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High-dose, post-transplantation cyclophosphamide to promote graft-host tolerance after allogeneic hematopoietic stem cell transplantation

Leo Luznik · Ephraim J. Fuchs

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Abstract Graft-versus-host disease, or GVHD, is a major complication of allogeneic hematopoietic stem cell transplantation (alloHSCT) for the treatment of hematologic malignancies. Here, we describe a novel method for preventing GVHD after alloHSCT using high-dose, post-transplantation cyclophosphamide (Cy). Post-transplantation Cy promotes tolerance in alloreactive host and donor T cells, leading to suppression of both graft rejection and GVHD after alloHSCT. High-dose, post-transplantation Cy facilitates partially HLA-mismatched HSCT without severe GVHD and is effective as sole prophylaxis of GVHD after HLA-matched alloHSCT. By reducing the morbidity and mortality of alloHSCT, post-transplantation Cy may expand the applications of this therapy to the treatment of autoimmune diseases and non-malignant hematologic disorders such as sickle cell disease.

Keywords Bone marrow transplantation · Graft-versus-host disease · Graft-versus-leukemia effect · Cyclophosphamide · Human leukocyte antigens · T lymphocytes

Introduction: the promise and challenges of allogeneic hematopoietic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation, or alloHSCT, is a potentially curative treatment for a variety of hematolymphoid malignancies including the acute and chronic leukemias, lymphoma, multiple myeloma, myelodysplastic syndrome, and the myeloproliferative disorders. The therapy comprises the administration of transplantation conditioning, consisting of high-dose chemotherapy with or without total body irradiation (TBI), followed by the intravenous transfusion of marrow or peripheral blood from a related or unrelated donor. Initially, intensive conditioning of the patient was felt to be necessary to

L. Luznik · E. J. Fuchs (✉)
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, 288 Bunting-Blaustein Cancer Research Building, 1650 Orleans Street, Baltimore, MD 21231, USA
e-mail: fuchsep@jhmi.edu

eradicate the hematologic malignancy, and the graft was given to rescue the patient from the lethal effects of conditioning on the bone marrow and on hematopoiesis [1]. However, pre-clinical and clinical studies conclusively demonstrated the existence of a "graft-versus-leukemia", or GVL, effect of alloHSCT mediated by donor T-cell recognition of histocompatibility antigens expressed by the recipient's normal and leukemia cells [2–4]. The GVL effect of alloHSCT is oftentimes, but not always, linked to the development of graft-versus-host disease (GVHD), a donor T cell attack on normal tissues resulting in skin rash, diarrhea, liver damage, and profound immunosuppression. Transplantation immunologists have attempted unsuccessfully for decades to develop methods of inducing GVL effects without causing GVHD. Nonetheless, the GVL effect of alloHSCT remains the best, if not only, evidence that T cells can shrink or eradicate advanced cancers in humans.

The choice of alloHSCT as treatment of a hematologic malignancy is immensely difficult for the treating physician and especially for the patient as it must balance the potential benefits of the GVL effect against the substantial toxicities of the conditioning regimen and of GVHD. The risk of dying from the transplant itself can be as high as 30% or greater, depending upon a variety of patient and disease characteristics. Recipients of a T-cell-replete graft must be treated with drugs to prevent GVHD; these drugs globally suppress the donor's immune system and increase the risk of serious infection and possibly relapse. Finally, HLA mismatching between donor and recipient has been associated with a markedly increased risk of severe GVHD and treatment-related mortality, and poor event-free survival (EFS) [5, 6]. These results militate in favor of choosing an HLA-matched sibling or unrelated donor over a partially HLA-mismatched related or unrelated donor. Unfortunately, only 30% of patients have an HLA-matched sibling, and the likelihood of finding an HLA-matched unrelated donor ranges from approximately 20% for African-Americans to as high as 80% for Caucasians of Northern European origin. Thus, an HLA-matched donor is not available for as many as half of patients referred for alloHSCT.

