Nonmyeloablative Allogeneic Stem Cell Transplant Strategies and the Role of Mixed Chimerism

THOMAS R. SPITZER
Bone Marrow Transplant Program, Harvard Medical School, Boston, Massachusetts, USA

Key Words. Nonmyeloablative therapy · Mixed chimerism · Graft-versus-host disease · Allogeneic transplant

ABSTRACT
Experimental and clinical experiences have demonstrated successful donor engraftment following nonmyeloablative preparative regimens. These less toxic conditioning strategies may be better tolerated with diminished transplant-related morbidity and mortality. Importantly, the intentional induction of mixed chimerism can be established following nonmyeloablative conditioning. This approach has the potential advantages of inhibiting graft-versus-host disease, presumably secondary to the persistence of host immunoregulatory cells, and providing a platform for the delivery of adoptive cellular immunotherapy with donor leukocyte infusions for patients with an underlying malignancy. This review will describe the preclinical evolution of nonmyeloablative transplant strategies, the rationale for considering these approaches, and the preliminary clinical experience with this novel allogeneic stem cell therapy. The Oncologist 2000;5:215-223

INTRODUCTION
Conventional allogeneic bone marrow or peripheral blood stem cell transplantation has utilized high-dose, allegedly myeloablative chemoradiotherapeutic preparative (conditioning) therapy. This strategy was founded on the principles of maximal tumor cytoreduction according to a well-demonstrated, dose-response relationship of chemoradiotherapy [1] and adequate immunosuppression to allow for engraftment, even following non-HLA-genotypically identical donor transplants [2-4]. While the latter objectives have been largely realized (rejection rates are generally less than 5% with non-T-cell-depleted allogeneic transplants), the former goal remains problematic. Despite maximally tolerated doses of chemoradiotherapy, relapse probabilities, especially for advanced hematologic malignancies, remain disappointingly high [4-6]. High-dose chemoradiotherapy is also associated with substantial transplant-related toxicities and a significant incidence of acute and chronic graft-versus-host disease (GVHD) [7]. The latter complications of allogeneic transplantation are, moreover, enhanced by the increasing intensity of the conditioning regimen [8].

Recently, attempts have been made to diminish transplant-related morbidity and possibly mortality by administering relatively nontoxic, nonmyeloablative doses of chemotherapy or radiation therapy prior to allogeneic transplantation [9-13]. Donor engraftment is feasible even following nonmyeloablative conditioning therapy [14-18], and these less toxic conditioning regimens are generally better tolerated, thus allowing for treatment of older patients and patients with co-morbid disease. The induction of mixed chimerism, moreover, may serve as a platform for later adoptive cellular immunotherapy (and conversion of the mixed chimeric state to one of fully donor hematopoiesis). Figure 1 is a depiction of the use of a nonmyeloablative preparative regimen to induce a state of mixed chimerism followed by a donor leukocyte infusion (DLI) for chimerism conversion and augmentation of a graft-versus-leukemia (GVL) effect. The effectiveness of these approaches, in terms of durable disease control, remains to be determined, however. This review will briefly describe the preclinical evidence for these nonmyeloablative transplant strategies, discuss the rationale for considering these approaches, and review their preliminary clinical experience with these transplant strategies.

NOMENCLATURE
Transplant strategies that employ nonmyeloablative preparative conditioning therapy have been popularly termed...
“mini-transplantation” or “transplant-lite” [10]. However, central to the issue of these strategies is what constitutes ablative versus nonablative conditioning therapy. Lymphohematopoietic recovery has been described following putatively ablative doses of whole-body irradiation and other seemingly ablative doses of high-dose chemotherapy, raising the question of whether conventional conditioning regimens are truly ablative of the bone marrow and immune system [19, 20]. Certain preparative regimens, particularly those containing whole-body irradiation in total doses of \(900\) cGy or high-dose busulfan, are associated with such profound marrow injury that meaningful hematologic recovery generally does not occur following graft rejection. On the other hand, minimal or transient myelosuppression usually follows the relatively low doses of chemotherapy or radiation therapy that are used for these “mini-transplants.” Following low-dose total-body irradiation (200 cGy, for example), most patients have not become neutropenic or transfusion dependent [20]. Following the more intensive cyclophosphamide-based regimens that we have employed (which include cyclophosphamide at a dose of 150-200 mg/kg), significant myelosuppression occurs but rapid hematologic recovery has uniformly been seen, even when donor hematopoiesis is not established (with early recovery of host hematopoiesis in these situations). Thus, nonmyeloablative conditioning regimens should be defined by the transient marrow injury that occurs with resultant complete and non-delayed hematologic recovery, even following graft rejection.

