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Busulfan Fludarabine and Melphalan is effective conditioning for pediatric and young adult patients with myeloid malignancies underdoing matched sibling or alternative donor transplantation

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Abstract

Background: Allogeneic hematopoietic cell transplantation (allo-HCT) remains a curative option for patients with high-risk myeloid malignancies. **Procedure:** We present our ten-year experience (October 2012-October 2021) of consecutive allo-HCT in patients with myeloid malignancies treated on the pediatric HCT service and conditioned with myeloablative targeted dose-busulfan (BU), fludarabine (FLU) and melphalan (MEL). Twenty-three children, adolescents, and young adult patients (CAYA) (median age 15.4yr) with acute myeloid leukemia (AML, n=17), myelodysplastic syndrome (MDS, n=4), or chronic myeloid leukemia (CML, n=2) underwent allo-HCT post-BU-FLU-MEL. Four patients had treatment-related AML/MDS. Donor/stem cell source was MSD PBSC (n=7), MUD PBSC (n=2), UCB (n=3) or haploidentical-BMT (n=11). Risk stratification was low (n=2), intermediate (n=15), high (=3) and very high risk (n=1). The two patients with CML had failed tyrosine kinase inhibitor therapies. **Results:** With a median follow-up of 39.6 months the relapse rate is only 4.5% with an OS 100%, PFS 95.5% and graft-versus-host-free-relapse-free survival (GRFS) 67.8%. The donor source and the GvHD prophylaxis regimen significantly impacted grades II-IV aGvHD 66.7% versus 19.2% (*P=0.039*) and cGvHD 66.7% versus 0% (*P=0.002*) in the patients receiving matched sibling (MSD) or matched unrelated donor (MUD) PBSC compared to haplo-BMT respectively, resulting in improved GRFS in haplo-BMT, 83.3% compared to 40% matched donor PBSCT (*P=0.025*). **Conclusions:** Our results demonstrate that BU-FLU-MEL is efficacious conditioning for disease control in young patients with myeloid malignancies undergoing MSD or alternative donor allo-HCT but in the setting of PBSC grafts with CSA-MTX GvHD prophylaxis it results in an unacceptably high incidence of GvHD.

Introduction

Standard treatment for children, adolescents, and young adult patients (CAYA) patients with acute myeloid leukemia (AML) includes up to five cycles of chemotherapy containing cytarabine and anthracyclines. Improvements in supportive care, including oral and skin care regimens, empiric broad spectrum antibiotics and prophylactic antifungals have had a meaningful impact on the treatment related toxicity associated with AML therapy[1, 2]. Despite this, more than 40% of patients ultimately die of refractory or relapsed disease or treatment related toxicity[3]. Patients with high-risk AML are candidates for allogeneic hematopoietic cell transplantation (allo-HCT) which similarly carries regimen related toxicities in addition to risk for graft-versus-host disease (GvHD). Advancements in supportive care have also led to improvement in survival for patients with myeloid malignancies undergoing allo-HCT[4].

The choice of conditioning regimen plays a major role in a patient's likelihood of achieving a graft-versus-host-free-relapse-free survival (GRFS), however there is no consensus on the optimal conditioning regimen. Historically, the most common preparative regimen used for patients with AML was total body irradiation

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(TBI) with cyclophosphamide (CY) which was later largely replaced by busulfan (BU) and CY[5]. BU-CY was associated with comparable disease control without the long-term secondary effects of irradiation[6]. BU-fludarabine (FLU) gained significant traction to reduce the toxicity of high dose CY, however it led to inferior progression-free-survival (PFS), when compared to BU-CY in adults[7]. To improve the efficacy of pre-transplant conditioning while keeping non-relapse mortality (NRM) low, three drug regimens were introduced with an additional alkylator. These included thiotepa (TT)-BU-FLU, BU-CY-melphalan (MEL) or BU-FLU-MEL. In an adult study, TT-BU-FLU demonstrated superior survival as compared to BU-FLU due to a reduced risk of relapse, with comparable NRM[8]. BU-FLU-Mel was also associated with better overall (OS) in adult patients without increase in non- NRM compared to FLU-BU due to decreased relapse[9]. There is limited pediatric data on the comparison of three drug regimens to the more conventional BU-CY or TBI-CY. A multi-center study by the European Group for Blood and Marrow Transplantation (EBMT) reported on AML patients between ages 2-18 years. Patients receiving BU-CY-MEL showed a lower incidence of relapse, higher OS and PFS with a comparable NRM when compared to those conditioned with BU-CY or TBI-CY[10]. We previously demonstrated that BU-FLU-MEL conditioning was safe and effective in a limited series of six pediatric patients with high-risk myeloid malignancies[11]. Herein, we expand our experience utilizing this regimen effectively in CAYA with myeloid malignancies undergoing matched or alternative donor allo-HCT.

