Front. Med. 2021, 15(1): 108–115
https://doi.org/10.1007/s11684-019-0730-8

RESEARCH ARTICLE

Fludarabine and intravenous busulfan conditioning with post-transplantation cyclophosphamide for allogeneic peripheral stem cell transplantation for adult patients with lymphoid malignancies: a prospective single-arm phase II study

Ling Wang*, Lining Wang*, Xing Fan*, Wei Tang, Jiong Hu (✉)

Department of Hematology, Blood & Marrow Transplantation Center, Collaborative Innovation Center of Hematology, Ruijin Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

Abstract **Post-transplantation cyclophosphamide (PT-Cy) alone or in combination with other immunosuppressive drugs has emerged as a promising strategy in the setting of allogeneic hematopoietic stem cell transplantation. Improved survival rate was reported in lymphoid malignancies following PT-Cy strategy compared with myeloid disease in non-myeloablative bone marrow transplant setting. Thus, we aimed to evaluate the safety and efficacy of PT-Cy combined with cyclosporine as graft-versus-host disease (GVHD) prophylaxis after myeloablative conditioning and T cell-replete peripheral stem cell transplantation in lymphoid malignancies. This single-arm phase II clinical trial (NCT01435447) involving 31 adult patients was conducted from January 2013 to June 2018. The donor-type neutrophil engraftment rate was 100%, and the overall incidence of grade II to IV and grade III to IV acute GVHD was 39% and 24%, respectively. The cumulative incidence rates of chronic GVHD (35%), including moderate to severe forms (10%), were reduced compared with those of the historical group ($P = 0.03$ and $P = 0.04$, respectively). With a median follow-up of 18 months, the estimated 2-year overall and event-free survival was 64.8% (95% confidence interval: 47.8%–86.7%) and 58.4% (95% CI: 41.9%–81.7%), respectively. The 2-year cumulative incidence rate of relapse was 19.5% (95% CI: 9.0%–35.8%), whereas the non-relapse mortality rate was 21.8% (95% CI: 11.3%–38.1%). These results demonstrated the feasibility of PT-Cy as GVHD prophylaxis in this clinical setting. This strategy could significantly reduce the incidence of chronic GVHD and its moderate to severe forms but not of acute GVHD and results in similar survival outcomes compared with the historical group. A prospective study with additional patients is warranted to confirm the role of PT-Cy in lymphoid malignancy.**

Keywords post-transplantation cyclophosphamide; allogeneic hematopoietic stem cell transplantation; lymphoid malignancies

Introduction

Graft-versus-host disease (GVHD) remains as a major complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1]. The standard regimen for GVHD prophylaxis include the combination of calcineurin inhibitor (CNI), cyclosporine (CsA), tacrolimus with methotrexate and/or mycophenolate mofetil (MMF), or sirolimus [1,2]. Post-transplantation cyclophosphamide (PT-Cy) that targets proliferating allo-reactive T cells after allo-HSCT has emerged as a new GVHD prophylaxis regimen [3]. The safety and efficacy of PT-Cy alone or combined with CNI have been previously demonstrated after T cell-replete bone marrow transplantation from human leucocyte antigen (HLA)-matched or partially HLA-mismatched related donors [4,5]. Notably, in the first clinical report of PT-Cy study in HLA-haploidentical bone marrow transplantation setting, patients with lymphoid malignancies have significantly improved EFS than those with myeloid disease [6]. Although the underlying mechanism remains unclear, the graft-versus-leukemia (GVL) effect may be diminished to some extent by PT-Cy, thereby exerting considerable effect on myeloid disease, which depends on the GVL effect in the allo-HSCT setting [7,8]. Another possible explanation

Received February 27, 2019; accepted October 25, 2019

Correspondence: Jiong Hu, hj10709@rjh.com.cn

*These authors contributed equally to the manuscript.

may be the additional anti-tumor effect of high-dose cyclophosphamide after transplantation in lymphoid malignancies.

On the basis of these data, we conducted a single-center phase II study to assess the safety and efficacy of PT-Cy as GVHD prophylaxis after myeloablative conditioning (MAC) and T cell-replete peripheral stem cell transplantation in adult patients with lymphoid malignancies.

