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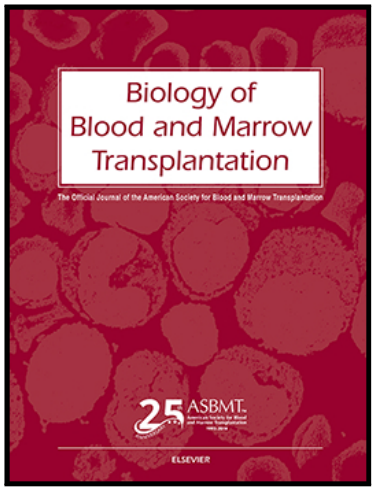

Graft cryopreservation does not impact overall survival allogeneic hematopoietic cell transplantation using post-transplant cyclophosphamide for GVHD prophylaxis

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PII: S1083-8791(20)30209-3
DOI: https://doi.org/10.1016/j.bbmt.2020.04.001
Reference: YBBMT 55977

To appear in: *Biology of Blood and Marrow Transplantation*

Received date: 31 March 2020
Accepted date: 2 April 2020

Please cite this article as: Mehdi Hamadani , Mei-Jie Zhang , Xiao-Ying Tang , Mingwei Fei , Claudio Brunstein , Saurabh Chhabra , Anita D'Souza , Filippo Milano , Rachel Phelan , Wael Saber , Bronwen E. Shaw , Daniel Weisdorf , Steven M. Devine , Mary M. Horowitz , Graft cryopreservation does not impact overall survival allogeneic hematopoietic cell transplantation using post-transplant cyclophosphamide for GVHD prophylaxis, *Biology of Blood and Marrow Transplantation* (2020), doi: https://doi.org/10.1016/j.bbmt.2020.04.001

Highlights

- In ptCY-based allo-HCT, graft cryopreservation was not associated with significantly higher mortality
- Cryopreserved grafts were not associated with significantly delayed hematopoietic recovery or higher acute GVHD risk.
- Cryopreserved grafts were associated with lower chronic GVHD risk and inferior DFS rate, but these differences were of only borderline statistical significance.

Graft cryopreservation does not impact overall survival allogeneic hematopoietic cell transplantation using post-transplant cyclophosphamide for GVHD prophylaxis.

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Abstract word count = 252, Text word count = 2611, Number of figures = 1, Number of tables = 3, Number of references = 28

Running head: Cryopreservation and ptCY

Keywords: Cryopreservation, ptCY, allogeneic hematopoietic cell transplant, COVID-19.

ABSTRACT

INTRODUCTION

The COVID-19 pandemic has created significant barriers to timely donor evaluation, cell collection and graft transport for allogeneic hematopoietic stem cell transplantation (allo-HCT). To ensure availability of donor cells on the scheduled date of infusion, many sites now collect cryopreserve grafts before the start of pretransplant conditioning. Post-transplant cyclophosphamide (ptCY), is an increasingly used approach for graft-versus-host disease (GVHD) prophylaxis but the impact of graft cryopreservation on the outcomes of allo-HCT using ptCY is not known. Using the Center for International Blood and Marrow Transplant Research (CIBMTR) database, we compared the outcomes of HCT using cryopreserved versus fresh grafts in patients undergoing HCT for hematologic malignancy with ptCY.

METHODS

We analyzed 274 patients with hematologic malignancy undergoing allo-HCT from 2013-2018 with cryopreserved grafts and ptCY. Eighteen received bone marrow and 256, peripheral blood grafts. These were matched for age, graft type, disease risk index (DRI) and propensity score to 1,080 patients that underwent allo-HCT with a fresh graft. The propensity score, which is an assessment of the likelihood of receiving a fresh versus cryopreserved graft, was calculated logistic regression to account for the following: disease histology, Karnofsky Performance Score (KPS), HCT-comorbidity index, conditioning regimen intensity, donor type and recipient race. The primary endpoint was overall survival (OS). Secondary endpoints included acute and chronic graft-versus-host disease GVHD, non-relapse mortality (NRM), relapse/progression and disease-free survival (DFS). Because of multiple comparisons, only p-values <0.01 were considered statistically significant.