Another outcome of some nonmyeloablative transplant strategies is the induction of mixed lymphohematopoietic chimerism. This state of mixed chimerism is operationally defined by the stable presence of both host and donor lymphohematopoietic elements in the peripheral blood and/or bone marrow. In some situations, “split-lineage” chimerism has also been observed, in which varying percentages of host and donor lymphoid and nonlymphoid cell subsets are seen following transplantation. These states of mixed chimerism have been intentionally induced in some transplant protocols [12, 13, 21]. This approach has the potential advantage of preventing GVHD by allowing for the persistence of host immunoregulatory cells, and at the same time establishing a platform for delivering adoptive cellular immunotherapy via delayed DLI.

**Preclinical Data**

In several animal models, mixed lymphohematopoietic chimerism can be established following nonmyeloablative transplant preparative regimens (Table 1). In a murine model, stable mixed lymphohematopoietic chimerism is reliably achieved following low-dose total-body irradiation (300 cGy) or cyclophosphamide (200 mg/kg), peri-transplant monoclonal anti-T-cell antibody therapy directed against both host and donor T-cells, thymic irradiation and major histocompatibility complex (MHC) fully mismatched donor bone marrow transplantation (BMT) [18, 22]. With the
addition of post-transplant cyclosporine (CYA), these animals are completely protected from acute and chronic GVHD. Remarkably, these mice are also resistant to the induction of GVHD following delayed DLI (beginning on day +35 post-transplant), despite a potent lymphohematopoietic graft-versus-host response that converts their state of mixed chimerism to one of fully donor hematopoiesis.

In large animals, mixed lymphohematopoietic chimerism has also been established following several different nonmyeloablative preparative strategies. In miniature swine, mixed chimerism has been achieved following low-dose whole body radiation therapy (300 cGy), anti-T-cell antibody therapy, thymic irradiation, and swine leukocyte antigen-matched donor BMT [23]. There is evidence in both murine and miniature swine models indicating that stable mixed chimerism can be achieved following regimens that employ only anti-T-cell antibody therapy and thymic radiation, even following stem cell transplants from MHC mismatched donors ([17] and Dr. D.H. Sachs, personal communication). Acute GVHD also appears to be less prominent than following transplants utilizing conventional ablative doses of chemoradiotherapy.

In a dog model, an effective regimen for inducing mixed chimerism employs very low-dose whole body radiation (300 cGy) as the sole preparative therapy and post-transplant immunosuppression with mycophenolate mofetil (MMF) and CYA, and dog leukocyte antigen-matched donor BMT [16]. Optimizing post-transplant immunosuppression to inhibit competing graft-versus-host and host-versus-graft reactions is believed to be a central focus of this treatment strategy.