The application of alloHSCT to treat hematologic malignancies, or even non-malignant hematologic disorders such as inherited hemoglobinopathies or autoimmune diseases, is limited by the substantial risks of conditioning regimen toxicity, GVHD, profound immunosuppression, and the lack of suitably matched donors for as many as half of patients. The challenge to transplantation immunologists is to improve the safety and efficacy of alloHSCT primarily by harnessing alloreactivity to induce graft-versus-tumor effects without graft rejection or GVHD.

Suppression of alloreactivity using drug-induced immunologic tolerance

Since T cells specific for histocompatibility antigens mediate GVHD after HLA-matched or partially HLA-mismatched BMT in humans, some of the earliest attempts to prevent GVHD after alloHSCT involved depleting mature T cells from the transplanted bone marrow [7, 8]. T-cell depletion of the graft significantly reduces the incidence and severity of GVHD after alloHSCT, but at the expense of increased graft rejection, infection, and relapse [9]. Consequently, T-cell depletion of the graft has not been shown to improve outcomes after HLA-matched or HLA-mismatched alloHSCT. GVHD may also be prevented by the administration of immunosuppressive drugs after transplantation. Methotrexate (MTX), an anti-metabolite drug with immunosuppressive properties, was one of the first drugs used to prevent GVHD after alloHSCT [10], but the real watershed in GVHD prophylaxis came with the advent of the calcineurin inhibitors (CNIs), cyclosporine A, and tacrolimus [11–13]. CNIs specifically inhibit intracellular signals that are generated by

ligation of the clonotypic T-cell antigen receptor and are required for T-cell activation [14]. By inhibiting the activation of alloreactive T cells, CNIs have reduced the incidence of acute GVHD and non-relapse mortality (NRM) after allogeneic BMT [12]. Current standard regimens of GVHD prophylaxis comprise the combination of a CNI, cyclosporine, or tacrolimus, and either MTX, mycophenolate mofetil (MMF), or sirolimus [15–18]. However, CNIs also block T-cell development [19] and the induction of T cell [20] and transplantation tolerance [21], leading some investigators to hypothesize that cyclosporine treatment and/or withdrawal itself could play a role in the development of chronic GVHD [22, 23]. Accordingly, chronic GVHD occurs in as many as 50% of allogeneic transplant recipients following GVHD prophylaxis with CNIs [15, 16, 18, 24, 25]. Potential toxicities of CNIs are substantial and include profound immunosuppression with risk of opportunistic infection, hypertension, renal dysfunction, reversible encephalopathy, and the hemolytic-uremic syndrome. CNIs also may increase the risk of relapse by blunting the GVL effect [26]. Clearly, there is a need to develop agents that prevent GVHD without causing global immunosuppression.

Our laboratories became interested in the protocol of drug-induced immunologic tolerance (DIT) as a method to suppress GVHD without causing global immunoincompetence. In the first description of DIT [27], rabbits were immunized with human serum albumin (HSA) and then treated daily for 1 week with the anti-metabolite 6-mercaptopurine. Rabbits treated this way were rendered specifically tolerant of HSA: they failed to make antibody against HSA even after repeated challenge with the protein, but were able to make antibodies in response to immunization with bovine gammaglobulin. In 1963, Berenbaum showed that cyclophosphamide (Cy) administration prolonged the survival of allogeneic skin grafts in mice, especially if the drug was given 1–3 days after placement of the skin graft [28]. Santos and Owens [29] found that Cy suppressed the incidence and severity of allogeneic GVHD in rats given allogeneic spleen cells, especially if the drug was commenced on day 2 after the spleen cell infusion. Finally, Mayumi et al. [30] developed a method for inducing tolerance to non-MHC, or "minor" histocompatibility antigens by giving mice an intravenous injection of MHC-matched, allogeneic spleen cells followed in 2–3 days by an intraperitoneal injection of high-dose Cy. Such animals were completely tolerant of the allogeneic cells, as demonstrated by persistent low levels of donor cell chimerism, but were able to reject skin grafts from third party allogeneic donors. These investigators postulated three distinct and sequential mechanisms for the induction and maintenance of tolerance [31]. The first mechanism was destruction of donor-Ag-stimulated T cells in the periphery by Cy treatment. This mechanism is illustrated in Fig. 1. Of note, Cy induces selective allodepletion by killing host and donor T cells proliferating in response to donor and host cells, respectively (Fig. 1, top), while sparing T cells that do not react to either host or donor alloantigens (Fig. 1, bottom). These cells may provide the transplant recipient with immunity to infection in the short term and immune reconstitution in the long term. The second mechanism was intrathymic clonal deletion of donor-reactive T cells, correlating strongly with intrathymic mixed chimerism. The third mechanism was generation of tolerogen-specific suppressor T cells, especially in the late stage of the tolerance [32].

