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Ask the Expert

Post-Transplant High-Dose Cyclophosphamide for the Prevention of Graft-versus-Host Disease

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Cyclophosphamide's lack of hematopoietic stem cell toxicity and its unique effects on the immune system have prompted several investigators to explore its potential for the prevention of graft-versus-host disease (GVHD). In haploidentical hematopoietic stem cell transplants, post-transplant cyclophosphamide together with standard prophylaxis reduces the incidence of GVHD to acceptable rates without the need for T cell depletion. In matched related and unrelated donor settings, cyclophosphamide alone has produced encouraging results. In particular, the low incidence of chronic GVHD is noteworthy. Here, we present a review of the current understanding of the mechanism of action of post-transplant cyclophosphamide and summarize the clinical data on its use for the prevention of GVHD.

INTRODUCTION

Although considerable advances have been made in HLA matching and donor selection, graft-versus-host disease (GVHD) remains a major impediment to the development and widespread applicability of allogeneic blood and marrow transplantation [1-3]. Despite the routine use of prophylactic therapies, acute GVHD complicates 40% to 50% of transplants, whereas chronic GVHD is encountered in 10% to 80% of cases [1-3]. The detrimental effect of current prophylactic regimens on immune reconstitution and the potential to abolish graft-versus-disease (GVD) effect have provided additional impetus to develop alternative regimens.

In 1963, based on personal work and that of others, Berenbaum [4] reported that skin allografts in mice have better survival after the administration of a single dose of cyclophosphamide 1 to 3 days after grafting. Subsequent animal experiments and clinical trials enhanced our understanding of the immune modulatory effects of cyclophosphamide and established a platform for innovation and progress in the field of transplantation. Here, we review the data on the use of post-transplant high-dose cyclophosphamide for the prevention of GVHD.

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RATIONALE AND MECHANISM OF ACTION

Cyclophosphamide is an alkylating agent of the nitrogen mustard category. It has been in use for many years and has an excellent toxicity profile [5]. Cyclophosphamide is oxidatively metabolized by the hepatic cytochrome P450 into 2 powerful metabolites, phosphoramide mustard and acrolein, and prevents cell division by crosslinking DNA strands. Although the alkylating effect of the active metabolites occurs throughout the cell cycle, it is most pronounced during the G1 and S phases of cell division [6]. Rapidly proliferating cells subjected to DNA crosslinking are more susceptible to cyclophosphamide because of their reduced ability to replicate damaged DNA. Conversely, hematopoietic stem cells that are rich in aldehyde dehydrogenase, the enzyme required for the conversion of phosphoramide mustard into the inactive metabolite carboxycyclophosphamide, are resistant to cyclophosphamide. Consequently, cyclophosphamide can be administered after allogeneic hematopoietic cell transplant without impairing engraftment [7].

A fundamental difference of the effect of cyclophosphamide on T cells in contrast to other immunosuppressive agents is its ability to induce apoptosis. Comparing the effects of different drugs on human peripheral blood T cells, cytotoxic cell lines, and Jurkat T cells, Strauss et al. [8] found that only cyclophosphamide (and methotrexate) triggered cell death. All other drugs, including steroids, calcineurin inhibitors, sirolimus, and mycophenolate mofetil (MMF)

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inhibited T cells without causing apoptosis. In addition, cyclophosphamide up-regulated Fas (CD95) expression, triggering activation-induced cell death within 6 days of activation. None of the other drugs affected CD95 expression.

Early animal experiments identified cyclophosphamide as the most effective drug to suppress antibody production in response to bacterial vaccines [9]. In addition, unlike other antimitotics, cyclophosphamide did not need to be used at low doses over prolonged periods of time [4,9]. In organ transplantation, pivotal experiments showed that cyclophosphamide administered intraperitoneally in single high dose early after skin allografts in mice was sufficient to delay graft rejection across MHC mismatches. The timing of cyclophosphamide administration was critical [4,10]. Although administration of cyclophosphamide to mice before or simultaneously with allogeneic stimuli suppressed antibody production, tolerance was not induced. The maximal effect in improving graft survival was observed when cyclophosphamide was given between grafting and day 4 afterward [4,10]. Furthermore, although cyclophosphamide only delayed graft rejection in MCH mismatches, it induced definitive tolerance across minor histocompatibility mismatches [11,12].

