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A Comparison of Hematopoietic Cell Transplant Conditioning Regimens for Hemophagocytic Lymphohistiocytosis Disorders

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AUTHOR CONTRIBUTION

RAM, KH, CCD, AG and ME designed the study. KH and SK prepared and analyzed the data. RAM, KH, SK, CCD, AG and ME interpreted the data. RAM and ME drafted the manuscript. All other authors critically reviewed and edited the manuscript. All authors approved the final version of the manuscript.

DISCLOSURE OF CONFLICT OF INTEREST

The authors declare that there are no relevant conflicts of interest.

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Abstract

Background: Allogeneic hematopoietic cell transplantation for hemophagocytic lymphohistiocytosis (HLH) disorders is associated with substantial morbidity and mortality.

Objective: The effect of conditioning regimen groups of varying intensity on outcomes after transplantation was examined to identify an optimal regimen(s) for HLH disorders.

Methods: Included are 261 patients with HLH disorders transplanted between 2005–2018. Risk factors for transplant outcomes by conditioning regimen groups were studied using Cox regression models.

Results: Four regimen groups were studied: 1) fludarabine (Flu), melphalan (Mel), n=123; 2) Flu, Mel, thiotepa (TT) n=28, 3) Flu, busulfan (Bu), n=14; and 4) Bu, cyclophosphamide (Cy), n=96. The day-100 incidence of veno-occlusive disease (VOD) was lower with Flu/Mel (4%) and Flu/Mel/TT (0%) compared to Flu/Bu (14%) and Bu/Cy (22%), p<0.001. The 6-month incidence of viral infections was highest after Flu/Mel (72%) and Flu/Mel/TT (64%) compared to Flu/Bu (39%) and Bu/Cy (38%), p<0.001. Five-year event-free survival (alive and engrafted without additional cell product administration) was lower with Flu/Mel (44%), compared to Flu/Mel/TT (70%), Flu/Bu (79%) and Bu/Cy (61%), p=0.002. The corresponding 5-year overall survival was 68%, 75%, 86% and 64% and did not differ by conditioning regimens (p=0.19). Low event-free survival with Flu/Mel is attributed to high graft failure (42%) compared to Flu/Mel/TT (15%), Flu/Bu (7%) and Bu/Cy (18%), p<0.001.

Conclusion: Given the high rate of graft failure with Flu/Mel, and the high rate of VOD with Bu/Cy and Flu/Bu, Flu/Mel/TT may be preferred for HLH disorders. Prospective studies are warranted.

Capsule Summary

This study demonstrates that fludarabine, melphalan, and thiotepa HCT conditioning regimen best optimizes the balance between limiting toxicities yet ensuring sustained donor engraftment for HLH disorders.

Keywords

Hemophagocytic Lymphohistiocytosis; HLH; Allogeneic Hematopoietic Cell Transplantation; HCT; Hematopoietic Stem Cell Transplantation; HSCT; Bone Marrow Transplantation; BMT

INTRODUCTION

Genetic hemophagocytic lymphohistiocytosis (HLH) disorders are a group of diseases that are characterized by hyperinflammation and present unique challenges to allogeneic

hematopoietic cell transplantation (HCT).[1] HLH disorders are typically caused by genetic defects which compromise cytotoxic lymphocyte cytotoxicity or dysregulate inflammasome function.[2] Patients commonly develop HLH and may experience other inflammatory disease manifestations such as inflammatory bowel disease in patients with XIAP deficiency and activating *NLRC4* mutations for example. Allogeneic HCT approaches for HLH disorders have evolved over time in efforts to reduce the risk of HCT toxicities and improve survival. Traditional fully myeloablative conditioning regimens such as busulfan (Bu; 16 mg/kg) and cyclophosphamide (Cy) were associated with very high risks of toxicities and mortality in patients with HLH. In a prior study on HCT for HLH that covered the period 1989–2005, the incidence of hepatic veno-occlusive disease (VOD) was 18% and early mortality was 35%.[3] Reduced intensity conditioning approaches including alemtuzumab, fludarabine (Flu), and melphalan (Mel; 140 mg/m^2) have offered remarkably low rates of regimen-related toxicity and early mortality, but the benefits came at a cost of high rates of mixed chimerism and graft failure including in a recent multi-center trial.[4–10] Other approaches have been developed that take an intermediate approach by using alternative agent combination and dosing regimens that maintain reduced chemotherapeutic exposure and toxicity risks but achieve myeloablation. In a recent report on 25 patients with HLH who received Flu/Bu regimen with alemtuzumab or ATG, all had sustained engraftment and 100% event-free and overall survival.[11] Despite targeted Bu dosing (cumulative area under the curve for Bu was 63.1 mg/L x h [range 48 – 77]) sinusoidal obstructive syndrome occurred in a third of patients, although all resolved after defibrotide.[11] In another approach that added thiotepa (TT) 10 mg/kg as single dose to the Flu/Mel/alemtuzumab regimen (Mel dose 140 mg/m^2), Naik and colleagues reported sustained engraftment in their 9 patients with HLH.[12]

A review of transplantations for HLH disorders reported to the Center for International Blood and Marrow Transplant Research (CIBMTR) showed transplant conditioning regimens for HLH disorders have evolved in an effort to balance the higher toxicity and mortality associated with the traditional Bu/Cy regimen and the higher graft failure associated with reduced intensity Flu/Mel regimen. However, randomized trials to directly compare the outcomes of the different conditioning regimens have not been performed as such trials are challenging to conduct. Thus, we sought to utilize data reported to a transplant registry to study the effects of the following common conditioning regimens: Flu/Mel, Flu/Mel/TT, Flu/Bu (median Bu dose 12 mg/kg), and Bu/Cy (median Bu dose 16 mg/kg) with careful adjustment for transplant period.