RESULTS

The two cohorts (cryopreserved versus fresh) were similar in patient age, KPS, diagnosis, DRI, HCT-comorbidity index, donor/graft source, and conditioning intensity. One-year probabilities of OS were 71.1% (95% confidence interval, 68.3-73.8%) with fresh grafts and 70.3% (64.6-75.7%) with cryopreserved grafts (p=0.81). Corresponding probabilities of OS survival at two years were 60.6% (57.3-63.8%) and 58.7% (51.9-65.4%) (p=0.62). In matched pair regression analysis, graft cryopreservation was not associated with a significantly higher risk of mortality (Hazard Ratio for cryopreserved versus fresh [HR] =1.05, 95% confidence interval, 0.86-1.29, p=0.60). Similarly, rates neutrophil recovery (HR=0.91, 0.80-1.02, p=0.12), platelet recovery (HR=0.88, 0.78-1.00, p=0.05), grade 3-4 acute GVHD (HR=0.78, 0.50-1.22, p=0.27), NRM (HR=1.16, 0.86-1.55, p=0.32) and relapse/progression (HR=1.21, 0.97-1.50, p=0.09) were similar with cryopreserved versus fresh grafts. There were somewhat lower rates chronic GVHD (HR=0.78, 0.61-0.99, p=0.04) and DFS (HR for treatment failure=1.19, 95%CI=1.01-1.29, p=0.04) with graft cryopreservation that were of marginal statistical significance after adjusting for multiple comparisons.

CONCLUSIONS

Graft cryopreservation does not significantly delay hematopoietic recovery, increase acute GVHD risk or NRM, or decrease overall survival after all-HCT using ptCY.

INTRODUCTION

Donor hematopoietic stem and progenitor cells for allogeneic hematopoietic cell transplantation (allo-HCT) are generally collected and infused fresh (i.e. without cryopreservation).[1] Limited data in patients undergoing HLA-matched related donor (MRD) allo-HCT using bone marrow (BM) as the graft source suggest that cryopreservation of the harvested marrow product does not impact hematopoietic recovery or risk of graft-versus-host disease (GVHD).[2-5] Among recipients of peripheral blood (PB) allografts, some[6] but not all studies[7] report delayed platelet recovery with cryopreserved grafts, but these studies show no impact of cryopreservation of PB allografts on neutrophil recovery, GVHD or survival outcomes.

The emergence of coronavirus disease 2019 (COVID-19) in Wuhan, China in December 2019[8] and its rapid evolution into a pandemic caused not only a serious healthcare crisis, but also impacted the world economy and disrupted travel across international borders and within countries. These travel restrictions combined with potentially reduced HCT donor availability (due to infection, quarantine and constraints on travel to collection centers) and complex allograft processing logistics (donor assessment, collection, on-schedule delivery for fresh infusion) directly impact a transplant center's ability to infuse fresh donor-cells into intended recipients on the scheduled day of transplantation. Recognizing these challenges, both the American Society for Transplantation and Cellular Therapy (ASTCT)[9] and the National Marrow Donor Program (NMDP)[10] initially issued strong recommendations that all unrelated donor (URD) products should be delivered and cryopreserved at transplant centers prior to initiation of patient conditioning. On March 23, 2020 the NMDP informed transplant centers that starting March 30, 2020, cryopreservation of URD grafts would be required prior to initiating conditioning on transplant recipients.[10] Many transplant centers have instituted similar practices for HCT using cells from related donors.

While published studies (all with limited patient numbers) suggest no significant impact of graft cryopreservation on outcomes of allo-HCT using conventional GVHD prophylaxis

platforms (e.g. calcineurin inhibitor based), no data are available on whether this strategy is feasible for HCT using post-transplant cyclophosphamide (ptCY)-based GVHD prophylaxis. Using the CIBMTR database we evaluated the outcomes of hematologic malignancy patients undergoing ptCY-based allo-HCT for hematologic malignancies with either fresh or cryopreserved grafts, to inform clinical practice during the ongoing COVID-19 pandemic.