Pre-clinical studies of post-transplantation Cy

The studies in rodents described earlier demonstrated that: (1) post-transplantation Cy is capable of inducing durable tolerance to MHC-compatible hematopoietic grafts differing in the expression of multiple minor histocompatibility antigens, analogous to HLA-matched

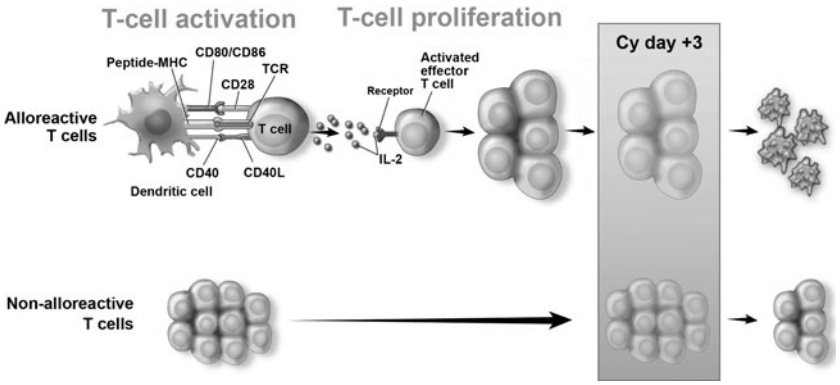

Fig. 1 Proposed mechanism for the induction of transplantation tolerance by high-dose cyclophosphamide. *Top*: alloreactive T-cell recognition of alloantigens on dendritic cells leads to T-cell activation, interleukin-2 production, and proliferation. Proliferating, alloreactive T cells are killed by a properly timed dose of Cy, an S phase-specific drug, given on day 3. *Bottom*: non-alloreactive T cells remain in a resting state and are resistant to being killed by high-dose Cy

BMT in humans; and (2) post-transplantation Cy mitigates GVHD after MHC-mismatched lymphocyte infusion. With these results as background, our group set out to develop a conditioning regimen that did not eradicate host hematopoiesis but would permit the sustained engraftment of partially MHC-mismatched grafts without severe GVHD. The use of a non-myeloablative conditioning regimen for partially HLA-mismatched alloHSCT in humans would provide the safeguard of recovery of autologous hematopoiesis in the event of rejection of the donor marrow graft. This objective was achieved in a mouse model of MHC-mismatched alloHSCT using pre-transplantation conditioning with fludarabine, a highly immunosuppressive purine analog, and 200 cGy TBI, and GVHD prophylaxis with high-dose, post-transplantation Cy (200 mg/kg intraperitoneally) [33]. This regimen was sufficient to induce stable engraftment of donor cells in 100% of transplanted mice. The conditioning did not ablate host hematopoiesis, since autologous hematopoietic recovery occurred in mice that received the conditioning and high-dose Cy but did not receive an allogeneic marrow infusion. Further, high-dose Cy given on day 2 after transplantation mitigated both the incidence and severity of acute GVHD in an MHC-mismatched donor–recipient combination. These results provided the pre-clinical rationale to proceed with a clinical trial of partially HLA-mismatched, or HLA-haploidentical, bone marrow transplantation with high-dose, post-transplantation Cy for patients with poor prognosis hematologic malignancies.