Methods

Patients: This is a single-center retrospective review of consecutive pediatric and young adult patients with myeloid malignancies who underwent allo-HCT on the pediatric service from October 2012 to October 2021. All patients received myeloablative (MAC) conditioning with BU-FLU-MEL, which has been our pediatric hematopoietic transplant program's standard of care conditioning for myeloid leukemias for the last ten years. Allo-HCT recipients met organ criteria allowing for MAC and had no evidence of active untreated infection. University of Arizona institutional review board approval was obtained to review and report our findings. Four of the eleven patients receiving haploidentical bone marrow transplantation (haplo-BMT) were treated as part of an institutional review board (IRB)–approved Phase Ia single-institution trial[12, 13].

Conditioning regimens: Allo-HCT conditioning for matched sibling donor (MSD) and unrelated allo-HCT consisted of BU at 0.8 mg/kg IV every 6 hours for a total of 16 doses (days -9 to -6). BU pharmacokinetics of the first dose were performed at the Seattle Cancer Care Alliance laboratory. The seventh and remaining doses were modified to achieve an average area under the curve (AUC) of 3.9 to 4.4 mg x hr/L for the duration of the course. The estimated median exposure was 4.0 for MSD and 4.2 mg x hr/L for unrelated allo-HCT. FLU was given at 30 mg/m^2 on days -5 to -2 and MEL 180 mg/m^2 divided into three equal doses and administered on days -4 to -2. One patient with AML who underwent matched unrelated donor (MUD) peripheral blood stem cell transplant (PBSCT) also received rabbit anti-thymocyte globulin (rATG) 1 mg/kg on days -3 to -1. The other patient with AML (evolving from myelodysplastic syndrome [MDS]) who received a MUD PBSCT had 32% myeloblasts prior to conditioning and was therefore given a higher total dose of MEL at 200 mg/m^2 split between days -3 and -2 without the addition of ATG[11].

All eleven patients undergoing T-replete haplo-BMT received BU at 0.8 mg/kg IV every 6 hours for a total of 12 doses (days -8 to -6). BU pharmacokinetics of the first dose were performed with the seventh and remaining doses modified to achieve average AUC exposure of 4.0 to 4.5 mg x hr/L for the duration of the course (median was 4.3). BU was followed by FLU 30 mg/m^2 for our first seven patients and FLU 40 mg/m^2, for the later four, given on days -5 to -2 and a single dose of MEL 100 mg/m^2 on day -2[12-15].

Graft-versus-host disease prophylaxis: All MSD and MUD PBSCT patients (n=9) received GvHD prophylaxis with methotrexate (MTX) 10 mg/m^2 IV infused on days +1, +3, +6 and +11 and cyclosporine A (CSA) starting on day -2 and targeting levels of 150-200 ng/ml. The three umbilical cord blood (UCB) patients received mycophenolate mofetil (MMF) 15 mg/kg q 8 h IV between day -3 and +28 and CSA also starting on day -3. In all patients and in the absence of GvHD, CSA taper occurred between days + 90 and +180. Seven haplo-BMT patients received PT-CY 50 mg/kg IV on days +3 and +4 and another four patients that were part of an IRB–approved phase I single institution clinical trial through the University

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of Arizona Cancer Center (NCT02996773) received PT-CY/bendamustine (BEN). One patient (cohort 2) received PT-CY 50 mg/kg on day +3 and PT-CY 20 mg/kg followed by PT-BEN 60 mg/m^2 on day +4. Another patient (cohort 3) was treated with PT-CY 50 mg/kg IV on day +3 and PT-BEN 90 mg/m^2 on day +4. Two patients (cohort 4) received PT-CY 40 mg/kg on day +3 followed by PT-BEN 20 mg/m^{2}on day +3 and PT-BEN 90 mg/m^2 on day +4. All patients were started on MMF on day +5 until day +28 and tacrolimus from day +5, targeting levels of 6-10 ng/ml. In the absence of GvHD, tacrolimus was weaned starting day +70 to +90 and discontinued by day +120 to +180. GvHD was graded according to the consensus criteria for grading acute and chronic GVHD[16, 17].