**PRELIMINARY CLINICAL DATA**

With this positive preclinical experience as background, clinical investigation of nonablative transplant regimens has begun in a number of transplant centers. Several published reports have demonstrated the feasibility of achieving allogeneic engraftment following nonmyeloablative conditioning therapy [9-12]. The diversity of the conditioning regimens, small numbers and heterogeneity of the patient populations, and limited goals of the sentinel experiments (e.g., reduction in transplant-related morbidity versus the intentional induction of mixed lymphohematopoietic chimerism), allow only preliminary interpretation of the data, particularly in terms of its effectiveness in achieving control of malignant disease. Nonetheless, taken together, these reports indicate the tolerability of most of these regimens, and demonstrate that mixed lymphohematopoietic chimerism can be intentionally induced, even across major HLA barriers; in some cases, the mixed chimerism led to potent antitumor response (perhaps enhanced by delayed DLI), a particularly exciting advance in the field of clinical allogeneic stem cell transplantation. Many transplant centers have initiated pilot trials evaluating nonmyeloablative conditioning regimens. The results of most of the trials are too preliminary to report. The clinical data from several trials, representing the diversity of conditioning strategies that have been employed, have been published. The results of the more notable of these preliminary trials are shown in Table 2 and discussed below.

Two series from the M.D. Anderson Cancer Center have employed fludarabine-based nonmyeloablative conditioning
regimens and recombinant growth factor-mobilized allogeneic peripheral blood stem cell transplants for treatment of hematologic malignancies [9, 10]. Khouri and colleagues [10] described 15 patients with chronic lymphocytic leukemia (CLL) or low to intermediate-grade non-Hodgkin’s lymphoma (NHL) who received fludarabine (30 mg/m²/d × 3 days) and cyclophosphamide (300 mg/m²/d × 3 days). Eleven of 15 patients had donor cell engraftment as measured by restriction fragment length polymorphism (RFLP) analysis of the bone marrow. The percentage of donor cells ranged from 50% to 100% at one month post-transplant. Seven patients had greater than or equal to 90% donor cells. Four patients had exclusively recipient marrow cells and recovered autologous hematopoiesis promptly. Only four patients received delayed DLI from 55 to 100 days post-transplant. One of the patients converted from 75% donor cells at six weeks post-transplant to 100% donor cells following the DLI. Five patients developed acute GVHD following the initial transplant. Two patients developed extensive chronic GVHD, which was fatal in one patient. Three patients developed GVHD following DLI; one of these patients had grade IV GVHD that was fatal. Eight patients achieved a complete response. At an initial median follow-up of 180 days, five of six patients (83.3%) with chemosensitive disease were alive compared to only two of nine patients (22.2%) who had chemorefractory or untreated disease.

Giralt and colleagues [9] treated 15 patients with acute myeloid leukemia or myelodysplastic syndrome. Preparative regimens were either fludarabine (30 mg/m²/d × 4 days) and idarubicin (12 mg/m²/d × 3 days) with either cytosine arabinoside (2,000 mg/m²/d × 4 days) or high-dose melphalan (140 mg/m²/d), or 2-chlorodeoxyadenosine (2-CDA), 12 mg/m²/d × 5 days) and cytosine arabinoside (1,000 mg/m²/d × 5 days). Thirteen patients received peripheral blood stem cell transplants from HLA-genotypically identical sibling donors and two from a one-antigen mismatched sibling donor. Chemotherapy was generally well tolerated with only one death from multiorgan failure. Thirteen patients had engraftment as measured by restriction fragment length polymorphism (RFLP) analysis of the bone marrow. The percentage of donor cells ranged from 50% to 100% at one month post-transplant. Seven patients had greater than or equal to 90% donor cells. Four patients had exclusively recipient marrow cells and recovered autologous hematopoiesis promptly. Only four patients received delayed DLI from 55 to 100 days post-transplant. One of the patients converted from 75% donor cells at six weeks post-transplant to 100% donor cells following the DLI. Five patients developed acute GVHD following the initial transplant. Two patients developed extensive chronic GVHD, which was fatal in one patient. Three patients developed GVHD following DLI; one of these patients had grade IV GVHD that was fatal. Eight patients achieved a complete response. At an initial median follow-up of 180 days, five of six patients (83.3%) with chemosensitive disease were alive compared to only two of nine patients (22.2%) who had chemorefractory or untreated disease.