Non-myeloablative, HLA-haploidentical BMT for hematologic malignancies

The major rationale for HLA-haploidentical BMT is to extend the potential benefits of alloHSCT and the graft-versus-tumor effect to patients who lack an HLA-matched donor, and the major challenge is to reduce the incidence of fatal graft rejection, severe GVHD, and treatment-related mortality. A non-myeloablative, outpatient conditioning regimen was developed based upon the pre-clinical modeling and is shown in Fig. 2. We recently reported the outcomes of 68 patients with poor-risk hematologic malignancies who were

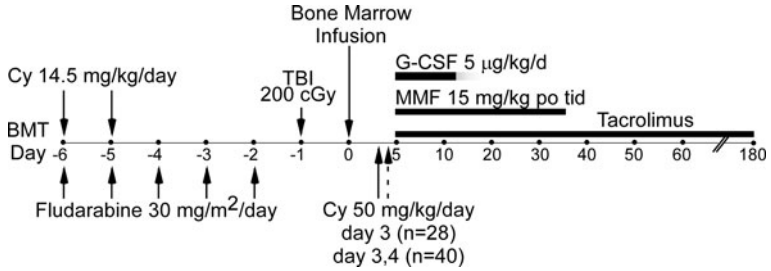

Fig. 2 Schema of non-myeloablative, HLA-haploidentical bone marrow transplantation with high-dose, post-transplantation cyclophosphamide. *Cy*, cyclophosphamide; *BMT*, bone marrow transplantation; *TBI*, total body irradiation; *G-CSF*, granulocyte-colony stimulating factor; *MMF*, mycophenolate mofetil

conditioned with fludarabine, Cy, and 2 Gy TBI prior to receiving T-cell-replete bone marrow from HLA-haploidentical, first-degree relatives [34]. Donors and recipients were mismatched at a median of 4 out of 5 HLA alleles (HLA-A, -B, -Cw, -DRB1, and -DQB1), indicating substantial donor–recipient histoincompatibility. GVHD prophylaxis comprised Cy 50 mg/kg IV on day 3 ($n = 28$) or on days 3 and 4 ($n = 40$) after transplantation, followed by Tacrolimus and MMF, each beginning on the day after the last dose of Cy. These two drugs were included to provide extra protection against graft failure and GVHD and were started after the completion of Cy because of pre-clinical evidence that CNIs block Cy-induced transplantation tolerance by blocking the activation and proliferation of alloreactive T cells [35]. Engraftment of neutrophils and platelets occurred at a median of 16 and 24 days, respectively, after transplantation. Graft failure occurred in nine patients (13%) but was fatal in only one. Acute grades II–IV and III–IV GVHD occurred in 34% and 6% of patients, respectively, and chronic GVHD developed in 15% of patients. These rates of GVHD development are comparable to or below the incidences of acute and chronic GVHD after HLA-matched alloHSCT without post-transplantation Cy [36]. The cumulative incidences of relapse and NRM at 1 year after transplantation were 15 and 51%, respectively, and overall survival and EFS at 2 years after transplantation were 36 and 26%. Only six patients died of infection ($n = 4$) or GVHD ($n = 2$). This trial established the safety and potential efficacy of high-dose, post-transplantation Cy in preventing GVHD and fatal graft rejection after HLA-haploidentical HSCT.

A subsequent report retrospectively compared the outcomes of patients with Hodgkin lymphoma (HL) treated with non-myeloablative conditioning and grafts from HLA-matched related ($n = 38$), unrelated ($n = 24$), or HLA-haploidentical related ($n = 28$) donors [37]. Recipients of HLA-haploidentical grafts were conditioned as in Fig. 2. Patients had received a median of five prior regimens, including autologous HSCT in 92%. With a median follow-up of 25 months, 2-year OS, EFS, and incidences of relapsed/progressive disease were 53, 23, and 56% (HLA-matched related), 58, 29, and 63% (unrelated), and 58, 51, and 40% (HLA-haploidentical related), respectively. NRM was significantly lower for HLA-haploidentical related recipients compared to HLA-matched related recipients ($P = .02$). There were also significantly decreased risks of relapse for HLA-haploidentical related recipients compared to HLA-matched related ($P = .01$) and unrelated ($P = .03$) recipients. In a recent report from the CIBMTR, patients with HL receiving reduced intensity, unrelated donor HSCT had a 2-year OS and EFS of 37 and 20%, respectively [38]. HLA-haploidentical HSCT may therefore be uniquely effective for patients with relapsed or refractory HL.