METHODS

Data Source

The CIBMTR is a working group of over 400 transplant centers that contribute data on consecutive allogeneic and autologous transplants. Patients are followed longitudinally until death or lost to follow-up. Accuracy of data reported to the CIBMTR and compliance are monitored by on-site audits. Consent is sought from patients and/or their legal guardians for research. The Institutional Review Board of the National Marrow Donor Program approved the study.

Patients

Eligible were 261 patients with HLH disorders who were aged ≤20 years at transplantation and received their first allogeneic HCT in the U.S.A. between 01/2005 and 12/2018. Transplant outcomes were followed through 06/2020. Excluded were patients who received total body irradiation (TBI) containing regimens (n=11), uncommon non-TBI regimens (treosulfan-containing conditioning regimens [n=4], other regimens [n=4]), *ex vivo* T-cell depletion (n=3) cord blood transplants mismatched at ≥3 HLA-loci (n=1), adult unrelated donor transplants mismatched at ≥2 HLA-loci (n=4) and mismatched related donor transplants (n=8). Three transplant centers that failed an audit of their data were excluded (n=4 patients). As there were only 5 patients older than 20 years who were transplanted for HLH they were excluded *a priori*.

Outcomes

The primary outcome was event-free survival (an event was defined as primary graft failure, secondary graft failure, or death). Primary graft failure was defined as failure to achieve absolute neutrophil recovery (ANC) $\geq 0.5 \times 10^9$/L for 3 consecutive days or whole-blood donor chimerism <5%. Secondary graft failure included ANC decline to $<0.5 \times 10^9$/L without recovery after having achieved ANC $\geq 0.5 \times 10^9$/L, cellular product intervention for mixed chimerism, second transplant or whole-blood donor chimerism <5%.[13] Other outcomes studied were overall survival (death from any cause), neutrophil recovery, and acute and chronic GVHD graded using standard criteria. [14,15]

Statistical Methods

The characteristics of patients by conditioning regimen were compared using the chi-square test for categorical variables. The incidence of graft failure (primary or secondary), acute and chronic GVHD, hepatic VOD, infections, pulmonary and renal complications were calculated using the cumulative incidence estimator to accommodate competing risks.[16] Risk factors associated with day-28 neutrophil recovery were examined using logistic regression method.[17] Risk factors associated with acute and chronic GVHD and graft failure were examined using the Fine and Gray method.[18] Event-free survival and overall survival were examined using the Cox proportional hazards model.[19] The probabilities of event-free and overall survival were generated from final Cox regression models.[20] Variables considered included conditioning regimen intensity, age at transplant, sex, performance score, HCT–comorbidity score, recipient cytomegalovirus serostatus, donor type and transplant period (Table 1). Models were built using stepwise forward selection and variables that met a significance level ≤0.05 were held in the final model. All variables met the assumption of proportional hazards and there were no first order interactions between the variables held in the final models. An effect of transplant center on disease-free survival was tested.[21] P-values are two-sided and analyses were done using SAS version 9.3 (Cary, NC).

RESULTS

Patient and transplant characteristics

Table 1 shows the characteristics of 261 patients with HLH disorders by conditioning regimen. Most transplant regimens included *in vivo* T-cell depletion. Four regimen groups were studied: 1) Flu/Mel (Mel 140mg/m^2 [60%], 100 mg/m^2 [40%]), n=123; 2) Flu,/Mel (Mel 140mg/m^2 [79%], 100 mg/m^2 [21%]), TT (8 mg/kg or 10 mg/kg), n=28, 3) Flu/Bu (Bu median dose 9mg/kg [IQR 6–14]), n=14; and 4) Bu (Bu median dose 15 mg/kg [IQR 12–17]), Cy, n=96. Alemtuzumab was most likely to be used with Flu/Mel and Flu/Mel/TT regimens and ATG was more likely to be used with Bu/Cy and Flu/Bu regimens. The median time from diagnosis to transplant for recipients of Bu/Cy and Flu/Bu was 4 months and for recipients of Flu/Mel and Flu/Mel/TT, 6 and 8 months, respectively. There were no differences in sex distribution, performance score or cytomegalovirus serostatus positivity between the treatment groups. The predominant donor type was HLA-matched and mismatched unrelated donors including umbilical cord blood. The use of umbilical cord blood varied with conditioning regimen. Umbilical cord blood was predominantly used with Bu/Cy and Flu/Mel/TT regimens. While a calcineurin inhibitor containing GVHD prophylaxis with methotrexate or mycophenolate was predominantly used with Bu/Cy, Flu/Mel/TT and Flu/Bu regimens, calcineurin inhibitor with prednisone was predominantly used with Flu/Mel conditioning regimen. Transplant regimens varied by transplant period with the Bu/Cy regimen being predominantly used in the period 2005 to 2010 and Flu/Mel/TT and Flu/Bu regimens being predominantly used in the period 2011 to 2018. Consequently, the median follow-up of surviving patients varied, 96 months (range 6–147), 72 (3–132), 36 (12–111) and 48 (13–128) months after Bu/Cy, Flu/Mel, Flu/Mel/TT and Flu/Bu regimens, respectively. The burden of morbidity (invasive fungal infection or mechanical ventilation) prior to transplantation did not differ by transplant period, 25% of patients transplanted 2005 to 2010 compared to 33% after 2010 (p=0.25).