Supportive care: Levofloxacin bacterial prophylaxis was started when the absolute neutrophil count (ANC) dropped below 0.5 x 10^9/L. Antifungal prophylaxis with voriconazole was initiated following completion of BU administration. For*Pneumocystis jirovecii* prophylaxis IV pentamidine was given on day -1 and then twice monthly until day +45 and monthly afterwards until one year after BMT. Acyclovir was also started on admission for herpes simplex and varicella virus prophylaxis. Bi-weekly polymerase chain reaction (PCR) monitoring for cytomegalovirus (CMV) and weekly for adenovirus, Epstein-Barr virus (EBV) and human herpes virus-6 (HHV-6) were performed until discharge from hospital and subsequently at least every other week during first 100 days and at least monthly for another 6 months. All patients were transplanted in HEPA filtered rooms on a HEPA filtered unit and encouraged to walk laps on the unit daily.

Donor selection: MSD and MUD PBSC donors were 10/10 HLA-A, -B, -Cw, -DRB1, and -DQB1 antigen matched at high resolution and -DPB1 matched or permissive. The UCB units were a 5/6 HLA-A, -B, -DRB1 antigen match (single) with all units being 4/6 in the two double UCB transplants. Haploidentical donors were first degree relatives who were HLA-haploidentical based on high-resolution typing at HLA-A, -B, -Cw, -DRB1, and -DQB1. Five of the donors were 5 of 10 antigen matches, five were 6 of 10 and one was 7/10 **(Table 1)** . None of the patients had anti-donor HLA antibodies.

Engraftment and Donor chimerism monitoring. For UCB transplants and haplo-BMT granulocyte-colony stimulating factor (G-CSF) was started on day +1 and +5 respectively at 5 µg/kg/day until an ANC of 2.5 x 10^9/L was achieved for three consecutive days. Day of myeloid engraftment was defined as the first of three consecutive days with an ANC of 0.5 x 10^9/L. Day of platelet engraftment was considered the first of three consecutive days with platelet counts of $>$ 20 x 10^9/L with no platelet transfusions administered in the previous 7 days.

Donor chimerism was evaluated on days +28 from bone marrow (BM) and +100, +180 and +365 from peripheral blood (PB) by short tandem repeats (STRs). Engraftment testing was performed using labeled primers to PCR-amplify STR polymorphic DNA markers followed by capillary electrophoresis to distinguish between the DNA contributed by the recipient versus the donor and estimate the percentage of the contribution. The Promega GenePrint 24 System which includes 24 polymorphic markers was used (Promega Corporation, Madison, WI)[18].

Statistical analysis: Time to event endpoints were estimated using cumulative incidence curves and Kaplan-Meier curves, with comparisons using log-rank tests[19, 20]. Cox proportional hazards models for aGvHD II-IV, aGVHD III-IV, cGVHD and severe cGVHD also were adjusted for competing risks due to disease relapse and graft failure. Analyses and graphs were performed using GraphPad Prism version 9.4.0.

Results

*Patient, disease and transplant characteristics:*Twenty-three pediatric and young adult patients with myeloid malignancies underwent allo-HCT following BU-FLU-MEL conditioning. The clinical characteristics of the patients are outlined in **Table 1** . The median age at transplant was 15.4 years (0.6 to 27.2 years). Seventeen patients were male. Ethnic and/or racial minorities constituted 48% of all patients, the majority of whom (35%) were Hispanic.

Seventeen patients had AML with fourteen being *de novo* , one evolving from MDS and two were treatment related (t-AML) secondary to osteogenic and Ewings sarcoma therapy. The remaining patients included four

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patients with MDS, two of which were treatment related secondary to acute lymphoblastic leukemia (ALL) therapy, and two patients had chronic myeloid leukemia (CML). Most AML patients were transplanted in first remission (76%) while five MDS patients (including an MDS patient evolving to AML) did not receive conventional chemotherapy prior to conditioning **(Table 1)** . Cytogenetics were unfavorable in the majority of AML/MDS patients **(Table 1)** while the pediatric disease risk index (DRI) was intermediate in 71%, and high or very high in 19%[21]. Two patients with CML are included in this analysis, with one undergoing allo-HCT following accelerated phase while the other had T315I mutation in the BCR-ABL1 kinase domain.

