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Anti-thymocyte globulin and post-transplant cyclophosphamide predisposes to inferior outcome when using cryopreserved stem cell grafts

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Abstract

During 2020, the concurrent novel COVID-19 pandemic lead to widespread cryopreservation of allogeneic hematopoietic cell transplant grafts based on National Marrow Donor Program and European Society of Blood and Marrow Transplantation recommendations, in order to secure grafts before the start of conditioning chemotherapy. We sought to examine the impact of this change in practice on patient outcomes. We analyzed the outcomes of 483 patients who received hematopoietic stem cell transplantation (HSCT) between August 2017 and August 2020, at Princess Margaret Cancer Centre, Canada, in the retrospective study, comparing the outcomes between those who received cryopreserved or fresh peripheral blood stem cell grafts. Overall compared with those who received fresh grafts (n = 348), patients who received cryopreserved grafts (n = 135) had reduced survival and GRFS, reduced incidence of chronic graft-versus-host disease (GvHD), delay in neutrophil engraftment, and higher graft failure (GF), with no significant difference in relapse incidence or acute GvHD. However, recipients of cryopreserved matched-related donor HSCT showed significantly worse OS, NRM, GRFS compared with fresh grafts. Multivariable analysis of the entire cohort showed significant impact of cryopreservation on OS, relapse, cGvHD, GF, and GRFS. We conclude that cryopreservation was associated with inferior outcomes post-HSCT, possibly due to the combination of ATG and post-transplant cyclophosphamide impacting differential tolerance to cryopreservation on components of the stem cell graft; further studies are warranted to elucidate mechanisms for this observation.

Jonas Mattsson and Fotios V. Michelis contributed equally.

Novelty Statements: 1. The COVID-19 pandemic led to increased cryopreservation of stem cell grafts to ensure availability for transplant. 2. In this study, cryopreservation was associated with inferior outcome overall and in particular for matched-related donor recipients. 3. Cryopreservation has been shown to impact lymphocyte viability more than stem cells, and combined with dual anti-lymphocyte GvHD prophylaxis, may lead to increased lymphocyte depletion and reduced alloreactivity.

KEYWORDS
allogeneic stem cell transplant, COVID-19, cryopreservation

1 | INTRODUCTION

In allogeneic hematopoietic stem cell transplantation (HSCT), donor hematopoietic stem and progenitor cells are typically collected and infused fresh and without cryopreservation. During 2020, the concurrent novel COVID-19 pandemic lead to widespread cryopreservation of allogeneic HCT grafts based on NMDP and EBMT recommendations, in order to secure grafts before the start of conditioning chemotherapy.[1]

Limited studies in patients undergoing HLA-matched-related donor (MRD) allo-HCT using bone marrow (BM) as stem cell source suggested that bone marrow product cryopreservation does not impact engraftment or the risk of graft-versus-host disease (GvHD).[2,3] Among recipients of peripheral blood stem cell (PBSC) HSCT, some studies[4] showed delayed platelet recovery with cryopreserved grafts, but without impact from cryopreservation on neutrophil recovery, GvHD, or survival outcomes. Other work has shown no differences in terms of time to neutrophil or platelet engraftment, the incidence of acute or chronic GvHD, or relapse, overall survival (OS), and non-relapse mortality (NRM).[5] Another study showed that cryopreserved grafts were associated with faster neutrophil engraftment and a higher incidence of grade II-IV acute GvHD, but despite this, similarly, there were no differences between the cryopreserved and the fresh groups in the incidence of chronic GvHD, disease-free survival (DFS), OS, NRM, and relapse rates.[6] This is in contrast to the results from our own group published recently, which showed that in patients with no or mild chronic GvHD, cumulative incidence of relapse (CIR) was less for fresh compared with cryopreserved grafts, while the incidence of chronic GvHD was higher in the cryopreserved cohort[7]; overall, however, survival rates were not affected. Importantly, this work studied patients transplanted over an 8-year period, with potential impact from changes in conditioning regimens and GvHD prophylaxis. Thus, we hypothesized that changes in transplant conditioning and GvHD prophylaxis may be associated with differences in outcomes when using cryospreserved stem cell grafts, given that differences in sensitivity to freeze-thaw have been reported for the components of stem cell grafts.[8]

The change in practice during this recent exceptional period thus allowed us to examine the outcomes in a single-center retrospective cohort and correlate outcomes between fresh and frozen grafts over a more recent 3-year period, including 2020.

2 | PATIENTS AND METHODS

We retrospectively reviewed 483 consecutive patients who underwent unrelated donor allogeneic HSCT between August 2017 and August 2020, at Princess Margaret Cancer Centre, Toronto, Canada. The research ethics board at the University Health Network approved this study. Median follow-up was 26.1 (12.3-47.9) months in patients who received fresh grafts (termed FRESH group) and 25.1 (11.9-46.9) months for patients who received cryopreserved grafts (CRYO group).

2.1 | Pretransplant characteristics

Baseline patient characteristics are summarized in Table 1. From the start of the COVID-19 pandemic (March 2020), until the end of this study in August 2020, 47 patients received cryopreserved grafts and 17 received fresh grafts. Of the entire cohort, median age was 58 years; 57% were males. Acute myeloid leukemia was the most common indication (n = 245, 50.7%) for HSCT. Disease risk index (DRI)[9] was high or very high in 103 (20.4%) of patients, and HCT-specific comorbidity index (HCT-CI)[10] was >3 in 174 (34.5%) of patients with no difference between FRESH and CRYO groups. Regarding transplant conditioning, 79 (23%) and 23 (17%) patients received myeloablative conditioning (MAC) in the FRESH and the CRYO groups, respectively (*P* not significant).

Donor type was significantly different between the FRESH and CRYO groups, with a higher proportion of MRD and haploidentical (Haplo), and relatively fewer MMUD and MUD receiving cryopreserved grafts ($P < .001$). Female donors to male recipient grafts occurred in 52 (14.9%) of FRESH and 33 (24.4%) of CRYO patients, $P = .02$. In the FRESH group, 253 patients (72.7%) received antithymocyte globulin (ATG) and post-transplant cyclophosphamide (PTCy) as GvHD prophylaxis, whereas 56 (16.1%) received ATG only and 36 (10.3%) received PTCy only, while in the CRYO group, 114 (84.4%) received ATG and PTCy as GvHD prophylaxis, whereas 17 (12.6%) received ATG only and 2 (1.5%) received PTCy only, $P = .01$.

CMV-negative recipients receiving grafts from CMV-negative donors occurred in 59 (17.0%) and 19 (14.1%) cases, while other combinations of CMV serostatus in donor and recipients occurred in 289 (83.0%) and 116 (85.3%) patients in the FRESH and the CRYO groups, respectively (*P* not significant).

Of the entire cohort, one patient transplanted during this period developed COVID-19 and experienced relatively mild disease.