In light of previous publications showing an adverse effect of HLA mismatching on the outcomes of myeloablative conditioning and HLA-haploidentical HSCT [5, 6, 39], we conducted a retrospective analysis of the effect of HLA mismatching on the outcome of 185 patients with hematologic malignancies treated with non-myeloablative, HLA-haploidentical SCT, and post-transplantation Cy [40]. Surprisingly, increasing HLA mismatch between donor and recipient did not have a detrimental impact on overall and EFS following non-myeloablative, HLA-haploidentical HSCT and even appeared to be beneficial. This apparent beneficial effect of increasing degree of HLA mismatch between donor and recipient is shown in Fig. 3a, which plots EFS according to the number of HLA antigen mismatches in either direction (host-versus-graft or graft-versus-host). The hazard ratio of .8 suggests that each increment of HLA antigen mismatch is associated with a 20% reduction in the risk of an event, either relapse or NRM. Interestingly, increasing HLA mismatch in the graft-versus-host direction was not associated with an increased risk of acute GVHD (Fig. 3b; HR .89, 95% CI .50–1.58, $P = .68$). We next analyzed the effect of HLA Class I or Class II antigen mismatches on the outcome of non-myeloablative, HLA-haploidentical BMT with post-transplantation Cy (Table 1). The presence of an HLA-DRB1 antigen mismatch in the GVH direction was associated with a significantly lower cumulative incidence of relapse ($P = .04$) and improved EFS ($P = .009$), whereas HLA-DQB1 antigen mismatch status had no effect (not shown). Additionally, the presence of two or more Class I antigen mismatches (composite of HLA-A, -B, and -Cw) in the host-versus-graft direction was associated with a significantly lower cumulative incidence of relapse ($P = .02$) and improved EFS ($P = .02$). These results should be taken with a healthy dose of skepticism owing to the retrospective nature of the analysis [41] and the small numbers of pairs with two or fewer HLA antigen mismatches ($n = 26$). Nonetheless, the results are remarkable in two respects. First, this is to our knowledge, the first report in which increasing HLA mismatch did not have a detrimental impact on overall or EFS following HLA-haploidentical HSCT. Although some studies of unrelated or mismatched related donor HSCT have found a lower relapse risk with increasing degrees of HLA mismatch or with specific mismatches, suggesting a graft-versus-tumor effect, this has generally been offset by higher rates of GVHD, graft failure, and NRM [5, 6, 39, 42–45]. In our study, the reduced risk of relapse with HLA-DRB1 or HLA Class I antigen mismatching was not offset by increased NRM, and so EFS improved with increasing mismatch (Table 1). Second, the

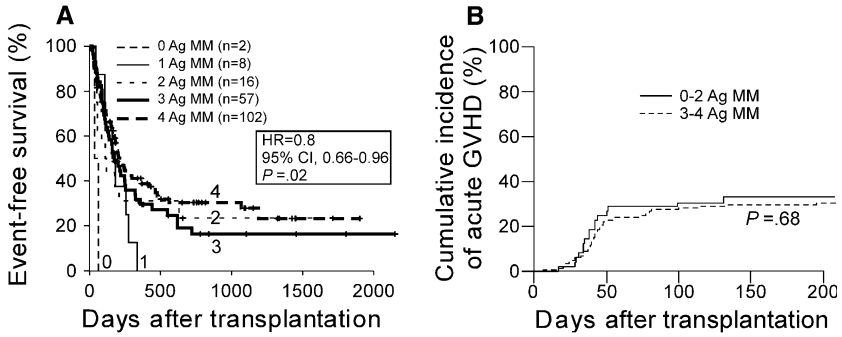

Fig. 3 **a** Event-free survival according to the number of HLA antigen mismatches in either the graft-versus-host or the host-versus-graft direction. *Ag MM*, antigen mismatch; *HR*, hazard ratio; *CI*, confidence interval. **b** Cumulative incidence of acute, grades II–IV GVHD according to the number of HLA antigen mismatches (composite of HLA-A, -B, -Cw, and -DRB1) in the graft-versus-host direction