Seven patients received PBSCT from an HLA-matched sibling. Unrelated donor allo-HCT included two patients who received a MUD PBSC graft, one patient who received a UCB unit and two patients who were infused with double UCB units. Eleven patients underwent T-cell replete haplo-BMT.

Engraftment and chimerism: All patients had trilineage engraftment with complete donor chimerism on their day +28 bone marrows biopsies. One patient who received haplo-BMT experienced secondary graft failure after early CMV reactivation and treatment with ganciclovir. He was salvaged 46 days after the initial transplant using a single day conditioning regimen followed by infusion of unmanipulated G-CSF mobilized PBSC from his original maternal donor and engrafted promptly (day +14)[22, 23]. All patients when re-assessed by peripheral blood chimerisms on days +100, +180 and +365 remained complete donor chimeras.

Graft-versus-host disease: The cumulative incidence of grades II-IV and III-IV acute GvHD (aGvHD) was 44.8% **(Figure 1A)** (MSD/MUD-PB 66.7% vs haplo-BMT 19.2%; *P=0.039*)**(Figure 1B)** and 17.8% respectively (MSD/MUD-PB 33.3% vs haplo-BMT 10%; *P=0.196*) **(Figure 1B)** . The cumulative incidence of chronic GvHD (cGvHD) was 27.3% **(Figure 1C)**(MSD/MUD-PB 66.7% vs haplo-BMT 0%; *P=0.002*) **(Figure 1D)** and severe cGvHD was 9.1% respectively (**Figure 1C**). Of the six patients with cGVHD (1 mild, 3 moderate, 2 severe) three previously had grade III-IV aGvHD, two had grade II aGvHD and one developed *de novo* cGvHD seven months after MSD PBSCT while off immunosuppression. Five of six patients required systemic steroid therapy and three were also given ruxolitinib with resolution/improvement of their cGvHD symptoms. Two patients continue on cGvHD therapy.

Survival: Two patients (8.7%) required admission to the intensive care unit (ICU) within their first 100 days post-allo-HCT. One patient developed hyperammonemia due to *Ureaplasma parvum* with metabolic encephalopathy necessitating intubation and mechanical ventilation[24]. The other patient was transferred to ICU because of mucositis needing brief intubation for airway support. Only one patient with infant AML who underwent haplo-BMT (paternal donor) in CR1 with 1.8% minimal residual disease (MRD)+ relapsed 13.6 months following transplantation resulting in a cumulative incidence of relapse of 4.5%. This patient received a second haplo-BMT (maternal donor) 16.6 months after the first and remains in remission without GvHD 8.2 months post second haplo-BMT. With a median follow-up of 39.6 months (range 11 to 115 months) the OS is 100% and PFS 95.5% (**Figure 1E**). Taking into consideration both relapse and grade III-IV acute or chronic GvHD requiring systemic treatment, the GRFS is 67.8% (**Figure 1E**) with haplo-BMT recipients having significantly improved GRFS 83.3% compared to 40% in patients receiving MSD or MUD PBSCT (*P=0.025*) (**Figure 1F**).

Discussion

We describe our ten-year institutional experience in CAYA patients with myeloid malignancies undergoing allo-HCT on our pediatric service. This includes twenty-three consecutive patients of all risk categories who were conditioned with BU-FLU-MEL and received grafts from a variety of donor and stem cell sources. Thirty-nine percent of our patients were young adults (19 to 27 years). At a median follow-up of greater than 37 months, we have a remarkable OS of 100% and PFS of 95.5%. Irrespective of age, risk index, or treatment related disease, our OS and PFS is noticeably higher that previous reports of AML/MDS in CAYA undergoing allo-HCT.

There are several indications for allo-HCT in AML, including poor cytogenetics such as the presence of high allelic *FLT3/ITD* ratio, monosomy 5, monosomy 7, or an *MLL/KMT2A* rearrangement. A pediatric DRI

