Haploidentical stem cell transplantation is a treatment option for the approximately 70% of patients who do not have an HLA-identical sibling donor. The availability of a haploidentical donor in most families is a potential advantage, both for avoiding the need to find an alternative unrelated donor, and for the potentially more potent graft-versus-tumor effect that can be induced. The early complications of severe graft-versus-host disease (GVHD) following T-cell replete stem cell transplantation (SCT), and graft failure and recurrent malignancy (after T-cell depleted SCT) have limited the applications of this approach. Newer strategies employing T-cell depletion of the graft, using either very high-dose peripheral blood stem cells and/or more intensive conditioning therapy have overcome some of the problems of conventional transplantation. Nonmyeloablative SCT approaches have overcome some of the morbidity and mortality associated with the early complications of SCT and have been associated with favorable engraftment and GVHD profiles. Induction of mixed lymphohematopoietic chimerism as a platform for adoptive cellular immunotherapy (via delayed donor lymphocyte infusions) may have important application in avoiding early GVHD, while ultimately capturing a very potent graft-versus-tumor effect. Current strategies are focusing on improvement of immune reconstitution and prevention of recurrence of the underlying malignancy.

Haploidentical related donor stem cell transplantation (SCT) has been evaluated over the past two to three decades as an alternative transplant option for the approximately 70% of patients who do not have an HLA-identical related donor1-8 (Table 1). The advantages of haploidentical SCT are that nearly all patients have an immediately available donor and that a stronger graft-versus-tumor effect can be realized with partial HLA disparity. The disadvantages of haploidentical SCT are the immunological consequences of crossing the major histocompatibility barrier, namely graft-versus-host disease (GVHD), graft rejection, and delayed or incomplete immune reconstitution. With very intensive conditioning therapy, graft rejection has been largely overcome. Severe acute or chronic GVHD, however, have been formidable obstacles to the success of T-cell replete transplants following myeloablative conditioning.1-3

**Historical Perspective**

The early post-transplant complications of myeloablative T-cell replete haploidentical bone marrow transplantation were well described by Powles and colleagues.1 Of 35 patients with advanced acute myeloid leukemia (AML) or acute lymphocytic leukemia (ALL) who received a one- to three-antigen mismatched bone marrow transplantation (BMT), 12 patients died from an early syndrome characterized by pulmonary edema, seizures, intravascular hemolysis, and acute renal failure. Ten of the 35 patients had engraftment failure, requiring regrafting from the same donor, though 11 patients were alive at the time of reporting. There was no difference in survival between HLA-1 to 2 antigen and 3 of 6 antigen mismatched transplants. There also was no impact of the addition of methotrexate to cyclosporine on the development of “hyperacute GVHD.”

Beatty et al described the outcomes (including the incidence of GVHD and leukemia-free and overall survival) of HLA-matched versus -mismatched donor bone marrow transplantation in patients with advanced hematologic malignancies who received myeloablative (total-body irradiation [TBI]-based) conditioning.2 The incidence of grades II-IV GVHD was higher after HLA-mismatched versus matched donor BMT. However, overall survival was similar after HLA-matched and 1-antigen mismatched donor BMT for patients with acute leukemia in remission. Despite the increase in GVHD following HLA-mismatched BMT, overall survival was not worse, likely owing to a more enhanced graft-versus-leukemia (GVL) effect. While the number of patients who received an HLA 2- or 3-antigen mismatched BMT was too small to reach conclusions regarding overall survival, there was very high, early transplant-related mortality in this subpopulation. Based on these results, it was long held that the outcomes of related donor 1-antigen mismatched BMT were similar to those of HLA-identical donor BMT. Two- or 3-antigen mismatched transplants were believed to be associated with a prohibitively high mortality risk, at least in the setting of T-cell replete transplants using pharmacologic GVHD prophylaxis.

An IBMTR analysis of a much larger population of patients with leukemia showed that the relative risk of treat-
ment failure for early leukemia and the overall risk of transplant-related mortality were significantly higher for patients who received a 1- or 2-antigen mismatched BMT compared to an HLA-identical sibling donor transplant. For standard-risk leukemic patients a significantly higher probability of leukemia-free survival was observed for patients who received an HLA-identical donor transplant compared with recipients of a 1- or 2-antigen mismatched donor transplant. For high-risk patients, however, any difference was obscured by a disappointing 15% leukemia-free survival probability in both matched and mismatched transplant recipients. Thus, the consequences of T-cell replete myeloablative BMT in which HLA barriers were crossed were readily apparent and, at least in the situation of standard-risk hematologic malignancies, were associated with a significantly lower leukemia-free survival.