Table 1 Effect of selected HLA mismatches on outcome of non-myeloablative, HLA-haploidentical HSCT with high-dose, post-transplantation Cy

	Hazard ratio (95% CI); *P* value for:	
	HLA-DRB1 antigen mismatch in graft-versus-host direction: 1 ($n = 131$) vs. 0 (reference; $n = 54$)	HLA Class I antigen mismatch in host-versus-graft direction: 2–3 ($n = 152$) vs. 0–1 (reference; $n = 33$)
Graft-versus-host disease	1.06 (.60–1.88); $P = .84$	–
Relapse	.65 (.43–.99); $P = .04$	.55 (.33–.91); $P = .02$
Non-relapse mortality	.87 (.40–1.93); $P = .74$	1.33 (.46–3.85); $P = .59$
Event-free survival	.62 (.43–.89); $P = .009$	.60 (.39–.92); $P = .02$

observation that HLA-DRB1 antigen mismatching in the GVH direction was associated with a decreased risk of relapse without an increased incidence of GVHD suggests that this transplantation regimen can separate the GVT effect from GVHD.

How could this regimen separate a GVT effect from GVHD when such an effect has not been apparent in prior HLA-haploidentical HSCT protocols? We can think of at least two possibilities. First, it is possible that post-transplantation Cy is more toxic to donor T cells that cause GVHD than to donor T cells that induce a GVT effect. For example, it is possible that Cy is more toxic to naïve T cells, which can cause severe GVHD, than to memory T cells, which induce a graft-versus-tumor effect without severe GVHD [46–48]. A second possibility is that this non-myeloablative HSCT regimen effectively unmasks an anti-tumor effect of host T cells, which are genetically tolerant of host histocompatibility antigens and so cannot mediate acute GVHD. The conditioning regimen could unmask host-mediated anti-tumor immunity by killing or inactivating regulatory T cells while sparing tumor-specific host $CD8^+$ T cells. According to this possibility, the dual benefit of HLA-DRB1 antigen mismatching in the GVH direction and HLA Class I antigen mismatching in the HVG direction may reflect a cooperation between donor $CD4^+$ T cells and host $CD8^+$ T cells in mediating anti-tumor immunity. This cooperation would end either upon acquisition of donor $CD4^+$ T-cell tolerance of the host or, more likely, upon elimination of host $CD8^+$ T cells by the GVH reaction. There is already evidence for the participation of host T cells, including $CD8^+$ T cells, in anti-tumor immunity after allogeneic HSCT or donor lymphocyte infusion, including: (1) involvement of host $CD8^+$ T cells in the anti-tumor response of mixed hematopoietic chimeras to post-transplantation tumor vaccines [49]; (2) tumor responses despite graft rejection following allogeneic HSCT [50]; (3) an anti-tumor effect of infusing recipient lymphocytes into mixed hematopoietic chimeras created by non-myeloablative alloHSCT [51]; and (4) participation of host T cells in the anti-tumor effect of transiently engrafting allogeneic donor lymphocyte infusions [52]. Potential mechanisms of a host T-cell-mediated anti-tumor effect include direct killing of tumor cells by tumor-specific T cells or secretion of tumoricidal cytokines by host T cells specific for donor alloantigens. Demonstrating that host T cells participate in anti-tumor immunity after alloHSCT would establish the more general principle that the adaptive immune system is not irreversibly tolerant of growing tumors and can be stimulated to mediate tumor regression contingent upon the creation of an appropriate immunologic environment, including the destruction or inhibition of regulatory T cells, presentation of tumor antigens in the context of immunologic "danger signals", and provision of $CD4^+$ T cell help.