Patients and methods

Patients and eligibility criteria for allo-HSCT

This study was an investigator-initiated, prospective, non-randomized, single-arm phase II clinical trial (NCT 01435447). This investigation was approved by the Human Ethics Committee of Ruijin Hospital and was conducted in accordance with the *Declaration of Helsinki*. The study was performed in the Blood and Marrow Transplantation Center, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. A total of 31 adult patients with lymphoid malignancies were enrolled from January 2013 to June 2018. The eligibility criteria for allo-HSCT were as follows: (1) adult patients (18–60 years) with acute lymphoid malignancies, including acute lymphoblastic leukemia (ALL) or various lymphomas; (2) status at transplantation should be first or second clinical remission (CR1 or CR2) for ALL and at least CR1, partial remission (PR) or CR2 for lymphoma disease; (3) patients received their first allo-HSCT from HLA-matched sibling (MSD), unrelated donor (MUD) or HLA-haploidentical related donor (Haplo); (4) at the transplantation, performance status was $\leqslant 2$ with normal renal and hepatic function (serum creatinine $\leqslant 1.5$ mg/100 mL, serum bilirubin $\leqslant 1.0$ mg/100 mL, serum alanine aminotransferase or aspartate aminotransferase < 3 times the upper normal limit), cardiac left ventricular ejection fraction $\geqslant 50\%$, and normal pulmonary function tests (including forced expiratory volume in 1 min); (5) negative serology for hepatitis B, C, and human immunodeficiency virus; and (6) all patients provided written informed consent prior to allo-HSCT.

Conditioning regimen

All patients received fludarabine (Flu) 30 mg/m^2 daily from day –6 to day –3 and i.v. busulfan (Busulfex, Bu) at 3.2 mg/kg daily from day –6 to day –3. The dose of Bu was based on actual body weight, except for overweight patients (BMI greater than or equal to 25 according to the World Health Organization), for whom the dose was adjusted based on ideal body weight. Peripheral blood stem cells (PBSC) mobilized by granulocyte colony-stimulating factor (G-CSF) were infused on day 0. GVHD prophylaxis consisted of cyclophosphamide 50 mg/kg on day +3 and +4, followed by 1.5 mg/kg CsA twice daily via i.v. infusion starting on day +5. The infusion was switched to oral formulation when it was tolerated. The CsA dose was monitored twice a week to maintain a therapeutic level of 200–250 mg/mL. When no acute GVHD was documented, the tapering of CsA was started on day +100 forward for more than 2–3 months and stopped at approximately 5–6 months after HSCT. All patients with Philadelphia chromosome ALL (Ph^+ ALL) resumed imatinib 2–3 months after transplantation until relapse or for at least 2 years.

Supportive care

For VOD prophylaxis, 0.5 mg/kg lipo-prostaglandin E1 (lipo-PGE1) was given regularly at the start of conditioning until day +28 as previously reported [9]. Mesna, antiemetics, blood components, and all other supportive care measures were provided in accordance with institutional guidelines. Starting on day +5 after allo-HSCT, 5 μg/kg G-CSF was administered until the absolute neutrophil count (ANC) exceeded 0.5×10^9/L.

GVHD and other toxicities

Acute GVHD (aGVHD) and chronic GVHD (cGVHD) were diagnosed and graded in accordance with standard guidelines [10,11]. The first-line therapy of clinically significant aGVHD consisted of i.v. 1–2 mg/kg/day methylprednisolone with full-dose tacrolimus or basiliximab for hepatic or gut aGVHD, respectively. The definition and grading of other toxicities were based on Common Terminology Criteria Adverse Events Version 4.0 (CTCAE v4.0) [12].

Study endpoints

The primary endpoint of the study was the incidence of aGVHD and cGVHD. Secondary endpoints included the median time to the recovery of neutrophils or platelet, 2-year overall survival (OS), 2-year event-free survival (EFS), 2-year cumulative incidence of relapse (2-year CIR), and 2-year cumulative incidence of non-relapse mortality (2-year NRM). For OS, failure was defined as death from any cause. For EFS, failure was defined as documented event of either disease relapse or death from any cause. Patients were considered to have died of NRM if no evidence indicated disease relapse or progression before death. Relapse was defined as $> 5\%$ blasts in bone marrow examination or extramedullary relapse documented by bone marrow aspiration and biopsy in patients with ALL or positive PET-CT scan, followed by biopsy in other lymphoid malignancies. Time to recovery of neutrophils was defined as the interval between transplantation and the

first of 3 consecutive days with an ANC of $> 0.5\times10^9$/L. Platelet recovery was defined as the time interval between transplantation and the first day of a platelet count $> 20\times10^9$/L without platelet transfusion in the preceding 7 days. GVHD-free, relapse-free survival (GRFS) was defined as patients without any event, including disease relapse and occurrence of III–IV aGVHD and cGVHD requiring systemic immune-suppression treatment.