Previous efforts to overcome the problem of GVHD following myeloablative haploidentical BMT using ex vivo T-cell depleted (TCD) bone marrow were complicated by a very high risk of graft failure and recurrent malignancy. Epstein-Barr virus (EBV)-related post-transplant lymphoproliferative disease was another important complication of TCD transplantation. Other attempts to reduce transplant-related mortality focused on prevention and treatment of opportunistic infections. Recognition of the importance of cytomegalovirus (CMV) serostatus on transplant outcomes, for example, led to the preferential selection of CMV-seronegative donors for CMV-seronegative recipients, whenever possible.

Table 1. Haploidentical stem cell transplantation (SCT): potential applications.

<table>
<thead>
<tr>
<th>Hematologic Malignancy</th>
<th>Non-Malignant Stem Cell Disorders</th>
<th>Tolerance Induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediate availability of donor →</td>
<td>Available (often parent) donors for children with inherited stem cell disorders (e.g., SCID)</td>
<td>Avoidance of long term immunosuppression</td>
</tr>
<tr>
<td>Expansion of transplant opportunities</td>
<td>Alternative donor source for acquired stem cell disorders (e.g., SAA)</td>
<td>Potential opportunities for cadaveric donor organ transplantation</td>
</tr>
<tr>
<td>Availability of donor for subsequent stem cells or DLI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potentially stronger GVT effect</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: DLI, donor lymphocyte infusion; GVT, graft-versus-tumor; SAA, severe aplastic anemia; SCID, severe combined immune deficiency.

Table 2. Haploidentical stem cell transplantation (SCT) strategies.

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Center</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYELOABLATIVE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-cell replete BMT</td>
<td>Royal Marsden</td>
<td>Hyperacute GVHD; frequent rejection</td>
<td>1</td>
</tr>
<tr>
<td>Pharmacologic GVHD Prophylaxis</td>
<td>Seattle</td>
<td>↑ GVHD with ↑ HLA disparity</td>
<td>2</td>
</tr>
<tr>
<td>Partial ex vivo TCD, Post-BMT Pharmacoprophylaxis</td>
<td>U. South Carolina</td>
<td>Good GVHD Protection DFS in ~ 20%</td>
<td>4</td>
</tr>
<tr>
<td>“Mega-dose” TCD PBSCT</td>
<td>Perugia</td>
<td>Minimal GVHD Favorable survival in remission AML/ALL</td>
<td>5,11</td>
</tr>
<tr>
<td></td>
<td>Canada (multi- center) Emory</td>
<td>Delayed immune reconstitution with high rates of relapse, infectious deaths</td>
<td>12,13</td>
</tr>
<tr>
<td>Ex vivo T-cell anergization</td>
<td>Children's/DFCI</td>
<td>Minimal GVHD</td>
<td>6</td>
</tr>
<tr>
<td>Donor selection according to feto-maternal chimerism*</td>
<td>Japan (multiple centers)</td>
<td>↓ GVHD with donor/recipient mismatched for NIMA</td>
<td>23-26</td>
</tr>
<tr>
<td>NON-MYELOABLATIVE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex vivo/in vivo TCD, delayed DLI</td>
<td>MGH</td>
<td>“split level” mixed chimerism, conversion of T-cell chimerism after DLI</td>
<td>7,8</td>
</tr>
<tr>
<td>Post-BMT high-dose cyclophosphamide</td>
<td>Johns Hopkins</td>
<td>Full donor chimerism in most, GVHD in ~ 50%</td>
<td>17</td>
</tr>
<tr>
<td>Ex vivo/in vivo TCD with Campath</td>
<td>Duke</td>
<td>Low incidence of acute GVHD, high incidence of GVHD after DLI</td>
<td>Personal communication N. Chao</td>
</tr>
</tbody>
</table>

* Reduced-intensity conditioning in a minority of patients.

Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BMT, bone marrow transplantation; DFCI, Dana-Farber Cancer Institute; DFS, disease-free survival; DLI, donor lymphocyte infusion; GVHD, graft-versus-host disease; MGH, Massachusetts General Hospital; NIMA, non-inherited maternal antigens; PBSCT, peripheral blood stem cell transplantation; TCD, T-cell depletion.
Newer Approaches to Haploidentical SCT

This early experience highlighted the need for new strategies to deal with the complications of haploidentical SCT, particularly GVHD and its attendant complications (Table 2). If ex vivo T-cell depletion approaches were to be used to prevent GVHD, more intensive conditioning and/or higher doses of stem cells (e.g., mobilized peripheral blood stem cells versus bone marrow) would be required. Mehta et al showed that a combination of in vitro and in vivo T-stem cells versus bone marrow) would be required. Mehta et al showed that a combination of in vitro and in vivo T-cell depletion resulted in an acceptably low incidence of GVHD and reasonable survival probabilities following BMT for advanced hematologic malignancy. One such analysis evaluated 201 patients with acute leukemia who were conditioned with TBI/etoposide/cyclophosphamide, cytarabine, anti-thymocyte globulin (ATG), and methylprednisolone. In vitro T-cell depletion was performed with a T10 B9 or OKT3 monoclonal antibody. GVHD prophylaxis consisted of ATG, corticosteroids, and cyclosporine. Greater than grade I GVHD and chronic GVHD occurred in 13% and 15% of patients, respectively. Five-year actuarial disease-free and overall survival probabilities were 18% and 19%.

In an effort to further decrease the incidence of GVHD and improve the outcomes of haploidentical SCT, Aversa et al evaluated a strategy in which “mega-dose,” vigorously T-cell depleted, granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells from haploidentical donors were transplanted following a TBI/thiotepa/fludarabine/ATG preparative regimen. Early results in patients with AML or ALL were remarkable for sustained donor chimerism in most patients and the absence of acute or chronic GVHD. Veto cells, within the high-dose CD34+ selected cell product, and NK cells that destroy residual host alloreactive cells were thought to be partially responsible for the favorable engraftment profile. Transplant-related mortality risk was 40%, with infection as the leading cause of death. Improved immune reconstitution and fewer deaths secondary to infection occurred when post-transplant G-CSF was discontinued. In a more recent report using automated devices for CD34+ cell selection, 94 of 101 patients achieved primary engraftment, and acute GVHD occurred in eight of 100 evaluable patients. Thirty-eight patients died due to nonleukemic causes. For patients with AML and ALL in remission, event-free survival probabilities were 48% and 46%, respectively.

Other approaches using myeloablative conditioning and high-dose CD34+ cell-selected grafts have described similarly favorable engraftment rates and protection from GVHD. However, recurrent malignancy and infection deaths due to poor immune reconstitution have been problematic. In a Canadian multi-center trial using an approach similar to that developed by Aversa et al, all 11 patients engrafted without GVHD. Ten of 11 patients died due to leukemic relapse or infection. Waller et al describe a 93% mortality in 28 patients who received a TCD, CD34+-enriched haploidentical SCT after an ATG-based regimen, with most deaths as a result of infection or relapse. Adoptive cellular therapy using antigen-specific donor T-cells has been proposed.

Another approach to myeloablative haploidentical stem cell transplantation by Guinan and colleagues has been the induction of alloantigen-specific anergy by the culturing of host and donor bone marrow in the presence of CTLA-4-IG. Of 12 patients with advanced hematologic malignancy, only 3 developed gastrointestinal GVHD. At the time of reporting, 5 of 12 patients were alive and disease-free from 5 to 29 months post-transplant. The induction of alloantigen-specific anergy was demonstrated by a substantial reduction in precursor helper T-cell frequency after in vitro culture of host and donor bone marrow compared to the precursor helper T-cell response to third-party antigen.

Nonmyeloablative Haploidentical SCT

Whereas the incidence of GVHD and related complications has likely been reduced by some form of ex vivo T-cell depletion or anergization, transplant-related mortality risk following myeloablative transplantation has remained high. In an effort to reduce the early regimen-related mortality risk while still capturing the potent graft-versus-tumor effect of the transplant, several recent clinical trials have evaluated the efficacy of nonmyeloablative conditioning for haploidentical SCT.

