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General review

Post-transplant cyclophosphamide in matched donor transplantation: are we there yet?

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ABSTRACT

Graft-versus-host disease (GvHD) is a frequent cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (alloHCT) and optimal approaches for its prevention have been recently updated. Post-transplant cyclophosphamide (PTCy) has demonstrated impressive results in the setting of haploidentical donor transplantation, allowing for a more widespread application of alloHCT. For this reason, over the years, several groups have implemented the use of PTCy in the context of transplantation from HLA-matched related and unrelated donors, as a replacement for standard GvHD prophylaxis based on calcineurin inhibitors and methotrexate. With increasing results from retrospective studies and new insights from prospective clinical trials, this comprehensive reevaluation of the literature aims to clarify the precise role of PTCy in this context. This review will summarize and critically discuss the overall results of the use of PTCy in alloHCT from HLA-matched donors, unmet needs, and future perspectives.

Introduction

Substantial improvements over recent decades, including new approaches for prophylaxis and management of graft-versus-host disease (GvHD), have contributed to the expanding use of allogeneic hematopoietic stem cell transplantation (alloHCT) across various disease indications [1,2]. Conventional backbone schemes for GvHD prevention, which historically include a calcineurin inhibitor (CNI) either with methotrexate (MTX) or mycophenolate mofetil (MMF) [3–7], effectively reduce acute and chronic GvHD rates only by indiscriminately targeting T-cells, thereby delaying immune reconstitution [8,9]. The incorporation of in vivo T-cell-depletion (TCD) using polyclonal anti-thymocyte globulin (ATG) and anti-T lymphocyte globulin (ATLG) has demonstrated significant efficacy in mitigating both acute and chronic graft-versus-host disease (GvHD) [10,11]. Although ATG and ATLG are effective in GvHD prevention, their use at higher doses – especially when combined with reduced-intensity conditioning (RIC) regimens – potentially slightly increase the incidence of relapse and infection-related mortality, without negatively affect overall survival (OS) [10–14].

Post-transplant cyclophosphamide (PTCy) was initially proposed to overcome human leukocyte antigen (HLA) barriers in the setting of haploidentical alloHCT [15]. Since the first trials, PTCy has demonstrated the ability to achieve rapid immune reconstitution combined with high GvHD prevention, yielding promising results in this high-risk context [16–18]. Based on these preliminary results, many groups have started to use the same strategy also for alloHCT from HLA-matched related (MRD) and unrelated donors (MUD).

Currently, as data from retrospective studies accumulate and new insights emerge from prospective trials, a critical reevaluation of the literature is needed to determine the exact role of PTCy in this setting.

Methods

Data for this literature review were identified by searching Pubmed and Cochrane database using the search terms “post-transplant cyclophosphamide”, “allogeneic hematopoietic cell transplant”, and “matched donor”. Only articles written in English, published in peer-reviewed journals from December 2001 to February 2024, and pertaining to human studies have been included. Additional reports were identified by checking the bibliographic lists of already eligible articles.

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PTCy: mechanism of action and toxicities

The mechanism of action of PTCy in mediating protection against GvHD is thought to be based on the selective elimination of actively expanding alloreactive T-cells infused within the graft, as well documented in murine models [19]. Later, several studies pointed out the importance of increased resistance to PTCy of regulatory T lymphocytes (Tregs) over $CD4^+$ and $CD8^+$ T-cells due to enhanced expression of aldehyde dehydrogenase (ALDH), the main detoxifying enzyme of cyclophosphamide [20]. A complementary role for myeloid-derived suppressor cells (MDSCs) in the expansion and augmented survival of Tregs has been postulated; PTCy induces MDSCs differentiation in patients undergoing alloHCT by increasing colony-stimulating factors that are crucial for MDSCs development. It was observed that depleting $Foxp3^+$ Tregs from the initial graft did not exacerbate GvHD after the subsequent infusion of donor splenocytes on day +5. However, depleting Tregs also in infused donor splenocytes induced fatal GvHD in mice treated with PTCy. For this reason, the effect of PTCy on $Foxp3^+$ Tregs seems to be indirect and dependent on other cells [21]. Other studies suggest that PTCy ultimately induces T-cell exhaustion with both a direct effect on alloreactive T lymphocytes, by modifying the quantity and quality of inhibitory molecules, and an indirect impact on suppressive T-cells [22,23].