Statistical analysis

The primary endpoint of the study was the incidence of aGVHD and cGVHD with expectation of II–IV aGVHD and/or moderate to severe cGVHD at 30% to calculate the sample size of the study. Survival rates were calculated using Kaplan–Meier estimates [13]. The cumulative incidence of aGVHD, cGVHD, relapse, and NRM were summarized using cumulative incidence estimates [14]. Death without relapse was a competing risk for relapse, and relapse was a competing risk for NRM. Graft failure, relapse, or death without GVHD were considered competing risks for aGVHD or cGVHD. Data for patients who were alive and in CR were censored during the last follow-up visit on September 30, 2018. Univariate comparison was used to compare the data of the study with historical group. The statistics were performed using SPSS and R software at Shanghai Clinical Research Center.

Results

Patients and characteristics

A total of 31 patients were enrolled in the study. All patients were followed up until at least day +100 or when the study endpoints were met with a median follow-up of 18 months (3.5–60). The demographic data for these patients are summarized in Table 1. Twenty-five patients were ALL in first CR1, including 8 patients with Ph^+ ALL receiving imatinib treatment before and after transplantation. For 4 patients with T cell lymphoma, 1 with peripheral T cell lymphoma in CR1 and 1 with anaplastic large cell lymphoma in CR2 relapsed after previous autologous HSCT with two other patients with NK/T cell lymphoma in CR1 and CR2, respectively. For two patients with B cell lymphoma, one with diffuse large B cell lymphoma (DLBCL), who has relapsed after previous auto-HSCT, received allo-HSCT in CR2, and the other patient was transplanted with Burkitt lymphoma in CR1. The donors were HLA-matched related siblings in 18, matched unrelated (10/10) in 10, and HLA-haploidentical in 3. A group of 48 patients of lymphoid malignancies transplanted before January 2013 with standard GVHD prophylaxis regimen was included in the analysis as the historical group. The characteristics of these patients are listed in Table 1.

Engraftment and chimerism

For all 31 patients, the median number of mononucleated cells and CD34 cells infused was 5.62×10^8/kg (range, $2.03\times10^8-16.46\times10^8$/kg) and 5.43×10^6/kg (range, $1.39\times10^8-12.5\times10^6$/kg), respectively. Neutrophil engraftment occurred in all 31 patients at a median of 13 days (11–17 days). The median time of platelet recovery was 12 days (0–70 days) in 30 patients, whereas 1 patient failed to achieve platelet recovery. The development of donor-derived hematopoiesis was further documented by short tandem-repeat polymerase chain reaction in bone marrow mononucleated cells and peripheral T cells. All 31 evaluable patients achieved full donor type ($\geqslant$ 99%) on day +28 to +30 post-transplantation.

Acute GVHD and chronic GVHD

All 31 patients were evaluable for aGVHD; 11 developed II-IV aGVHD, whereas 6 developed III–IV aGVHD. The overall incidence was 39.0% ± 8.8% for II–IV aGVHD and 23.6% ± 7.6% for III–IV aGVHD. A total of 11 patients developed cGVHD, including 3 with moderate to severe cGVHD, 2 of which had bronchiolitis obliterans (BO). The 2-year cumulative incidence of cGVHD and moderate to severe cGVHD was at 35.4% ± 9.5% and 10.1% ± 6.8%, respectively.

Notably, the overall incidence of II–IV aGVHD or III–IV aGVHD was similar ($P = 0.14$) when compared with the historical group transplanted with MAC and standard GVHD prophylaxis with CsA-based regimen for MSD or adding anti-thymoglobulin (ATG) in MUD settings. The overall incidence of cGVHD and moderate to severe cGVHD was reduced ($P = 0.03$ and $P = 0.04$, respectively; Table 2).

