Loss of Mismatched HLA in Leukemia after Stem-Cell Transplantation

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BACKGROUND
Transplantation of hematopoietic stem cells from partially matched family donors is a promising therapy for patients who have a hematologic cancer and are at high risk for relapse. The donor T-cell infusions associated with such transplantation can promote post-transplantation immune reconstitution and control residual disease.

METHODS
We identified 43 patients who underwent haploidentical transplantation and infusion of donor T cells for acute myeloid leukemia or myelodysplastic syndrome and conducted post-transplantation studies that included morphologic examination of bone marrow, assessment of hematopoietic chimerism with the use of short-tandem-repeat amplification, and HLA typing. The genomic rearrangements in mutant variants of leukemia were studied with the use of genomic HLA typing, microsatellite mapping, and single-nucleotide–polymorphism arrays. The post-transplantation immune responses against the original cells and the mutated leukemic cells were analyzed with the use of mixed lymphocyte cultures.

RESULTS
In 5 of 17 patients with leukemia relapse after haploidentical transplantation and infusion of donor T cells, we identified mutant variants of the original leukemic cells. In the mutant leukemic cells, the HLA haplotype that differed from the donor’s haplotype had been lost because of acquired uniparental disomy of chromosome 6p. T cells from the donor and the patient after transplantation did not recognize the mutant leukemic cells, whereas the original leukemic cells taken at the time of diagnosis were efficiently recognized and killed.

CONCLUSIONS
After transplantation of haploidentical hematopoietic stem cells and infusion of donor T cells, leukemic cells can escape from the donor’s antileukemic T cells through the loss of the mismatched HLA haplotype. This event leads to relapse.
TRANSPANTATION OF HEMATOPOIETIC stem cells from a haploidentical family do-
nor who shares only one HLA haplotype
with the recipient is a potentially curative option for
patients with high-risk hematologic cancers who
lack an HLA-matched donor. The major limita-
tion of this strategy is the risk of severe graft-ver-
sus-host disease (GVHD), which can result from
alloreactions mediated by donor T cells against the
recipient’s unshared HLA haplotype. Since the pub-
lication of studies on extensively T-cell–depleted
grafts, a variety of strategies have been developed
to prevent or control GVHD after transfer of haplo-
identical T cells. The feasibility and efficacy of
infusions of haploidentical donor T cells have been
established and new immunosuppressive drugs
have allowed for the transplantation of haploidenti-
cal grafts without depleting them of T cells.

The infusion of donor T cells promotes rapid
reconstitution of the immune system after trans-
plantation. In addition, the graft-versus-leukemia
effect mediated by such infusions is an effective
form of immunotherapy for hematologic cancers.
However, relapses still occur, and the mechanisms
involved in such relapses remain elusive.

Genomic or phenotypic alterations of HLA and
the antigen-presenting machinery are frequent-
ly observed in patients with solid tumors. Studies in animal models have shown that these
phenomena can be the direct consequence of
selective pressure mediated by T cells. Moreover,
loss of HLA class I surface antigens has been
described in patients with melanoma after
a partial response to cellular immunotherapy. Con-
versely, alterations involving HLA are rare at
the time of diagnosis in patients with hematologic cancers.

Here we show that genomic loss of the re-
cipient’s mismatched HLA haplotype, which in
principle is targeted by donor T cells, can occur
in the leukemic cells of patients who have under-
gone transplantation of haploidentical he-
matopoietic stem cells. We suggest that this
phenomenon is a mechanism of tumor escape
from the selective pressure of a patient-specific
graft-versus-leukemia reaction.