Transient cardiac dysfunction, hemorrhagic cystitis, and gastrointestinal symptoms are among the most common toxicities associated with the use of cyclophosphamide [24–26]. Additionally, PTCy has been linked to the risk of infections [26–28]. The use of PTCy displayed a significantly higher risk of viral infections in MUD and haploidentical donor alloHCT, particularly early cytomegalovirus (CMV) reactivations compared to CNI-based protocols, and human herpesvirus-6 compared to ATG/ATLG strategies [29–34]. Moreover, PTCy-induced damage of the mucosal barrier combined with delayed neutrophil engraftment predisposes to bacteremias and increases the risk of multidrug-resistant infections in colonized patients [35,36]. In contrast, the higher rate of invasive fungal diseases observed in haploidentical donor alloHCT was confirmed only for mold infections when PTCy was applied in the HLA-matched setting [27,37,38].

Retrospective data reported faster immune reconstitution of $CD8^+$, NK, NKT and gdT cells with ATG/ATLG, and of $CD4^+$ T and B cells with PTCy. This pattern was associated with a reduced incidence of infections and moderate/severe chronic GvHD (cGvHD) in the ATG/ATLG group, without any impact on others long-term outcomes [39]. Furthermore, the incorporation of PTCy into standard prophylaxis revealed a global bottleneck in post-transplant T-cell receptor diversity. [40] The fraction of T-cells originating from transplant infusion was similar between the PTCy and standard prophylaxis arm, but a substantial deficit in naïve T cells was found in PTCy-exposed patients at all timepoints after alloHCT. This portends to a significantly less diverse T-cell receptor repertoire, offering protection from cGvHD but simultaneously increasing the risk of infections in this group of recipients.

PTCy: results in HLA-matched donor transplantation

Prospective studies in HLA-matched alloHCT using single-agent PTCy and PTCy in combination with other immunosuppressants are outlined in Table 1 and Table 2, respectively.

PTCy as a single agent

Following the demonstration of the safety and efficacy of high-dose PTCy (100 mg/Kg) combined with tacrolimus (TAC) and MMF after non-myeloablative bone marrow (BM) alloHCT from haploidentical donors [16,17], a single-center phase 1/2 study was conducted by Luznik et al to assess PTCy as sole GvHD prophylaxis in HLA-matched alloHCT [41]. A total of 117 recipients (78 MRD, 39 MUD; 58% not in complete remission) were included in the study; conditioning was myeloablative (MAC) using busulfan/cyclophosphamide and BM was selected as the sole source of stem cells. The 100-day cumulative incidence (CI) of grade II-IV and grade III-IV acute GvHD (aGvHD) were 43% and 10%, respectively, and 2-year CI of cGvHD was 9%, without significant differences between donor types. OS, disease-free survival (DFS), and non-relapse mortality (NRM) at 2 years were 55%, 39%, and 15%, respectively. The 2-year CI of relapse was 44% for the overall cohort and 26% for those transplanted in remission.

Two similar studies from Kanakry et al using different MAC regimens reported similar results in high-risk hematologic malignancies [42,43]. In the first one, a multicenter phase 2 trial, they tested the use of busulfan/fludarabine conditioning followed by BM allograft and single-agent PTCy [42]. Overall, 92 patients (45 MRD, 47 MUD) were enrolled in the study. The CI of grade II-IV and grade III-IV aGvHD at 100 days was 51% and 15%, respectively; only grade II-IV aGvHD was significantly higher in patients receiving MUD compared to MRD. The 2-year CI of cGvHD was 14%, with recipients of MRD allografts displaying a significant lower incidence compared to those transplanted using MUD. In the second study, using a larger single-center retrospective cohort (209 patients; 116 MRD, 93 MUD), they reported similar CI of aGvHD and cGvHD [43]. Here, the primary focus was to analyze disease-specific outcomes in this context. Despite having 30% of patients with active disease, DFS and CI of relapse at 3 years were 46% and 26%, respectively. Chronic GvHD-free/relapse-free survival (CRFS) reflected DFS due to the low rate of cGvHD, with nearly half of patients receiving no further immunosuppression after PTCy.

In the reported studies, along with similar aGvHD rates and survival outcomes for both MRD and MUD alloHCT, the CI of cGvHD using PTCy was lower than that reported using T-cell-depleted strategies [44,45] or standard CNI-based prophylaxis, with or without ATG [4,46,47]. These favorable preliminary results lead to a phase 3 trial (CTN 1301) with the aim of comparing two different CNI-free approaches (PTCy and ex vivo CD34-selected peripheral blood graft) with standard TAC/MTX prophylaxis [25]. CRFS was the primary endpoint of the study and, at 2 years, was 50.6%, 48.1%, and 41% for CD34-selection, PTCy, and TAC/MTX, respectively. PTCy was associated with an increased 100-day grade II-IV and grade III-IV aGvHD, but also a trend towards lower disease relapse and better DFS compared with controls. NRM, CI of moderate/severe cGvHD, and OS were similar between PTCy and TAC/MTX. On the contrary, PTCy was associated with a slower engraftment compared with the other arms. Both experimental arms resulted in similar but not better outcomes compared with standard prophylaxis, questioning the role of single-agent PTCy in myeloablative HLA-matched alloHCT.