2.2 | Conditioning regimens and GvHD prophylaxis

MAC regimens included fludarabine 50 mg/m^2 × 4 days, busulfan 3.2 mg/kg ×4 days, TBI 400 cGy in two fractions (FBT400), or fludarabine 30 mg/m^2 × 4 days followed by intravenous busulfan 3.2 mg/kg of actual or adjusted ideal body weight × 4 days (FB4). Reduced intensity conditioning (RIC) regimens included fludarabine 30 mg/m^2 × 4 days, busulfan 3.2 mg/kg ×2 days, and TBI 200 cGy in a single fraction (FBT200). GvHD prophylaxis included any of or a combination of the following: cyclosporin A (CSA, 2.5 mg/kg IV q12h),

TABLE 1 Pretransplant characteristics

	Fresh n = 348	Frozen n = 135	*P*-value
Median age: Years (range)	58 (18-76)	58 (18-75)	.76
Males	198 (57)	77 (57)	.98
Diagnosis			
Acute myeloid leukemia	179 (51)	69 (51)	.80
Myelodysplastic syndrome	48 (14)	17 (13)	
Myeloproliferative neoplasm	28 (8)	16 (12)	
Acute lymphoblastic leukemia	37 (11)	13 (10)	
Lymphoma	13 (4)	10 (7)	
Chronic myeloid leukemia	10 (3)	0	
Chronic lymphocytic leukemia	7 (2)	2 (1)	
Chronic myelomonocytic leukemia	9 (3)	2 (1)	
Mixed phenotype acute leukemia	14 (4)	4 (3)	
Non-malignant	3 (1)	2 (1)	
AML Stage			
CR1	148 (83)	60 (86)	.88
CR2	24 (13)	5 (7)	
CRi	2 (1)		
Not in CR	3 (2)	5 (7)	
Disease Risk Index (DRI)			
Non-malignant (not applicable)	3 (1)	2 (1)	.21
Low	20 (6)	5 (4)	
Intermediate	243 (70)	104 (77)	
High-Very high	81 (23)	22 (16)	
Unknown	1 (0.3)	2 (1.5)	
HCT-CI			
0	95 (27)	26 (19)	.17
1-2	127 (36)	53 (39)	
>3	122 (35)	52 (39)	
Missing	4 (1)	4 (3)	
Donor type			
MRD	65 (19)	47 (35)	<.001
MUD 10/10	197 (57)	36 (27)	
MUD 9/10	40 (11)	8 (6)	
Haplo	46 (13)	42 (31)	
Donor age	29 (8-70)	35 (14-74)	<.001

(Continues)

TABLE 1 (Continued)

	Fresh n = 348	Frozen n = 135	*P*-value
Donor sex: female to male	52 (15)	33 (24)	.02
Conditioning			
MAC	79 (23)	23 (17)	.21
RIC	269 (77)	112 (83)	
GVHD prophylaxis:			
ATG only	56 (16)	17 (13)	.01
PTCy only	36 (10)	2 (1)	
PTCy+ATG	253 (73)	114 (84)	
No ATG or PTCy	3 (1)	2 (1)	
CMV serology (R/D)			
Negative/Negative	59 (17)	19 (14)	.96
Positive/Negative	126 (36)	46 (34)	
Negative/Positive	16 (5)	13 (10)	
Positive/Positive	147 (42)	57 (42)	
$CD34^+$/kg cell dose count (Median)	7.6 (1.5-25.2)	7.5 (0.25-28.6)	.27
Stem cell source			
PBSC	348	135	
Follow-Up (months)	26.1 (12.3-47.9)	25.0 (11.9-46.9)	

Abbreviations: ATG, anti-thymocyte globulin; BM, bone marrow; CMV, cytomegalovirus; CP, chronic phase; CR, complete remission; GvHD, graft-versus-host disease; HCT-CI, Hematopoietic cell transplant comorbidity index; HLA, human leukocyte antigen; MAC, myeloablative conditioning; PBSC, peripheral blood stem cells; PTCy, post-transplant cyclophosphamide; RIC, reduced intensity conditioning.

methotrexate (MTX, 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, and +11), mycophenolate mofetil (15 mg/kg q8h × 30 days), anti-thymocyte globulin (rabbit-ATG [Thymoglobulin; Genzyme-Sanofi, Lyon, France], 4.5 mg/kg: 0.5 mg/kg IV on day −3 and 1.5 mg/kg IV on days −2 and −1 or 2 mg/kg: 0.5 mg/kg IV on day −2 and 1.5 mg/kg IV on days −1), and post-transplant cyclophosphamide (PTCy, 50 mg/kg IV on days +3 and +4).

Acute GvHD (aGvHD) was graded according to the Keystone criteria,[11] and chronic GvHD (cGvHD) was graded in accordance with the National Institutes of Health (NIH) Chronic Graft-versus-Host Disease Consensus criteria.[12] cGvHD was only evaluated in patients surviving more than 100 days after HSCT. The HCT-CI[10] score was calculated for all patients and categorized into three risk groups: low risk defined as score of 0, intermediate risk defined as score of 1–2, and high risk defined as score of 3 or greater.

2.3 | Cryopreservation of grafts

Cryopreservation was used in order to facilitate transplant scheduling (predominantly from local related donors), when needed for unexpected delays requiring temporary postponement of transplant, or due to the exigencies imposed by the COVID-19 pandemic. All patients received PBSC grafts. Cryopreservation of grafts was undertaken using the uncontrolled-rate freezing (dump freezing) method. Quality control was performed on pilot tubes from cryopreserved grafts by calculating cellular viability using the trypan blue exclusion test on approximately 30% of randomly selected grafts until December 2019, with a mean viability of 74% (95% CI: 73.5-74.5). From January 2020, a flow cytometry-based assay was used to evaluate 60 (44.4%) cryopreserved grafts, with mean viability 82.4% (95% CI: 80.0%-84.7%).

Neutrophil and platelet engraftments were defined as the time from the date of PBSC infusion to the first of three consecutive days with an absolute neutrophil count of 0.5×10^9/L or higher, and to the first of seven consecutive days with an untransfused platelet count of 20×10^9/L or higher.

Primary and secondary graft failures were diagnosed as per accepted definitions.[13]

2.4 | Statistical analysis

Overall survival, relapse-free survival (RFS), and GvHD and RFS (GRFS) were calculated using the Kaplan-Meier method and compared with the log-rank test. GRFS was defined accounting death, relapse, grade III-IV acute GvHD, and severe chronic GvHD as an event.

Non-relapse mortality (NRM), GvHD, and relapse incidence (RI) were estimated using a non-parametric estimator of cumulative incidence curves taking competing events into consideration. NRM and RI were competing events for each other. cGvHD was only evaluated in patients surviving more than 100 days after SCT.

For OS, RFS, NRM, and GRFS, multivariate analyses were carried out using Cox regression models (to estimate hazard ratios (HRs). All *P*-values were two-tailed. The following factors were included in the multivariate analyses: recipient and donor age, recipient/donor CMV serostatus, HCT-CI, DRI, Karnofsky Performance Score (KPS) before HSCT, cryopreservation, conditioning (RIC/MAC), GvHD Prophylaxis, $CD34^+$ cell dose, type of donor, and female donor to male recipient by backward step-wise elimination method.