METHODS

Data sources

The CIBMTR® is a working group of more than 380 transplantation centers worldwide that contribute detailed data on HCT to a central coordinating center managed by the NMDP and the Medical College of Wisconsin (MCW). Participating centers are required to report all transplantations consecutively and compliance is monitored by on-site audits. Computerized checks for discrepancies, physicians' review of submitted data, and on-site audits of participating centers ensure data quality. Observational studies conducted by the CIBMTR are performed in compliance with all applicable federal regulations pertaining to the protection of human research participants. The NMDP, Institutional Review Board (IRB), which is the IRB of record for the CIBMTR's database protocols, approved this study.

The CIBMTR collects data at two levels: Transplant Essential Data (TED) and Comprehensive Report Form (CRF) data. TED-data include disease type, age, gender, pre-HCT disease stage and chemotherapy-responsiveness, date of diagnosis, graft type, conditioning regimen, post-transplant disease progression and survival, development of a new malignancy, and cause of death. All CIBMTR centers contribute TED-data. More detailed disease and pre- and post-transplant clinical information is collected on a subset of registered patients selected for CRF data by a weighted randomization scheme. TED- and CRF-level data are collected pre-transplant, 100-days, and six months post-HCT and annually thereafter or until death. Data for the current analysis were retrieved from CIBMTR (TED and CRF) report forms,

considering all patients for whom a CRF 2006 form (which collects details of graft manipulation and composition) was submitted.

Patients

Included in this analysis are adults (≥18 years) undergoing an allo-HCT from 2013 through 2018 for hematologic malignancies with ptCY (+/- calcineurin inhibitor and/or mycophenolate mofetil) as the GVHD prophylaxis. Diagnosis was limited to acute leukemias in first or second complete remission (CR1/CR2), chronic leukemias or myelodysplastic syndrome (with <5% blasts at HCT) and lymphomas. Donors included MRD, haploidentical related donors, matched URD or mismatched URD. Umbilical cord blood grafts, due to universal cryopreservation were not included.

Definitions and Study Endpoints

The primary endpoint was overall survival (OS); death from any cause was considered an event and surviving patients were censored at last contact. Secondary endpoints included hematopoietic recovery, acute and chronic GVHD, non-relapse mortality (NRM), progression/relapse and disease-free survival (DFS). NRM was defined as death without evidence of disease relapse/progression; relapse was considered a competing risk. Relapse/progression was defined as morphologic, cytogenetic, or molecular disease recurrence for leukemias and myeloid malignancies, and as progressive lymphoma after HCT or lymphoma recurrence after a CR; NRM was considered a competing risk. For DFS, a patient was considered a treatment failure at the time of relapse/progression or death from any cause. Patients alive without evidence of disease relapse or progression were censored at last follow-up.

Neutrophil recovery was defined as the first of 3 successive days with absolute neutrophil count (ANC) ≥500/μL after post-transplantation nadir. Platelet recovery was considered to have occurred on the first of three consecutive days with platelet count 20,000/μL

or higher, in the absence of platelet transfusion for 7 consecutive days. For neutrophil and platelet recovery, death without the event was considered a competing risk. The intensity of allo-HCT conditioning regimens was categorized as myeloablative (MAC) or reduced-intensity/non-myeloablative conditioning (RIC/NMA) using consensus criteria.[11] Disease risk index (DRI) was assigned as previously reported.[12] Acute GVHD[13] and chronic GVHD[14] were graded using standard criteria. For calculation of acute and chronic GVHD incidences, death without the event was considered a competing risk.