Genetic mutational data was collected after 2013. Thus, of the 215 HLH cases, only 70 (33%) were transplanted between 2014 and 2018. Of these, 7 patients were not tested for genetic mutation. Of those for whom genetic mutational data were available, *PRF1* (n=22), *UNC13D* (n=15) and *STXBP2* (n=6) mutations were the most frequent. The mutation was not reported for the remaining 20 patients. As these are recent transplants, patients received Flu/Mel (n=47), Flu/Mel/TT (n=17) or Flu/Bu (n=6). Categorical diagnoses for patients in the entire dataset included HLH (n=215), X-linked lymphoproliferative disease (n=34), Chediak-Higashi syndrome (n=11), and Griscelli syndrome (n=1). Thirty of 34 patients with XLP confirmed presence or not of genetic mutation; *SH2D1A* (n=19), *XIAP/BIRC4* (n=3) and not reported (n=8). Seventeen XLP patients had prior EBV-virus infection and 1 patient, Burkitt lymphoma (EBV-negative) prior to transplant. Of the 17 patients with prior EBV infection, 4 reported HLH, 8 reported lymphoproliferative disease prior to transplantation and remaining 5 patients did not report HLH or lymphoproliferative disease.

Event-free survival and overall survival

Event-free survival was lowest in patients who received Flu/Mel regimen compared to the other regimens (Table 2). The 5-year probability of event-free survival was 44%, 70%,

79% and 61% after Flu/Mel, Flu/Mel/TT, Flu/Bu, and Bu/Cy regimens (Figure 1, Table 3). None of the other variables tested were associated with event-free survival (Table 2). We specifically tested for an effect of donor type on event-free survival and did not find an association (Table 2). We tested for an effect of transplant period and did not find an association (data not shown). A subset analysis was undertaken to examine whether event-free survival differed by melphalan dose when Flu/Mel was the conditioning regimen. Of the 123 patients who received Flu/Mel, 74 patients received 140 mg/m^2, and the remaining 49 patients, 100 mg/m^2. The 5-year probability of event-free survival was lower with melphalan dose 100 mg/m^2 (32%, 95% CI 19–46) compared to 140 mg/m^2 (53%, 95% CI 41–64), p=0.021. A subset analysis of HLH patients with genetic mutational data confirmed lower event-free survival with Flu/Mel (41%, 95% CI 27–56) compared to Flu/Mel/TT or Flu/Bu (73%, 95% CI 53–89) regimens, p=0.019. Examination by sub disease also confirmed lower event-free survival with Flu/Mel regimens (Supplemental Table 1, Supplemental Table 2).

Overall survival did not differ by conditioning regimen intensity (Table 2). The 5-year probability of overall survival was 68%, 75%, 86% and 64% after Flu/Mel, Flu/Mel/TT, Flu/Bu, and Bu/Cy regimens, respectively (Figure 1, Table 3). Compared to HLA-matched sibling donor transplants, mortality was higher after HLA-matched and mismatched unrelated donor transplants although the difference did not meet the level of significance set for the analysis (Table 2). Mortality was also higher in patients with performance score (play score) ≤80 (Table 2). In a subset analysis, 5-year overall survival was not associated with melphalan dose in the Flu/Mel regimen: 69% (95% CI 58–79) and 67% (95% CI 52–80) after Mel 140 mg/m^2 and 100 mg/m^2, respectively, p=0.83. A subset analysis of HLH patients with genetic mutational data did not show differences in survival with Flu/Mel (66%, 95% CI 51–79) compared to Flu/Mel/TT or Flu/Bu (78%, 95% CI 60–92) regimens, p=0.52. Examination by sub disease did not show differences in overall survival by conditioning regimen groups (Supplemental Table 1, Supplemental Table 2).

Graft failure

Graft failure was highest in patients who received Flu/Mel regimen compared to the other regimens (Table 2). The 5-year incidence of graft failure was 42%, 15%, 7% and 18% after Flu/Mel, Flu/Mel/TT, Flu/Bu and Bu/Cy regimens, respectively (Figure 2, Table 3). None of the other variables tested including donor type were associated with graft failure. Relatively few graft failures were primary, accounting for 5 of 51 graft failures after Flu/Mel, 1 of 5 after Flu/Mel/TT and 6 of 18 after Bu/Cy regimens. Subset analysis of the group that received Flu/Mel regimen confirmed the 5-year incidence of graft failure was higher with melphalan dose 100 mg/m^2 (59%, 95% CI 45–73) compared to 140 mg/m^2 (30%, 95% CI 20–41), p=0.001. A subset analysis of HLH patients with genetic mutational data confirmed higher graft failure with Flu/Mel (46%, 95% CI 31–60) compared to Flu/Mel/TT or Flu/Bu (9%, 95% CI 1–25) regimens, p<0.001. Review of graft failure by sub disease confirm higher graft failure with Flu/Mel regimen (Supplemental Table 1, Supplemental Table 2).