The use of PTCy was also tested in the setting of RIC alloHCT but with unfavorable results in terms of aGvHD and NRM [48–51]. Alousi at al conducted a phase 2 trial of single-agent PTCy after RIC and BM or peripheral blood (PB) alloHCT [48]. ATG was initially included in the preparative regimen for recipients of MUD based on institutional practice. The CI of grade II-IV aGvHD was significantly higher in recipients of PTCy when compared with a retrospective cohort treated with TAC/MTX, resulting in a worse NRM and OS. It remains controversial in this context whether to attribute this detrimental effect to the use of RIC alone versus the addition of ATG and PB stem cells in a proportion of the patients, who were older and with overall more comorbidities than those from other studies. In another study, 11 subjects with lymphoma or myeloma transplanted using single-agent PTCy and PB grafts displayed a CI of grade II-IV and grade III-IV aGvHD of 45% and 27%, respectively, leading to an unacceptably high NRM (36% at 2 years) and a premature closure of the trial [49]. In a third smaller trial of RIC transplantation using PB grafts, 4 out of 5 subjects developed severe aGvHD [50]. Overall, these studies suggest that PTCy should not be used as sole GvHD prophylaxis after RIC transplantation from HLA-matched donors, especially with PB as the source of stem cells.

Table 1
List of published prospective studies using single-agent post-transplant cyclophosphamide for graft-versus-host disease prophylaxis after matched allogeneic hematopoietic stem cell transplantation.

Author/Date	Reference	Study phase	Number of patients	MRD / MUD (%)	Conditioning	Source	Primary endpoint	aGvHD	cGvHD	CRFS	NRM	Relapse
Luznik L et al (2010)	41	Phase 1/2	117	67 / 33	MAC (Bu/Cy)	BM	CI of grade II-IV aGvHD	Grade II-IV (at 100-d): 43% Grade III-IV: 10%	At 2-y: 9% MRD vs 11% MUD	/	9% (at 100-d), 17% (at 2-y)	44% (at 2-y)
Kanakry CG et al (2014)	43	Phase 2	92	49 / 51	MAC (Bu/Flu)	BM	CI of grade III-IV aGvHD	Grade II-IV: 51% (MUD>MRD; p=0.027)	All grades (at 2-y): 14% (MUD>MRD, p=0.04)	/	9% (at 100-d), 16% (at 1-y)	22% (at 2-y)
Alousi AM et al (2015)	48	Phase 2	49	31 / 69	RIC (Bu/Flu)	BM (11) PBSC (38)	CI of grade II-IV aGvHD	Grade II-IV: 46% (versus 19% in a retrospective cohort of patients who received TAC/MTX)	All grades (at 1-y): 18%	/	14% (at 100-d); 39% (at 2-y)	35% (at 2-y)
Bradstock KF et al (2015)	50	Phase 2	5	40 / 60	RIC (Flu/ BCNU/Mel)	PBSC	CI of grade III-IV aGvHD	Grade III-IV: 4 out of 5 pts	/	/	/	/
Holtick U et al (2016)	49	Phase 2	11	55 / 45	RIC (Bu/Flu)	PBSC	% of patients not requiring additional IST within 100 days after alloHCT	Grade II-IV (at 100-d): 45% Grade III-IV: 27%	2 cases (NIH mild)	/	18% (at 100-d), 36% (at 1 and 3-y)	18% (at 1-y), 30% (at 2 and 3-y)
Luznik L et al (2021)	25	Phase 3	CD34-selected: 114 PTCy: 114 TAC/ MTX: 118	38 / 62	MAC	PBSC (121) BM (206)	CRFS	Grade II-IV: 16.3% (CD34-selected), 37.6% (PTCy), 29.8% (TAC/ MTX) Grade III-IV: 2.9% (CD34-selected), 10.1% (PTCy), 3.5% (TAC/ MTX)	Moderate/severe (at 2-y): 8.9% (CD34-selected), 27% (PTCy), 33.7% (TAC/MTX)	At 2-y: 50.6% (CD34-selected), 48.1% (PTCy), 41% (TAC/MTX)	At 2-y: 21.5% (CD34-selected), 15.7% (PTCy), 7.9% (TAC/MTX)	At 2-y: 21.4% (CD34-selected), 13.9% (PTCy), 25.6% (TAC/ MTX)
Le Bourgeois A et al (2023)	51	Phase 2	38	32 / 68	RIC (Baltimore platform)	PBSC	CI of grade III-IV aGvHD	Grade II-IV (at 100-d): 52.6% Grade III-IV: 21.1%	Moderate-severe (at 2-y): 15.7%	46.9% (at 2-y)	21.1% (at 2-y)	18.5% (at 2-y)