Khouri [10] described 15 patients with chronic lymphocytic leukemia (CLL) or low to intermediate-grade non-Hodgkin’s lymphoma (NHL) who received fludarabine (30 mg/m²/d × 3 days) and cyclophosphamide (300 mg/m²/d × 3 days) or fludarabine (30 mg/m²/d × 2 days), cisplatin (25 mg/m²/d × 4 days), and cytosine arabinoside (500 mg/m²/d × 2 days). Eleven of 15 patients had donor cell engraftment as measured by restriction fragment length polymorphism (RFLP) analysis of the bone marrow. The percentage of donor cells ranged from 50% to 100% at one month post-transplant. Seven patients had greater than or equal to 90% donor cells. Four patients had exclusively recipient marrow cells and recovered autologous hematopoiesis promptly. Only four patients received delayed DLI from 55 to 100 days post-transplant. One of the patients converted from 75% donor cells at six weeks post-transplant to 100% donor cells following the DLI. Five patients developed acute GVHD following the initial transplant. Two patients developed extensive chronic GVHD, which was fatal in one patient. Three patients developed GVHD following DLI; one of these patients had grade IV GVHD that was fatal. Eight patients achieved a complete response. At an initial median follow-up of 180 days, five of six patients (83.3%) with chemosensitive disease were alive compared to only two of nine patients (22.2%) who had chemorefractory or untreated disease.

Table 2. Nonmyeloablative conditioning regimens for stem cell transplantation

<table>
<thead>
<tr>
<th>Investigator</th>
<th>n of Pts</th>
<th>Regimen</th>
<th>Diagnosis</th>
<th>% Donor Chimerism</th>
<th>GVHD Acute/Chronic</th>
<th>Outcome(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Giralt [9]</td>
<td>15</td>
<td>F + I + A</td>
<td>AML</td>
<td>≥90% (n = 7)</td>
<td>Alive: 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F + I + M</td>
<td>MDS</td>
<td>30% (n = 2)</td>
<td>Disease free: 2</td>
<td>Median 100 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-CDA + A</td>
<td></td>
<td>0% (n = 4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Khouri [10]</td>
<td>15</td>
<td>F + CY</td>
<td>CLL</td>
<td>≥50% (n = 11)</td>
<td>Alive: 5/6 CSD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F + C + A</td>
<td>NHL</td>
<td>0% (n = 4)</td>
<td>Alive: 2/9 RD/UTD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GD</td>
<td>Complete (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McSweeney [21]</td>
<td>44</td>
<td>TBI (200 cGy) post-transplant</td>
<td>HM</td>
<td>42/42 patients with full donor chimerism @ 2 mos</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MMF + CYA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Childs [24]</td>
<td>15</td>
<td>Cy + F ± ATG</td>
<td>HM</td>
<td>T-cell chimerism</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ST</td>
<td>40%-100% @ day 14</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100% in 10/12 @ day 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100% in 6/6 @ day 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spitzer [12, 13]</td>
<td>M (n = 28)</td>
<td>CY, ATG, TI</td>
<td>HM</td>
<td>Stable mixed M (n = 20) MM (n = 7)</td>
<td>M = 8/3</td>
<td>Alive: 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MM = 12/0</td>
<td>Disease free: 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Median 13.5 mos</td>
</tr>
</tbody>
</table>

F = fludarabine; I = idarubicin; A = cytosine arabinoside; M = melphalan; 2-CDA = 2-chloro-deoxyadenosine; B = busulfan; CY = cyclophosphamide; ALG = anti-T-lymphocyte globulin; TBI = total body irradiation; MMF = mycophenolate mofetil; CYA = cyclosporine; TI = thymic irradiation; AML = acute myeloid leukemia; MDS = myelodysplastic syndrome; CLL = chronic lymphocyte leukemia; NHL = non-Hodgkin’s lymphoma; HM = hematologic malignancies; GD = genetic diseases; M = matched; MM = mismatched; ST = solid tumors; CSD = chemosensitive disease; RD/UTD = refractory or untreated disease; NA = not available.
relapse (between 43 and 127 days) post-transplant. Six of 15 patients were alive between 34 and 175 days post-transplant. Acute GVHD occurred in three patients. None of the five evaluable patients had evidence of chronic GVHD.