Overall outcome

A total of 10 patients died during the follow-up for an overall estimated 2-year OS of 64.8% (95% CI: 47.8%–86.7%, Fig. 1A). A total of 11 patients experienced events, including 5 relapses and 6 deaths without relapse, with a 2-year EFS of 58.4% (95% CI: 41.9%–81.7%, Fig. 1B).

A total of 5 patients relapsed at 2.4–17.1 months after allo-HSCT, with an overall accumulated 2-year CIR of 19.5% (95% CI: 9.0%–35.8%, Fig. 1C). Among these patients, 2 out of 8 Ph^+ ALL relapsed 2.4 and 4.0 months after allo-HSCT; one experienced bone marrow relapse, and the other had CNS relapse followed by subsequent bone marrow relapse 9 months later even after salvage therapy, such as intrathecal chemotherapy, withdrawal and

Table 1 Patient characteristics of the study group and compared with those of the historical group

	PT-Cy	Historical group	*P* value
No. of patients	31	48	
Median follow-up (month)	18 (3.5 – 60)	39 (3.0–108)	<0.001
Age (year, median, range))	30 (18–57)	23 (16–49)	0.38
Sex			0.63
Male	21 (67.7%)	30 (62.5%)	
Female	10 (32.3%)	18 (37.5%)	
Diagnosis			0.96
ALL	25 (80.6%)	43 (89.6%)	
Ph^- ALL	17	33	
Ph^+ ALL	8	10	
Lymphoid malignancies	6 (19.4%)	5 (10.4%)	
PTCL	1	1	
ALCL	1	1	
NK/T cell lymphoma	2	0	
DLBCL	2	2	
HD	0	1	
Disease status			0.58
CR1	27 (87.1%)	41 (85.4%)	
PR/CR2 or beyond	4[a] (12.9%)	7[b] (14.6%)	
Donor type			
MSD	18 (58.1%)	20 (41.7%)	<0.001
MUD	10 (32.3%)	28 (58.3%)	
Haplo	3 (9.6%)	0	
Graft source			
PBSC	31 (100%)	45 (93.7%)	0.16
Bone marrow	0	3 (6.3%)	
Conditioning			
Flu-Bu4	31 (100%)	0	<0.001
Bu4-Cy	0	48 (100%)	
GVHD prophylaxis			
PT-Cy + CsA	31 (100%)	0	<0.001
CsA + MTX + MMF±ATG	0	48 (100%)	

[a] Including 2 patients receiving allo-HSCT with previous auto-HSCT.
[b] Including 1 patient receiving allo-HSCT with previous auto-HSCT.
PT-Cy, post-transplantation cyclophosphamide; ALL, acute lymphoblastic leukemia; Ph, Philadelphia; PTCL, peripheral T cell lymphoma; ALCL, anaplastic large cell lymphoma; DLBCL, diffuse large B cell lymphoma; HD, Hodgkin's lymphoma; CR, complete remission; PR, partial remission; MSD, matched sibling donor; MUD, matched unrelated donor; Haplo, haploidentical donor; PBSC, peripheral blood stem cell; FLU, fludarabine; BU, busulfan; GVHD, graft-versus-host disease; CsA, cyclosporine A; MTX, methotrexate; MMF, mycophenolate mofetil; ATG, anti-thymoglobulin.

Table 2 Transplantation outcomes of patients in the study group and compared with those in the historical group

	PT-Cy	Historical group	*P* value
2-year OS	64.8%±9.7%	59.0%±7.3%	0.93
2-year EFS	58.4%±6.7%	57.8%±7.2%	0.96
2-year NRM	21.8%±6.7%	12.9%±5.8%	0.34
2-year CIR	19.5%±6.7%	33.0%±3.4%	0.27
aGVHD			
II–IV aGVHD	39.0%±8.8%	40.4%±7.2%	0.88
III–IV aGVHD	23.6%±7.6%	10.6%±4.6%	0.14
cGVHD			
cGVHD	35.4%±9.5%	65.1%±8.0%	0.03
moderate/severe cGVHD	10.1%±6.8%	33.6%±9.1%	0.04
2-year GRFS	42.0%±9.7%	33.1%±6.8%	0.78

PT-Cy, post-transplantation cyclophosphamide; OS, overall survival; EFS, event-free survival; NRM, non-relapsed mortality; CIR, cumulative incidence of recurrence; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; GRFS, GVHD-free, relapse-free survival.