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has been developed to risk stratify children and adolescents with leukemia prior to transplant[21]. For AML, stratification into low, intermediate, high, and very high-risk groups is based upon cytogenetic risk, but also age and disease status complete remission (CR) and MRD. Using this stratification, 5-year leukemia free survival (LFS) was 78%, 53%, 40%, and 25% respectively with OS of 80%, 64%, 50%, and 33%[21]. Applying this DRI to our AML/MDS patient cohort, we had 2 low risk patients, 15 intermediate risk, 3 high, and 1 very high for which we would anticipate OS and PFS of 64% and 54% respectively at 36 months. Four of our patients had treatment related MDS/AML. A multicenter study of 273 pediatric patients with treatment-related MDS/AML undergoing MAC allo-HCT found the 5-year OS and EFS to be 49.9% and 41.2%[25]. A retrospective review of pediatric patients with poor cytogenetics who underwent allo-HCT (55 MSD and 55 unrelated donor [URD]), demonstrated a 5-year OS of 46% with MSD, 50% with URD[26]. These results are in line with what would be predicted based on the DRI. In a review of Center for International Blood and Bone Marrow Transplant Research (CIBMTR) data comparing allo-HCT outcomes of pediatric (<15 years) and adolescent and young adult (AYA) patients (15-40 years), similar survivals were noted[27]. For patients receiving MSD allo-HCT in first complete remission (CR1) the 5-year OS was 69% for pediatric patients compared to 52% for AYAs. The PFS was 65% and 51% respectively. For those receiving URD allo-HCT, decreased OS was seen with 34% for pediatric patients and 41% for AYAs. More recent CIBMTR data have shown improvement in 3-year probabilities of OS at 70% for AML patients <18 years receiving MSD in CR1 and 67% in those in CR2 or greater and 61% following MUD allo-HCT.

As there is still no consensus on optimal conditioning regimen for allo-HCT in CAYA with myeloid malignancies there have been several reports examining various regimens. A large multicenter study by Pediatric Disease Working Party of the European Group for Blood and Marrow Transplantation reported on pediatric patients with AML receiving TBI-CY (n=109), BU-CY (n=389) or BU-CY-MEL (n=133)[10]. The addition of MEL to BU-CY resulted in a reduced incidence of relapse of 14.7% when compared to 31.5% with BU-CY and 30% with TBI-CY as well as improved OS, 76.6% vs 64% with BU-CY, and 64.5% with TBI-CY as well as PFS 74.5 vs 58% vs 61.9% respectively with comparable NRM. There have been limited studies applying BU-FLU-MEL as conditioning in pediatric transplantation. We have previously reported on six patients receiving alternative donor allo-HCT conditioned with BU-FLU-MEL that was found to be safe and effective[11]. No grade 4 toxicities were seen, with grade 3 mucositis being the most common adverse effect. In a single institution study, pediatric (n=17) and adult patients with AML/MDS received BU-MEL-FLU and ATG followed by a T-depleted PBSC graft. The dose of BU and MEL was lower, 8-9.6 mg/kg and 140 mg/m^2 when compared to our regimen of 12.8 mg/kg and 180 mg/m^2 respectively for MSD and URD transplants[28]. The PFS was only 47.2% for pediatric patients. The incidence of grade III-IV aGvHD and cGvHD was low as one would expect from a T-cell depleted graft at 3.9% and 5.9% respectively with a relapse rate of 28%[28]. In a large multicenter retrospective report of 846 adult patients compared FLU-BU to FLU-BU-MEL conditioning in myeloid and lymphoid malignancies and found a significant improvement in the 5-year OS of 34.2% vs 30.1% and a decrease in relapse 30.8% vs 38%[9]. Similarly, a retrospective single-center review of 39 adult patients reported on their use of FLU-BU-MEL in myeloid and lymphoid malignancies with BM, PB and UCB as stem cell sources, with a 2-year OS of 62% for their entire cohort and 44% in their high risk to very high-risk subset. The authors concluded that this was a safe and successful regimen considering the highest risk group included many chemo-resistant patients with active disease at the time of transplant[29].

We observed a significantly higher incidence of grades II-IV aGvHD in MSD and MUD PBSCT compared to patients undergoing haplo-BMT**(Figure 1B).** As expected with PBSC grafts, we saw a significantly increased cumulative incidence of cGvHD (1 mild, 2 moderate and 1 severe) and MUD allo-HCT (1 moderate, 1 severe) when compared to the anticipated lower occurrence in haplo-BMT with PT-CY, with none of the haplo-BMT patients developing cGvHD **(Figure 1D)** . Similarly, none of the three unrelated UCB transplant patients showed signs of cGvHD. Consequently, GRFS was superior in haplo-BMT patients when compared to our MSD/MUD PBSCT recipients **(Figure 1F)** . While aGvHD, and especially cGvHD, is well-known to occur more frequently with the use of PBSC grafts, the addition of MEL has also been associated with increased aGvHD when compared to non-MEL containing conditioning regimens. In an

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adult report in patients with hematologic malignancies receiving MUD or MSD PBSC, BM, or UCB grafts and either tacrolimus or cyclosporine GvHD prophylaxis, an increase in grade II-IV aGvHD was seen with BU-FLU-MEL compared to FLU-BU (52.6% *vs*40.8%) with a analogous protection from relapse (30.8% vs 39%)[9]. Moreover, pediatric AML patients receiving BU-CY-MEL had a higher incidence of grade III-IV aGvHD (17.6%) compared to those conditioned BU-CY alone (9.6%) with a parallel reduction in relapse of 14.7% and 31.5% respectively. Chronic GvHD was higher in PBSCs (34.9%) vs BM recipients (25.6%) as was observed in our series.