Based on earlier experiments performed by Nirmul et al. [11,12], Mayumi et al. [13] established a "cells-followed-by-cyclophosphamide" scheme in which administration of allogeneic spleen cells followed by cyclophosphamide administration 2 days thereafter induced tolerance to allografts in different mouse models. Several studies have examined the mechanism of action of cyclophosphamide. $V_{\beta 6}$-bearing T cells directed to minor lymphocyte stimulating-1^a (Mls-1^a) were examined in a skin allograft experiment in which BALB/c mouse recipient (Mls-1^b) were rendered tolerant to DBA/2 mouse donor (Mls-1^a) with donor spleen cells and cyclophosphamide [14]. In the early phase, the donor-reactive proliferative $CD4^+$-$V_{\beta 6}$ T cells in the lymph nodes were destroyed (clonal destruction), whereas the resting $CD8^+$-$V_{\beta 6+}$ T cells were spared. Furthermore, neither immature $CD4^+$-$V_{\beta 6}$ nor immature $CD8^+$-$V_{\beta 6}$ present in the thymus on day 14 were detectable by day 35 (clonal deletion). In contrast to clonal destruction, clonal deletion was long-lasting. These findings were supported by similar experiments based on different models [15-17]. In one similar study, the complete disappearance of $CD4^+$ cells in the periphery was delayed until thymus depletion occurred [18].

In hematopoietic stem cell transplantation, the same 2 mechanisms of action of cyclophosphamide were confirmed to prevent GVHD by affecting host-reactive cells. When AKR/J-derived Thy-1.1 cells expressing $V_{\beta 3}$ directed against Mls-2^a were examined in a model using a CH3/He mouse recipient (H-2^k, Mls-1^b, Mls-2^a) and AKR/J mouse donor (H-2k, Mls-1^a, Mls-2^b), cyclophosphamide similarly induced early clonal destruction of donor cells followed by clonal deletion of host-reactive cells [19]. These results were supported by the findings by Huyan et al. [20], who examined the effect of intraperitoneal administration of cyclophosphamide on peripheral blood lymphocyte counts in mice. Although 50 mg/kg produced no significant effect, higher doses induced a dose-dependent decrease in $CD4^+$ cells. The decrease began on day +1 and reached nadir on day +4. After a transient rebound, cell numbers decreased again after day +10. The same pattern was observed for B lymphocytes. In contrast, $CD8^+$ cells increased on day 4 [20]. Conceivably, the early decrease in $CD4^+$ but not $CD8^+$ counts was due to clonal destruction, whereas the later decrease in $CD4^+$ was secondary to thymocyte depletion.

Nirmual et al. [11,12] observed that timely administration of cyclophosphamide prevented sensitization of mice receiving large dose of donor spleen cells to skin allografts and prolonged the survival of the grafts. They postulated that cyclophosphamide acted on proliferating cells triggered into cycling after contact with antigen. Their hypothesis was recently confirmed by Ross et al. [21]. In a GVHD mouse model, donor cells were labeled with proliferation dyes and traced in syngeneic and allogeneic transplantation experiments. Mice received increasing doses of cyclophosphamide 3 to 4 days after transplantation. In the syngeneic model, lymphopenia-induced T cell proliferation was slow, sparing a large fraction of cells; the nondeleted cells retained functionality. In the allogeneic model, 22 mg/kg cyclophosphamide failed to prevent GVHD when antigen-stimulated T cells undergoing multiple divisions remained present. However, when the dose was increased to 66 mg/kg, GVHD was alleviated, and only the first 2 to 3 generations of the rapidly dividing alloreactive T cells remained. Cells undergoing more divisions did not survive, whereas slowly dividing cells were also preserved. In a different study published in abstract form, Cieri et al. [22] studied immune reconstitution during the first month in a series of patients undergoing T cell–replete peripheral blood haploidentical transplantation. GVHD prophylaxis consisted of post-transplant cyclophosphamide combined with MMF and sirolimus. Although proliferating alloreactive T lymphocytes were abated, memory cells, including stem memory cells ($CD45RA^+$, $CD62^+$, and $CD95^+$), were increased. The authors postulated that the increase in stem cell memory cells was due to differentiation from naïve T cells. These cells escaped the cyclophosphamide purging effect because of their delayed proliferation in comparison with the alloreactive T cells.