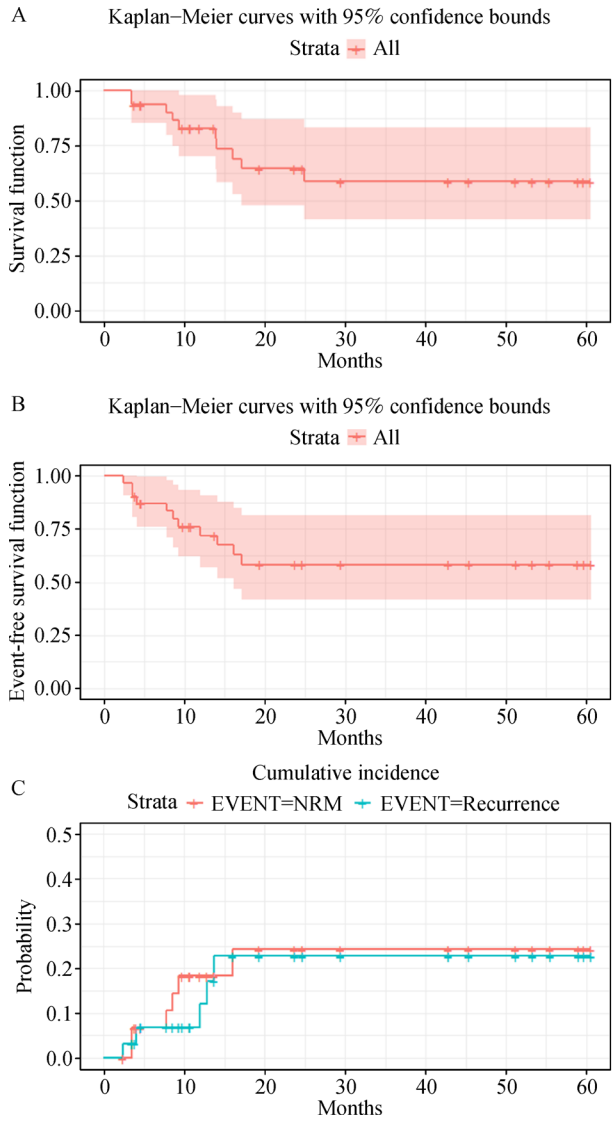

Fig. 1 (A) Kaplan–Meier curves with 95% confidence bounds for overall survival. (B) Kaplan–Meier curves with 95% confidence bounds for event-free survival. (C) Cumulative incidence of relapse and non-relapse mortality.

stopping of immunosuppression, and tyrosine kinase treatment with dasatinib. A total of 2 out of 17 patients with Philadelphia chromosome negative ALL (Ph^- ALL) relapsed at 10.0 and 13.0 months, with one extramedullary relapse and one bone marrow relapse. One patient with T cell lymphoma relapsed at 17.1 months.

A total of 6 patients died in remission 3.5–16.1 months after allo-HSCT. Two patients with III–IV aGVHD died due to severe infection at 3.5 months. Two patients with moderate or severe cGVHD, one with liver cGVHD and the other with BO, died at 8.6 and 16.1 months, respectively. Another patient with mild liver cGVHD responded well to tacrolimus treatment but experienced sudden death without prominent cause during drug tapering phase at 7.8 months after allo-HSCT. Although cytomegalovirus (CMV) reactivation was documented in 11 patients, no CMV disease and EBV reactivation was documented. The only patient with lethal infectious complication was a possible diagnosis of pulmonary aspergillosis at 9 months after allo-HSCT. In summary, the estimated 2-year NRM was 21.8% (95% CI: 11.3%–38.1%) with low NRM at day +180 at 7% (Fig. 1C).

By contrast, 16 patients remained free of relapse and III–IV aGVHD and cGVHD requiring systemic immunosuppression treatment, with an estimated 2-year incidence of GRFS at 41.9% (95% CI: 26.6%–66.1%).