METHODS

PATIENTS AND TRANSPLANTATION PROCEDURE
We retrospectively identified adults with hemat-
ologic cancers who had undergone one or more
haploidentical hematopoietic stem-cell trans-
plantations at the San Raffaele Hospital in Milan
between 2002 and 2007 and in whom immune
reconstitution (defined as an absolute CD3+ cell
count >100 per cubic millimeter) had been
achieved after infusion of donor T cells. All 43
patients who fulfilled these criteria were includ-
ed in the study. All had high-risk hematologic
myeloid cancers (36 with acute myeloid leukemia
and 7 with high-risk myelodysplastic syndrome);
26 patients underwent one transplantation, and
17 underwent more than one. Of these 43 pa-
tients, 25 had refractory or relapsing disease at
the time of transplantation. In all patients, the
conditioning regimen for the first haploidentical
hematopoietic stem-cell transplantation was my-
eloablative. Eight patients received melphalan
(140 mg per square meter of body-surface area),
thiotepa (13 mg per kilogram of body weight),
fludarabine (200 mg per square meter), and ant-
thymocyte globulin (ATG) (Fresenius) (25 mg per
kilogram). Thirty-five patients received treosul-
phane (42 g per square meter), fludarabine (150 mg
per square meter), ATG (25 mg per kg), rituximab
(500 mg), and total-body irradiation (200 cGy).
The median dose of CD34+ cells was 10.2×10^6
per kilogram (range, 2.1×10^6 to 15.5×10^6). Twenty-two
patients received donor T cells in one or more in-
fusion after transplantation of CD34+ purified
hematopoietic stem cells (median total T-cell dose,
10×10^6 per kilogram; range, 0.01×10^6 to 90×10^6;
median time of first infusion, 43 days after
transplantation; range, 14 to 61). No prophylaxis
against GVHD was administered to those patients
either after transplantation or after the T-cell in-
usions. The remaining 21 patients received an
infusion of donor T cells with the stem-cell
graft (median total T-cell dose, 438×10^6 per
kilogram; range, 83×10^6 to 796×10^6). All 21 patients
received prophylaxis for GVHD — 12 patients
received 15 mg of methotrexate per square meter
for 3 days plus 2 mg of intravenous cyclosporine
per kilogram per day, and the remaining 9 pa-
tients received 15 mg of mycophenolate per kilo-
gram three times a day plus sirolimus (at a start-
ing dose of 4 mg per day, which was adjusted to
achieve a target serum concentration of 8 to 15
mg per milliliter). All participants gave written
informed consent in accordance with the proto-
cols approved by the local ethics committee.

CHIMERISANALYSIS
Hematopoietic chimerism was assessed monthly
in samples of bone marrow aspirate with the use
of short-tandem-repeat amplification and genomic HLA typing in parallel, as previously reported [20] (also detailed in the Supplementary Appendix, available with the full text of this article at NEJM.org). For Patients 7, 16, and 43, the analyses were also performed on leukemic blasts purified by a fluorescence-activated cell-sorter (FACS). Results were always compared with those obtained from donor and patient cells before transplantation, which were used as reference controls.

**Loss of Heterozygosity**

We studied loss of heterozygosity and copy-number variations with the use of polymerase-chain-reaction amplification of 12 highly polymorphic short-tandem-repeat markers spanning the entire length of chromosome 6 and the use of the Illumina Human CNV370-Quad BeadArray or the Affymetrix Human SNP Array 6.0 single-nucleotide-polymorphism (SNP) array. (Details of these methods are provided in the Supplementary Appendix.) For Patients 7, 16, and 43, short-tandem-repeat mapping and SNP analysis were performed on FACS purified leukemic blasts, whereas for Patients 13 and 33, only samples of bone marrow aspirate containing leukemic blasts were available.

**In Vitro Evaluation of Graft-versus-leukemia Effect**

With the use of Ficoll–Hypaque centrifugation, we separated peripheral-blood mononuclear cells obtained from the stem-cell donor for Patient 16, from Patient 16, 85 days after the first hematopoietic stem-cell transplantation and 96 days after the second transplantation, and from a healthy HLA-mismatched subject. For the cells obtained under each of these conditions, 5×10^7 cells were used and plated with 5×10^6 irradiated mononuclear cells (radiation dose, 3000 rad) taken from Patient 16 at the time of diagnosis of leukemia (30% blasts) in 1 ml of Iscove’s Modified Dulbecco’s medium, supplemented with 10% human serum and 300 IU per milliliter of recombinant human interleukin-2. New medium was added to the cultures every 2 to 3 days, and responder cells were rechallenged with the original stimulator cells at a 1:1 ratio every 10 days. The function of responder cells from the mixed lymphocyte culture was tested after each stimulation with the use of 51Cr-release, enzyme-linked immunospot (ELISpot) and [3H]thymidine-incorporation assays (for details, see the Supplementary Appendix); the target cells were leukemic blasts obtained from Patient 16 at the time of diagnosis or when loss of the patient-specific HLA haplotype was documented.