Taken together, these findings suggest that cyclophosphamide can prevent GVHD without compromising timely immune reconstitution. This selective action of cyclophosphamide on proliferating T cells has a number of practical implications. The first implication relates to its potential use in combination with other immunosuppressive agents. For instance, the concomitant use of drugs that induce T cell anergy and may prevent cells from cycling might be theoretically counterproductive [13]. The addition of such drugs must rather be delayed. Pan T cell antibodies, on the other hand, might be synergistic and enhance the deletion of the relevant T cell clones [13]. On the downside, the selective action of cyclophosphamide allows memory T cells induced by prior alloimmunization of the recipient to escape cyclophosphamide and increase the risk of graft failure.

Finally, the effect of cyclophosphamide on regulatory T cells (Tregs) has been the subject of intense investigation. Although early animal studies suggested a negative effect of cyclophosphamide on Tregs, different results were obtained in human studies. North [23] demonstrated that cyclophosphamide can facilitate adoptive immunotherapy of established tumors in mice. Although the administration of cyclophosphamide failed to cause tumor regression, the combination of cyclophosphamide and spleen cells permanently abolished the tumor. The authors postulated that "suppressor but not immune T cells" were sensitive to cyclophosphamide. Similarly, Ghiringhelli et al. [24] demonstrated that the administration of a single dose of cyclophosphamide to rats with established tumor depleted

$CD4^+CD25^+$ cells and rendered the tumor sensitive to weak immunotherapies. A study by Kanakry et al. [25] in humans receiving myeloablative matched related-donor allogeneic blood and marrow transplants yielded contrary results. That study examined the effect of post-transplant cyclophosphamide on the different subsets of Tregs. The numbers of naïve Tregs ($CD4^+CD45RA^+Foxp3^{+lo}$) were lower in patients compared with donors, possibly because of their conversion to activated Tregs ($CD4^+CD45RA^-Foxp3^{+hi}$). Indeed, activated Tregs increased as did the total number of naïve and activated cells. The authors also demonstrated that the content of different Tregs in aldehyde dehydrogenase increased upon activation by allogeneic stimuli, rendering them resistant to mafosfamide. The cells were conversely sensitized by aldehyde dehydrogenase inhibition. The same group studied the role of Tregs in the cyclophosphamide GVHD prophylactic effect. Using a xenogenic GVHD mouse model, when peripheral blood mononuclear cell grafts were depleted of Tregs, the mice had higher GVHD scores and died earlier. These findings emphasize the requirement for Tregs for the optimal effect of cyclophosphamide.

To summarize, Mayumi et al. and Luznik et al. [13,26] proposed a 3-step scheme to explain the mechanism for induction of tolerance by post-transplant cyclophosphamide. Early, proliferating alloreactive donor and recipient T cells are selectively destroyed. Next, the increased Tregs counterbalance the effect of any remaining alloreactive mechanisms. Finally, the delayed but long-lasting intrathymic clonal deletion of anti-host T cells maintains long-term tolerance.

CLINICAL STUDIES

Haploidentical Transplant

Most early clinical studies on the use of post-transplant cyclophosphamide for the prevention of GVHD were performed in the setting of haploidentical transplantation (Table 1). O'Donnell et al. [27] at Johns Hopkins University in Baltimore conducted a pivotal clinical trial that set the stage for others. Patients with high-risk hematological malignancies received T cell–replete haploidentical bone marrow grafts after nonmyeloablative conditioning with 150 mg/m^2 fludarabine on days −6 to −2 and 2-Gy total body irradiation (TBI) on day −1 followed by 50 mg/kg cyclophosphamide on day +3 in combination with MMF from days +4 to +35 and tacrolimus from day +4 to at least day +50. Two of the first 3 patients developed graft rejection. Therefore, 14.5 mg/kg/day of cyclophosphamide was added on days −6 and −5. Eight of the 10 subsequent patients achieved sustained engraftment. Overall, 6 of 13 patients (46%) developed acute GVHD at a median of 99 days after transplant, with fatal acute GVHD in 1 patient. This study provided proof of principle that post-transplantation cyclophosphamide can be safely administered in the setting of haploidentical hematopoietic stem cell transplantation, eliminating the need for T cell–depleted mega cell dose grafts [27].