MAC: myeloablative conditioning; RIC: reduced intensity conditioning; CI: cumulative incidence; aGvHD: acute graft versus host disease; cGvHD: chronic graft versus host disease; NRM: non-relapse mortality; HCT: hematopoietic stem cell transplantation; BM: bone marrow; MUD: matched unrelated donor; MRD: matched related donor; PBSC: peripheral blood stem cells; Bu: busulfan; PTCy: post-transplant cyclophosphamide; Cy: cyclophosphamide; Flu: fludarabine; TBI: total body irradiation; BCNU: carmustine; Mel: melphalan; IST: immune suppressor; TAC: tacrolimus; MTX: metotrexate; NIH: national institutes of health; CFRS: moderate-severe chronic GVHD/disease-free survival; d: days; y: year

Table 2
List of published prospective studies using post-transplant cyclophosphamide combined with other agents for graft-versus-host disease prophylaxis after matched allogeneic hematopoietic stem cell transplantation.

Author/ Date	Reference	Study phase	Number of patients	MRD / MUD + MMUD (%)	Drug combinations	Conditioning	Source	Primary endpoint	aGvHD	cGvHD	GRFS	NRM	Relapse
Solomon SR et al (2014)	55	Phase 2	26	65 / 35	PTCy/ Sirolimus	RIC	PBSC	Donor engraftment, incidence and severity of GvHD, NRM	Grade II-IV (at 100-d): 46% Grade III-IV: 15%	Extensive (at 1-y): 31%	/	13% (at 2-y)	32% (at 2-y)
Mielcarek M et al (2016)	52	Phase 2	43	28 / 72	PTCy/CSA	MAC (25 Bu/ Flu, 18 TBI)	PBSC	CI of cGvHD requiring systemic treatment (compared with the historic rate of 35%)	Grade II-IV (at 100-d): 77% Grade III-IV: 0%	Requiring treatment (at 1-y): 16% Extensive: 30%	/	14% (at 2-y)	17% (at 2-y)
Moiseev IS et al (2016)	53	Phase 2	PTCy group: 86 Control group: 125	0 / 100	PTCy/TAC/ MMF Control: ATG based	MAC (21; 32) RIC (54; 93)	PBSC	Incidence of aGvHD and cGvHD requiring treatment (compared with historical patients who received ATG/TAC/MTX or MMF)	Grade II-IV (at 100-d): 19% (PTCy), 45% (ATG), $p<0.0001$ Grade III-IV: 4% (PTCy), 27% (ATG), $p<0.0001$	Moderate-severe (at 1-y): 16% (PTCy), 65% (ATG), $p<0.0001$	At 2-y: 52% (PTCy), 12% (ATG), $p<0.0001$	16% (PTCy), 36% (ATG), $p=0.005$	19% (PTCy), 27% (ATG)
Carnevale-Schianca F et al (2017)	54	Phase 2	35	29 / 29 + 43	PTCy/TAC/ MMF	Disease oriented	PBSC	CI of acute and chronic GvHD	All grades: 17% (no grade IV)	Requiring treatment (at 2-y): 7%	/	3% (at 2-y)	46% (at 2-y)
Shah MV et al (2019)	58	Pilot study	22	0 / 100	PTCy/TAC/ MMF	RIC	BM (86%) PBSC (14%)	Safety and NRM	Grade II-IV: 32% Grade III-IV: 4%	0%	/	43% (at 2-y)	9% (at 2-y)
Bolaños-Meade J et al (2019)	72	Phase 2	Experimental: 273 (PTCy 92, BOR 84, MVC 92) Control: 224	PTCy: 32 / 54 + 10 BOR: 33 / 60 + 11 MVC: 36 / 52 + 7 Control: 40 / 53 + 6	PTCy/TAC/ MMF TAC/MTX/ BOR TAC/MTX/ MVC TAC/MTX (control)	RIC	PBSC	GRFS at 12 months	Grade II-IV (at 180-d): 30% (control), 27% (PTCy), 26% (BOR), 32% (MVC) Grade III-IV: 13% (control), 2% (PTCy) $p<0.01$, 8% (BOR), 9% (MVC)	At 1-y: 38% (control), 28% (PTCy), 39% (BOR), 43% (MVC)	At 1-y: 34% (control), 43% (PTCy) $p<0.05$, 35% (BOR), 28% (MVC)	At 1-y: 16% (control), 11% (PTCy), 17% (BOR), 16% (MVC)	At 1-y: 25% (control), 28% (PTCy), 24% (BOR), 31% (MVC)
García-Cadenas I et al (2020)	57	Pilot study	Cohort 1: 29 Cohort 2: 27	30 / 32 + 37	PTCy + TAC (Sirolimus in case of renal disorders)	MAC (16%) RIC (84%)	PBSC	Graft failure rate ≤35% at day +180	Grade III-IV: 8%	Severe: 2.5% (at 1-y)	/	18% (at 1-y)	/
Broers AEC et al (2022)	74	Phase 3	PTCy/CSA: 99 CSA/MMF: 52	PTCy/ CSA: 30 / 70 CSA/ MMF: 33 / 67	PTCy/CSA CSA/MMF	MAC (CSA/ MMF 4%) RIC (CSA/ MMF 2%) NMA (CSA/ MMF 94%, PTCy 99%)	PBSC	Proportion of patients with non-severe GvHD at day +180	Grade II-IV: 56% (CSA/ MMF), 33% (PTCy), $p=0.007$ Grade III-IV: 16% (CSA/ MMF), 6% (PTCy)	Extensive (at 2-y): 48% (CSA/MMF), 16% (PTCy), $p<0.001$	At 1-y: 21% (CSA/MMF), 45% (PTCy), $p<0.001$	At 3-y: 14% (CSA/MMF), 10% (PTCy)	At 3-y: 24% (CSA/MMF), 32% (PTCy)