Based on murine models established by Sykes and colleagues, clinical trials at Massachusetts General Hospital (MGH) have been performed using nonmyeloablative conditioning (cyclophosphamide with or without fludarabine, in vivo T-cell depletion using polyclonal or monoclonal anti-T cell antibodies, pre-transplant thymic irradiation, and most recently, ex vivo T-cell depletion) in an effort to induce stable mixed lymphohematopoietic chimerism as an immunological platform for adoptive cellular immunotherapy. The rationale for this approach has included 1) the reduction of regimen-related toxicities with nonmyeloablative conditioning, 2) the prevention of GVHD by in vivo and ex vivo T-cell depletion, and 3) the capture of an optimal graft-versus-tumor effect by delayed donor lymphocyte infusions (DLI). Murine studies by Mapara et al showed that DLI-mediated GVL effects were more potent in mixed chimeras than in full donor chimeras. The preservation of host antigen-presenting cells was shown to be responsible for the enhanced GVL effect.

In initial trials, the risk of severe GVHD was high following T-cell replete bone marrow transplantation. However, impressive and durable antitumor responses in some patients with chemorefractory aggressive lymphomas were observed. The current MGH protocol includes cyclophosphamide and fludarabine, MEDI-507 (a monoclonal anti-CD2 antibody), and thymic irradiation. This has resulted in a high incidence of mixed chimerism without early GVHD and the observation that conversion of T-cell chimerism could occur with manageable or no GVHD (shown sche-
Recurrent malignancy and late infections have been the chief reasons for treatment failure with this approach. Efforts are underway to optimize the ex vivo T-cell depletion of the product and to explore different doses of delayed DLI.

O’Donnell et al have performed nonmyeloablative haploidentical BMT with high-dose posttransplant cyclophosphamide, 50 mg/kg on day 3, to improve GVHD prophylaxis. Thirteen patients with hematologic malignancy who received low-dose TBI/fludarabine (with or without post-transplant cyclophosphamide) and tacrolimus/mycophenolate mofetil (MMF) for GVHD prophylaxis were recently described. Acute GVHD developed in 6 of the 13 patients. Six of the 13 patients were alive, 5 of whom were in a complete remission at a median of 191 days post-transplant.

Using a non-radiation (cyclophosphamide/fludarabine) based chemotherapy preparative regimen and a combination of in vivo and ex vivo T-cell depletion (in vivo Campath plus Campath-treated peripheral blood stem cells), Chao and colleagues at Duke University treated 35 patients with a variety of hematologic malignancies and solid tumors with haploidentical SCT (N. Chao, personal communication; manuscript submitted). Three patients experienced primary graft failure. The incidence of severe acute and chronic GVHD was 9% and 23%, respectively. Progressive disease and infection were the leading causes of mortality. Five of 12 patients (42%) developed severe GVHD following DLI.

**Haploidentical SCT: Special Considerations and Implications for Donor Selection**

**Killer immunoglobulin-like receptor mismatching**

Lysis of tumor cells by natural killer cells is mediated in part by mismatching of the killer immunoglobulin-like receptor (KIR) ligand between the NK cell and its target. For this reason, mismatching of the KIR ligand in the GVH direction might enhance a graft-mediated antitumor effect following haploidentical SCT. A clinically meaningful impact of donor NK cell alloreactivity following haploidentical stem cell transplantation has been suggested by the lower relapse risks for patients with AML who received a haploidentical SCT according to the strategies developed by Ruggeri et al. The probability of relapse at 5 years (75% vs 0%) was reported for 57 patients with AML who received KIR ligand-matched versus mismatched transplant. The importance of KIR ligand compatibility on relapse and survival probabilities after alternative donor transplantation remains to be defined, as conflicting results have been reported regarding the effect of KIR ligand matching on these outcomes following unrelated donor transplantation. Nonetheless, considerable potential exists to enhance an antitumor effect of haploidentical SCT among selected hematologic malignancies. NK cell alloreactivity in the GVH direction is not associated with increased GVHD and may have a positive impact on the donor engraftment.

**Haploidentical stem cell transplantation based on fetomaternal microchimerism**

Evidence exists that exposure to non-inherited maternal antigens during pregnancy may result in lasting fetomaternal microchimerism and tolerance induction. Data from an IBMTR analysis showed that stem cell transplants from a non-inherited, maternal antigen-mismatched sibling result in a reduced incidence of acute GVHD (compared to non-inherited, paternal antigen-mismatched sibling donor transplants) and reduced transplant-related mortality (compared to parental donor transplantation). Favorable outcomes of haploidentical SCT using T-cell replete grafts from sibling donors mismatched for noninherited maternal antigens have been recently published. These reports have demonstrated the occurrence of sustained chimerism with an acceptably low incidence of GVHD after myeloablative or reduced-intensity conditioning and up to 3-HLA antigen mismatched T-cell replete SCT. In a study of 35 patients with hematologic malignancy from Kyoto, Japan, who received a 2- or 3-antigen mismatched SCT from a microchimeric, non-inherited maternal antigen-mis-
matched donor after myeloablative or reduced-intensity conditioning, a significantly reduced incidence of severe GVHD was observed compared to transplants where the donor and recipient were mismatched for inherited paternal antigens. Thus, choice of donor within families, both donor and recipient were mismatched for inherited paternal antigens, may improve survival outcomes following haploidentical SCT.

