</td>
</tr>
<tr>
<td>HindIII</td>
<td>15</td>
<td>12.5, 2.5</td>
</tr>
<tr>
<td>Bgl III</td>
<td>3.1, 6</td>
<td>3.1, 4.6, 1.4</td>
</tr>
<tr>
<td>EcoRI</td>
<td>4.6, 5, 4</td>
<td>4.6, 5, (0.7), 3.3</td>
</tr>
</tbody>
</table>

* The 3' fragment identified by the 19 genomic probe is underlined. The 0.7-kb fragment in EcoRI digests is unlikely to be detected with a cDNA probe in view of its size and limited exon content.
Correspondence

To the Editor:

The role of unrelated donor bone marrow transplantation (BMT) in the treatment of leukemia has been an area of controversy for some years. Although there has never been any doubt that occasional patients are cured by this procedure, sometimes at advanced stages of their disease, the place of such transplants in the routine management of children and adults with leukemia has not been defined clearly. To address the current problems in this area and to identify, if possible, areas of future development, a Consensus Conference was organized. This conference brought together experts in the management of leukemia, using both conventional and transplantation techniques in adults and children, health economics, and medical ethics. New concepts such as the use of umbilical cord blood cells and immunotherapy were also addressed.

A panel, under the chairmanship of Prof. E.C. Gordon-Smith (St. George's Hospital, London, UK) was convened to hear the presentations and to prepare a consensus statement. The panel members (see below) included an expert in BMT, a general hematologist, a pediatrician, patient advocates, and a medical journalist. The panel was asked to consider, if possible, four questions about unrelated donor BMT. These were to identify the current indications for such transplants in adults and children, to consider what was appropriate in terms of donor care, and to consider what studies might contribute to improved evaluation of the procedure. In addition to the presentations given at the conference, the panel also had received in advance the four background papers published subsequently in Blood Reviews. These papers were sent to referees for comment and were amended in the light of these comments before being distributed to the panel members.

During the 2-day conference, the Consensus Panel produced the Consensus Statement, which follows. In addition to making recommendations about how published reports on unrelated donor BMT should present results, the statement makes strong recommendations with regard to the introduction of molecular typing at both HLA class I and II to ensure optimal matching between donor and recipient and, in addition, proposes that current differences be removed between the management of family and unrelated donors with regard to the use of hematopoietic growth factor mobilized peripheral blood stem cells (PBSC).

The conclusions and statements are based largely but not exclusively on information provided at the Consensus Conference.

CONSENSUS CONFERENCE ON UNRELATED DONOR BMT

OCTOBER 29-30, 1996

Its Use in Leukemias and Allied Disorders

BMTs from unrelated donors for leukemias are increasing greatly in number and also in proportion to matched sibling donor transplants. The panel has considered unrelated donor transplant (UD-BMT) on the basis of efficacy, toxicity, and indications in leukemias. The conclusions and statements are based largely but not exclusively on information provided at the Consensus Conference.

Efficacy

(1) Unrelated BMTs for some types of leukemia can produce prolonged quiescence and, in some cases, eradication of disease.

(2) Data based on serologically matched donors at HLA A,B and DR suggest that matched unrelated transplants may have similar survival to sibling transplants in comparable disease states. This is accepted as a reasonable statement but begs the question of what is implied by matched in unrelated transplants. Much of the data concerning the survival and toxicity in unrelated transplants have come from studies using serologic typing. The effect of molecular typing on outcome may alter indications.

(3) Information on the place of sibling transplants compared with chemotherapy and autologous transplants in the management of some leukemias has been provided by randomized studies organized by the EORTC and the MRC. These studies define the place of sibling BMT in the management of acute leukemias. Conclusions drawn from these studies on the presence or absence of benefit of sibling transplants may apply to unrelated transplants.

(4) In a few situations, the evidence for efficacy is based on the level 1 documentation of zero survival after conventional therapy but with some survivors after transplant (eg, childhood acute lymphoblastic leukemia [ALL] with early bone marrow relapse). However, in situations in which alternative therapies occasionally succeed, level 1 evidence from randomized trials is rarely available to help in decision making.

(5) There is variation in outcome reported from different sources for particular conditions. In part, this may be because subdivisions of different types of leukemia are not always accurately defined.

References

1. Aplan PD, Chervinsky DS, Stanulla M, Burhans WC: Site-specific DNA cleavage within the MLL breakpoint cluster region induced by Topoisomerase II inhibitors. Blood 87:2649, 1996