Our OS and PFS were similar, regardless of donor and stem cell source. In a recent single institution retrospective pediatric study, the outcomes of patients with hematologic malignancies (40% AML/MDS) undergoing MSD BMT (n=31) and MUD BMT (n-47) where compared to their haplo-PBSCT with PT-CY (n=26) recipients[30]. The OS (80.9%, 83.3% and 80.7%), PFS (66.5%, 70.2% and 73.8%) and the GRFS (62.1%, 44.6% and 61%) were not different between MSD, MUD and haplo respectively. However, cGvHD was significantly higher in MUD BMT recipients 31.7% compared to MSD BM 10% and haplo 9.2% despite use of PBSC grafts in the latter group. We have recently updated our experience of 31 CAYA patients with hematologic malignancies receiving haplo-BMT, which compares favorably to the above study with OS 85.6%, PFS 76.1% and GRFS 58.2% with our median follow-up of 32 months compared to only 12 months in the above study[13].

Our current study along with our previous reports and those of others confirm that haplo-BMT in CAYA is a viable alternative to MSD and MUD allo-HCT[12-15, 30, 31]. This study expands our experience with myeloablative BU-FLU-MEL in allo-HCT for myeloid malignancies. As we are a smaller pediatric HCT center, this report is limited by the relatively small number of patients, their heterogeneous transplant characteristics in terms of stem cell source, type of donor and GvHD prophylaxis regimen used and the retrospective nature of our study. Nonetheless, the favorable outcomes remain striking considering the disease risk in this ethnically and racially diverse population with myeloid malignancies undergoing allo-HCT. Of caution is the unacceptably high aGvHD and cGvHD seen in patients receiving MSD and MUD PBSC transplants with MTX + CSA GvHD prophylaxis. It remains to be determined whether the evolving application of PT-CY in the non-haplo setting will offer better control of GvHD in CAYA PBSCT recipients following BU-FLU-MEL without increasing their risk of relapse.

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Conflict of interest disclosure

There are no conflicts of interest, financial or otherwise, involving any of the authors regarding the submission or publication of this manuscript.

Authorship

LT, HP, MD, MM, SH treated the patients before HCT and co-wrote the manuscript. LS and KS co-managed the patients during HCT and edited the manuscript. MP reviewed the blood and bone marrow results and edited the manuscript. EK managed the patients throughout their HCT course, collected and analyzed the data and co-wrote the manuscript.

References

1. Creutzig U, Zimmermann M, Reinhardt D, Dworzak M, Stary J, Lehrnbecher T. Early deaths and treatment-related mortality in children undergoing therapy for acute myeloid leukemia: analysis of the multicenter clinical trials AML-BFM 93 and AML-BFM 98. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* . Nov 1 2004;22(21):4384-93. doi:10.1200/JCO.2004.01.191

2. Jastaniah W, Abrar MB, Khattab TM. Improved outcome in pediatric AML due to augmented supportive care. *Pediatric blood & cancer* . Nov 2012;59(5):919-21. doi:10.1002/pbc.24195

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3. Woods WG. Curing childhood acute myeloid leukemia (AML) at the half-way point: promises to keep and miles to go before we sleep.*Pediatric blood & cancer* . May 1 2006;46(5):565-9. doi:10.1002/pbc.20646

4. Flower A, Cairo MS. The evolution of allogeneic stem cell transplant for children and adolescents with acute myeloid leukemia. *Clin Adv Hematol Oncol* . Jan 2017;15(1):52-62.