Effect of donor type on outcome

In the study group, we further analyzed the potential effect of donor type on the incidence of GVHD and long-term outcome, as shown in Table 3. Patients who received transplantation with MUD and Haplo donor presented increased incidence of II–IV aGVHD (P = 0.02) and moderate to severe cGVHD (P = 0.029) but not III–IV aGVHD and overall cGVHD. Further analysis revealed that MUD transplantation had higher incidence of moderate to severe cGVHD than MSD transplantation in the study group (Table S1). When compared with the historical group, no significant difference was found in the incidence of overall aGVHD, cGVHD, and moderate to severe cGVHD with PT-Cy prophylaxis for MSD or MUD transplantation (Tables S2 and S3). The PT-Cy strategy did not affect overall treatment outcomes in terms of OS, EFS, CIR, and NRM (Tables S4–S7).

Discussion

The standard GVHD prophylaxis strategy of CNI-based regimen with ATG added to MUD and Haplo settings resulted in an incidence of 25%–40% II–IV aGVHD and 40%–60% in patients undergoing allo-HSCT [1,2]. The feasibility of PT-Cy alone or in combination with other immunosuppressive (IS) drugs confirmed in Haplo setting with low incidence of aGVHD and cGVHD has prompted its use as a GVHD prophylaxis strategy in patients receiving allogeneic bone marrow transplantation (allo-BMT) from MSD or MUD [3–6]. On the basis of these initial reports, we conducted this phase II prospective study to use PT-Cy combined with CsA as GVHD prophylaxis in patients with lymphoid malignancies receiving PBSCT with MAC.

We made two major observations from the present study: (1) the overall incidence of II–IV aGVHD was not significantly reduced as we expected when compared with

Table 3 Effect of donor type on treatment outcome

	MSD (n = 18)	Non-MSD group* (n = 13)	P value
2-year OS	61.6%±12.6%	70.1%±14.7%	0.94
2-year EFS	57.8%±12.4%	60.6%±15.7%	0.98
2-year NRM	12.9%±8.9%	28.9%±14.7%	0.57
2-year CIR	26.1%±13.3%	13.0%±9.5%	0.45
aGVHD			
II–IV aGVHD	25.6%±9.9%	63.6%±14.5%	0.02
III–IV aGVHD	11.2%±7.4%	23.8%±14.8%	0.79
cGVHD			
cGVHD	25.8%±11.2%	50.0%±15.8%	0.28
moderate/severe cGVHD	0	35.2%±16.5%	0.029
2-year GRFS	50.0%±15.8%	38.9%±11.8%	0.81

* Including 10 MUD and 3 Haplo patients
MSD, matched sibling donor; MUD, matched unrelated donor; Haplo, haplo-identical donor; OS, overall survival; EFS, event-free survival; NRM, non-relapsed mortality; CIR, cumulative incidence of recurrence; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; GRFS, GVHD-free, relapse-free survival.

the historical group using CsA-based combination regimen (MTX + MMF) for MSD and ATG (6 mg/kg) for MUD transplantation [9]. (2) No moderate or severe cGVHD was observed in the MSD group, whereas moderate/severe cGVHD was significantly higher in the non-MSD group than that in the MSD group, with a significantly higher overall incidence than expected.

With regard to aGVHD, a possible reason that explains the observation may be due to lymphoid malignancies. In the recent study of Acute Leukemia Working Party of the EBMT (ALWP-EBMT), ALL (HR 0.57, 95% CI: 0.32–0.84, $P < 0.001$) was independently associated with increased risk of II–IV aGVHD using PT-Cy as GVHD prophylaxis [15]. A second possibility may be due to MAC and the use of PBSC as graft [16,17]. A meta-analysis demonstrated that reduced intensity conditioning (RIC) had a considerably low II–IV aGVHD although patients receiving RIC were old and received PBSC graft [18]. Similarly, the ALWP-EBMT report revealed that in the PT-Cy setting, PBSC alone was associated with high and extensive cGVHD but not for aGVHD [15]. The incidence of overall aGVHD was low in both adults and elderly patients receiving PBSC as graft, especially in the setting of non-myeloablative or reduced intensity regimen with Haplo donor transplantation [19,20]. A third explanation is that PT-Cy alone or combined only with CsA is not sufficient to decrease aGVHD in an HLA-matched setting. With MAC, such as Bu-Cy or Flu-Bu with bone marrow graft from MSD or MUD donor, grade II–IV aGVHD ranged from 43% to 51% with PT-Cy prophylaxis [20–22]. Mielcarek *et al.* reported that in PT-Cy followed by CsA alone in PBSC transplantation from an HLA-matched related and unrelated donor settings, the cumulative incidence estimates of grade II–IV aGVHD reached as high as 77% [23]. By contrast, Law *et al*. reported a promising result with the combination of ATG and PT-Cy as GVHD prophylaxis [24]. In 50 patients undergoing allo-HSCT with PBSC from Haplo donors with reduced intensity regimen composed of Flu (30 mg/m^2/day on days –5 to day –2), Bu (3.2 mg/kg/day on days –3 and –2), and total body irradiation (200 cGy) on day –1, the cumulative incidence of aGVHD of any grade and III–IV aGVHD on day +100 was 38.3% and 5.2%, respectively. The combination of PT-Cy with tacrolimus (FK506) combined with MMF achieved grade II–IV aGVHD as low as ~20% and grade III–IV aGVHD of ~5% in both HLA-matched sibling or unrelated donor transplantation [25,26]. Despite myeloablative Flu-Bu conditioning, which may be associated with less aGVHD [27], our observation suggests that for PBSC with MAC conditioning regimen, PT-Cy with CsA alone may not be the optimal regimen. However, combination with tacrolimus + MMF or ATG may improve outcomes in aGVHD prophylaxis.