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Table 2 (*continued*)

Author/ Date	Reference	Study phase	Number of patients	MRD / MUD + MMUD (%)	Drug combinations	Conditioning	Source	Primary endpoint	aGvHD	cGvHD	GRFS	NRM	Relapse
Bolaños-Meade J et al (2023)	73	Phase 3	PTCy: 214 Control: 217	PTCy: 28 / 69 + 3 Control: 31 / 65 + 4	PTCy/TAC/ MMF TAC/MMF	RIC	PBSC	GRFS at 12 months	Grade II-IV: 53.8% (PTCy), 51.9% (control) Grade III-IV: 6.3% (PTCy), 14.7% (control)	All grades (at 1-y): 21.9% (PTCy), 35.1% (control)	At 1-y: 52.7% (PTCy), 34.9% (control), p=0.001	At 1-y: 12.3% (PTCy), 17.2% (control)	At 1-y: 20.8% (PTCy), 20.2% (control)
Brissot E et al (2024)	75	Phase 2	PTCy: 44 ATG: 37	PTCy: 39 / 61 ATG: 40.5 / 59.5	PTCy/CSA/ MMF ATG/CSA/ MMF	RIC (Bu/Flu)	PBSC	GRFS at 12 months	Grade II-IV (at 6-mo): 26.4% (PTCy), 24.3% (ATG) Grade III-IV: 6.8% (PTCy), 5.4% (ATG)	At 1-y: 32.5 % (PTCy), 36.1% (ATG)	At 5-y: 43.2% (PTCy), 37.8% (ATG)	At 5-y: 18.6% (PTCy), 10.6% (ATG), p=0.057	At 5-y: 27.3% (PTCy), 37.6% (ATG)

MAC: myeloablative conditioning; RIC: reduced intensity conditioning; CI: cumulative incidence; aGvHD: acute graft-versus-host disease; cGvHD: chronic graft-versus-host disease; NRM: non-relapse mortality; HCT: hematopoietic stem cell transplantation; BM: bone marrow; MUD: matched unrelated donor; MMUD: mismatched unrelated donor; MRD: matched related donor; PBSC: peripheral blood stem cells; Bu: busulfan; PTCy: post-transplant cyclophosphamide; Flu: fludarabine; TBI: total body irradiation; TAC: tacrolimus; MTX: metotrexate; GRFS: GvHD-free/relapse-free survival; ATG: anti-thymocyte globulin; BOR: bortezomib; MVC: maraviroc; MMF: micofenolate mofetil; CSA: cyclosporine; DFS: disease-free survival; OS: overall survival; d: days; y: year; mo: months.

PTCy combined with other agents

After these disappointing results, combinations of PTCy with other immunosuppressive drugs have been explored in the setting of mobilized PB alloHCT. To demonstrate an advantage in the CI of cGvHD compared to standard CNI-based prophylaxis, a study was conducted by Mielcarek et al in 43 patients with high-risk hematologic malignancies transplanted from HLA-matched donors (12 MRD, 30 MUD) using MAC regimens [52]. Cyclosporin (CSA) was added to PTCy with the aim of reducing the previously reported high rate of grade III-IV aGvHD. Possibly due to the addition of CSA, no severe manifestations of aGvHD were observed and the 1-year CI of cGvHD requiring systemic immunosuppressive treatment was low at 16%. However, the reported CI of grade II aGvHD and extensive cGvHD reached 77% and 30%, respectively.