5. Santos GW, Tutschka PJ, Brookmeyer R, et al. Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. *The New England journal of medicine* . Dec 1 1983;309(22):1347-53. doi:10.1056/NEJM198312013092202

6. Nagler A, Rocha V, Labopin M, et al. Allogeneic hematopoietic stem-cell transplantation for acute myeloid leukemia in remission: comparison of intravenous busulfan plus cyclophosphamide (Cy) versus total-body irradiation plus Cy as conditioning regimen–a report from the acute leukemia working party of the European group for blood and marrow transplantation. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* . Oct 1 2013;31(28):3549-56. doi:10.1200/JCO.2013.48.8114

7. Lee JH, Joo YD, Kim H, et al. Randomized trial of myeloablative conditioning regimens: busulfan plus cyclophosphamide versus busulfan plus fludarabine. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* . Feb 20 2013;31(6):701-9. doi:10.1200/JCO.2011.40.2362

8. Sora F, Grazia CD, Chiusolo P, et al. Allogeneic Hemopoietic Stem Cell Transplants in Patients with Acute Myeloid Leukemia (AML) Prepared with Busulfan and Fludarabine (BUFLU) or Thiotepa, Busulfan, and Fludarabine (TBF): A Retrospective Study. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* . Apr 2020;26(4):698-703. doi:10.1016/j.bbmt.2019.12.725

9. Shimomura Y, Hara M, Yamamoto H, et al. Adding melphalan to fludarabine and a myeloablative dose of busulfan improved survival after allogeneic hematopoietic stem cell transplantation in a propensity score-matched cohort of hematological malignancies. *Bone marrow transplantation* . Jul 2021;56(7):1691-1699. doi:10.1038/s41409-021-01217-w

10. Lucchini G, Labopin M, Beohou E, et al. Impact of Conditioning Regimen on Outcomes for Children with Acute Myeloid Leukemia Undergoing Transplantation in First Complete Remission. An Analysis on Behalf of the Pediatric Disease Working Party of the European Group for Blood and Marrow Transplantation. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* . Mar 2017;23(3):467-474. doi:10.1016/j.bbmt.2016.11.022

11. Katsanis E, Sapp LN, Pelayo-Katsanis L, Whitney K, Zeng Y, Kopp LM. Alternative Donor Hematopoietic Cell Transplantation Conditioned With Myeloablative Busulfan, Fludarabine, and Melphalan is Well Tolerated and Effective Against High-risk Myeloid Malignancies. *Journal of pediatric hematology/oncology* . Nov 2016;38(8):e315-e318. doi:10.1097/MPH.0000000000000621

12. Katsanis E, Maher K, Roe D, Simpson RJ. Progressive substitution of post-transplant cyclophosphamide with bendamustine: A phase I study in haploidentical bone marrow transplantation. *eJHaem* May 26, 2020 2020;1(1)(May 26):286-292. doi:10.1002/jha2.20

13. Katsanis E, Stea B, Kovacs K, et al. Feasibility and efficacy of partially replacing post-transplant cyclophosphamide with bendamustine in pediatric and young adult patients undergoing haploidentical bone marrow transplantation. *Transplant Cell Ther* . Apr 20:S2666-6367(22)01230-1 2022;Apr 20:S2666-6367(22)01230-1; 10.1016/j.jtct.2022.04.015doi:10.1016/j.jtct.2022.04.015

14. Katsanis E, Sapp LN, Varner N, Koza S, Stea B, Zeng Y. Haploidentical Bone Marrow Transplantation with Post-Transplant Cyclophosphamide/Bendamustine in Pediatric and Young Adult Patients with Hematologic Malignancies. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* . Oct 2018;24(10):2034-2039. doi:10.1016/j.bbmt.2018.06.007

15. Katsanis E, Sapp LN, Reid SC, Reddivalla N, Stea B. T-Cell Replete Myeloablative Haploidentical Bone

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Marrow Transplantation Is an Effective Option for Pediatric and Young Adult Patients With High-Risk Hematologic Malignancies. *Front Pediatr* . 2020;8:282. doi:10.3389/fped.2020.00282

16. 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. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* . Mar 2015;21(3):389-401 e1. doi:10.1016/j.bbmt.2014.12.001

17. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone marrow transplantation* . Jun 1995;15(6):825-8.

