mia during follow-up between 5 months and 4 years. The other 60 patients remained negative in GBV-C PCR.

One important route of GBV-C transmission is blood products (blood plasma and erythrocyte and thrombocyte concentrates). Therefore, we examined the number of blood products administered to these patients in connection with liver transplantation. We did not find a significant difference between the number of blood products administered to the 42 patients who acquired GBV-C infection perioperatively (median, 142 U) and the 60 patients without GBV-C infection (median, 137.5 U).

From our results we conclude that GBV-C infection is not linked to fulminant hepatitis. Our data further show that patients who undergo liver transplantation are at high risk for acquiring GBV-C infection. The prevalence after transplantation was 41.2%, in contrast to 6.4% before transplantation. Transfusion-transmitted GBV-C infection has to be assumed due to the great number (median, 140 U) required for liver transplantation. This is in agreement with previously published data in patients with hematologic malignancy.

Clinical studies are already in progress to evaluate the clinical impact of GBV-C infection in liver transplant recipients.

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### 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-

![Fig 1. Southern blot analysis of BamHI-, EcoRI-, and BglII-digested DNA from patients no. 593 and 975, compared with a placenta negative control (NC). The sizes (in kilobases) of the germline bands are indicated by arrows and the rearranged bands as lines. The 2 faint ~4- and ~4.3-kb bands in the BamHI digest from patient no. 593 are probably due to star activity, because they are not seen in other digests.](image-url)
cDNA probe in view of its size and limited exon content.

zymes, with identically sized
January 1994 and January 1996 were studied retrospectively. MLL T-ALL.
hybridization of
in de novo acute myeloid leukemia (AML) of diverse French- scripts was analyzed in patient no. 593, but RT-PCR analysis failed
induction with Topo II inhibitors. sent star activity. The possible presence of a minor translocation
identical MLL DNA cleavage occurs relatively frequently in de 1), which are compatible with a second cleavage site, but these
lines and 1 of 6 solid tumor cell lines. We show here that an
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, 3 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 11 3 (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 Janu-
ary 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 en-
zymes, with identically sized BamHI fragments to those identified
by Aplan 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, B9, 2 showed
that the MLL breakpoint localized to the 2.5-kb EcoRI-Bgl II geno-
mic 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 repre-
sent star activity. The possible presence of a minor translocation
leading to transcription of one of the common AML fusion tran-
scripts 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 Aplan 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 M4/5 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
diagnosis. The original case described by Aplan et al 1 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 signifi-
cance 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 inhibi-
tors to induce secondary leukemias with MLL rearrangement, as
discussed by Aplan 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 prefer-
entially 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.

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P. Bourquelot
D. Leboeuf

Table 1. Clinical (A) and Molecular (B) Characteristics of AML Patients With MLL Cleavage

<table>
<thead>
<tr>
<th>Restriction Enzyme</th>
<th>Germline Fragments (kb)</th>
<th>Fragments Induced by 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, 7.5</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.
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).

Consensus Conference on Unrelated Donor Bone Marrow Transplantation: Royal College of Physicians of Edinburgh, October 29th and 30th, 1996

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.

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