Building on these findings, Luznik et al. [28] compared the outcome of patients with advanced hematological malignancies receiving 1 (n = 28) or 2 (n = 40) doses of cyclophosphamide administered on days +3 or +3 and +4, respectively, in addition to MMF and tacrolimus. All 68 patients underwent nonmyeloablative conditioning as described above, followed by T cell–replete haploidentical bone marrow grafts. Nine of 66 assessable patients (13%) had graft failure. The remainder engrafted with a median time to neutrophil recovery of 15 days and to platelet recovery of 24 days. The cumulative incidence of treatment-related mortality (TRM) of the entire group was 15%, whereas the relapse rate at 1 year was 51%. The 2-year overall survival (OS) and event-free survival (EFS) rates were 36 and 26%, respectively. Thirty-four percent of patients developed acute GVHD grades II to IV and 6% developed grades III to IV. The only difference between the 2 groups was a trend toward a lower incidence of extensive chronic GVHD (5% versus 25%; $P = .05$) for patients receiving 2 doses of cyclophosphamide. The robust suppression of alloreactivity by post-transplant cyclophosphamide was further supported by a retrospective analysis of 3 similar trials that revealed the number of HLA mismatches at A, B, C, and DRB1 loci had no impact on EFS and the incidence of grades II to IV acute GVHD [29].

More recently, Munchel et al. [30] reported on behalf of the Johns Hopkins University group the results of a large phase II study enrolling 210 patient with advanced hematological malignancies. Patients received haploidentical bone marrow transplants using the group's original nonmyeloablative conditioning regimen and GVHD prophylaxis protocol. Eighty-seven percent of patients experienced sustained engraftment. The incidence of 5-year TRM was 18%. The incidence of disease relapse was 55%, with EFS and OS rates of 35% and 27% respectively. The incidence of acute grades II to IV GVHD and chronic GVHD was 27% and 13%, respectively, confirming the notably low incidence of chronic GVHD.

The Bone Marrow Transplant Clinical Trials Network sponsored a trial treating in parallel 2 groups of patients at two different centers. The first group received haploidentical blood and marrow transplantation with post-transplant cyclophosphamide, and the second group received umbilical cord blood transplant in patients with high-risk hematological malignancies [31]. The first group received the same nonmyeloablative conditioning regimen used by the Johns Hopkins University group. The second group received a conditioning regimen combining fludarabine 160 mg/m^2, cyclophosphamide 50 mg/kg, and TBI 2 Gy. The OS and EFS rates were 62% and 48%, respectively, for the haploidentical transplants and 54% and 46%, respectively, for the group receiving cord blood transplant. The incidence of acute GVHD grades II to IV and III to IV was 32% and 0%, respectively, for the first group and 40% and 21%, respectively, for the second group. The 1-year rate of chronic GVHD was 13% and 25%, respectively. The authors concluded that both approaches were useful and provided outcomes similar to the outcome of patients receiving reduced-intensity conditioning transplants from matched related or matched unrelated donors. This conclusion is consistent with Bashey et al.'s [32] retrospective analysis of 53 patients receiving haploidentical bone marrow or peripheral blood stem cells with post-transplant cyclophosphamide in comparison with patients treated at the same center with matched related (n = 117) or unrelated (n = 101) donor transplants receiving standard GVHD prophylaxis. This latter study also demonstrated no difference in TRM, relapse, or survival rates, with a similar incidence of GVHD.

Although most initial studies focused on the use of bone marrow, several recent studies explored the role of post-transplant cyclophosphamide in permitting the use of peripheral blood instead of bone marrow and to omit the need for mega cell dose grafts originally introduced to induce immune tolerance [33]. Solomon et al. [34] studied 20 patients with hematological malignancies, 11 of whom had

Table 1
Summary of the Published Studies Using Post-Transplant Cyclophosphamide for Prophylaxis of GVHD