An additional experience in the setting of MUD alloHCT using PB as a source was reported in a single-center trial which enrolled 200 patients [53]. The final study only included 86 patients with acute leukemia who received GvHD prophylaxis using TAC/MMF combined with PTCy. Results were compared with those from a historical cohort of patients who received ATG with TAC or MTX/MMF. Of note, 21% of patients received a mismatched unrelated donor alloHCT and 76% a RIC regimen. Even considering these statistical limitations, significant advantages were observed with the use of PTCy compared to ATG in terms of grade II-IV (19% versus 45%), grade III-IV (4% versus 27%), steroid-refractory aGvHD (13% versus 49%), and moderate/severe cGvHD (16% versus 65%). OS, DFS, NRM, and especially GRFS (52% versus 12%) were significantly better in the PTCy group. Furthermore, the use of PTCy displayed a better safety profile in terms of reduced incidence of sinusoidal obstruction syndrome, cytomegalovirus reactivations, invasive fungal infections, and severity of mucositis. Incidence of primary graft failure was not different between the two groups, but engraftment was significantly slower in the PTCy cohort. In a similar trial, Carnevale-Schianca et al tested the same combination of drugs in MRD and MUD alloHCT, demonstrating a satisfactory control of aGvHD (17%, with no grade IV) and low NRM (3%) at 2 years [54].

To exert a possible synergistic effect on the expansion regulatory T-cells, different group applied a CNI-free approach using sirolimus combined with PTCy in PB alloHCT. Solomon et al tested a brief course of sirolimus in addition to PTCy in 26 patients receiving HLA-matched transplantation after a fludarabine/busulfan/cyclophosphamide RIC regimen [55]. The 100-day CI of grade II-IV and grade III-IV aGvHD, and 1-year extensive cGvHD were 46%, 15%, and 31%, respectively; 1-year NRM was 4%. This combination allowed a rapid withdrawal of immunosuppression, with only 11% of patients still requiring immunosuppression at 2 years. Our group reported results of 249 patients (comprising 48 MRD and 50 MUD) with different hematological malignancies transplanted using a sirolimus-PTCy prophylaxis, with the addition of MMF only in unrelated donor alloHCT [56]. In the matched cohort, the 100-day CI of aGvHD was respectively 23% for grade II-IV and 9% for grade III-IV; overall, the CI of cGvHD was 25% at 2 years, with severe forms occurring in only 5% of patients. García-Cadenas et al conducted a prospective single-center study which enrolled 56 patients who underwent HLA-matched alloHCT using double agents GvHD prophylaxis with TAC or sirolimus combined with PTCy [57]. Here, the CI of grade II-IV and grade III-IV aGvHD was 24% and 8%, respectively, while severe cGvHD occurred in only 1 of the 43 evaluable patients.

PTCy has also been explored in the context of alloHCT for older patients, in an attempt to mitigate the risks associated with GvHD and reduce NRM. A prospective trial was conducted in older patients (>55 years) undergoing MUD alloHCT using melphalan-based RIC and PTCy plus TAC/MMF prophylaxis [58]. BM was the primary source of stem cells, used in 18 out of 22 patients. The 100-day CI of grade II-IV and grade III-IV aGvHD was 32% and 4%, respectively; no cases of cGvHD were noted. However, late aGvHD was observed in 6 patients and was partially responsible for a higher NRM (43% at 2 years). However, these

results compared favorably with those from an EBMT retrospective analysis that employed RIC conditioning with standard CNI-based GvHD prophylaxis in older patients [59]. Another retrospective study aimed to compare outcomes of PTCy plus TAC versus standard prophylaxis in patients with a median age of 60 years [60]. Patients received PB grafts from MRD (28.9%), MUD (46.5%), and also mismatched unrelated donors; 114 patients (70.8%) underwent RIC alloHCT, and 88 (54.7%) received PTCy. Time to neutrophil and platelet engraftment was longer in the PTCy group: 20 and 19 days compared to 16 and 11 days, respectively. In multivariate analysis, PTCy/TAC significantly reduced the probability of grade II-IV aGvHD and moderate/severe cGvHD.

Retrospective studies have attempted to define the best combination of agents to be used alongside PTCy and evaluate their effectiveness compared to standard GvHD prophylaxis in HLA-matched alloHCT. One EBMT study collected data from 423 patients transplanted using HLA-matched donors and PTCy, alone or in combination with other immunosuppressants [61]. The main result was that only the addition of two drugs (CSA/MTX or CSA/MMF) to PTCy was associated with a reduced risk of extensive cGvHD, lower NRM, and better OS and GRFS in multivariate analysis, without differences in the incidence of aGvHD. Furthermore, the use of BM and the addition of ATG were associated with lower rates of cGvHD. Another retrospective two-center study assessed the impact on transplant outcomes of adding CSA/MMF or Sirolimus/MMF to PTCy in HLA-matched alloHCT and found no significant differences between the two approaches [62]. Additional retrospective studies have tested these combinations in comparison to the conventional CNI-based prophylaxis, either with or without ATG. Overall, the results were inconclusive: some studies reported better outcomes with PTCy, especially in terms of GvHD control, GRFS, and NRM [26,63–67], while others showed similar findings in both MRD and MUD alloHCT [68–70]. On the contrary, in another EBMT analysis, the addition of ATG to standard prophylaxis was associated with a lower incidence of cGvHD compared with those receiving PTCy [71].