Analysis was performed using Statistica 13 software (StatSoft, Tulsa, OK) and the EZR statistical software version 1.50 (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[14]

3 | RESULTS

3.1 | Post-HSCT outcomes

The median $CD34^+$ cell doses infused per kg were 7.6 (1.5-25.2) and 7.5 (0.25-28.6) $\times 10^6$ $CD34^+$ cell/kg recipient body weight, in the FRESH and the CRYO groups, respectively (*P* not significant), Table 1. Thirty-three patients experienced graft failure (GF), 17 from the FRESH (4.9%) and 16 from CRYO (11.9%) groups (*P* = .01), having all exclusively received RIC, Table 2.

TABLE 2 Post-transplant characteristics

	Fresh N = 348	Frozen N = 135	*P*-value
Graft Failure	17 (4.9)	16 (11.9)	.01
Primary	6 (1.7)	5 (3.7)	
Secondary	11 (3.2)	11 (8.1)	
Acute GvHD			
None	169 (48.6)	63 (46.7)	.85
Grade 1	58 (16.7)	25 (18.5)	
Grade 2	75 (21.6)	30 (22.2)	
Grade 3	36 (10.3)	13 (9.6)	
Grade 4	10 (2.9)	4 (3.0)	
Chronic GvHD[a]			
None	197 (62.5)	91 (76.5)	.01
Mild	60 (19.0)	13 (10.9)	
Moderate	52 (16.5)	10 (8.4)	
Severe	6 (1.9)	5 (4.2)	
Engraftment			
CI neutrophils at 30 d	99.7% (96.6-100)	96.3% (91.0-98.5)	**.01**
CI Platelets at 30 d	79.6% (74.9-83.5)	77.0% (68.9-83.3)	.23

Abbreviations: CI, cumulative incidence; GvHD, graft-versus-host disease.
[a]Patients who died before 100 days are not included in this outcome.

Cumulative incidence (CI) of neutrophil engraftment at 30d was 99.7% and 96.3% in the FRESH and the CRYO groups, respectively (*P* = .01), while CI of platelet engraftment 30d was 79.6% and 77.2% in the FRESH and the CRYO groups, respectively (*P* not significant), Table 2.

Grade II-IV aGvHD occurred in 121 (34.8%) and 47 (34.8%) patients in the FRESH and the CRYO groups, respectively (*P* not significant), while no aGvHD occurred in 169 (48.6%) and 63 (46.7%) patients, respectively (*P* not significant), Table 2. Patients who received fresh grafts from 10/10 MUDs (n = 197) had CI of aGvHD Grade II-IV at 100 days of 25.4%, compared with the CRYO group 10/10 MUDs (n = 36) 25.0%, *P* not significant, whereas those who received grafts from FRESH MRD (n = 65) had CI 18.5% and CRYO MRD 16.3% (n = 49), respectively, Table 3.

Mild cGvHD occurred in 60 (19.0%) patients and 13 (10.9%) patients in the FRESH and the CRYO groups, respectively, while moderate/severe cGvHD occurred in 58 (18.4%) patients and 15 (12.6%) patients, respectively; 197 (62.5%) FRESH patients and 91 (76.5%) CRYO patients, respectively, experienced no cGvHD (*P* = .01, Table 2). Forty-nine patients (10.1%) died within 100 days of SCT and were not evaluated for cGvHD.

TABLE 3 Post-transplant outcome

Outcome	Group	Fresh n	Fresh %	Fresh Lower 95% CI	Fresh Upper 95% CI	Frozen n	Frozen %	Frozen Lower 95% CI	Frozen Upper 95% CI	P-value
Acute GvHD Grade II-IV at D100	Whole Cohort	348	25.3	20.8	30.0	135	21.5	15.0	28.8	.99
	MRD	65	18.5	10.1	28.8	49	16.3	7.6	28.0	.38
	MUD 10/10	197	25.4	19.5	31.6	36	25.0	12.3	40.0	.78
	Haplo	46	34.8	21.3	48.6	42	21.4	10.5	34.9	.06
	MMUD	40	25.0	12.8	39.2	8	37.5	7.2	69.4	.08
Moderate / Severe Chronic GvHD at 1 year[a]	Whole Cohort	314	16.6	12.7	20.9	119	10.9	6.1	17.3	.20
	MRD	63	23.8	14.1	34.9	45	8.9	2.8	19.5	.08
	MUD 10/10	180	13.3	8.8	18.8	35	11.0	3.5	24.5	.65
	Haplo	40	25.0	12.8	39.3	32	15.6	5.6	30.3	.48
NRM at 1 year	Whole Cohort	348	17.8	14.0	22.0	135	20.0	13.7	27.1	.51
	MRD	65	1.5	0.1	7.4	49	14.3	6.2	25.6	.005
	MUD 10/10	197	15.7	11.0	21.2	36	13.9	5.0	27.3	.56
	Haplo	46	32.6	19.6	46.3	42	28.6	15.8	42.7	.80
GRFS at 2 y	Whole Cohort	345	51.4	45.9	56.6	133	41.2	32.1	50.1	.04
	MRD	65	73.8	61.3	82.8	49	41.7	27.5	55.2	<.001
	MUD 10/10	195	50.5	43.2	57.4	35	58.4	36.9	74.9	.37
	Haplo	45	42.3	26.9	57.0	42	33.0	18.7	48.0	.22
Overall Survival at 2 y	Whole Cohort	348	65.8	60.4	70.6	135	53.5	44.0	62.1	.04
	MRD	65	79.4	67.1	87.5	49	47.6	32.8	61.0	<.001
	MUD 10/10	197	68.0	60.9	74.1	36	80.6	63.5	90.2	.22
	Haplo	46	54.6	38.5	68.2	42	45.1	29.0	59.9	.29
ATG-PTCy Overall Survival at 2 y	ATG-PTCy	253	65.5	59.3	71.1	114	51.9	41.8	61.1	<.05
	ATG	56	63.6	49.3	74.8	17	64.7	37.7	82.3	.86
Graft Failure	Whole Cohort	348	4.9	3.0	7.5	135	11.1	6.5	17.1	.01
	MUD 10/10	197	3.0	1.3	6.2	36	11.1	3.4	23.8	.03
	Haplo	46	6.5	1.7	16.2	42	19.0	8.8	32.2	.08
Relapse	Whole Cohort	345	23.2	18.8	27.9	133	28.5	20.7	36.8	.08
	MRD	65	21.5	12.5	32.2	49	37.1	23.6	50.7	.10
	MUD	195	24.1	18.3	30.4	35	19.7	5.3	40.7	.50
	Haplo	45	15.7	5.8	29.9	42	27.0	14.2	41.5	.05

(Continues)

TABLE 3 (Continued)

Causes of death	Group	Fresh			Frozen		
		Cause	Number	%	Cause	Number	%
	Whole Cohort	Alive	221	63.5	Alive	75	55.5
		Relapse	56	16.1	Relapse	29	21.5
		Infection	29	8.3	Infection	9	6.7
		GvHD	18	5.2	GvHD	8	5.9
		Graft failure	12	3.4	Graft failure	8	5.9
		Respiratory Failure	6	1.7	Liver Failure	1	0.7
		Bleeding	2	0.6	Multi-organ Failure	3	2.2
		Other	4	1.1	Other	2	1.5

Abbreviations: ATG, anti-thymocyte globulin; CI, confidence interval; GRFS, GvHD-relapse-free survival; GvHD, graft-versus-host disease; Haplo, haploidentical donor; MMUD, mismatched unrelated donors; MUD, matched unrelated donor; na, not available; NRM, non-relapse mortality; NRM, non-relapse mortality; OS, overall survival; PTCy, post-transplant cyclophosphamide.