Reference First author	Type of Study	No. of Patients	Median Patient Age (yr)	Conditioning Regimen	Donor and Graft Source	Graft Failure	GVHD Prophylaxis	Median Follow-Up (mo)	Acute GVHD Grades II-IV	Chronic GVHD	TRM, EFS, OS
27 O'Donnell	Prospective	13	53	JH (first 3 patients without Cy on days −6 and −5)	HID-BM	4 (30%) (2 of the first 3 patients)	Cy50 × 1 + MMF + TAC	6.4	46%	NR	18%, 38%, 46%
28 Luznik	Prospective	68	Range .5-70	JH	HID-BM	9 (14%)	Cy50 × 1 (28) or Cy50 × 2 (40) + MMF + TAC	24	34%	Cy50 × 1: 25% Cy50 × 2: 5% $P = .05$	15%, 26%, 36%
29 Kasamon	Retrospective from 3 trials (67 patients were included in reference 23)	185	50	JH	HID-BM	29 (16%)	Cy50 × 1 (48) or Cy50 × 2 (137) + MMF + TAC	6	31%	15%	15%, 35% at 1 yr, NR
30 Munchel	Prospective	210	52	Flu + Cy + TBI (2) NMA	HID-BM	Of 204: 27 (13%)	Cy50 × 2 + MMF + TAC		27%	13%	18%, 32% at 3 y, 41% at 3 yr
31 Brunstein	Comparison of 2 separate prospective conducted in parallel	100	HID: 48 CB: 58	HID: JH CB: Flu + Cy + TBI (2 Gy)	HID-BM (in 1 study) CB (in the second study)	HID: 1 (2%) CB: 6 (12%)	HID: Cy50 × 2 + MMF + TAC CB: MMF + CSA	12	HID: 32% CB: 40%	HID: 13% CB: 25%	HID: 7%, 48% at 1 y, 62% at 1 yr CB: 24%, 46% at 1 y, 54% at 1 yr Relapse HID: 45% CB: 31%
32 Bashey	Retrospective comparison between 2 groups of patients	271	HID: 46 MRD: 50 MUD: 51	HID: JH (35) or Flu + Bu + Cy (MA) (18) MRD: MA (70) or NMA (47) MUD: MA (47) or NMA (54)	HID: BM (32), HID: PB(21) MRD and MUD: PB (most)	NR	HID: Cy50 × 2 + MMF + TAC	24	HID: 30% MRD: 27% MUD: 39%	HID: 4% MRD: 11% MUD: 12% $P = .05$ in favor of HID vs. MRD or MUD *all were severe	HID: 7%, 60% at 2 y, 64% at 2 yr MRD: 13%, 53% at 2 y, 76% at 2 yr MUD: 16%, 52% at 2 y, 67% at 2 yr Relapse HID: 33% MRD and MUD: 34%
34 Solomon	Prospective	20	44	Flu + Bu + Cy (MA)	HID-PB	0	Cy50 × 2 + MMF + TAC	20	30%	35%	10%, 50% at 1 yr, 69% at 1 yr
35 Bhamidipati Abstract	Retrospective	18	41	JH	HID-PB	2 (11%)	Cy50 × 2 + MMF + TAC (16) Cy50 × 2 + MTX + TAC (2)	8.4	40.7%	8%	17%, 53% at 1 yr, 62% at 1 yr
36 Ciurea	Retrospective comparison between 2 groups of patients	65	TCR: 45 TCD: 36	TCR: MA (26) or NMA (6) TCD: MA	TCR: HID-BM (31) HID-PB (1) TCD: HID-PB	TCR 6% TCD: 19%	TCR: Cy50 × 2 + MMF + TAC TCD: ATG	TCR: 11 TCD: 48	TCR: 20% TCD: 11%	TCR: 7% TCD: 18% $P = .03$	TCR: 16%, 50% at 1 yr, 64% at 1 yr TCD: 42%, 21% at 1 yr, 30% at 1 yr $P = .02$
37 Castagna	Retrospective comparison between 2 groups of patients	69	HID-BM: 44 HID-PB: 54	JH	HID-BM (46) HID-PB (23)	Reported as similar between the 2 groups	Cy50 × 2 + MMF + CSA or TAC	18	HID-BM: 25% HID-PB: 33%	HID-BM: 13% HID-PB: 13%	2 y HID: BM 22% PB 12%, reported as similar between 2 groups (62%), reported as similar between 2 groups (68%)
38 Gayoso Abstract	Retrospective review: Hodgkin disease	29	31	Flu + Cy + TBI 2 (NMA) or Flu + Cy + Bu (NMA)	HID-BM (15) HID-PB (14)	NR	Cy50 × 2 + MMF + CSA or TAC	9	25%	16%	17% 59%, NR Relapse 21%