Utilizing a busulfan-based preparatory regimen, Slavin and colleagues [11] demonstrated excellent tolerability and favorable survival probabilities in 22 patients with hematologic malignancies and four patients with genetic diseases. Preparative therapy consisted of busulfan at a dose of 8 mg/kg, plus fludarabine 180 mg/m2 and anti-T-lymphocyte globulin. Fourteen of the 22 patients with hematologic malignancy had low-risk disease (acute myeloid leukemia in first remission or chronic myeloid leukemia [CML] in chronic phase). G-CSF-mobilized peripheral blood stem cells were used as the source of stem cell support. GVHD prophylaxis consisted of CYA. Twenty-five patients received HLA-genotypically identical sibling donor transplants. One patient received stem cells from a donor with a single antigen mismatch at the A and C locus. Chimerism was evaluated by standard cytogenetic analysis, by analysis of residual Ph+ cells in patients with CML, or by polymerase chain reaction (PCR)-based variable number of tandem repeat (VNTR) sequence analysis in sex matched donor recipient combinations. Treatment was generally well tolerated. All patients had evidence of donor engraftment. In 9 of 26 evaluable patients, transient mixed chimerism was observed. Acute GVHD occurred in 12 of 26 patients. Six patients developed grade III-IV GVHD which was the sole cause of mortality in four patients. Limited chronic GVHD developed in 9 of 25 evaluable patients. At a median of eight months post-transplant, 22 of 26 patients (85%) were alive, 21 of whom (81%) were clinically disease free.

Based on their dog model showing that mixed chimerism is reliably achieved following low-dose total body irradiation (200 cGy) and post-transplant immunosuppression (MMF and CYA), McSweeney and colleagues have initiated a clinical trial utilizing this treatment. Forty-four patients with hematologic malignancies, ineligible for conventional allografting due to age, prior therapy or organ dysfunction were treated. Of 42 evaluable patients, all had persistent donor chimerism at two months post-transplant [21]. The regimen was remarkable for its excellent tolerability (lack of significant myelosuppression, gastrointestinal toxicities or alopecia) and the achievement of major disease responses in 70% of the patients.

Childs and colleagues described the engraftment kinetics after nonmyeloablative therapy with cyclophosphamide (60 mg/kg/d × 2 days) and fludarabine (25 mg/m2/d × 5 days) and HLA-matched or one-antigen mismatched donor peripheral blood stem cell transplantation for hematologic malignancy (n = 8) or solid tumors (melanoma, n = 4; renal cell cancer [RCC], n = 3). Patients who achieved mixed chimerism were eligible for monthly escalating doses of DLI. Full donor T-cell engraftment was more rapid than donor myeloid engraftment and was a prerequisite for acute GVHD and disease regression. Ten patients had a response (three patients with metastatic RCC; two with CML in chronic phase; one with chronic myelomonocytic leukemia; one with myelodysplastic syndrome; one with diffuse large-cell NHL; one with melanoma; and one with extramedullary plasmacytoma), five of whom were in a sustained complete remission (one metastatic RCC, three with CML and one with NHL). Eight were alive between 121 and 409 days post-transplant [24].

Utilizing a similar nonmyeloablative preparatory regimen to that used in the murine model of Sykes and colleagues [14, 18], we have reliably induced mixed lymphohematopoietic chimerism in patients with chemoradiotherapy refractory hematologic malignancies [12, 13]. Preparative therapy consisted of cyclophosphamide 150-200 mg/kg, antithymocyte globulin (ATG) 15-30 mg/kg/d on days –2, –1 and +1 or –1, +1, +3 and +5, and thymic irradiation in patients who had not previously received mediastinal radiation therapy. Post-transplant CYA was given as GVHD prophylaxis. Twenty-eight patients received an HLA-matched (HLA genotypically matched in 27 and phenotypically matched in 1) donor transplant while 16 received an HLA-mismatched donor transplant (one HLA 3-antigen, 11 HLA 2-antigen, and four HLA 1-antigen mismatch). Therapy has been well tolerated. Cyclophosphamide-induced cardiac toxicity has been observed in six patients and has been reversible in five. Severe ATG toxicities at a dose of 30 mg/kg in two patients necessitated a dose reduction to 15 mg/kg. Chimerism assays were performed using PCR analysis of VNTR or short tandem repeat sequences. In recipients of HLA-mismatched donor transplants, flow cytometric analysis of chimerism was performed using allele specific anti-HLA monoclonal antibodies (mAbs). Analyses were performed on weekly peripheral blood samples through day 100 post-transplant (then every six months) and on day +28, +100 and yearly bone marrow samples.