[a]Patients who died before 100 days are not included in this outcome.

3.2 | Multivariate analysis

3.2.1 | Survival

Survival at 2 years for the whole cohort (n = 483) was 62.5%.

The probability of survival at the 2-year time point, in patients who received FRESH grafts (n = 348), was 65.8% (60.4%-70.6%) compared to 53.5% (44.0%-62.1%) for patients in the CRYO group (n = 135), *P* = .04, Table 3, Figure 1A.

This was primarily due to the outcomes in the MRD cohort; the probability of survival at the 2-year time point, in patients who received FRESH grafts (n = 65), was 79.4% (67.1%-87.5%) compared to 47.6% (32.8%-61.0%) for patients in the CRYO group (n = 49), *P* <.001, Table 3, Figure 2A.

On multivariate analysis, significant factors affecting survival were patient age, HR 1.02, *P* = .005; DRI high/very high, HR 2.22, *P* < .001; Donors: Haplo HR 1.60, *P* = .02; MMUD HR 2.28, *P* < .001; and cryopreservation of the graft HR 1.49, *P* = .02, Table 4.

3.2.2 | Non-Relapse Mortality (NRM)

NRM at 1 year for FRESH (n = 348) was 17.8% (14.0-22.0) vs CRYO (n = 135) 20.0% (13.7-27.1), p not significant, Table 3. However, in the MRD cohort, NRM at 1 year in patients who received FRESH grafts (n = 65) was 1.5% (0.1%-7.4%) compared to 14.3% (6.2%-25.6%) for patients in the CRYO group (n = 49), *P* = .005, Table 3, Figure 2B.

On multivariate analysis, significant factors for NRM were patient age, HR 1.03, *P* < .001; DRI high or very-high HR 1.68, *P* = .02; Donors: Haplo HR 2.32, *P* < .001; and MMUD HR 2.67 *P* < .001, Table 4.

3.2.3 | Relapse

Cumulative incidence of relapse for graft groups at 2 years: whole cohort was 24.8%, for FRESH 23.2% (18.8-27.9) vs CRYO 28.5% (20.7-36.8), *P* = .08 Table 3.

On multivariate analysis, significant factors correlated with relapse were DRI high and very-high HR 2.90, *P* < .001 and cryopreservation HR 1.61, *P* = .02, Table 4

3.2.4 | GRFS

GvHD and relapse-free survival (GRFS) at 2 years for the whole cohort was 48.8%, for FRESH 51.4% (45.9-56.6) vs CRYO 41.2% (32.1-50.1), *P* = .04. Table 3, Figure 1B

However, in the MRD cohort, GRFS at 2 years in patients who received FRESH grafts (n = 65) was 73.8% (61.3%-82.8%) compared to 41.7% (27.5%-55.2%) for patients in the CRYO group (n = 49), *P* < .001, Table 3, Figure 2C. For other donor types studied, there was no significant difference in outcome between the two graft types.