High-dose Cy as sole GVHD prophylaxis after HLA-matched alloHSCT

Despite precise molecular matching of donor and recipient HLA alleles, post-transplantation administration of immunosuppressive drugs is still required to prevent graft rejection or severe GVHD after HLA-matched sibling or unrelated donor HSCT. The most commonly used GVHD prophylaxis after myeloablative alloHSCT comprises the combination of MTX and a CNI, either cyclosporine A (CsA) or Tacrolimus. However, acute GVHD still occurs in 35–55% of BMT recipients from HLA-matched siblings, and more frequently in unrelated donor BMT recipients [18, 24, 53, 54]. While CNIs inhibit acute GVHD, they are not as effective in reducing the incidence of chronic GVHD even if their administration is prolonged up to 24 months [55]. Moreover, CNIs induce global immunosuppression, impair immune reconstitution by inhibiting T-cell development, and may increase the risk of disease relapse. Thus, reduction or even elimination of the use of CNIs after alloHSCT may be quite beneficial for patients with hematologic malignancies. Furthermore, elimination of CNIs may result in reduced renal and neurologic toxicity, speedier immune reconstitution with a decreased incidence of opportunistic infection such as cytomegalovirus or invasive fungal infection, and overall reduced morbidity.

By blocking intracellular signals that result from T-cell recognition of antigen, the CNIs block T-cell tolerance as well as T-cell activation. In contrast, the ideal agent for GVHD prevention would cause apoptotic death of host-reactive donor lymphocytes shortly after alloBMT while leaving other donor T cells intact. Strauss et al. [56] have shown that Cy and MTX are the only agents capable of inducing apoptosis of alloactivated T cells, thereby promoting the induction of transplantation tolerance. Since the toxicity of MTX does permit dose escalation sufficient for the induction apoptosis of alloreactive T cells in vivo, high-dose Cy was the most reasonable choice to examine in the clinic.

We studied whether high-dose Cy alone is sufficient prophylaxis of GVHD after myeloablative HLA-matched related or unrelated donor BMT. We have recently reported the results of one hundred and seventeen consecutive patients with hematologic malignancies treated on a phase I/II clinical trial; 78 patients received HLA-matched related and 39 matched unrelated donor allografts [57]. Transplantation conditioning consisted of oral or intravenous busulfan from days -7 to -4 (target AUC 800-1400) and Cy 50 mg/kg on days -3 and -2, followed by an infusion of donor marrow obtained in a targeted collection of 4×10^8 nucleated cells/kg (Fig. 4). No growth factors were administered. The median patient age was 50 years with 14 patients being 60 years or older, the most common diagnosis was acute myeloid leukemia (AML; $n = 58$, or 50%), and 68 patients (58%)

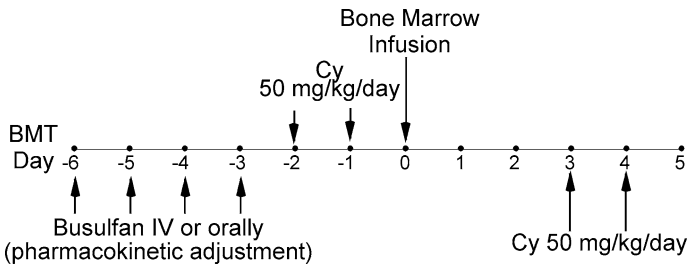

Fig. 4 Schema of myeloablative conditioning and HLA-matched bone marrow transplantation with high-dose, post-transplantation cyclophosphamide as sole prophylaxis of GVHD. *BMT*, bone marrow transplantation; *IV*, intravenously; *Cy*, cyclophosphamide

were not in remission at the time of transplant. Sustained engraftment of donor cells occurred in 114 patients (98%), with the median time to neutrophil recovery of 23 days for recipients of related and 25 days for those that received unrelated allografts. High-dose post-transplantation Cy was well-tolerated; the most common toxicities were transient mild renal dysfunction or elevations of serum liver enzymes, while hepatic veno-occlusive disease, a severe complication of alloHSCT, developed in 10 patients (9%) and was lethal in 2. NRM at day 100 and 1 year after transplantation was 9 and 17%, respectively. The cumulative incidence of moderate-severe acute GVHD by day 200 after transplantation was 42 and 46% among recipients of related or unrelated donor grafts, respectively. The incidence of severe acute GVHD for all patients was 10%. At 2 years after transplantation, the cumulative incidences of chronic GVHD for recipients of related versus unrelated donor grafts were 9 and 11%, respectively. Of 11 patients with chronic GVHD, seven had classic limited and three had classic extensive forms of the disease. The efficacy of high-dose Cy in preventing GVHD is further supported by the fact that more than half of the patients never required additional immunosuppressive medications. At the time of last follow-up, only three patients, all with chronic GVHD, remain on systemic immunosuppressive agents. In multivariate analysis, the only variable associated with an increased risk of developing moderate-severe acute GVHD was a male recipient of a female donor graft ($P = .05$).