Of 23 evaluable recipients of HLA-matched donor transplantation, 20 have achieved stable mixed lymphohematopoietic chimerism. Ten patients with stable mixed chimerism, who had no evidence of GVHD, received DLI beginning on day +35 post-transplant. Conversion of mixed chimerism to fully donor hematopoiesis has occurred in six of the ten patients. Full donor T-cell chimerism was not necessary for the development of acute GVHD (or antitumor response).

Seven of 10 evaluable recipients of HLA-mismatched donor marrow transplants have achieved stable mixed
chimerism. Flow cytometric determinations of cell subsets exhibit discrepancies in the level of chimerism in various lineages. This “split lineage” chimerism has been seen in all evaluable recipients of HLA-2 antigen mismatched donor marrow transplants. Because of the presence of acute GVHD, none of the recipients of HLA-mismatched donor marrow received prophylactic DLI.

Striking antitumor responses have been seen in the majority of these patients with refractory hematologic malignancies, including three patients who received a prior autologous stem cell transplant. Of 23 evaluable patients with chemorefractory Hodgkin’s disease or NHL, seven (29%) achieved a partial remission and eight (33%) a complete response. Twenty-two patients are presently alive. Thirteen of these 22 patients are evaluable for response, and eight are clinically progression free. Notably, of the 10 patients who received delayed DLI beginning at day +35 post-transplant for conversion of their chimerism (and enhancement of their graft-versus-malignancy effect), six patients have achieved a complete remission and seven patients remain progression free.

Thus, the nonmyeloablative regimen that we have employed has been associated with a low incidence of acute GVHD following HLA-matched donor allogeneic BMT and in approximately one-half of the patients, a state of stable mixed chimerism without GVHD is induced allowing for the early administration of DLI. The majority of these patients have achieved a complete remission and are progression free. Several patients have had conversion of chimerism and achievement of a complete remission without the development of severe GVHD, confirming the findings in the Sykes murine model in which a potent lymphohematopoietic GVHD could be achieved following delayed DLI without clinical GVHD.

We have also demonstrated for the first time that stable mixed lymphohematopoietic chimerism can be achieved with this regimen following HLA-mismatched donor allogeneic BMT. The presence of GVHD, although manageable in most cases, has impeded the use of delayed DLI for chimerism conversion and enhancement of GVL effect in this population. This GVHD is believed to result from suboptimal in vivo donor T-cell depletion with ATG. A clinical trial evaluating a novel anti-CD2 mAb, which has been shown in preclinical models to affect a profound and sustained T-cell depletion, is in progress.