Donor leukocyte infusion for mixed chimerism and second transplants occurred in 25 of 123 (20%) and 14 of 123 (11%) recipients of Flu/Mel regimen, respectively. The median time to donor leukocyte infusion from transplantation was 4 months and most infusions

occurred within 6 months of transplantation. Eighteen of 25 (72%) patients are alive. Of the 14 patients who received a second transplant after a Flu/Mel regimen, 9 (64%) are alive. The median time to second transplant from the first was 11 months and 4 out of 14 received their graft from the same donor as their first transplant. One patient who received the Flu/Mel/TT regimen received a second transplant (from a different donor) and is alive. Three of 96 (3%) patients who received Bu/Cy received a second transplant and only 1 is alive. Of the 16 second transplantations, 12 received Flu/Bu or Bu/Cy for the second transplant. Two received total body irradiation (200 cGy and 300 cGy) with fludarabine and the remaining 2 patients, Flu/Mel regimen.

Acute and Chronic GVHD

Grade II-IV acute GVHD was not associated with conditioning regimen (Table 2). No other factors were associated with acute GVHD risks. The day-100 probability of grade II-IV acute GVHD was 24% (95% CI 17–32), 21% (95% CI 10–35), 21% (95% CI 8–39) and 30% (95% CI 21–39) after Flu/Mel, Flu/Mel/TT and Bu/Cy regimens, respectively. On the other hand, chronic GVHD risks were higher with Flu/Mel/TT, Flu/Bu and Bu/Cy regimens compared to Flu/Mel regimen and after HLA-matched and mismatched unrelated donor compared to HLA-matched sibling (Table 2). No other factors were associated with chronic GVHD. The 5-year probability of chronic GVHD was 13% (95% CI 7–20), 29% (95% CI 13–46), 31% (95% CI 9 – 59) and 35% (95% CI 25–45) after Flu/Mel, Flu/Mel/TT, Flu/Bu and Bu/Cy regimens, respectively (p=0.001).

Post-transplant Complications

The day-100 incidence of hepatic VOD was lower with Flu/Mel (n=5; 4%) compared to Flu/Bu (n=2; 14%) and Bu/Cy regimen (n=21; 22%), p<0.001 (Table 4). None of the patients who received Flu/Mel/TT developed VOD. Seventeen patients are dead, with median time to death less than 2 months from transplant in all groups. The median Bu dose for VOD patients treated with Bu/Cy regimen was 15 mg/kg and the doses of Bu for the 2 Flu/Bu patients were 14 mg/kg and 12 mg/kg. The 6-month incidence of systemic viral infections was higher with Flu/Mel (72%) and Flu/Mel/TT (64%) compared to Flu/Bu (39%) and Bu/Cy (38%), p<0.001 regimens (Table 4). There was no difference in the 6-month incidence of systemic bacterial or fungal infections (Table 4). An incidence of lymphoproliferative disease (EBV positive) after transplantation could not be calculated as there were very few events; two recipients of the Flu/Mel regimen developed lymphoproliferative disease and none for the other regimen groups. There was no difference in the incidence of pulmonary (interstitial pneumonitis, acute respiratory distress syndrome, pulmonary hemorrhage) complications, but renal complications (dialysis or renal transplant) were higher with Flu/Bu (7%) compared to 1% after Flu/Mel, 4% after Flu/Mel/TT and none after Bu/Cy regimens (Table 4).

Umbilical Cord Blood Transplant

Of the 112 umbilical cord blood transplantations, 62% (n=69) received Bu/Cy regimen and none received alemtuzumab. Twenty-one percent (n=24) received Flu/Mel and the remaining 17% (n=18), Flu/Mel/TT and (n=1) Flu/Bu regimens. Alemtuzumab was the predominant *in vivo* T-cell depleting agent used with Flu/Mel (19 of 24) and Flu/Mel/TT (15 of 18).

Consistent with the main analyses, graft failure was higher and event-free survival lower after Flu/Mel regimen; 42% (95% CI 23–62) and 50% (95% CI 31–70). The corresponding rates after Flu/Mel/TT were 25% (95% CI 9–46) and 60% (95% CI 38–80) and after Bu/Cy were 19% (95% CI 10–29) and 69% (95% CI 57–79). The single patient who received Flu/Bu has sustained engraftment and is alive. Viral infections were higher after Flu/Mel (54%, 95% CI 34–74) and Flu/Mel/TT (65%, 95% CI 42–85) compared to Bu/Cy (38%, 95% CI 26–50). Other outcomes were also consistent with the main analyses (data not shown). Of note, viral infections were also higher after Flu/Mel (77%, 95% CI 68 – 85) and Flu/Mel/TT 50%, 95% CI 29 – 71) compared to Bu/Cy (38%, 95% CI 19 – 60) for bone marrow and peripheral blood grafts.

DISCUSSION

Identifying an optimal transplant conditioning regimen is best accomplished by performing prospective studies with randomized allocation of patients to the treatment groups of interest. However, in the absence of randomized trials, we used available resources to better understand the risks associated with regimen-related toxicity, graft failure, and survival after allogeneic HCT for HLH disorders. We studied outcomes for patients with HLH disorders treated with Flu/Mel, Flu/Mel/TT, Flu/Bu, and Bu/Cy regimens over a period of 13 years to accommodate clinical practice changes in conditioning regimen preference and considered a period effect in all analyses. An effect of transplant period was not observed, and the 5-year overall survival was comparable across the treatment groups. There were remarkable differences in event-free survival (the likelihood of being alive with sustained engraftment in the absence of cellular interventions for mixed chimerism). The risk of graft failure was more than double with the Flu/Mel regimen and attributed mostly to secondary graft failure. With a 5-year graft failure rate of 42% with the Flu/Mel regimen we advise that this regimen should be avoided. While we do not know why some patients received melphalan dose 140 mg/m^2 and others, 100 mg/m^2, graft failure was high with both doses and many patients were rescued with donor leukocyte infusion and second transplant. Notably, all graft failure occurred within 2 years after transplantation and calls for frequent monitoring of donor chimerism at pre-defined intervals for early intervention.