**Haploidentical Stem Cell Transplantation: Other Applications**

**Specific transplantation tolerance induction**
The major obstacle to successful solid organ transplantation (SOT) is graft rejection. Substantial morbidity accompanies lifelong immunosuppression following SOT. Several preclinical models have shown that sustained donor-reactive mixed lymphohematopoietic chimerism is sufficient to induce durable tolerance. Favorable experience has been observed with combined HLA-matched bone marrow and kidney transplantation for patients with end-stage renal disease secondary to multiple myeloma. Durable anti-myeloma responses and sustained renal allograft tolerance despite, in some cases, only transient donor chimerism have been observed. At the MGH, a protocol was developed for combined haploidentical bone marrow and kidney transplantation for end-stage renal disease without an underlying malignancy. By using a cyclophosphamide, MED1-507, thymic irradiation preparation similar to that used for transplantation of multiple myeloma, and simultaneous bone marrow/kidney transplantation, sustained specific transplantation tolerance has been documented (clinically and by in vitro studies of alloreactivity) in 2 of 3 evaluable patients (manuscript in preparation). The sustained tolerance despite only transient (< 14 days) lymphohematopoietic chimerism suggests that, in addition to the mechanism of central deletional tolerance demonstrated in the pre-clinical models, peripheral mechanisms of tolerance induction are important in the maintenance of tolerance. Such tolerance approaches will be investigated in cadaveric kidney transplantation and transplantation of other solid organs.

**Antitumor response following hematopoietic graft rejection: a new paradigm for transplantation?**
A striking and unexpected observation from our nonmyeloablative HLA-matched and mismatched stem cell transplant protocols has been the substantial response rate and the occurrence of durable antitumor responses in some patients with chemorefractory hematologic malignancies, even following hematopoietic graft rejection. Dey et al described a 41% response rate among patients who rejected their grafts, following nonmyeloablative HLA-matched or -mismatched SCT. While the mechanism of this antitumor response has not been fully defined, preclinical and clinical evidence suggests that a host-specific antitumor response may be generated by the graft rejection. Clinically, this is supported by the observation of ongoing tumor regression following multiple sequential DLI in some patients who previously rejected their grafts. This has been accompanied in some cases by transient increases in the number of host CD8+ cells. In a murine model, both spontaneous rejection and recipient lymphocyte infusions (RLI) to intentionally induce graft rejection provide protection from tumor-related mortality. This protection is dependent on recipient-derived interferon-gamma and on the generation of tumor-specific cytotoxic cells. Colvin and colleagues have reported responses among patients with refractory AML following low-dose TBI and haploidentical SCT in which graft rejection reliably occurs. Sequential DLIs are now given in an effort to enhance the antitumor effect associated with graft rejection.

**Haploidentical Stem Cell Transplantation: Conclusions and Future Direction**
A potentially huge upside of haploidentical SCT exists: namely, an expansion of transplant opportunities for patients without an HLA-matched donor and the potential of a graft-versus-tumor effect of the transplant. Haploidentical stem cell transplant strategies may also be important for specific transplantation tolerance induction. Whereas some of the important challenges of haploidentical SCT have been addressed, and at least partially ameliorated (i.e., severe GVHD), other problems, such as delayed immune reconstitution and recurrent malignancy, particularly for patients with advanced disease at the time of transplant, remain significant hurdles limiting long-term success. Given these unresolved issues, the role and timing of haploidentical SCT, especially in relation to other potential alternative donor stem cell sources (cord blood, mismatched unrelated donors), remain to be defined. The most promising approaches to haploidentical SCT involve graft engineering to deplete cells capable of causing GVHD while preserving (or adding back later) cells that are responsible for a graft-versus-tumor effect and for restoring T-cell immunity. In this regard, approaches that employ delayed adoptive cellular immunotherapy, including infusion of specific regulatory cells and/or tumor or pathogen-specific cytotoxic T-lymphocytes, appear particularly promising.

**References**