Statistical analysis

Two hundred seventy-seven patients were identified who met the eligibility criteria described above and who received cryopreserved grafts and 4,083 patients who met eligibility criteria and received fresh graft infusion. A mixed method of direct matching and propensity score matching was applied prior to analyses to obtain a control groups with similar clinical characteristics. The propensity score is the probability of a given patient to receive the cryopreserved graft, based on the observed covariates of the patient. The propensity score was predicted for each patient using logistic regression accounting for following risk factors: disease histology (acute myeloid leukemia vs. acute lymphocytic leukemia vs. chronic myeloid leukemia vs. chronic lymphocytic leukemia vs. myelodysplastic syndrome vs. non-Hodgkin lymphoma vs. Hodgkin lymphoma), Karnofsky Performance Score (KPS) (≥90 vs. <90%), HCT-comorbidity index (0 vs. 1-2 vs. ≥3), conditioning intensity (MAC vs. RIC/NMA), donor type (MRD vs. haploidentical related donor vs. 8/8 matched URD vs. ≤7/8 URD) and recipient race. Two patients with equal propensity scores meant they had similar probabilities of receiving a cryopreserved graft. The distributions of estimated propensity scores between cryopreserved and fresh grafts were examined. We then matched each recipient of a cryopreserved graft with controls receiving fresh grafts, by matching on four covariates including graft type (BM vs. PB), DRI (low risk vs. intermediate risk vs. high risk), recipient age (within 5-years) and propensity

score (within 1 standard deviation from pooled sample). The following procedure was adopted to find a maximum of four fresh graft controls for each cryopreserved graft cases:

1. Identify all potential matched controls for each case.
2. Assign the control with the smallest age difference with the case. If there were multiple controls with the same age difference, assign one at random.
3. Repeating steps 1 and 2 four times, to identify 4 controls.

As a result, we matched a total of 1,080 controls to 274 cases, including 266 cases matched to 4 controls, 3 matched to 3 controls, 2 matched to 2 controls, and 3 matched to a single control. Three cases with no matched controls were excluded.

Patient-, disease- and transplant-related factors were compared between matched cases and controls using the Chi-square test for categorical and Mann-Whitney test for continuous variables. The Kaplan-Meier estimator was used to evaluate the probability of OS and DFS.[15] Cumulative incidence rates were calculated for hematopoietic recovery, GVHD, NRM and relapse, while accounting for competing events.[16] The marginal Cox model was applied to evaluate the main treatment effect, while adjusting for the potential correlation within each matched pair. The assumption of proportional hazards for the main risk factor (cryopreserved graft vs. fresh graft) for each outcome was tested by adding a time-dependent covariate. Hazard ratios (HR) (95%CI) and p-values were reported for each clinical outcomes of interest comparing the cryopreserved graft treatment group with the fresh graft group. Because of the large number of comparisons performed, only **p-values < 0.01** were considered statistically significant *a priori*. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Baseline Characteristics

A total of 1,354 patients were included in the analysis, of whom 1,080 received fresh grafts and 274 patients received cryopreserved grafts. The baseline patient-, disease- and

transplantation-related characteristics are shown in **Table 1**. The two cohorts were similar in median patient age, gender, race, KPS, diagnosis, DRI, HCT-comorbidity index, donor/graft source, conditioning intensity and donor-recipient CMV serostatus. Acute leukemias constituted the most common diagnosis, and haploidentical related donors were the most common donor-source across both cohorts. BM was the graft source in only ~6% of procedures. Median follow-up of survivors was 24 months (range: 3-77 months) in the fresh graft cohort and 23 months (range: 3-68 months) in the cryopreserved graft cohort.

Overall Survival

Two-year OS rates were 60.6% (95%CI=57.3-63.8%) and 58.7% (95%CI=51.9-65.4%) with fresh and cryopreserved grafts, respectively, p=0.62 (**Figure 1a, Table 2**). In matched pair regression analysis, graft cryopreservation was not associated with a significantly higher risk of mortality (HR for cryopreserved vs. fresh=1.05, 95%CI=0.86-1.29%, p=0.60) (**Table 3**).