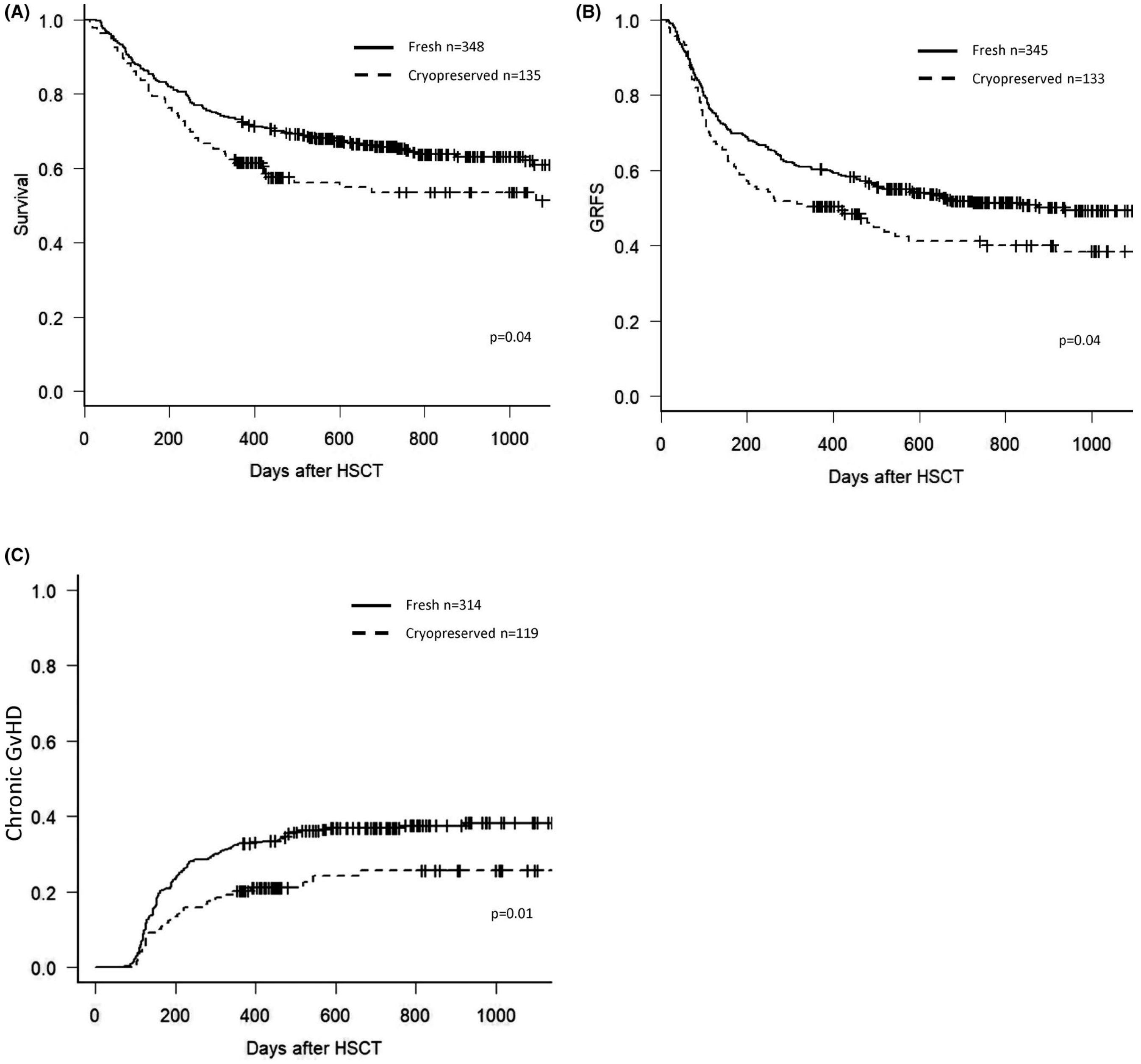

FIGURE 1 Outcomes segregated by either fresh or cryopreserved grafts for A, survival at 2 years following HSCT. B, GRFS at 2 years following HSCT, C, cumulative incidence of cGvHD at 2 years following HSCT

On multivariate analysis, significant factors correlated with worse GRFS were DRI high and very-high HR 1.99, $P < .001$; cryopreservation HR 1.47, $P < .01$; and Donors: MMUD HR 1.93, $P < .001$, Table 4

3.2.5 | Graft failure

Graft failure occurred in 6.8% of patients. Significant donor factors for GF on multivariate analysis were Haplo donor HR 2.61, $P = .03$; MMUD HR 4.26, $P = .002$; and cryopreservation of the graft HR 2.59, $P = .01$.

3.2.6 | Chronic GvHD

The CI of any cGvHD at 1 year for patients in the FRESH group (n = 314) was 32.8% (27.7-38.0) and 20.2% (13.5-27.8) in the CRYO group (n = 119), $P = .01$, Figure 1C. Patients who received fresh grafts from 10/10 MUDs (n = 180) had CI of any cGvHD at 1 year of 28.9% (22.4-35.6) compared with the CRYO group 10/10 MUDs (n = 35), CI 25.7% (12.6-41.0) $P = .62$, whereas those who received fresh grafts from MRD (n = 63) CI was 46.0% (33.3-57.8) vs CRYO MRD (n = 45) CI 15.6% (6.7-27.7), $P = .002$.

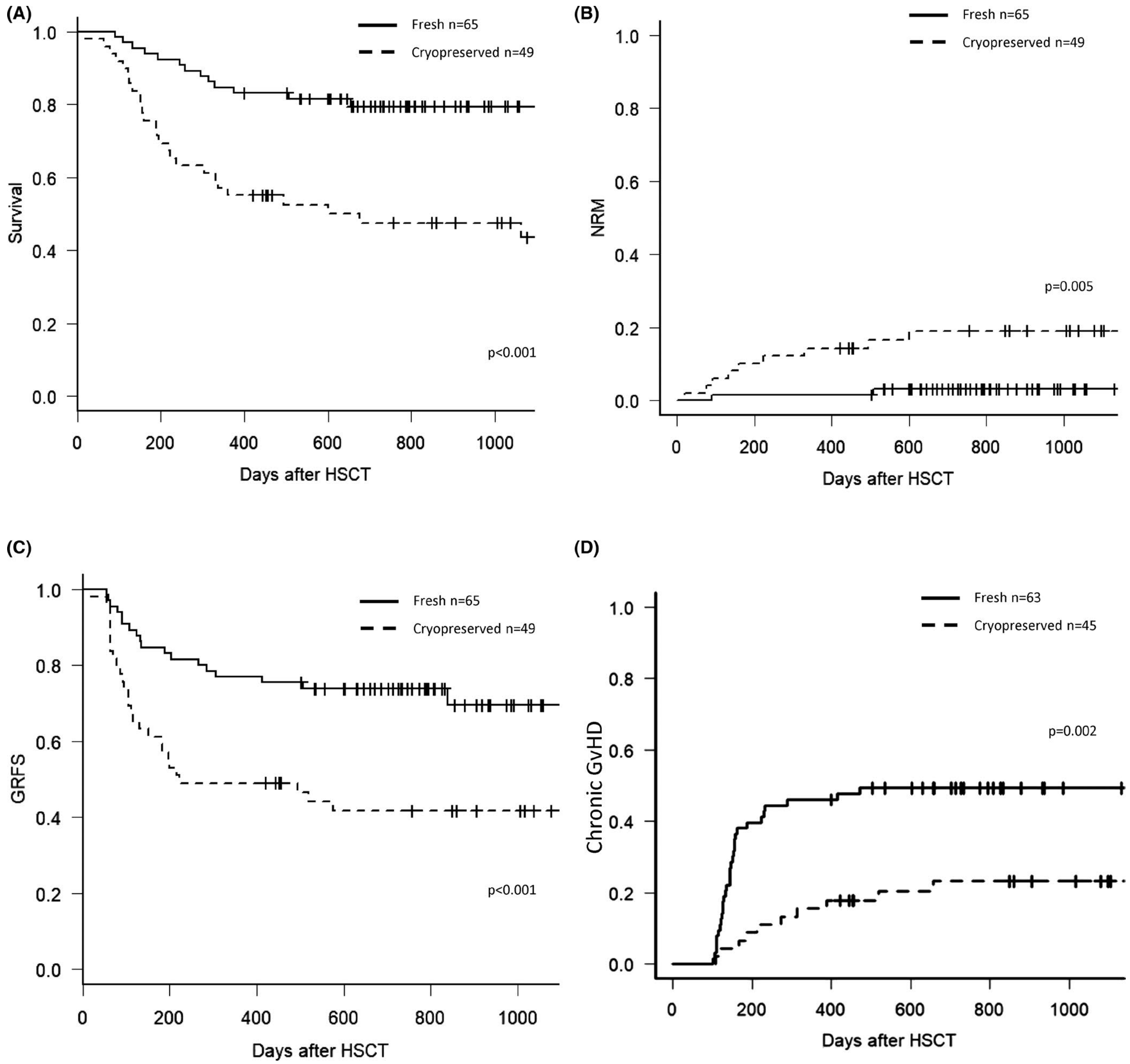

FIGURE 2 Outcomes of MRD segregated by either fresh or cryopreserved grafts for A, Survival at 2 years following HSCT. B, NRM at 1 year following HSCT. C, GRFS at 2 years following HSCT. D, cumulative incidence of cGvHD at 2 years following HSCT

The CI of moderate-severe cGvHD at 1 year for patients in the FRESH group (n = 314) was 16.6% and 10.9% in the CRYO group (n = 119), *P* = .20. Patients who received fresh grafts from 10/10 MUDs (n = 180) had CI of moderate-severe cGvHD at 1 year of 13.3% compared with the CRYO group 10/10 MUDs (n = 35) of CI 11.0%, p not significant, whereas those who received fresh grafts from MRD (n = 63) had CI 23.8% vs CRYO MRD (n = 45) CI 8.9%, *P* = .08 for MRD, Table 3.