**OTHER APPLICATIONS OF NONMYELOABLATIVE PREPARATIVE REGIMENS FOR ALLOGENEIC STEM CELL TRANSPLANTATION**

Given the excellent tolerance of these nonmyeloablative regimens and the high rates of alloengraftment, even following transplants from HLA-mismatched donors, there has been considerable interest in extending these transplant strategies to patients with nonmalignant disease [11, 25, 26]. These include the genetic diseases described by Slavin et al. (beta-thalassemia major, Fanconi’s anemia, Blackfan Diamond anemia and Gaucher’s disease) [11]. In a separate report [25] the transplant group from Hadassah University Hospital reported a child with Fanconi’s anemia and leukemic transformation who underwent successful transplantation following a nonmyeloablative conditioning regimen consisting of fludarabine, cyclophosphamide and ATG. Two patients with primary T-cell immunodeficiency have been described who received only post-transplant immunosuppression with MMF and CYA [26]. Stable multilineage mixed chimerism was seen in both patients. Both patients developed grade II acute GVHD that responded to prednisone therapy. Studies of immune reconstitution in one patient showed a significant increase in numbers of T-cells, T-cell subsets and T-cell proliferative responses in vitro. We have shown that stable mixed erythroid chimerism is achievable following ABO mismatched BMT, raising the prospect of mixed chimerism transplant approaches for disorders such as sickle cell anemia and the thalassemias.

Given the therapeutic dilemmas that have surrounded the application of allogeneic BMT for conditions such as sickle cell anemia and thalassemia major, particularly the early transplant-related mortality risk among a group of patients who may have prolonged survival with medical therapy alone, these nonmyeloablative transplant approaches may have particular benefit. These advantages include a low transplant-related mortality risk, the development of stable mixed erythroid chimerism and the lack of a need to enhance a GVL effect by giving delayed DLI, all of which make this strategy particularly attractive for nonmalignant disorders.

Another potential application of nonmyeloablative transplant strategies and induction of mixed chimerism is the induction of donor-specific tolerance for solid organ grafts [27, 28]. We recently treated a 56-year-old patient with end-stage renal disease secondary to multiple myeloma with a combined HLA-matched bone marrow and sibling donor bone marrow and kidney transplant following a preparative regimen consisting of cyclophosphamide (120 mg/kg), thymic irradiation and peri-transplant ATG [28]. The patient achieved normal renal function, which has been sustained approximately ten months after withdrawal of all immunosuppression. While donor chimerism was lost after day +105 post-transplant, the patient remains without evidence of disease progression approximately one year post-transplant. This patient has, moreover, had no evidence of acute or chronic GVHD. Based on a series of animal models demonstrating that donor-specific tolerance can be induced for solid organ allografts following the achievement of mixed lymphohematopoietic chimerism, several clinical trials are
under way to further test this hypothesis. Since sustained tolerance can be induced even in the presence of transient hematopoietic chimerism, delayed DLI may be necessary only in the situation of an underlying malignant disease (where optimal GVL effects are desirable).

**REMAINING QUESTIONS AND FUTURE DIRECTION**

There have been many fascinating observations, both in preclinical models and clinical trials, about the safety and efficacy of nonmyeloablative allogeneic stem cell transplant strategies. The achievement of stable mixed lymphohematopoietic chimerism, even following transplants from HLA-mismatched donors, and the use of this mixed chimerism as a platform for subsequent adoptive cellular immunotherapy, mark significant advances in the field of transplantation, patient biology and immunology. However, there are likely as many questions remaining regarding the utility of these approaches as have been answered. These questions and the studies designed to answer them include:

1. **Is there a true distinction between myeloablative and nonmyeloablative regimens?**

   While there is still controversy regarding whether permanent marrow ablation occurs following the most widely utilized traditional transplant conditioning regimens, ample evidence indicates that host hematologic recovery occurs promptly in a small subset of patients, following these nonmyeloablative transplant regimens. The degree of myelosuppression is highly dependent upon the intensity of the regimen with, for example, less myelosuppression following very low-dose TBI compared to those employing high-dose cyclophosphamide. It is unclear from the published data whether prompt host hematologic recovery will occur if donor hematopoiesis is not established following more intensive conditioning regimens, such as those that employ intermediate-dose busulfan (i.e., the regimen developed by Slavin and colleagues incorporating busulfan 8.0 mg/kg) [11].