**Results**

**Clinical Observations**

Studies of donor–host hematopoietic chimerism were carried out monthly after transplantation in all 43 patients with the use of short-tandem-repeat amplification and HLA typing in order to look for a reappearance of the host hematopoiesis in the bone marrow, which often predicts relapse. [20] Among the 43 patients, 17 patients — 14 of whom received transplants when they had persistent disease — had a leukemia relapse. In all 17 patients, relapse was confirmed to be of host origin on the basis of short-tandem-repeat chimerism. Surprisingly, in five of these patients, genomic HLA typing of bone marrow cells did not detect host-specific HLA alleles (Fig. 1). In all five patients (Table 1), the leukemic cells at the time of relapse had the same immunophenotype and the same cytogenetic features found at diagnosis, and no new cytogenetic abnormalities were observed. Patient 7 and Patient 43 had GVHD at the time of leukemia relapse (consensus grade 2.
Loss of Mismatched HLA in Leukemia after Stem-Cell Transplantation

**Patient 13**

<table>
<thead>
<tr>
<th>No. of Days after Transplantation</th>
<th>Leukemic Blasts in Bone Marrow (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20%</td>
</tr>
<tr>
<td>103</td>
<td>80%</td>
</tr>
<tr>
<td>114</td>
<td>0%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HLA STR</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSCT</td>
</tr>
<tr>
<td>Bone marrow aspirate</td>
</tr>
<tr>
<td>Bone marrow biopsy</td>
</tr>
<tr>
<td>Host chimerism (%)</td>
</tr>
<tr>
<td>Donor chimerism (%)</td>
</tr>
</tbody>
</table>

**Patient 33**

<table>
<thead>
<tr>
<th>No. of Days after Transplantation</th>
<th>Leukemic Blasts in Bone Marrow (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>60%</td>
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<tr>
<td>58</td>
<td>0%</td>
</tr>
<tr>
<td>114</td>
<td>80%</td>
</tr>
</tbody>
</table>

**Patient 43**

<table>
<thead>
<tr>
<th>No. of Days after Transplantation</th>
<th>Leukemic Blasts in Bone Marrow (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>33</td>
<td>60%</td>
</tr>
<tr>
<td>61</td>
<td>10%</td>
</tr>
<tr>
<td>92</td>
<td>0%</td>
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</tbody>
</table>

**Patient 16**

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<th>No. of Days after Transplantation</th>
<th>Leukemic Blasts in Bone Marrow (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>101</td>
<td>80%</td>
</tr>
<tr>
<td>127</td>
<td>0%</td>
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</tbody>
</table>

**Patient 7**

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<th>No. of Days after Transplantation</th>
<th>Leukemic Blasts in Bone Marrow (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>80%</td>
</tr>
<tr>
<td>131</td>
<td>0%</td>
</tr>
<tr>
<td>219</td>
<td>100%</td>
</tr>
</tbody>
</table>

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*The New England Journal of Medicine*