On multivariate analysis, significant factors for chronic GvHD were increasing donor age, HR 1.01, *P* = .03; female donor to male recipient HR 1.85, *P* = .002, while graft cryopreservation protected from cGvHD, HR 0.53, *P* = .003, Table 4.

3.2.7 | GvHD prophylaxis

We undertook a subset analysis of the patients who received ATG with or without PTCy as GvHD prophylaxis, comparing patients that received grafts given fresh and those that were cryopreserved.

For the patients that received ATG-PTCy GvHD prophylaxis, the survival probability at the 2-year time point, in patients who received FRESH grafts (n = 253), was 65.5% (59.3%-71.1%) compared to 51.9% (41.8%-61.1%) for patients in the CRYO group (n = 114), *P* <.05 (Table 3).

In contrast, for the patients that received ATG without PTCy, the survival probability at the 2-year time point, in patients who

TABLE 4 Multivariable analysis

Outcome	Variable		HR	Lower 95% CI	Upper 95% CI	*P*-value
Survival	Age	(Continuous)	1.02	1.00	1.03	.005
	DRI	Low	0.49	0.18	1.33	.16
		High or very high	2.22	1.62	3.04	<.001
		Intermediate	Reference			
	HCT-CI	≥3	1.34	0.96	1.88	.08
		2	1.44	0.96	2.18	.08
		0-1	Reference			
	Donor	MRD	1.12	0.75	1.69	.57
		Haplo	1.60	1.07	2.39	.02
		MMUD	2.28	1.47	3.53	<.001
		MUD	Reference			
	Cryopreserved	No	Reference			
		Yes	1.49	1.06	2.09	.02
NRM	Age		1.03	1.01	1.05	<.001
	Donor	MRD	0.63	0.32	1.24	.18
		Haplo	2.32	1.44	3.73	<.001
		MMUD	2.67	1.53	4.64	<.001
		MUD	Reference			
	DRI	Low	0.71	0.22	2.27	.56
		High or very high	1.68	1.09	2.58	.02
		Intermediate	Reference			
Relapse	DRI	Low	0.50	0.16	1.59	.24
		High or very high	2.90	1.98	4.23	<.001
		Intermediate	Reference			
	Cryopreserved	No	Reference			
		Yes	1.61	1.09	2.38	.02
Graft failure	Donor	MRD	0.45	0.12	1.67	.23
		Haplo	2.61	1.09	6.29	.03
		MMUD	4.26	1.68	10.8	.002
		MUD	Reference			
	Cryopreserved	No	Reference			
		Yes	2.59	1.25	5.34	.01
cGvHD	Donor Age	(Continuous)	1.01	1.00	1.03	.03
	Female donor to	No	0.54	0.37	0.80	.002
	Male recipient	Yes	Reference			
	Cryopreserved	No	Reference			
		Yes	0.53	0.35	0.81	.003
GRFS	Donor	MRD	0.75	0.53	1.07	.11
		Haplo	1.32	0.94	1.85	.11
		MMUD	1.93	1.31	2.83	<.001
		MUD	Reference			
	Cryopreserved	No	Reference			
		Yes	1.47	1.10	1.97	<.01
	DRI	Low	0.99	0.54	1.85	.99
		High or very high	1.99	1.51	2.62	<.001
		Intermediate	Reference			

Note: Abbreviations: ATG, anti-thymocyte globulin; BM, bone marrow; cGvHD, chronic GvHD; CI, confidence interval; CMV, cytomegalovirus; GvHD, graft-versus-host disease; Haplo, haploidentical donor; HCT-CI, hematopoietic cell transplant comorbidity index; HLA, human leukocyte antigen; MVA, multivariate analysis, MMUD, mismatched unrelated donors; PBSC, peripheral blood stem cells; PTCy, post-transplant cyclophosphamide received FRESH grafts (n = 56) was 63.6% (49.3%-74.8%) compared to 64.7% (37.7%-82.3%) for patients in the CRYO group (n = 17), p not significant.

3.2.8 | Outcomes of cryopreserved transplants prior to and after declaration of COVID-19 pandemic

We compared the outcomes of 47 transplants undertaken with cryopreserved grafts from March 2020 to August with those (n = 88) undertaken prior to March 2020. The analysis was done at 1 year in order to take into account follow-up duration. While there was no difference in NRM, moderate-severe cGvHD and GRFS, there was a significant difference in survival (prepandemic 53.4% [42.5-63.2] vs pandemic 76.6% [61.7-86.3], *P* = .013). This is due to a significantly heterogenous cohort.

4 | DISCUSSION

In the current single-center retrospective analysis, we show that patients who received cryopreserved grafts had significantly reduced OS, worse GRFS, less cGvHD higher risk of GF, and delay in neutrophil engraftment. The poorer outcomes were due primarily to worse outcomes in the cryopreserved MRD group. The MRD cryopreserved subgroup had significantly higher NRM and lower CI of chronic GvHD, and there was a trend toward higher CIR in the cryopreserved MRD group, culminating in inferior GRFS. Patients who received MRD cryopreserved grafts had a CI of any cGvHD at 1 year of 15.6% vs 46%, *P* = .002, for those who received fresh grafts. We observed an increased likelihood of blood stream infections at day 30 post-transplant in the CRYO group (46% vs 36% for FRESH group, *P* = .05), of borderline significance. This may have contributed to the significantly higher NRM seen in the MRD CRYO group (14.3%) compared with the MRD FRESH group (1.5%, *P* = .005). We speculate this suggests that MRD patients who received cryopreserved grafts experienced significantly less alloreactivity as manifest by the trends toward higher infection and the significantly lower cGvHD incidence.

In the modern era of transplantation, alternative donors such as haploidentical have become increasingly viable given novel developments in transplant conditioning. The Johns Hopkins group pioneered the use of post-transplant cyclophosphamide (PTCy) in the Haplo donor setting, with significant improvements in the rates of GvHD, NRM, and engraftment.[15] PTCy now represents an established GvHD-prophylaxis approach, having recently been extended to HLA-matched unrelated and related HSCT with favorable results.[16]

Utilizing the benefits of PTCy in both reducing risks from acute and chronic GvHD, and ATG (both followed by cyclosporin), our group has developed the use of the dual T-cell depletion strategy ATG-PTCy as GvHD prophylaxis for related and unrelated donors[16-19] with reduced acute GvHD[20] and acceptable levels of cytokine release syndrome,[21] such that it is now our standard GvHD prophylaxis.

Regarding effects of cryopreservation on transplant outcomes, we previously reported our single-center experience including 958 patients using PBSC for various hematological malignancies, of which 349 (36.4%) received ATG-PTCy, where fresh grafts were received by 648 (68%) patients and 310 (32%) received cryopreserved.[7] We found that moderate/severe chronic GvHD was observed in 176 (27%, fresh grafts) vs 123 (40%, cryopreserved grafts) patients, respectively (*P* <.001). Of note, for patients with no or mild chronic GvHD, CIR was less for fresh compared with cryopreserved grafts (HR=0.67 for fresh [0.48-0.92] *P* = .01). Overall, survival and engraftment data were not significantly different, and MRD was not associated with differential outcomes.