Our findings are consistent with a smaller multi-center phase II trial that used Flu/Mel (Mel dose 140 mg/m^2) with alemtuzumab that recorded 1-year event-free survival of 39%.[9] Similarly, the recorded 5-year event-free and overall survival in the current analyses with the more intense regimens (Flu/Mel/TT, Flu/Bu, and Bu/Cy) are in keeping with that reported in the HLH-2004 study.[22] The addition of thiotepa to the Flu/Mel backbone led to a 5-year event-free survival of 70% and offer a compelling reason to recommend this regimen for HLH disorders. Only 6 patients received Mel dose of 100 mg/m^2 and 4 are alive with sustained remission. A substantially larger cohort is needed to determine whether a Mel dose of 100 mg/m2 with TT is sufficient for sustained event-free survival. While the details of conditioning regimens in HLH-2004 were not reported, the majority of transplantations employed Bu-containing or treosulfan-containing regimens.[22] We were unable to examine treosulfan-containing conditioning regimens as treosulfan can only be used in the United States under an IND (Investigational New Drug).

We did not observe an association between conditioning regimen intensity and donor type/donor-recipient HLA match. Consistent with published literature, survival was lower after transplantation of unrelated HLA-matched and HLA-mismatched transplants, though the modest sample size prevented detection of a statistically significant difference ($p<0.05$).[23,24] There were no significant differences in acute GVHD rates among the regimen groups. Although the Flu/Mel regimen was associated with low chronic GVHD the high rate of mixed chimerism and graft failure confounds this observation.

With regard to the risk of toxicities, Flu/Bu and Bu/Cy regimens were associated with the highest risk of hepatic VOD. Pharmacokinetic data was not collected consistently during the study period so the effect of cumulative exposure could not be evaluated. In the European report on 25 patients with HLH who received Flu/Bu with pharmacokinetic monitoring and dose adjustment, it is notable that a third of patients still developed VOD, implying the high rate of VOD may be attributable to the underlying disease.[11] Indeed, endothelial injury has been demonstrated in untransplanted patients with HLH who developed concurrent thrombotic microangiopathy, which may contribute to the apparent increased propensity to develop VOD.[25] A report of transplantation for non-malignant diseases that only included 12 patients with HLH disorders treated with Flu/Bu with pharmacokinetic monitoring of Bu report a low incidence of VOD.[26] Similarly, others have reported VOD or diffuse alveolar hemorrhage only in HLH patients compared to other primary immune deficiency disorders who received Flu/Bu with pharmacokinetic monitoring of Bu.[27] None of the patients who received Flu/Mel/TT in the current analyses developed VOD and this approach may offer an advantage for this disease for this reason. We did not observe differences in pulmonary toxicity by conditioning regimen in our current study but recorded higher renal toxicity with Flu/Mel/TT and Flu/Bu regimens. With regard to infectious complications, the high rate of viral infections with Flu/Mel likely reflects the predominant use of alemtuzumab for *in vivo* T-cell depletion. A subset analyses of umbilical cord blood transplants also demonstrated higher viral infections associated with alemtuzumab.

Our real-world data support that Flu/Mel (140 mg/m^2 or 100 mg/m^2) should be avoided due to the high risk of secondary graft failure. This is relevant information as over half of transplantations in the United States between 2011 and 2018 used this regimen. The data support Flu/Mel/TT is the optimal regimen amongst the four regimens studied in the current analysis. The addition of TT to Flu/Mel regimen lowered graft failure and improved event-free survival and none of the patients developed VOD which remains a challenge with Flu/Bu despite pharmacokinetic monitoring. Others have added low dose irradiation to the Flu/Mel regimen to overcome graft failure although this approach is not been adopted widely.[7,28] A plausible explanation is a desire to avoid irradiation containing regimens and its sequela in very young children.[29] The reported success with treosulfan-containing regimen and Flu/Mel/TT regimen reported here-in merit further testing through carefully controlled trials to optimize sustained engraftment and survival for HLH diseases. Additional efforts aimed at controlling interferon gamma activity and other pre-transplant factors may also lead to improved patient outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

HLH	Hemophagocytic Lymphohistiocytosis
HCT	Allogeneic Hematopoietic Cell Transplantation
Bu	Busulfan
Cy	Cyclophosphamide
Flu	Fludarabine
Mel	Melphalan
TT	Thiotepa
ATG	Anti-thymocyte Globulin
VOD	veno-occlusive disease
GVHD	Graft Versus Host Disease
TBI	Total Body Irradiation
ANC	Absolute Neutrophil Count
HLA	Human Leukocyte Antigen

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Key Message

- Five-year event-free survival for HLH disorders treated with HCT was only 44% with fludarabine and melphalan compared to 70% with fludarabine, melphalan, and thiotepa conditioning regimen.
- Fludarabine, melphalan, and thiotepa regimen may best optimize the balance between limiting toxicities yet ensuring sustained donor engraftment for HLH disorders.