Hematopoietic recovery and GVHD

The day 28 cumulative incidences of neutrophil recovery were 93.8% (95%CI=92.3-95.1%) and 93.3% (95%CI=90-96%) with fresh and cryopreserved grafts, respectively (p=0.80; **Table 2**). The corresponding median times to neutrophil recovery were 16 days (range: 5-69 days) and 17 days (range: 8-48 days; p=0.05). The day 100 cumulative incidences of platelet recovery were 88.8% (95%CI=86.8-90.6%) and 87.7% (95%CI=83.4-91.4%) with fresh and cryopreserved grafts, respectively (p=0.62; **Table 2**). The corresponding median times to platelet recovery were 24 days (range: 1-321 days) and 26 days (range: 7-351 days; p=0.007). In matched pair regression analysis, graft cryopreservation was not associated with significantly delayed neutrophil (HR=0.91, 95%CI=0.80-1.02%, p=0.12) or platelet recovery (HR=0.88, 95%CI=0.78-1.00%, p=0.05; **Table 3**).

The cumulative incidences of grade 2-4 acute GVHD at day 100 (**Table 2**) were 31.3% (95%CI=28.5-34.1%) and 34% (95%CI=28.5-39.8%) with fresh and cryopreserved grafts, respectively (p=0.40). Corresponding rates of grades 3-4 acute GVHD were 9.4% (95%CI=7.7-11.3%) and 6.3% (95%CI=3.7-9.5%), respectively (p=0.07). In matched pair regression analysis (**Table 3**), the two cohorts had similar risks of grade 2-4 (HR=1.10, 95%CI=0.87-1.38%, p=0.43) and grade 3-4 acute GVHD (HR=0.78, 95%CI=0.50-1.22%, p=0.27). The cumulative incidences of chronic GVHD at 1-year (**Figure 1b, Table 2**) were 30.7% (95%CI=27.9-33.5%) and 26.8% (95%CI=21.5-32.5%) with fresh and cryopreserved grafts, respectively (p=0.22). In matched pair regression analysis (**Table 3**), cryopreserved grafts were associated with a lower risk of chronic GVHD (HR=0.78, 95%CI=0.61-0.99%) but this was of only borderline statistical significance (p=0.04).

NRM, relapse/progression and DFS

The 2-year rates of NRM were 19.0% (95%CI=16.5-21.5%) and 22.0% (95%CI=16.8-27.7%) with fresh and cryopreserved grafts, respectively (p=0.32). Corresponding rates of relapse/progression were 30.7% (95%CI=27.7-33.7%) and 36.3% (95%CI=29.9-42.9%) (p=0.13) and corresponding rates of DFS were 50.4% (95%CI=47-53.7%) and 41.7% (95%CI=35-48.6%) (p=0.03) (**Table 2**). In matched pair regression analysis (**Table 3**), the HRs for NRM (HR=1.16, 95%CI=0.86-1.55%, p=0.32) and relapse/progression (HR=1.21, 95%CI=0.97-1.50%, p=0.09) were not statistically significant while the HR for treatment failure (inverse of DFS) was of borderline significance (HR=1.19, 95%CI=1.01-1.29, p=0.04).

DISCUSSION

Prospective, randomized data comparing outcomes of ptCY-based allo-HCT using fresh versus cryopreserved grafts are not available. Using the CIBMTR database, we evaluated both approaches retrospectively, using available data to adjust for known covariates. The most

important finding our analysis is that, OS out to two years was virtually identical with fresh and cryopreserved grafts. Second, there was no evidence of significantly delayed hematopoietic recovery or higher risks of either acute or chronic GVHD with cryopreservation. Marginal increases in relapse/progression and marginal decreases in chronic GVHD and DFS are of uncertain significance given the multiple comparisons in the study and the fact that this was not a randomized study. In fact, a key piece of information was unavailable to us, which is the reason that these grafts were cryopreserved. One can reasonably assume that this was not a random decision. While some delays might be precipitated by donor scheduling issues, many were likely influenced by clinical events requiring delay in the transplant and that these events themselves might be indicators of prognosis (e.g. delay because of need for chemotherapy to achieve better pretransplant disease control). Given this background, the very similar survival outcomes are particularly reassuring.