The current work complements and supports these findings. A major difference between our prior publication[7] and the current study is that there has been a significant change in GvHD prophylaxis, with the ATG-PTCy protocol adopted as primary GvHD prophylaxis in our center. Over the longer time period of the previous study,[7] there were also other changes in transplant conditioning that may have led to increased variability in outcomes not detectable in that sample. The present study comprises transplants undertaken over a shorter time period (3 years) and also overlapped with the COVID-19 pandemic, which necessitated a rapid change in stem cell graft manipulation. ATG-PTCy has served well in reducing GvHD both acute and chronic, and has worked well across all donor types. The subgroup analysis with other GvHD prophylaxis did not yield a significant difference in outcomes; the non-ATG-PTCy subgroup showed wide variation in outcome and may have been too small for meaningful statistical results.

There is an extensive literature describing optimization of cryopreservation methods, which were primarily established in the autologous transplant setting.[22-24] There are few studies comparing uncontrolled and controlled rate freezing, although both methods are considered adequate for cryopreservation of hematopoietic stem cells for subsequent transplantation.[25-27] In the autologous setting, one prospective study showed an overall equivalence of both methods, although there was borderline statistically increased CFU-GM recovery in the controlled rate freezing group.[28] Notably, compared with our previous publication[7] which spanned over a longer time period and included more diverse conditioning regimens and GvHD prophylaxis, we have continued to use the same cryopreservation method (dump or uncontrolled-rate freezing); the main difference with the present study is the primary use of ATG-PTCy as GvHD prophylaxis. Thus, we conclude that cryopreservation and ATG-PTCy GvHD prophylaxis may interact depending on donor type to give the outcomes presented in this study.

The findings of the trend toward increased likelihood of relapse and significantly reduced chronic GvHD (Figure 1D) in the MRD CRYO subgroup is supported by previous work examining showing statistically significant differences for $CD34^+$ cell recovery (93.0 ± 20.7%) when compared to $CD3^+CD4^+$ cell (83.1 ± 15.4%, *P* = .014) and $CD3^+CD8^+$ cell recovery (83.3 ± 13.9%, *P* = .001). Similarly, $CD19^+$

cell recovery (98.6 ± 15.1%) was higher than $CD3^+CD4^+$ cell ($P = 2.5 \times 10^{-7}$) and $CD3^+CD8^+$ cell recovery ($P = 1.2 \times 10^{-8}$). Post-thaw recovery rates of all cell populations were not impaired in G-CSF–mobilized products compared with non-mobilized products nor in unrelated compared with related donor products. This work suggested a lower tolerance of $CD3^+$ cells for cryopreservation and demonstrated that freezing-thawing resistance is cell-specific and independent from other factors that affect the post-thaw recovery of cryopreserved cells.[8]

There may be clinical correlates to these findings, whereby a loss of T cells impacts the development of alloreactivity. A CIBMTR analysis compared outcomes between 1080 patients who received fresh grafts and 274 who received cryopreserved grafts. There were lower rates of chronic GvHD and DFS with graft cryopreservation that were of marginal statistical significance, but overall graft cryopreservation did not significantly delay hematopoietic recovery, increase the risk of acute GvHD or NRM, or decrease OS after allo-HCT using PTCy.[29] In contrast, a study undertaken during the COVID-19 pandemic showed higher incidence of GF and higher mortality in patients with aplastic anemia who received cryopreserved grafts,[30] which is of notable interest given the interplay in aplastic anemia between host and donor immunity. Murine mechanistic and clinical HSCT studies have shown a role for regulatory T-lymphocytes (T-regs) in the suppression of GvHD without impact on the graft-versus-leukemia effect.[31] Cryopreservation may have a significant impact on T-reg numbers and their functional activity, with implications for immune regulation in the post-HSCT period.[32] Complicating the dissection of the role of T-regs in explaining the observed phenomena, relative Treg resistance to PTCy compared with other T-cell subsets may contribute to the clinical activity of PTCy in preventing GvHD.[33] Regarding the outcomes of the MRD CRYO group in our study, we speculate that while ATG-PTCy serves to reduce the naïve T-cell burden and promote immune regulation by selective preservation of T-regs, the additional step of cryopreservation may lead to excessive T-cell killing, manifesting the results observed in this subgroup.

The limitation of the present study is the non-randomized, retrospective nature. The small numbers of patients limited the power of the analysis, as did the relatively short follow-up. Additionally, a comparison of data on the tempo of immune reconstitution in both groups is of interest and will be considered in future studies.

Comparison of the outcomes of 47 transplants undertaken with cryopreserved grafts from March 2020 to August with those (n = 88) undertaken prior to March 2020 showed a significant difference in survival, but the two groups were significantly heterogenous and this may account for the findings.

In summary, we report that the combination of cryopreservation and ATG-PTCy GvHD prophylaxis is associated with inferior outcomes, likely due to over immunosuppression as a result of reduced T-cell viability post-thaw.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

ETHICS APPROVAL

The research ethics board at the University Health Network approved this study.

DATA AVAILABILITY STATEMENT

Data are available on request.

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REFERENCES

1. Dholaria B, Savani BN. How do we plan hematopoietic cell transplant and cellular therapy with the looming COVID-19 threat? *Br J Haematol*. 2020;189(2):239-240. https://doi.org/10.1111/bjh.16597
2. Eckardt JR, Roodman GD, Boldt DH, et al. Comparison of engraftment and acute GVHD in patients undergoing cryopreserved or fresh allogeneic BMT. *Bone Marrow Transplant*. 1993;11(2):125-131.
3. Stockschlader M, Hassan HT, Krog C, et al. Long-term follow-up of leukaemia patients after related cryopreserved allogeneic bone marrow transplantation. *Br J Haematol*. 1997;96(2):382-386. https://doi.org/10.1046/j.1365-2141.1997.d01-2032.x
4. Medd P, Nagra S, Hollyman D, Craddock C, Malladi R. Cryopreservation of allogeneic PBSC from related and unrelated donors is associated with delayed platelet engraftment but has no impact on survival. *Bone Marrow Transplant*. 2013;48(2):243-248. https://doi.org/10.1038/bmt.2012.118
5. Kim DH, Jamal N, Saragosa R, et al. Similar outcomes of cryopreserved allogeneic peripheral stem cell transplants (PBSCT) compared to fresh allografts. *Biol Blood Marrow Transplant*. 2007;13(10):1233-1243. https://doi.org/10.1016/j.bbmt.2007.07.003
6. Parody R, Caballero D, Marquez-Malaver FJ, et al. To freeze or not to freeze peripheral blood stem cells prior to allogeneic transplantation from matched related donors. *Eur J Haematol*. 2013;91(5):448-455. https://doi.org/10.1111/ejh.12140
7. Alotaibi AS, Prem S, Chen S, et al. Fresh vs. frozen allogeneic peripheral blood stem cell grafts: a successful timely option. *Am J Hematol*. 2020;96:179-187. https://doi.org/10.1002/ajh.26033
8. Berens C, Heine A, Müller J, et al. Variable resistance to freezing and thawing of CD34-positive stem cells and lymphocyte subpopulations in leukapheresis products. *Cytotherapy*. 2016;18(10):1325-1331. https://doi.org/10.1016/j.jcyt.2016.06.014
9. Armand P, Kim HT, Logan BR, et al. Validation and refinement of the Disease Risk Index for allogeneic stem cell transplantation. *Blood*. 2014;123(23):3664-3671. https://doi.org/10.1182/blood-2014-01-552984
10. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood*. 2005;106(8):2912-2919. https://doi.org/10.1182/blood-2005-05-2004
11. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995;15(6):825-828.
12. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow*