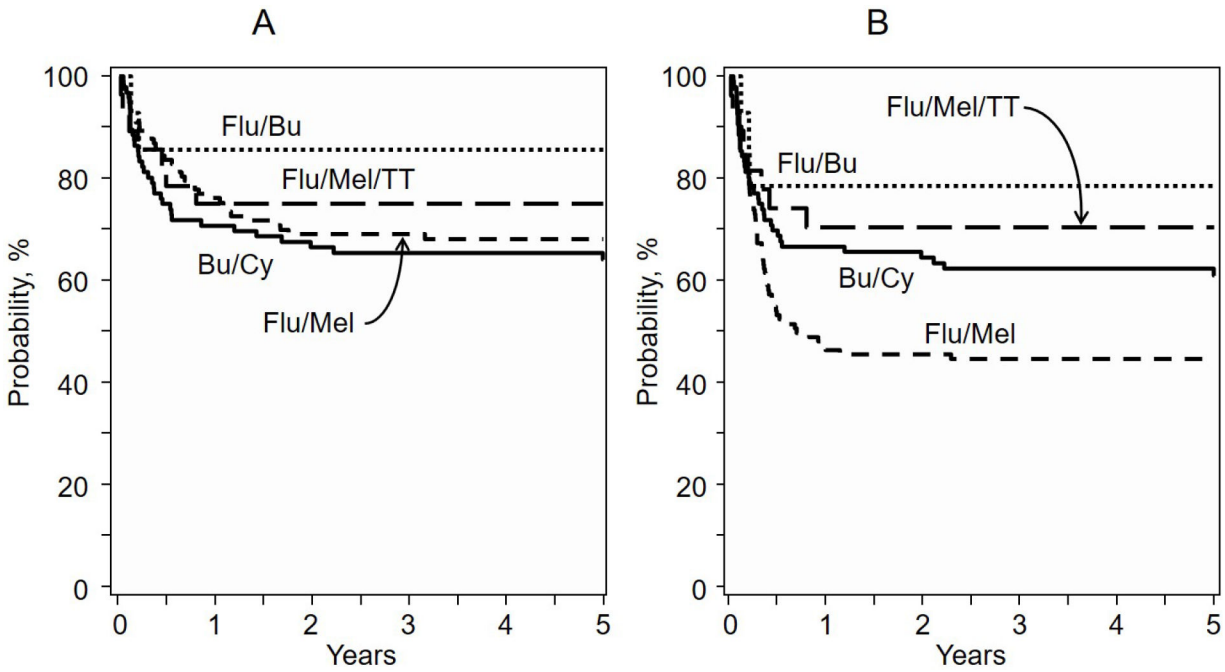

Figure 1: Overall (A) and event-free (B) survival.

Figure 1A: *Overall survival*: The 5-year probability of overall survival was 68% (95% CI 60–76), 75% (95% CI 58–89), 86% (95% CI 63–98) and 64% (95% CI 54–74) after Flu/Mel, Flu/Mel/TT, Flu/Bu and Bu/Cy regimens, respectively (p=0.19).

Figure 1B: *Event-free survival*: The 5-year probability of event-free survival was 44% (95% CI 36–53), 70% (95% CI 52–86), 79% (95% CI 54–95) and 61% (95% CI 51–71) after Flu/Mel, Flu/Mel/TT, Flu/Bu and Bu/Cy regimens, respectively (p=0.002).