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Table 1
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Reference First author	Type of Study	No. of Patients	Median Patient Age (yr)	Conditioning Regimen	Donor and Graft Source	Graft Failure	GVHD Prophylaxis	Median Follow-Up (mo)	Acute GVHD Grades II-IV	Chronic GVHD	TRM, EFS, OS
39 Bolaños-Meade	Prospective: sickle cell disease	14	23.5	Flu + Cy + TBI (2 Gy) + ATG	HID-BM	6 (43%)	Cy50 × 2 + MMF + CSA or TAC	23.7	0%	0%	0%, 57% abatement of sickle cell crisis, 100%
40 Luznik	Prospective	117	50	Bu + Cy (MA)	MRD-BM (78) MUD-BM (39)	MRD: 1 (1%) MUD: 2 (5%)	Cy50 × 2	26.3	43%	10%	17%, 39% at 2 yr, 55% at 2 yr
41 Kanakry Abstract	Prospective	92	NR	Flu + Bu (MA)	MRD-BM (45) MUD-BM (47)	NR	Cy50 × 2	19.2	51%	14%	16%, 62% at 2 yr, 67% at 2 yr
42 Alousi Abstract	Comparison of 2 matched groups of patients (Cy for GVHD prophylaxis versus standard)	74	Cy: 61 Control:62	Cy: Flu + Bu (ATG if MUD) Control: Flu + Bu or Flu + Mel (ATG if MUD)	MRD or MUD-BM 26 MRD or MUD-PB 11 in each group	NR	Cy: Cy50 × 2 Control: MMF or MTX + TAC	NR	CY: 46% Control: 19% $P = .02$	CY: 14% Control: 21%	Cy: 36%, 22%, 26% Control: 16%, 33%, 46% $P = .08$
45 Dezern	Case report: aplastic anemia	2	54.5	Cy + Bu (MA)	MRD-BM	0	Cy50 × 2 + MMF + TAC	NR	0%	0%	NR

JH indicates Johns Hopkins University regimen; Cy, cyclophosphamide; HID, haploidentical donor; BM, bone marrow; MMF, mycophenolic acid; TAC, tacrolimus; NR, not reported; NMA, nonmyeloablative; CB, cord blood; Flu, fludarabine; CSA, cyclosporine A; MRD, matched related donor; MUD, matched unrelated donor; Bu, busulfan; MA, myeloablative; PB, peripheral blood; Mel, melphalan; MTX, methotrexate; ATG, antithymocyte globulin.

relapsed disease. Patients received a busulfan-based myeloablative conditioning regimen followed by T cell–replete peripheral blood stem cells from haploidentical donors. The maximal CD34 cell dose was 5×10^6/kg. GVHD prophylaxis consisted of cyclophosphamide 50 mg/kg on days +3 and +4 followed by tacrolimus and MMF starting on day +5. All patients engrafted. One-year OS and EFS rates were 69% and 50%, respectively, for the entire group and 88% and 67% for the 9 patients in remission at the time of transplant. The overall incidences of grades II to IV and grades III to IV acute GVHD were 30% and 10%, respectively. At a median follow-up of 20 months, the incidence of chronic GVHD was 35% (5% severe). These results were substantiated by Bhamidipati et al. [35] for nonmyeloablative conditioning. The incidence of extensive chronic GVHD in this study was also 5%.

Furthermore, 2 additional studies reported the results of retrospective comparisons between haploidentical transplants using bone marrow or peripheral blood as the source of grafts. First, Ciurea et al. [36] analyzed 65 consecutive patients with hematological malignancies receiving T cell–replete peripheral blood stem cell transplants (n = 32, TCR group) as opposed to T cell–deplete bone marrow transplants (n = 33, TCD group). Both groups received the same conditioning regimen combining fludarabine, melphalan, and thiotepa. The first group was administered post-transplant cyclophosphamide along with tacrolimus and MMF for GVHD prophylaxis. For the TCD group, the only GVHD prophylaxis consisted of 6 mg/kg rabbit antithymocyte globulin. There was no difference in engraftment between the 2 groups. However, TRM was lower in the TCR group (16% versus 42%; $P = .02$). OS and EFS rates were 64% versus 30% ($P = .02$) and 50% versus 21% ($P = .02$), respectively, in favor of the TCR group. There was no significant difference in the incidence of acute GVHD grades II to IV (20% in the TCR versus 11% in the TCD group; $P = .2$), whereas chronic GVHD was less frequent in the TRC group (7% versus 18%; $P = .03$). Recovery of $CD4^+$ and $CD8^+$ cells was significantly faster in the TCR transplant patients. This was also true for naïve and memory T cells and translated into a lower incidence of viral and fungal infections. The authors concluded that the outcome of TCR transplants with post-transplant cyclophosphamide was superior to that of TCD transplants. In the second study, Castagna et al. [37] compared 2 groups receiving T cell–replete grafts. The first group received bone marrow grafts (n = 46), whereas the second group received peripheral blood grafts (n = 23). No differences in OS or EFS were observed. The incidences of acute and chronic GVHD were also statistically similar.