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Table 1. Characteristics of the Patients in Whom Loss of the Patient-Specific HLA Haplotype Was Documented.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient 7</th>
<th>Patient 13</th>
<th>Patient 16</th>
<th>Patient 33</th>
<th>Patient 43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>41</td>
<td>33</td>
<td>65</td>
<td>54</td>
<td>53</td>
</tr>
<tr>
<td>Donor characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>54</td>
<td>55</td>
<td>42</td>
<td>45</td>
<td>25</td>
</tr>
<tr>
<td>Relationship</td>
<td>Cousin</td>
<td>Mother</td>
<td>Son</td>
<td>Brother</td>
<td>Son</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Acute myeloid leukemia</td>
<td>Acute myeloid leukemia</td>
<td>Acute myeloid leukemia</td>
<td>Myelodysplastic syndrome</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>Status at first HSCT</td>
<td>Relapse</td>
<td>Relapse</td>
<td>Relapse</td>
<td>Relapse</td>
<td>Relapse</td>
</tr>
<tr>
<td>No. of haploidentical HSCTs from the same donor</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>T-cell–replete graft</td>
<td>No (both HSCTs)</td>
<td>Yes</td>
<td>No (first HSCT), yes (second HSCT)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Donor–T-cell add-backs</td>
<td>Yes (both HSCTs)</td>
<td>No</td>
<td>Yes (first HSCT), no (second HSCT)</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Total T-cell dose received (×10^6 CD3+ cells/kg body weight)</td>
<td>113</td>
<td>583</td>
<td>90</td>
<td>289</td>
<td>246</td>
</tr>
<tr>
<td>Time from first HSCT to HLA haplotype loss (days)</td>
<td>244</td>
<td>103</td>
<td>278</td>
<td>344</td>
<td>285</td>
</tr>
<tr>
<td>CD3+ cell count at time of HLA haplotype loss (per mm³)†</td>
<td>1860</td>
<td>107</td>
<td>407</td>
<td>3559</td>
<td>2676</td>
</tr>
<tr>
<td>Final clinical outcome</td>
<td>Death</td>
<td>Death</td>
<td>Death</td>
<td>Survival — complete response‡</td>
<td>Death</td>
</tr>
<tr>
<td>Cause of death</td>
<td>Relapse§</td>
<td>Relapse</td>
<td>Relapse</td>
<td>—</td>
<td>Multiorgan failure¶</td>
</tr>
</tbody>
</table>

* HSCT denotes hematopoietic stem-cell transplantation.
† CD3+ cell counts were determined within 30 days of documentation of HLA haplotype loss, except in the case of Patient 13, in whom it was determined 75 days before documentation of HLA haplotype loss (28 days after HSCT).
‡ Leukemia remission was achieved after a subsequent haploidentical HSCT from a new donor, who was mismatched for the HLA haplotype retained by the mutant leukemia.
§ Patient 7 received a subsequent haploidentical HSCT from a new donor (matched for the HLA haplotype retained by the mutant leukemia), did not have a response to the treatment, and died in relapse.
¶ Multiorgan failure occurred in this patient during preparation for receipt of a haploidentical HSCT from a new donor, who was mismatched for the HLA haplotype retained by the mutant leukemia.
Figure 2. Genomic Loss of the Patient-Specific HLA Haplotype by Leukemic Cells after Transplantation in Patient 16. Panel A shows electropherograms of the polymerase-chain-reaction products (amplified with the AmpFISTR Profiler Plus kit) used to interrogate nine highly polymorphic short-tandem-repeat loci on different chromosomes. Patient-specific markers, identified on the basis of comparison with samples from the stem-cell donor, are shown in red. At the time of both diagnosis and relapse, the short-tandem-repeat profile of leukemic blasts was patient-specific. Panel B shows the results of strip-based genomic HLA-DRB probing for Patient 16 (Dynal RELI SSO kit). Note the absence of signals for the patient-specific HLA-DRB alleles (corresponding to the red vertical lines) in the leukemic cells obtained at relapse. Band 39 of the typing strip (indicated by the asterisk) can be accounted for by both DRB3*0101 (donor-specific) and DRB1*0701 (patient-specific) and was thus negative only in the mutant leukemic cells at relapse, in accordance with loss of DRB1*0701. Similar results were obtained for the genomic typing of HLA-A, HLA-B, HLA-C, and HLA-DQB1 in Patient 16, as well as for all five HLA loci in the other four patients with leukemic relapse that was not detected by HLA typing. HSCT denotes hematopoietic stem-cell transplantation.
and grade 1, respectively). None of the other three patients had GVHD after transplantation.

**Genomic Characterization of the Mutant Leukemic-Cell Variants**

To determine why recipient HLA alleles were not detected at the time of relapse, leukemic blasts from the five patients, obtained at the time of diagnosis and at the time of relapse, were purified with the use of FACS and subjected to genomic HLA typing and short-tandem-repeat analysis. Although the blasts obtained at the time of relapse were of patient origin (Fig. 2A), they did not harbor any of the patient-specific HLA alleles for the five loci tested (Fig. 2B). Instead, they carried only the HLA haplotype shared by the donor and the recipient. In contrast, blasts obtained at the time of diagnosis were heterogeneous for the same loci. These findings show that genomic loss of the patient-specific HLA haplotype occurred in vivo after transplantation.