A rapidly growing body of literature shows good outcomes of ptCY-based allo-HCT in patients with both myeloid[17-20] and lymphoid malignancies,[21-25] validating the seminal observations from the Johns Hopkins group.[20] Administration of ptCY potentially mitigates the risk of GVHD by targeting alloreactive T-cells rapidly proliferating early after stem cell infusion, and by relatively sparing regulatory T-cells and leaving unaffected the non-dividing hematopoietic stem and progenitor cells.[20] Whether the proliferation kinetics of thawed alloreactive T-cells are different from fresh cells is not known. Murine data suggest that freeze and thaw of regulatory T-cells results in loss of CD62L expression and a reduced capacity to protect against GVHD.[26] In addition, limited data indicate that cryopreservation can increase the sensitivity of porcine PB mononuclear cells (stimulated by phorbol myristate acetate) for IFN-gamma production, but not for interleukin-6 production.[27] Despite these preclinical observations, our analysis did not show any clinically relevant differences in hematopoietic recovery kinetics, acute GVHD risk or OS between fresh vs. cryopreserved grafts for patient undergoing ptCY-based allo-HCT. Limited data in allo-HCT (with non ptCY-based GVHD prophylaxis) using either BM[2-5] or PB[6,7] as graft source, also show no impact of cryopreservation on hematopoietic

recovery, GVHD or survival outcomes. Though of uncertain clinical significance, the observations of lower chronic GVHD and DFS warrants further investigation, especially probing the impact on freeze-thaw cycle on functional profile of immune effector cells. However, in the ongoing COVID-19 pandemic, necessitating cryopreservation of all URD grafts and the majority of related donor products, our data do not show a net safety signal against using ptCY-based platforms with frozen products. In this ongoing global outbreak, the ability to cryopreserve allografts has obvious logistical advantages; the most important being the ability to secure a graft before myeloablative therapy in a transplant recipient. Even under normal circumstances, it is sometimes advantageous to ensure availability of an optimal stem cell dose before the start of conditioning (e.g., in the setting of major donor/recipient weight discrepancy and/or advanced donor age).

Our current analysis, also underscore the value of observational transplant registries like CIBMTR, that can be quickly leveraged to examine critical clinical questions to inform practice and improve patient care, even in unexpected emergencies. We must acknowledge the limitations of our analysis. Despite propensity score matching, our analysis cannot adjust for unknown clinical parameters influencing the decision to use cryopreservation. We cannot assess the impact of cryopreservation on graft viability (compared to a fresh graft) or examine functional characteristics of thawed immune effector cells. We also acknowledge that chronic GVHD rates across both cohorts in the current analysis are higher than those originally reported with ptCY,[20] likely a reflection of increased use of PB grafts in clinical practice.[28] Bone marrow grafts were underrepresented in our study, and thus we suggest caution in interpreting these results.

In conclusion, our analysis provides evidence that for patients undergoing ptCY-based allo-HCT, cryopreservation of donor allografts, though not fully understood, appear safe and thus suitable for patients during the current worldwide crisis, and perhaps in other settings more broadly.