The use of PBSC as graft is substantially associated with high incidence of and extensive cGVHD in the ALWP-EBMT analysis [15]. In the bone marrow transplantation from both MSD and MUD, 10%–14% overall incidence of cGVHD was reported with PT-Cy alone [21,22]. In the PBSC setting, PT-Cy combined with FK506 + MMF was a more effective approach than PT-Cy alone to reduce the overall cGVHD to 7%–16% in both MSD and MUD transplantation [25,26]. A recent report of combined ATG with PT-Cy also resulted in a total of 15.5% mild cGVHD without moderate/severe cGVHD [28]. Considering these data, we may speculate that PT-Cy alone or with CsA may be sufficient to prevent cGVHD in bone marrow transplantation but may not be sufficient in a PBSCT setting, particularly in non-MSD patients with MAC.

Czerw *et al.* reported that the use of ATG considerably reduced grades II–IV and III–IV aGVHD and ext cGVHD

but increased the risk of relapse in adult patients with Ph$^-$ ALL undergoing allo-PBSCT [28]. Similarly, a major concern of PT-Cy is the relatively high incidence of relapse in earlier reports in various hematological malignancies, particularly with low incidence of cGVHD [29]. For ALL, Sayehmiri *et al*. conducted a long-term follow-up of 425 patients who underwent bone marrow or stem cell transplantation and reported that cGVHD positively influenced the survival rate, owing mostly to the reduction of relapse [30]. With regard to our series, we observed a decrease in both cGVHD and moderate or severe cGVHD with PT-Cy compared with the historical group. The 2-year CIR in the PT-Cy group was not higher than that of the historical group. Our observation at least showed that the PT-Cy strategy was not associated with increased risk of relapse.

In summary, this prospective study on PT-Cy prophylaxis in patients with lymphoid malignancy undergoing allo-HSCT with PBSC graft and MAC demonstrated a decrease in cGVHD and moderate/severe cGVHD compared with the historical group. The results did not translate into any benefit of overall HSCT outcome in terms of OS, EFS, and GRFS when compared with the historical group. However, significant differences in donor type and conditioning regimen were found. Although the limited number of patients enrolled in the present study prevents us from arriving at any conclusion, our data at least support the feasibility of further investigation of PT-Cy as GVHD prophylaxis. Considering that recent studies suggest promising outcomes from the combination of PT-Cy with tacrolimus + MMF or ATG, prospective studies are warranted to confirm its role in lymphoid malignancy.

Acknowledgements

We thank all clinical fellows and the nursing staff from Blood and Marrow Transplantation Center, Department of Hematology, Ruijin Hospital. This study was supported by the National Natural Science Foundation of China (No. 81770187) and the National Key Research and Development Plan of China (No. 2017YFA0104500).

Compliance with ethics guidelines

Ling Wang, Lining Wang, Xing Fan, Wei Tang, and Jiong Hu declare no conflict of interest. This observational study was conducted in accordance with the *Declaration of Helsinki*, International Conference on Harmonization Good Clinical Practice, and nationally mandated ethical requirements. The study protocol and informed consent document were reviewed and approved by the ethics committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. All study participants provided informed consent.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-019-0730-8 and is accessible for authorized users.

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