Enrolling only patients with relapsed or refractory Hodgkin disease, Gayoso et al. [38] presented the outcome of 29 patients conditioned with a combination of fludarabine, cyclophosphamide, and either busulfan (6.4 mg/kg) or TBI 2 Gy. Most patients (72%) had active disease at the time of transplant. With a short follow-up, 59% of patients were in complete remission. The rates of acute and chronic GVHD were comparable with those reported in other studies.

Finally, the John Hopkins University group reported the outcome of haploidentical transplantation in nonmalignant illnesses using their original regimen [39]. Most patients in this study had sickle cell disease. Six of 14 patients (43%) had graft failure. The incidence of acute and chronic GVHD was very low, with only 1 patient developing skin GVHD but requiring no therapy. However, the low incidence of GVHD might be due in part to the high rate of graft failure. At a

Table 2
Summary of Selected Registered Trials Including Post-Transplant Cyclophosphamide for GVHD Prevention

Study Identification	Study Type	Intervention	Responsible Party
NCT01749111	Phase III randomized	PTCy versus MTX and CNI for GVHD prevention in MRD and MUD transplants	Paulo V. Campregher, MD Hospital Israelita Albert Einstein
NCT01860170	Phase I	PTCy and bortezomib for GVHD prevention in MRD and MUD transplants	A. Samer Al-Homsi, MD Spectrum Health Hospitals
NCT01349101	Phase II	PTCy, MMF, and tacrolimus for GVHD prevention in haploidentical and MUD transplants	John L. Wagner, MD Thomas Jefferson University
NCT02065154	Phase II	PTCy for GVHD prevention in MUD and mismatched UD transplants	Racquel Innis-Shelton, MD University of Alabama at Birmingham
NCT00622895	Phase I/II	PYCy, MMF, and tacrolimus for GVHD prevention in MRD and MUD transplants in severe systemic sclerosis	George Georges, MD Fred Hutchinson Cancer Research Center
NCT01350232		PTCy for GVHD prevention in haploidentical and MRD transplants for sickle cell disease. Alloimmunized patients receive rituximab and bortezomib with or without plasma exchange. Patients receive donor lymphocyte infusion followed by PTCy followed by CD34 selected graft.	Joanne Flilicko-O'Hara, MD Thomas Jefferson University
NCT02167958	Phase I	PTCy, MMF, and tacrolimus for GVHD prevention in haploidentical transplants using peripheral blood	Rafic Farah, MD University of Pittsburgh
NCT01374841	Phase II	PTCy, MMF, and tacrolimus for GVHD prevention in haploidentical transplants	Rocco Pastano, MD European Institute of Oncology
NCT01028716	Phase II	PTCy, MMF, and tacrolimus for GVHD prevention in haploidentical transplants using peripheral blood	Rachel Salit, MD Fred Hutchinson Cancer Research Center
NCT02053545	Phase I/II	PTCy, MMF, and tacrolimus for GVHD prevention in haploidentical transplants	Reggie E. Duerst, MD Children's Hospital of Chicago
NCT01435447	Phase II	PTCy for GVHD prevention in transplants for adult ALL	Jiong Hu, MD Shanghai Jiao Tong University School of Medicine
NCT01707004	Phase II	PTCy, MMF, and tacrolimus for GVHD prevention in haploidentical transplants	Mark Juckett, MD University of Wisconsin, Madison
NCT02049580	Phase II	PTCy for prevention of GVHD in MRD and MUD transplants for poor-risk lymphoma	Luca Castgna, MD Istituto Clinico Humanitas
NCT02208037	Phase II randomized	PTCy, MMF, and tacrolimus, MTX, tacrolimus, and bortezomib, or MTX, tacrolimus, and maraviroc for prevention of GVHD in MRD, MUD, and mismatched UD transplants	Javier Bolaños-Meade, MD Johns Hopkins University John Koreth, MBBS Dana-Farber Cancer Institute Ran Reshef, MD University of Pennsylvania
NCT01719341	Phase II	PTCy for GVHD prevention in haploidentical transplants	Zi Yi Lim, MB ChB National University Hospital, Singapore
NCT02169791	Phase II	PTCy, tacrolimus, and ixazomib for GVHD prevention in haploidentical transplants.	Scot Solomon, MD Northside Hospital

PTCy indicates post-transplant cyclophosphamide; CNI, calcineurin inhibitor; UD, unrelated donor; ALL, acute lymphoblastic leukemia.

median follow-up of almost 2 years, 11 patients were asymptomatic and 6 were no longer on immunosuppressive therapy. The high rate of graft failure in hemoglobinopathies is not unique to this study. This can be explained by the lack of prior cytotoxic chemotherapy or decreased sensitivity to cyclophosphamide of effector T cells induced by alloimmunization secondary to multiple transfusions. The authors also speculated that ABO incompatibility may be a factor [39]. Intensifying the degree of immunosuppression of the conditioning regimen and performing transplantation earlier in the course of disease before alloimmunization occurs can conceivably overcome these barriers.