The actuarial OS and EFS for all patients at 1 year after transplantation were 63 and 48% and at 2 years after transplantation were 55 and 39%, respectively. Consistent with other recent studies, EFS and OS did not differ according to the donor type. The cumulative incidence of relapse for patients transplanted in remission was 26% at 2 years after transplantation. Among patients with myelodysplastic syndrome or AML in remission at the time of transplantation, the one and 2 year EFS were 61 and 52%, respectively. In conclusion, high-dose, post-transplantation Cy is effective as sole prophylaxis of GVHD after HLA-matched alloHSCT and may not significantly compromise the allogeneic GVT effect.

HLA-matched alloHSCT with high-dose, post-transplantation Cy was marked by prompt immune reconstitution and a low incidence of opportunistic infections. Among 54 patients studied consecutively, the median absolute lymphocyte counts on day 30 and 60 after transplantation were 440 and >700/μl, respectively. The median $CD4^+$ T-cell count on day 60 after transplantation was 119/μl. Levels of Foxp3 mRNA, the signature transcript of regulatory T cells, in the peripheral blood of patients who did not develop GVHD were more than 100-fold greater than those detected in patients who developed GVHD. These results correlated with significantly lower levels of $CD4^+$ $CD25^+$ $Foxp3^+$ cells in the blood of patients who developed GVHD. The frequency of cells secreting interferon gamma in response to stimulation with pentadecapeptides of the immunodominant CMV protein, pp65, at day 30–60 after alloHSCT did not differ from pre-transplant specimens from CMV-seropositive donor/recipient pairs. Reactivation of CMV occurred in 32% of patients, and there was only one documented case of CMV disease and no CMV-associated mortality. The absence of post-transplant Epstein–Barr virus-associated lymphoproliferative disease is another indicator of the prompt immunologic recovery with post-transplantation Cy.

Ongoing studies and future directions

The clinical trials described here demonstrate that high-dose Cy can be administered safely after allogeneic HSCT without causing prolonged aplasia from toxicity to donor stem cells.

Moreover, the results suggest that high-dose, post-transplantation Cy is uniquely effective in preventing chronic GVHD, perhaps by inducing tolerance in the T cells that cause this disease. The first step in the ongoing development of post-transplantation Cy for GVHD prophylaxis is to determine whether these data can be replicated at other transplantation centers via recently initiated multi-center trials. The major problem associated with the partially HLA-mismatched alloHSCT protocol is a persistently high relapse rate. We plan to explore strategies to reduce the incidences of graft failure and relapse without increasing transplant-related toxicity. Examples of potential approaches include enhancing the anti-tumor activity of the conditioning regimen, immunizing donors against tumor-specific antigens, or infusing alloreactive donor natural killer cells after transplantation. The advantage of high-dose Cy as sole GVHD prophylaxis after HLA-matched BMT is that the elimination of CNIs permits the reconstitution of the immune system in an environment free of ongoing pharmacologic immunosuppression. This environment may be ideal for preventing post-transplantation infection or relapse using novel strategies of active immunotherapy, such as vaccinating donors before transplantation and recipients early after transplantation with viral or tumor vaccines. By mitigating alloreactivity after allo-HSCT with post-transplantation Cy, we ultimately hope to apply alloHSCT to treat immunodeficiencies or non-malignant disorders of hematopoiesis. We have recently reported the successful treatment of sickle cell disease and paroxysmal nocturnal hemoglobinuria with non-myeloablative, HLA-haploidentical HSCT, and post-transplantation Cy [58]. Additional diseases that are amenable to the application of alloHSCT with post-transplantation Cy include autoimmune disease or the acquired immunodeficiency syndrome, using donors lacking a functional CCR5 receptor for HIV [59].

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