To determine the extent and the mechanism of the loss of heterozygosity in the patients with relapsed leukemia, we performed microsatellite mapping and SNP arrays of chromosome 6, including the HLA region. In all five patients, the analyses showed loss of heterozygosity involving the short arm of chromosome 6, encompassing the HLA region and causing loss of the patient-specific haplotype (Fig. 3, and Fig. 1 of the Supplementary Appendix). Analysis of copy-number variations showed no deletions in chromosome 6p, a finding that is in line with the observed karyotype. On the basis of these results, we concluded that loss of the HLA haplotype was due to acquired partial uniparental disomy of chromosome 6 (i.e., substitution, for the “lost” haplotype, of a corresponding region from the homologous chromosome).

**Functional Study of the Graft-Versus-Leukemia Response**

Since HLA mismatches can elicit robust T-cell responses, we investigated whether loss of the HLA haplotype allowed the mutant leukemic cells to escape immunosurveillance by the donor’s T cells. We stimulated mononuclear cells obtained at different times after hematopoietic stem-cell transplantation from a representative patient (Patient 16) with cells obtained from the patient at the time of diagnosis. Mononuclear cells from the stem-cell donor and from a healthy HLA-mismatched subject served as controls. After three rounds of stimulation with leukemic cells, T cells accounted for more than 85% of the cultures (data not shown). These T cells consistently produced a robust response to the original leukemic cells, as determined by tests for cytotoxicity, interferon-γ release, and proliferation. Leukemia-reactive T cells from the stem-cell donor and the patient after transplantation specifically targeted the patient-specific HLA molecules, as could be seen when we tested them against a panel of HLA-typed target-cell lines (data not shown). The same T cells did not respond to leukemic blasts harvested at relapse, whereas T cells from the healthy HLA-mismatched subject did respond to the blasts harvested at both time points (Fig. 4).

Figure 3 (facing page). Loss of the Patient-Specific HLA Haplotype through Extensive Rearrangements in Chromosome 6, Leading to Acquired Uniparental Disomy.

A schematic reference map (Panel A, left side) shows 12 highly polymorphic short-tandem-repeat (STR) markers spanning chromosome 6 that were used to assign the patient-specific (dark red and light red) and shared (black and dark gray) haplotype of the original leukemia, along with an expanded view of the HLA region (yellow). STR mapping of leukemic cells at relapse is shown in ideogram representations (Panel A, right side) of both chromosomes 6, in the five patients with documented loss of the HLA haplotype. Chromosome regions for which no unequivocal reconstruction was possible are shown in light gray and white. In all five patients, genomic rearrangements encompassing the entire HLA region (yellow) led to homozygosity for the HLA haplotype shared between donor and patient. Results from the Human CNV370Quad BeadArray (Panel B) show the leukemic blasts obtained at diagnosis and at relapse, with the loss of the patient-specific HLA haplotype, in Patient 16. Two plots are included for each time point. The upper plot shows the frequency of the B allele, which indicates the allelic composition of each single-nucleotide polymorphism (SNP). BB genotypes have a B allele frequency of 1, AB genotypes a frequency of 0.5, and AA genotypes a frequency of 0. The lower plot shows the log R ratio, which is a measure of the copy number for each SNP. The mean of the log R ratio is shown as a red line. The HLA region in chromosome 6p21.3 (yellow) shows a smeared signal pattern because of the high density of highly homologous SNPs, as is usually observed in samples from healthy donors. The lack of heterozygous genotypes across the p arm of chromosome 6 in the absence of an alteration in copy number in the leukemic cells at relapse indicates a large area (>50 Mb) of uniparental disomy (outlined in red).
Loss of Mismatched HLA in Leukemia after Stem-Cell Transplantation

A

STR Marker

D6S1600
D6S1574
D6S277
D6S276
D6S265
D6S291
D6S1610
D6S4828
D6S257
D6S402
D6S434
D6S311

HLA

Class I

Class II

Class III

Patient's Leukemic Blasts at Relapse

Patient's Leukemic Blasts at Diagnosis

B

Patient 16

Patient 7

Patient 13

Patient 16

Patient 33

Patient 43

HLA

Donor–patient shared

Could not be determined

Patient specific

Chromosome 6 STR Mapping of Leukemia at Relapse

Log R Ratio

B Allele Frequency

Log R Ratio

B Allele Frequency

Uniparental Disomy Region

Mb

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Genomic instability is a hallmark of myeloid cancers, and it has been shown to be associated with loss of heterozygosity without loss of genetic material (uniparental disomy), even in leukemic cells with a normal karyotype.\textsuperscript{23-25} Our data indicate that this loss of heterozygosity can