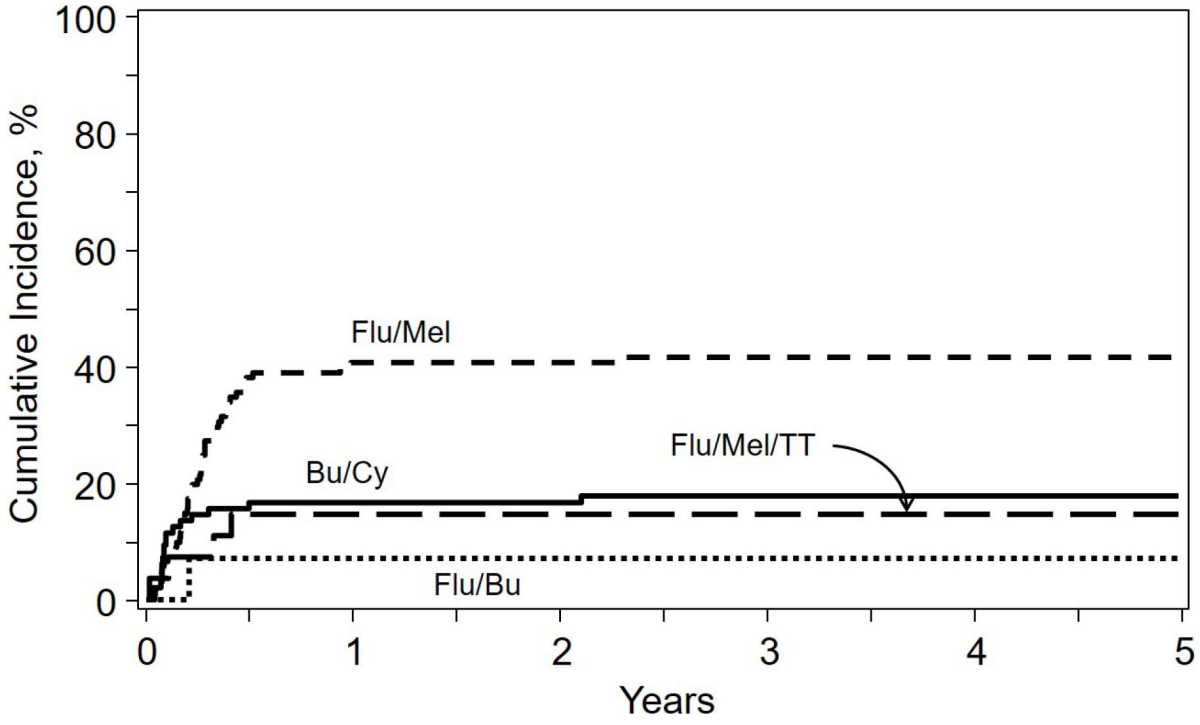

Figure 2: Cumulative incidence of graft failure.

The 5-year incidence of graft failure was 42% (95% CI 33–51), 15% (95% CI 4–31), 7% (95% CI <1–27) and 18% (95% CI 11–26) after Flu/Mel, Flu/Mel/TT, Flu/Bu and Bu/Cy regimens, respectively ($p<0.001$).

Table 1.

Patient and transplant characteristics.

Variable	Conditioning regimen*				P-value
	Bu/Cy	Flu/Mel	Flu/Mel/TT	Flu/Bu	
Number	96	123	28	14	
Transplant age, median (range), years	1 (<1–20)	2 (<1–20)	1 (<1–18)	2 (<1 – 17)	0.003
≤ 1 year	67 (70%)	56 (46%)	15 (54%)	7 (50%)	
2–5 years	21 (22%)	28 (23%)	6 (21%)	3 (21%)	
6–10 years	5 (5%)	16 (13%)	3 (11%)	1 (7%)	
≥ 11 years	3 (3%)	23 (19%)	4 (14%)	3 (21%)	
Sex					0.25
Male	51 (53%)	80 (65%)	18 (64%)	10 (71%)	
Female	45 (47%)	43 (35%)	10 (36%)	4 (29%)	
Performance score					0.65
90 – 100	72 (75%)	87 (71%)	23 (82%)	10 (71%)	
≤80	19 (20%)	32 (26%)	5 (18%)	4 (29%)	
Not reported	5 (5%)	4 (3%)	__	__	
Recipient cytomegalovirus serostatus					0.53
Negative	36 (38%)	33 (27%)	12 (43%)	5 (36%)	
Positive	58 (60%)	88 (72%)	16 (57%)	9 (64%)	
Not reported	2 (2%)	2 (2%)	__	__	
Disease type					0.02
HLH	83 (86%)	101 (82%)	22 (79%)	9 (64%)	
XLP	7 (7%)	19 (15%)	6 (21%)	2 (14%)	
Chediak-Higashi syndrome	6 (6%)	2 (2%)	__	3 (21%)	
Griscelli syndrome	__	1 (<1%)	__	__	
Donor type					0.003
HLA-matched sibling	4 (4%)	18 (15%)	2 (7%)	2 (14%)	
HLA-matched unrelated donor	35 (36%)	59 (48%)	4 (14%)	8 (57%)	
HLA-mismatched unrelated donor	57 (59%)	46 (37%)	22 (79%)	4 (29%)	
Graft type					<0.001
Bone marrow	23 (24%)	93 (76%)	7 (25%)	5 (36%)	
Peripheral blood	4 (4%)	6 (5%)	3 (11%)	8 (57%)	
Umbilical cord blood	69 (72%)	24 (20%)	18 (64%)	1 (7%)	
Serotherapy					<0.001
Anti-thymocyte globulin	75 (78%)	2 (2%)	3 (11%)	9 (64%)	
Alemtuzumab	1 (1%)	105 (85%)	24 (86%)	3 (21%)	
None	20 (21%)	16 (13%)	1 (4%)	2 (14%)	
Graft vs. host disease prophylaxis					<0.001

Variable	Conditioning regimen*				P-value
	Bu/Cy	Flu/Mel	Flu/Mel/TT	Flu/Bu	
Calcineurin inhibitor + MMF	36 (38%)	28 (23%)	20 (71%)	7 (50%)	
Calcineurin inhibitor + MTX	24 (25%)	17 (14%)	4 (14%)	6 (43%)	
Calcineurin inhibitor + steroid	36 (38%)	78 (63%)	4 (14%)	1 (7%)	
Transplant period					<0.001
2005 – 2010	85 (89%)	66 (54%)	3 (11%)	6 (43%)	
2011 – 2018	11 (11%)	57 (46%)	25 (89%)	8 (57%)	

Abbreviation

Bu = busulfan; Cy = cyclophosphamide; Flu = fludarabine; TT = thiotepa

HLH = hemophagocytic lymphohistiocytosis XLP = X-linked lymphoproliferative disease

MMF = mycophenolate MTX = methotrexate

* Drug dose

Bu/Cy: median Bu dose 15 mg/kg; inter-quartile range 12 – 17 mg/kg

Flu/Mel: 74 of 123 melphalan dose 140 mg/m^2; 49 of 123, melphalan dose 100 mg/m^2

Flu/Mel/TT: 22 of 28 melphalan dose 140 mg/m^2; 6 of 28, melphalan dose 100 mg/m^2 Flu/Bu: Bu dose 9 mg/kg; mg/m^2; inter-quartile range 6 – 14 mg/kg

Table 2.