Matched Related and Unrelated Transplant

Having established the role of post-transplant cyclophosphamide in the setting of haploidentical transplantation, the Johns Hopkins University group examined its role in matched related and unrelated transplants as the only GVHD prophylactic therapy. After performing a pilot study, Luznik et al. [40] reported the results of a large cohort of patients with advanced hematological malignancies who received myeloablative conditioning followed by grafts from matched related (n = 78) or unrelated (n = 39) donors. Overall, TRM was 17%. OS and EFS rates at 2 years were 55% and 39%, respectively, for the entire group and 63% and 54%, respectively, for patients with no evidence of disease at the time of transplantation. Acute GVHD grades II to IV occurred in 43% of patients. The incidence of chronic GVHD was again low at 10%.

Kanakry et al. [41] performed a similar multi-institutional study using a combination of fludarabine and busulfan at myeloablative doses as the conditioning regimen. The source of the grafts was T cell–replete bone marrow from matched related (n = 45) or unrelated (n = 47) donors. Twenty-five patients had active hematological malignancy at the time of transplantation. Cyclophosphamide was the sole GVHD prophylaxis. With a median follow-up of 1.6 years, the TRM was 16%, and OS and EFS rates were 62% and 67%, respectively. The incidence of grades II to IV and III to IV acute GVHD was 51% and 15%, respectively, whereas the incidence of chronic GVHD was again low at 14%.

Finally, a matched controlled analysis of 2 groups receiving either post-transplant cyclophosphamide or tacrolimus and methotrexate as GVHD prophylaxis after

matched related and unrelated donor reduced-intensity transplants was reported in an abstract [42]. The TRM was 36% and 16% ($P = .1$), respectively; progression-free survival was 22% and 33% ($P = .4$), respectively; and OS was 26% and 46% ($P = .08$), respectively. The incidence of acute GVHD grades II to IV was 46% and 19% ($P = .02$), respectively, and that of chronic GVHD was 14% and 21% ($P = .7$), respectively. The unfavorable outcomes of patients receiving post-transplant cyclophosphamide can be explained by the surprisingly high TRM. In addition, the incidence of acute GVHD in the control group was lower than traditionally encountered.

ONGOING AND FUTURE CLINICAL TRIALS

A number of trials have been initiated to confirm the current data and also to compare haploidentical transplant made practical by post-transplant cyclophosphamide with matched sibling, matched unrelated, and cord blood transplants. Confirmatory studies examining the role of cyclophosphamide are also underway in matched related and unrelated donor transplants. In this setting, cyclophosphamide is an appealing platform to be used in combination with other agents. The options include combinations with calcineurin and m-TOR inhibitors or with pan T cell antibodies. Other combinations using agents that affect other cellular components involved in GVHD pathogenesis, such as dendritic cells or cytokines, are attractive. The combinations with ruxolitinib, a potent suppressor of dendritic cells and cytokines [43], or vorinostat, which possesses anti-GVHD activity, also merit examination [44]. Table 2 summarizes selected registered clinical trials using cyclophosphamide for prevention of GVHD (other trials can be found at clinicaltrials.gov).

CONCLUSION

Cyclophosphamide has a unique mechanism of action. It selectively destroys rapidly proliferating donor- and host-alloreactive $CD4^+$ cells while sparing slowly proliferating cells. This is followed by clonal deletion of immature donor $CD4^+$ and $CD8^+$ cells in the chimeric thymus. Subsequently, although the preservation of naïve and activated Tregs further sustains its immunosuppressive effect, the selective nature of cyclophosphamide activity permits rapid immune reconstitution and conceivably preserves the GVD effect. In haploidentical settings, cyclophosphamide allows the use of unmanipulated peripheral blood grafts, omitting the need for any special expertise. In matched related and unrelated donor transplants, cyclophosphamide alone is active, with a notably low incidence of chronic GVHD. Confirmatory and comparative studies are warranted to examine the effect of such approach on disease relapse and immune reconstitution. Post-transplant cyclophosphamide also offers the opportunity to explore its use in conjunction with other agents in innovative approach.

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