ACKNOWLEDGEMENTS

CIBMTR Support List

The CIBMTR is supported by Public Health Service Grant/Cooperative Agreement U24-CA076518 from the National Cancer Institute (NCI), the National Heart, Lung and Blood Institute (NHLBI) and the National Institute of Allergy and Infectious Diseases (NIAID); a Grant/Cooperative Agreement 5U10HL069294 from NHLBI and NCI; a contract HHSH250201200016C with Health Resources and Services Administration (HRSA/DHHS); two Grants N00014-13-1-0039 and N00014-14-1-0028 from the Office of Naval Research; and grants from *Actinium Pharmaceuticals; Allos Therapeutics, Inc.; *Amgen, Inc.; Anonymous donation to the Medical College of Wisconsin; Ariad; Be the Match Foundation; *Blue Cross and Blue Shield Association; *Celgene Corporation; Chimerix, Inc.; Fred Hutchinson Cancer Research Center; Fresenius-Biotech North America, Inc.; *Gamida Cell Teva Joint Venture Ltd.; Genentech, Inc.;*Gentium SpA; Genzyme Corporation; GlaxoSmithKline; Health Research, Inc. Roswell Park Cancer Institute; HistoGenetics, Inc.; Incyte Corporation; Jeff Gordon Children's Foundation; Kiadis Pharma; The Leukemia & Lymphoma Society; Medac GmbH; The Medical College of Wisconsin; Merck & Co, Inc.; Millennium: The Takeda Oncology Co.; *Milliman USA, Inc.; *Miltenyi Biotec, Inc.; National Marrow Donor Program; Onyx Pharmaceuticals; Optum Healthcare Solutions, Inc.; Osiris Therapeutics, Inc.; Otsuka America Pharmaceutical, Inc.; Perkin Elmer, Inc.; *Remedy Informatics; *Sanofi US; Seattle Genetics; Sigma-Tau Pharmaceuticals; Soligenix, Inc.; St. Baldrick's Foundation; StemCyte, A Global Cord Blood Therapeutics Co.; Stemsoft Software, Inc.; Swedish Orphan Biovitrum; *Tarix Pharmaceuticals; *TerumoBCT; *Teva Neuroscience, Inc.; *THERAKOS, Inc.; University of Minnesota; University of Utah; and *Wellpoint, Inc. The views expressed in this article do not reflect the official policy or position of the National Institute of Health, the Department of the Navy, the Department of Defense, Health Resources and Services Administration (HRSA) or any other agency of the U.S. Government.

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Manuscript writing: First draft prepared by Mehdi Hamadani. All authors helped revise the manuscript.

Final approval of manuscript: All authors

Conflict of interest or Competing interests: **MH reports** Research Support/Funding: Takeda Pharmaceutical Company; Spectrum Pharmaceuticals. Consultancy: Incyte Corporation; ADC Therapeutics; Pharmacyclics, Omeros, Tenebio. Speaker's Bureau: AstraZeneca; Sanofi Genzyme. SC report honorarium from Takeda Pharmaceutical Company (Advisory Board).

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Table 1. Baseline characteristics of propensity score matched patient population (2013-18).

Characteristic	Fresh graft	Cryopreserved graft	P Value
No. of patients	**1080**	**274**	
Median age at transplant, yrs (range)	52 (19-80)	55 (22-75)	0.33
Male gender (%)	620 (57.4)	163 (59.5)	0.53
Karnofsky performance score ≥90 (%)	536 (49.6)	137 (50)	0.71
Not reported	13 (1.2)	5 (1.8)	
Race - no. (%)			0.98
Caucasian	738 (68.3)	187 (68.2)	
African-American	205 (19)	53 (19.3)	
Others	66 (6.1)	15 (5.5)	
Not reported	71 (6.6)	19 (6.9)	
Disease (%)			0.66
Acute myeloid leukemia	431 (39.9)	107 (39.1)	
Acute lymphoblastic leukemia	228 (21.1)	55 (20.1)	
Chronic leukemias	78 (7.2)	28 (10.2)	
Myelodysplastic syndrome	172 (15.9)	46 (16.8)	
Lymphoma	171 (15.9)	38 (13.8)	
Disease risk index (%)			1.00
Low	114 (10.6)	29 (10.6)	
Intermediate	715 (66.2)	181 (66.1)	
High	251 (23.2)	64 (23.4)	
HCT-CI (%)			0.17
0	192 (17.8)	36 (13.1)	
1-2	324 (30)	90 (32.8)	
≥3	564 (52.2)	148 (54)	
Donor/recipient CMV serostatus (%)			0.41
-/+	289 (26.8)	76 (27.7)	
Other combinations	786 (72.8)	197 (71.9)	
Not reported	5 (0.5)	1 (0.4)	
Conditioning intensity (%)			0.80