Risk factors associated with transplant outcomes.

	Number	Hazard Ratio (95% confidence interval)	P-value
Event-free survival			
Conditioning regimen			
Flu/Mel	123	1.00	
Bu/Cy	96	0.57 (0.37 – 0.89)	0.013
Flu/Mel/TT	27	0.46 (0.22 – 0.98)	0.044
Flu/Bu	14	0.34 (0.11 – 1.09)	0.069
Donor type			
HLA-matched sibling	26	1.00	
HLA-matched unrelated donor	85	0.90 (0.47 – 1.73)	0.76
HLA-mismatched unrelated donor	150	1.04 (0.55 – 1.97)	0.90
Overall survival			
Conditioning regimen			
Flu/Mel	123	1.00	
Bu/Cy	96	1.14 (0.69 – 1.85)	0.62
Flu/Mel/TT	28	0.84 (0.36 – 1.92)	0.67
Flu/Bu	14	0.47 (0.11 – 1.94)	0.29
Performance score			
90 – 100	192	1.00	
≤ 80	60	1.76 (1.08 – 2.85)	0.022
Donor type			
HLA-matched sibling	26	1.00	
HLA-matched unrelated donor	85	2.76 (0.83 – 9.16)	0.10
HLA-mismatched unrelated donor	150	2.99 (0.96 – 9.81)	0.069
Graft failure			
Conditioning regimen			
Flu/Mel	123	1.00	
Bu/Cy	96	0.33 (0.18 – 0.62)	0.0005
Flu/Mel/TT	27	0.27 (0.10 – 0.75)	0.012
Flu/Bu	14	0.16 (0.02 – 1.15)	0.068
Donor type			
HLA-matched sibling	26	1.00	
HLA-matched unrelated donor	85	0.55 (0.27 – 1.12)	0.10
HLA-mismatched unrelated donor	150	0.99 (0.50 – 1.99)	0.99
Grade II–IV acute graft-versus host disease			
Conditioning regimen			
Flu/Mel	123	1.00	

	Number	Hazard Ratio (95% confidence interval)	P-value
Bu/Cy	96	1.17 (0.71 – 1.93)	0.54
Flu/Mel/TT	28	0.91 (0.41 – 2.05)	0.83
Flu/Bu	14	0.79 (0.24 – 2.65)	0.71
Chronic graft versus host disease			
Conditioning regimen			
Flu/Mel	123	1.00	
Bu/Cy	96	2.89 (1.57 – 5.33)	0.0006
Flu/Mel/TT	28	2.59 (1.08 – 6.19)	0.033
Flu/Bu	14	2.88 (0.91 – 9.08)	0.071

Abbreviation: Bu = busulfan; Cy = cyclophosphamide; Flu = fludarabine; TT = thiotepa

Table 3.

Event-free survival, overall survival, and graft failure.

Outcomes	Conditioning regimen				P-value
	Bu/Cy (N=96)	Flu/Mel (N=123)	Flu/Mel/TT (N=28)	Flu/Bu (N=14)	
Event-free survival					
2-years	65% (95% CI 55–74)	45% (95% CI 37–54)	70% (95% CI 52–86)	79% (95% CI 54–95)	0.002
5-years	61% (95% CI 51 – 71)	44% (95% CI 36–53)	70% (95% CI 52–86)	79% (95% CI 54–95)	0.002
Overall survival					
2-years	67% (95% CI 57–76)	69% (95% CI 61–77)	75% (95% CI 58–89)	86% (95% CI 63–98)	0.29
5-years	64% (95% CI 54–74)	68% (95% CI 60–76)	75% (95% CI 58–89)	86% (95% CI 63–98)	0.19
Graft failure					
2-years	17% (95% CI 10–25)	41% (95% CI 32–50)	15% (95% CI 4–31)	7% (95% CI <1–27)	<0.001
5-years	18% (95% CI 11–26)	42% (95% CI 33–51)	15% (95% CI 4–31)	7% (95% CI <1–27)	<0.001

Abbreviation: Bu = busulfan; Cy = cyclophosphamide; Flu = fludarabine; TT = thiotepa

Table 4.

Incidence of organ-specific and infectious post-transplant complications.

Outcomes	Conditioning regimen				P-value
	Bu/Cy N=96	Flu/Mel N=123	Flu/Mel/TT N=28	Flu/Bu N=14	
Day-100 veno-occlusive disease	22% (14 – 31)%	4% (1 – 8)%	0%	14% (1 – 38)%	<0.001
6-month bacterial infection	54% (43 – 64)%	58% (49 – 67)%	39% (22 – 58)%	46% (20 – 74)%	0.27
6-month viral infection	38% (28 – 49)%	72% (64 – 80)%	64% (45 – 81)%	39% (14 – 67)%	<0.001
6-month fungal infection	14% (8 – 22)%	12% (7 – 19)%	7% (1 – 20)%	0%	0.77
2-year pulmonary complications *	29% (21 – 39)%	22% (15 – 30)%	36% (19 – 55)%	7% (<1 – 27)%	0.13
2-year renal failure **	0%	1% (<1 – 3)%	4% (<1–14)%	7% (<1 – 27%)	0.070

Abbreviation: Bu = busulfan; Cy = cyclophosphamide; Flu = fludarabine; TT = thiotepa

* interstitial pneumonitis, acute respiratory distress syndrome, diffuse alveolar hemorrhage

** dialysis or renal transplant