\textbf{Figure 4. Immune Escape of Mutant Leukemic Cells in Patient 16.}

Mononuclear cells harvested from Patient 16 at two time points after hematopoietic stem-cell transplantation (HSCT) (Panel A), from the haploidentical stem-cell donor (Panel B), and from a healthy HLA-mismatched subject (Panel C) were tested against leukemic blasts taken from the patient at diagnosis (black) and at relapse (red) after three rounds of specific stimulation. T-cell–specific lysis of leukemic blasts (top row) was determined with the use of a \textsuperscript{51}Cr-release assay at different effector:target ratios. T lymphocytes releasing interferon-γ in response to leukemia were detected as specific spots in an enzyme-linked immunospot (ELISpot) assay (middle row). Cell proliferation in response to the relevant stimulator cells (bottom row) was determined after a 72-hour coculture by means of 16 hours of pulsing with \textsuperscript{3}Hthymidine. Counts per minute at the time of harvest are shown on the y axis. In all three assays, T cells from the patient after transplantation and from the stem-cell donor reacted to the leukemia at diagnosis but not to the leukemia at relapse, which had lost the patient-specific HLA haplotype. Conversely, T cells from the healthy HLA-mismatched subject responded equally to leukemia cells harvested at both time points. T bars denote the standard deviation among experimental replicates.
confer a selective advantage on leukemic cells, which become able to escape immunologic pressure from alloreactive donor T cells.

Genomic loss of the patient-specific HLA haplotype occurred in 5 of 17 patients (29%) whose disease relapsed. The frequency of this event suggests the value of assessing the HLA genotype of the leukemic cells in cases of relapse after transplantation to identify alternative donors whose T cells could eliminate escape mutants. The last two patients in whom we documented loss of the patient-specific HLA haplotype were candidates for a subsequent transplantation of haploidentical hematopoietic stem cells from a different donor, who was mismatched for the HLA haplotype retained in leukemic cells. Remarkably, one of the two patients is alive and in complete remission more than 16 months after the second transplantation.

Previous reports have proposed that natural killer cells are the main determinant of the graft-versus-leukemia effect after haploidentical hematopoietic stem-cell transplantation with T-cell depletion.\textsuperscript{26,27} The escape mechanism we describe, which relies on uniparental disomy, did not affect the overall expression of cell-surface class I HLAs (see Fig. 2 of the Supplementary Appendix), nor did it consistently evoke reactivity by the total population of natural killer cells (see Fig. 3 of the Supplementary Appendix). In losing specific HLA alleles, leukemic blasts may have gained susceptibility to alloreactive natural killer cells that carry as their sole inhibitory receptors immunoglobulin-like receptors that are specific for the lost haplotype.\textsuperscript{28} The reportedly low frequency of this subpopulation of natural killer cells in adults, particularly during the initial months after haploidentical hematopoietic stem-cell transplantation with infusion of donor T cells,\textsuperscript{29} may explain why natural killer–cell alloreactivity failed to prevent a disease relapse.

In the five patients we describe, other mechanisms of T-cell–mediated alloreactivity, such as reactions against minor histocompatibility antigens\textsuperscript{30} or immunization against inherited paternal antigens,\textsuperscript{31} apparently did not provide protection against the mutated variants of leukemic cells, suggesting that in these patients, major HLA mismatches were the pivotal targets of the anti-leukemic response, and their loss was sufficient to allow relapse.

Taken together, our data indicate that immune escape by leukemic cells from a graft-versus-leukemia effect after haploidentical hematopoietic stem-cell transplantation can lead to relapse. The phenomenon we observed is likely to be the consequence of selective pressure mediated by alloreactive donor T cells, further strengthening the biologic rationale for the use of T-cell adoptive immunotherapy. Loss of the patient-specific HLA haplotype is easy to diagnose and has important implications for selecting a treatment that is suitable for relapse after transplantation.

References