Characteristic	Fresh graft	Cryopreserved graft	P Value
Myeloablative	515 (47.7)	133 (48.5)	
Reduced-intensity/non-myeloablative	565 (52.3)	141 (51.5)	
Donor type (%)			0.18
Matched related donor	152 (14.1)	49 (17.9)	
Haploidentical related donor	659 (61)	169 (61.7)	
8/8 unrelated donor	182 (16.9)	34 (12.4)	
≤7/8 unrelated donor	87 (8.1)	22 (8)	
Graft type (%)			1.00
Bone marrow (BM)	71 (6.6)	18 (6.6)	
Peripheral blood (PB)	1009 (93.4)	256 (93.4)	
TNC dose infused in BM grafts (x10^8/kg recipient body weight), median (range)	3.1 (1.2-26.3)	2.9 (1.8-4.6)	0.85
CD34+ cell dose infused in PB grafts (x10^6/kg recipient body weight), median (range)	5.3 (1-24.5)	5.2 (1.1-13.7)	0.03

Abbreviations: CMV=cytomegalovirus; HCT-CI=hematopoietic cell transplantation-comorbidity index; TNC=total nucleated cells

Table 2. Univariate outcomes of matched population.

	Fresh (N = 1080)		Cryopreserved (N = 274)		
Outcomes	**N**	**Prob (95% CI)**	**N**	**Prob (95% CI)**	**P Value**
Neutrophil recovery	1075		270		
28-day		93.8 (92.3-95.1)%		93.3 (90-96)%	0.80
Platelet recovery	1076		270		
100-day		88.8 (86.8-90.6)%		87.7 (83.4-91.4)%	0.62
Grade 2-4 acute GVHD	1040		271		
100-day		31.3 (28.5-34.1)%		34 (28.5-39.8)%	0.40
Grade 3-4 acute GVHD	1040		271		
100-day		9.4 (7.7-11.3)%		6.3 (3.7-9.5)%	0.07
Chronic GVHD	1077		272		
1-year		30.7 (27.9-33.5)%		26.8 (21.5-32.5)%	0.22
2-year		36.4 (33.4-39.6)%		29.5 (23.8-35.5)%	0.04
Relapse/Progression	1062		273		
1-year		24.1 (21.6-26.8)%		24.7 (19.7-30.1)%	0.85
2-year		30.7 (27.7-33.7)%		36.3 (29.9-42.9)%	0.13
Non-relapse mortality	1062		273		
1-year		15.8 (13.7-18.1)%		16.9 (12.6-21.7)%	0.67
2-year		19 (16.5-21.5)%		22 (16.8-27.7)%	0.32
Disease-free survival	1062		273		
1-year		60 (57-63)%		58.4 (52.4-64.3)%	0.63
2-year		50.4 (47-53.7)%		41.7 (35-48.6)%	0.03
Overall survival	1080		274		
1-year		71.1 (68.3-73.8)%		70.3 (64.6-75.7)%	0.81
2-year		60.6 (57.3-63.8)%		58.7 (51.9-65.4)%	0.62

Table 3. Matched pair analysis by marginal Cox model

Outcomes	Cryopreserved graft HR (95% CI)	Fresh graft HR (Reference)	P-value
Neutrophil recovery	0.91 (0.80-1.02)	1	0.12
Platelet recovery	0.88 (0.78-1.00)	1	0.05
Grade 2-4 acute GVHD	1.10 (0.87 -1.38)	1	0.43
Grade 3-4 acute GVHD	0.78 (0.50-1.22)	1	0.27
Chronic GVHD	0.78 (0.61-0.99)	1	0.04
Relapse/progression	1.21 (0.97-1.50)	1	0.09
Non-relapse mortality	1.16 (0.86-1.55)	1	0.32
Disease-free survival	1.19 (1.01 -1.40)	1	0.04
Overall survival	1.05 (0.86-1.29)	1	0.60

Figure Legend:

Figure 1. Outcomes of post-transplant cyclophosphamide based allogeneic transplant patients receiving ether fresh or cryopreserved grafts. 1a. Overall survival, 1b. Cumulative incidence of chronic GVHD.