Therefore, prospective trials were undertaken to determine the optimal combination in this context. In a prospective multicenter phase 2 study, adult patients transplanted using RIC regimens and PB grafts were randomly assigned to PTCy/TAC/MMF, TAC/MTX plus bortezomib, or TAC/MTX plus maraviroc. Results from each arm were compared to a contemporary non-randomized prospective cohort of patients treated with standard TAC/MTX [72]. TAC/MMF/PTCy was the most promising intervention, yielding the best GRFS benefit, and it was selected to be prospectively compared with TAC/MTX in a phase 3 randomized trial (CTN 1703). Overall, 431 patients were enrolled in this subsequent study and 196 received the experimental prophylaxis. The 1-year adjusted GRFS, which was the primary endpoint of the study, was 52.7% with TAC/MMF/PTCy and 34.9% with TAC/MTX [73]. This benefit derived primarily from a decrease in both severe acute and chronic GvHD in the 3-drugs arm, leading to a higher incidence of immunosuppression-free survival. Apart from a longer time to platelet recovery and a higher incidence of grade 2 infections in PTCy-exposed recipients, other outcomes did not differ substantially between the groups. The inclusion of 7/8 HLA-mismatched unrelated donors, even if limited (3.5% of the total), and the absence of high-resolution HLA--typing for sibling donors could represent limitations of this trial. Another phase 3 trial compared CSA prophylaxis with PTCy or MMF after RIC alloHCT and reported similar results in terms of GRFS: 45% at 1 years for the PTCy group versus 21% in the standard CSA/MMF prophylaxis cohort [74]. These substantial improvements in GRFS, a survival outcome that reflects the quality of life without long-term complications related to GvHD, support the endorsement of this combination as a possible new standard for GvHD prophylaxis in RIC alloHCT. However, both studies present potential limitations in terms of a short follow-up period and the absence of ATG/ATLG in the control group, even though this type of in vivo T-cell depletion is not routinely recommended when RIC regimens are employed due to concerns about an increased risk of relapse [10].

Recently, a prospective randomized phase 2 trial tested the combination of CSA/MMF GvHD prophylaxis with either PTCy or ATG, demonstrating similar overall results in RIC alloHCT from HLA-matched donors [75]. The primary endpoint of the study, GRFS, along with the other survival outcomes and the incidence of aGvHD/cGvHD, demonstrated no significant differences between PTCy and ATG, even when follow-up was extended to 5 years after alloHCT. Furthermore, the safety profiles of PTCy and ATG were comparable, with no disparity in the incidence of CMV and EBV reactivations or hemorrhagic cystitis. The only notable difference was a longer median time to neutrophil and platelet recovery in the PTCy compared to the ATG group (21 versus 19 days for neutrophils; 20 versus 11 days for platelets). A phase 3 clinical trial (NCT05153226) is currently underway to elucidate this debate in MUD alloHCT (Table 3).

Conclusions and future perspectives

PTCy, initially developed for haploidentical donor alloHCT, has raised as the most promising strategy for GvHD prevention also in the HLA-matched setting. Its use, in combination with other immunosuppressants, has gained further evidence from retrospective and prospective studies and should no longer be considered experimental.

In RIC alloHCT, the encouraging results obtained with PTCy-based GvHD prophylaxis in related and unrelated HLA-matched PB alloHCT were finally confirmed in the CTN 1703 trial. This study demonstrated a clear benefit in terms of GRFS for those receiving PTCy/TAC/MMF, without major disadvantages in other endpoints. Although further evidence and longer follow-up are needed, this scheme has been recognized by the EBMT as a new standard for GvHD prophylaxis in the context of PB alloHCT from unrelated donors, together with ATG/ATLG [76].

Expanding these results to MAC alloHCT is currently not possible as the few available data do not highlight a substantial difference in GvHD prevention with the use of PTCy. Therefore, due to high-quality evidence demonstrating its efficacy in reducing the rate of cGvHD, the addition of ATG/ATLG to CNI/MTX prophylaxis remains the established standard in combination with MAC regimens [77–81]. This apllies not only to MUD but also to HLA-identical sibling alloHCT when PB is used as the graft source [82]. However, controversies remain on optimal dosing, formulation, and time of administration of ATG/ATLG, as well as in-depth quantitative and functional characterization of immune reconstitution [76,81].