Transplant 2015;21(3):389-401 e381. https://doi.org/10.1016/j.bbmt.2014.12.001
13. Valcarcel D, Sureda A. Graft failure. In: Carreras E, Dufour C, Mohty M, Kroger N, eds. *The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies*. Springer; 2019:307-313.
14. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant*. 2013;48(3):452-458. https://doi.org/10.1038/bmt.2012.244
15. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using non-myeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14(6):641-650. https://doi.org/10.1016/j.bbmt.2008.03.005
16. Salas MQ, Prem S, Atenafu EG, et al. Reduced intensity allogeneic stem cell transplant with anti-thymocyte globulin and post-transplant cyclophosphamide in acute myeloid leukemia. *Eur J Haematol*. 2019;103(5):510-518. https://doi.org/10.1111/ejh.13321
17. Salas MQ, Prem S, Atenafu EG, et al. Dual T-cell depletion with ATG and PTCy for peripheral blood reduced intensity conditioning allo-HSCT results in very low rates of GVHD. *Bone Marrow Transplant*. 2020;55(9):1773-1783. https://doi.org/10.1038/s41409-020-0813-9
18. Alanazi W, Chen S, Lipton JH, et al. Post-transplant cyclophosphamide combined with anti-thymocyte globulin as graft-versus-host disease prophylaxis for allogeneic hematopoietic cell transplantation in high-risk acute myeloid leukemia and myelodysplastic syndrome. *Acta Haematol*. 2020;144(1):66-73. https://doi.org/10.1159/000507536
19. Pasic I, Lipton JH, Kim DD, et al. Post-transplant cyclophosphamide combined with anti-thymocyte globulin for graft-vs-host disease prophylaxis improves survival and lowers non-relapse mortality in older patients undergoing allogeneic hematopoietic cell transplantation. *Ann Hematol*. 2020;99(6):1377-1387. https://doi.org/10.1007/s00277-020-04033-2
20. Deotare U, Atenafu EG, Loach D, et al. Reduction of severe acute graft-versus-host disease using a combination of pre transplant anti-thymocyte globulin and post-transplant cyclophosphamide in matched unrelated donor transplantation. *Bone Marrow Transplant*. 2018;53(3):361-365. https://doi.org/10.1038/s41409-017-0053-9
21. Salas MQ, Lam W, Al-Shaibani Z, Viswabandya A, Law AD. Dual T cell depletion with anti-thymocyte globulin and post-transplant cyclophosphamide results in low rates of cytokine release syndrome in peripheral blood haplo-hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2019;25(12):e387-e388. https://doi.org/10.1016/j.bbmt.2019.09.013
22. Douay L, Gorin NC, Mary JY, et al. Recovery of CFU-GM from cryopreserved marrow and in vivo evaluation after autologous bone marrow transplantation are predictive of engraftment. *Exp Hematol*. 1986;14(5):358-365.
23. Douay L, Lopez M, Gorin NC. A technical bias: differences in cooling rates prevent ampoules from being a reliable index of stem cell cryopreservation in large volumes. *Cryobiology*. 1986;23(4):296-301. https://doi.org/10.1016/0011-2240(86)90035-0
24. Gorin NC. Collection, manipulation and freezing of haemopoietic stem cells. *Clin Haematol*. 1986;15(1):19-48. https://doi.org/10.1016/s0308-2261(86)80004-2
25. Berz D, McCormack EM, Winer ES, Colvin GA, Quesenberry PJ. Cryopreservation of hematopoietic stem cells. *Am J Hematol*. 2007;82(6):463-472. https://doi.org/10.1002/ajh.20707
26. Takaue Y, Abe T, Kawano Y, et al. Comparative analysis of engraftment after cryopreservation of peripheral blood stem cell autografts by controlled- versus uncontrolled-rate methods. *Bone Marrow Transplant*. 1994;13(6):801-804.
27. Halle P, Tournilhac O, Knopinska-Posluszny W, et al. Uncontrolled-rate freezing and storage at -80 degrees C, with only 3.5-percent DMSO in cryoprotective solution for 109 autologous peripheral blood progenitor cell transplantations. *Transfusion*. 2001;41(5):667-673. https://doi.org/10.1046/j.1537-2995.2001.41050667.x
28. Perez-Oteyza J, Bornstein R, Corral M, et al. Controlled-rate versus uncontrolled-rate cryopreservation of peripheral blood progenitor cells: a prospective multicenter study. Group for Cryobiology and Biology of Bone Marrow Transplantation (CBTMO), Spain. *Haematologica* 1998;83(11):1001-1005.
29. Hamadani M, Zhang MJ, Tang XY, et al. Graft cryopreservation does not impact overall survival after allogeneic hematopoietic cell transplantation using post-transplantation cyclophosphamide for graft-versus-host disease prophylaxis. *Biol Blood Marrow Transplant*. 2020;26(7):1312-1317. https://doi.org/10.1016/j.bbmt.2020.04.001
30. Eapen M, Zhang MJ, Tang XY, et al. Hematopoietic cell transplantation with cryopreserved grafts for severe aplastic anemia. *Biol Blood Marrow Transplant*. 2020;26(7):e161-e166. https://doi.org/10.1016/j.bbmt.2020.04.027
31. Lussana F, Di Ianni M, Rambaldi A. Tregs: hype or hope for allogeneic hematopoietic stem cell transplantation? *Bone Marrow Transplant*. 2017;52(9):1225-1232. https://doi.org/10.1038/bmt.2017.30
32. Golab K, Leveson-Gower D, Wang XJ, et al. Challenges in cryopreservation of regulatory T cells (Tregs) for clinical therapeutic applications. *Int Immunopharmacol*. 2013;16(3):371-375. https://doi.org/10.1016/j.intimp.2013.02.001
33. Kanakry CG, Ganguly S, Zahurak M, et al. Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide. *Sci Transl Med*. 2013;5(211):211ra157. https://doi.org/10.1126/scitranslmed.3006960

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