2. **Has the optimal nonmyeloablative regimen been determined?**

   A wide range of nonmyeloablative conditioning regimens has been employed. Most of these have been tested only in the setting of HLA-matched donor transplants. Some degree of donor engraftment has been achieved with each of these regimens. The presence of stable mixed lymphohematopoietic chimerism, if that was an original goal of the treatment program, has not been well documented in most series. Mixed chimerism is achievable with as little as 200 cGy of whole body radiation (with post-transplant immunosuppression) and can also be achieved following high-dose cyclophosphamide, peri-transplant ATG and thymic irradiation, even across major HLA barriers. It is not likely that a single conditioning regimen will be proven to be superior to others or applicable to all situations. Rapidly progressive hematologic malignancies in most situations require an initial cytoreduction of the tumor (thus likely requiring a reasonably aggressive chemotherapeutic preparation) in order to test whether a later GVL effect (as, for example, induced or potentiated by later DLI) will be operative. On the other hand, indolent hematologic malignancies (for example, early CLL) or nonmalignant disease may be optimally managed with conditioning regimens of lesser intensity. Long-term follow-up will also be required to determine the toxicities of these regimens. Concern, for example, has been raised about the potential for inducing secondary malignancies following low-dose whole body irradiation.

3. **Is mixed chimerism beneficial (or necessary) for GVHD prevention and optimal antitumor effect?**

   BMIs in which nonmyeloablative preparative therapy has been used appear to be associated with significantly less transplant-related morbidity, and possibly mortality. It does not, however, appear that these regimens are associated with significantly less acute GVHD. Thus, GVHD prophylaxis, using, for example, either CYA-based pharmacoprophylaxis or in vivo T-cell depletion using antibody-based therapy, is required. Mixed chimerism may create an important platform for the administration of adoptive cellular immunotherapy (DLI) and for optimization of the GVL effect. This two-step procedure may involve the initial induction of mixed chimerism with relatively little toxicity and manageable GVHD. A very potent effect of DLI can then be captured in the setting of less proinflammatory cytokines that may be in large part responsible for much of the early GVHD following conventional allogeneic transplantation. This strategy may be preferable for older patients, those with co-morbid disease, and those with malignancies already determined to be refractory to chemoradiotherapy.

4. **What is the optimal source of stem cells?**

   Both bone marrow and growth factor-mobilized peripheral blood stem cells have been used as sources of stem cell support following nonmyeloablative preparative regimens. There is a suggestion that immunologic recovery is faster following transplants in which peripheral blood stem cells are used compared to bone marrow [29, 30]. However, hematologic recovery is usually rapid following nonmyeloablative preparative regimens, regardless of which stem cell source is utilized. Similarly, a comparison of antitumor efficacy, whether bone marrow or peripheral blood stem cells are used, needs to be performed. Finally, if stable mixed lymphohematopoietic chimerism is a desirable goal,
the efficacy of mobilized peripheral blood stem cells in achieving this goal needs to be established. Theoretically, the markedly increased number of T-cells in a peripheral blood stem cell allograft could promote earlier and more complete donor chimerism and obviate the potential platform for delivering DLI. Other factors to be evaluated are the risks of chronic GVHD (which are likely increased following conventional conditioning therapy and allogeneic peripheral blood stem cell transplantation [31-33]) and relative risks to the donor of bone marrow versus peripheral blood stem cell collection [34].

5. What is the overall efficacy of nonmyeloablative transplant strategies versus conventional allogeneic BMT?

The published series of nonmyeloablative stem cell transplants have primarily involved either patients with refractory hematologic malignancies or who were otherwise poor candidates for conventional allogeneic BMT (because of age or co-morbid disease). For more standard indications (e.g., acute leukemia in first or subsequent remission or CML in chronic phase), prospective randomized trials will be necessary to determine the comparative safety and efficacy of these approaches. For other indications, (e.g., older patients with CLL who are not believed to be suitable candidates for conventional allogeneic BMT) these nonmyeloablative transplant strategies may become the transplant strategy of choice, provided that reasonable safety is confirmed and durable disease-free survival is achievable. Further study of the efficacy of nonmyeloablative transplant strategies for HLA-mismatched donor transplantation will also be required. These studies will be important, both because of the frequent need for alternative donor sources and the enhanced antitumor (i.e., GVL) effect that may occur in the setting of HLA incompatibility.

REFERENCES