The addition of PTCy to standard prophylaxis enhances GvHD prevention at the cost of an increased risk of grade $\geq$2 infections, a result explained by the underlying mechanism of action of PTCy [40] and partly related to the delayed neutrophil engraftment associated with this strategy. This risk may not be confined to the first months after alloHCT but apparently shows no correlation with increased NRM and can be mitigated through the use of prophylactic antimicrobial agents [27,83–85]. Further studies are needed to elucidate the exact impact of PTCy on the risk of viral reactivations, bacterial, and fungal infections, particularly when compared with the use of ATG within the same context and in the current era of letermovir primary prophylaxis of CMV.

The most effective PTCy platform is not yet defined. Studies are ongoing to evaluate the optimal dose and timing of cyclophosphamide administration, with growing evidence supporting the safety and preserved efficacy of a de-escalation approach (Table 3) [86–88]. Others have focused on the combination of PTCy and low-dose ATG, the latter administered during conditioning [89,90], after alloHCT [91], or based on the $CD3^{+}$ infused within the graft [92], with encouraging results in the ability to reduce both acute and chronic GvHD. Incorporation of novel agents, such as ruxolitinib [93], itacitinib (NCT05364762), or abatacept (NCT05289167) with PTCy is currently being tested in prospective studies (Table 3) with the aim of moving from a uniform GvHD prophylaxis to a more individualized strategy that takes into consideration donor options, disease characteristics, and comorbidities of each transplant recipient.

Table 3
List of ongoing prospective trials using post-transplant cyclophosphamide for graft-versus-host disease prophylaxis after matched allogeneic hematopoietic stem cell transplantation.

NCT number	State	Phase	Status	Enrollment	Treatment	Primary endpoint
NCT05120570	USA	Phase 1/2	Recruiting	75	PTCy/sirolimus plus VIC-1911	Otimal dose of VIC-1911 and CI of grade III-IV acute GvHD and relapse
NCT04669210	Russia	Phase 2	Active, not recruiting	128	PTCy/TAC/MMF versus PTCy/ Ruxolitinib	CI of grade II-IV acute GvHD
NCT05622318	USA	Phase 2	Recruiting	56	TAC/MMF plus low-dose PTCy and Ruxolitinib	GvHD-free survival at 1-y
NCT05895201	USA	Phase 1/2	Not yet recruiting	72	PTCy/Bortezomib/Sitagliptin	DLT and CI of grade II-IV acute GvHD
NCT05364762	USA	Phase 2	Recruiting	50	PTCy/TAC plus Itacitinib	Safety and efficacy of shortening TAC period to 60 days after alloHCT and GRFS at 1-y
NCT05289167	USA	Phase 1/2	Recruiting	100	PTCy/Bortezomib/Abatacept	DLT and CI of grade III-IV acute GvHD
NCT03246906	USA	Phase 2	Recruiting	160	CSA/Sirolimus plus MMF or PTCy after NMA conditioning	Chronic GvHD-free/relapse-free survival (CRFS) at 1-y
NCT03852407	Belgium	Phase 2	Recruiting	114	Flu-Mel plus PTCy versus ATG	Current GRFS for patients in the 2 arms
NCT04888741	UK	Phase 2	Recruiting	400	PTCy/CSA or PTCy/Sirolimus versus ATG	GRFS at 1-y
NCT05153226	Germany	Phase 3	Recruiting	540	PTCy versus ATG	Relapse- and immunosuppression-free survival (RIFS) at 1-y for patients receiving unrelated donor PB alloHCT
NCT03357159	Israel	Phase 2	Recruiting	30	PTCy plus ATLG	Overall and disease-free survival at 2-y
NCT05436418	USA	Phase 1/2	Recruiting	240	PTCy (25 mg/Kg on days +3 and +4) plus Sirolimus/MMF	Graft failure, CI of grade III-IV aGvHD, and GRFS at 1-y
NCT06000982	China	Phase 3	Not yet recruiting	138	PTCy 40 versus 50 mg/Kg on days +3 and +4	CI of grade II-IV acute GvHD
NCT04959175	USA	Phase 1/2	Recruiting	320	PTCy 25 mg/kg on days +3 and +4 (in older and/or unfit recipients)	CI of grade III-IV aGvHD

HCT: hematopoietic stem cell transplantation; PTCy: post-transplant cyclophosphamide; TAC: tacrolimus; MMF: micofenolate mofetil; CSA: cyclosporin; ATLG: anti-human T-lymphocyte immunoglobulin; ATG: anti-thymocyte globulin; GvHD: graft-versus-host disease; GRFS: GvHD-free/relapse-free survival; Flu: fludarabine; Mel: melphalan; DLT: dose limiting toxicities; CI: cumulative incidence; y:year.

Author contributions

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Additional information

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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Declaration of competing interest

The authors declare no conflict of interest related to the present submission.

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