18. Clark JR, Scott SD, Jack AL, et al. Monitoring of chimerism following allogeneic haematopoietic stem cell transplantation (HSCT): technical recommendations for the use of short tandem repeat (STR) based techniques, on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. *British journal of haematology* . Jan 2015;168(1):26-37. doi:10.1111/bjh.13073

19. Kaplan EL, Meier P. Nonparametric Estimation from Incomplete Observations. *Journal of the American Statistical Association* . 1958/06/01 1958;53(282):457-481. doi:10.1080/01621459.1958.10501452

20. Peto R, Peto J. Asymptotically Efficient Rank Invariant Test Procedures. *Journal of the Royal Statistical Society Series A (General)* . 1972;135(2):185-207. doi:10.2307/2344317

21. Qayed M, Ahn KW, Kitko CL, et al. A validated pediatric disease risk index for allogeneic hematopoietic cell transplantation. *Blood* . Feb 18 2021;137(7):983-993. doi:10.1182/blood.2020009342

22. Kanda J, Horwitz ME, Long GD, et al. Outcomes of a 1-day nonmyeloablative salvage regimen for patients with primary graft failure after allogeneic hematopoietic cell transplantation. *Bone marrow transplantation* . May 2012;47(5):700-5. doi:10.1038/bmt.2011.158

23. Katsanis E, Hennig T, Robinson JE, et al. Revisiting a single day salvage conditioning following graft failure *Bone marrow transplantation* . 2022;in press

24. Graetz R, Meyer R, Shehab K, Katsanis E. Successful resolution of hyperammonemia following hematopoietic cell transplantation with directed treatment of Ureaplasma parvum infection. *Transpl Infect Dis* . Apr 2018;20(2):e12839. doi:10.1111/tid.12839

25. Sharma A, Huang S, Li Y, et al. Outcomes of pediatric patients with therapy-related myeloid neoplasms. *Bone marrow transplantation* . Dec 2021;56(12):2997-3007. doi:10.1038/s41409-021-01448-x

26. Kelly MJ, Horan JT, Alonzo TA, et al. Comparable survival for pediatric acute myeloid leukemia with poor-risk cytogenetics following chemotherapy, matched related donor, or unrelated donor transplantation.*Pediatric blood & cancer* . Feb 2014;61(2):269-75. doi:10.1002/pbc.24739

27. Majhail NS, Brazauskas R, Hassebroek A, et al. Outcomes of allogeneic hematopoietic cell transplantation for adolescent and young adults compared with children and older adults with acute myeloid leukemia. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* . Jun 2012;18(6):861-73. doi:10.1016/j.bbmt.2011.10.031

28. Spitzer B, Jakubowski AA, Papadopoulos EB, et al. A Chemotherapy-Only Regimen of Busulfan, Melphalan, and Fludarabine, and Rabbit Antithymocyte Globulin Followed by Allogeneic T-Cell Depleted Hematopoietic Stem Cell Transplantations for the Treatment of Myeloid Malignancies. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* . Dec 2017;23(12):2088-2095. doi:10.1016/j.bbmt.2017.07.004

29. Edahiro T, Kawase T, Nagoshi H, et al. Allogeneic hematopoietic cell transplantation using fludarabine plus myeloablative busulfan and melphalan confers promising survival in high-risk

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hematopoietic neoplasms: a single-center retrospective analysis. *Hematology* . Dec 2021;26(1):186-198. doi:10.1080/16078454.2021.1881228

30. Srinivasan A, Raffa E, Wall DA, et al. Outcome of Haploidentical Peripheral Blood Allografts Using Post-Transplantation Cyclophosphamide Compared to Matched Sibling and Unrelated Donor Bone Marrow Allografts in Pediatric Patients with Hematologic Malignancies: A Single-Center Analysis. *Transplant Cell Ther* . Mar 2022;28(3):158 e1-158 e9. doi:10.1016/j.jtct.2021.11.009

31. Symons HJ, Zahurak M, Cao Y, et al. Myeloablative haploidentical BMT with posttransplant cyclophosphamide for hematologic malignancies in children and adults. *Blood Adv* . Aug 25 2020;4(16):3913-3925. doi:10.1182/bloodadvances.2020001648

Legends to figures

Figure 1. Kaplan-Meier estimates of acute and chronic graft-versus host disease and survival. **(A)** Cumulative incidence of grades II-IV and III-IV aGvHD; **(B)** Comparisons of cumulative incidence of grades II-IV and III-IV aGvHD between MSD and MUD PBSCT versus haplo-BMT; **(C)** Cumulative incidence of all cGvHD and severe cGvHD; **(D)** Comparisons of cumulative incidence of cGvHD and severe cGvHD between MSD and MUD PBSCT versus haplo-BMT;**(E)** Probability of OS, PFS, and GRFS; **(F)** Comparison of probability of GRFS between MSD and MUD PBSCT versus haplo-BMT

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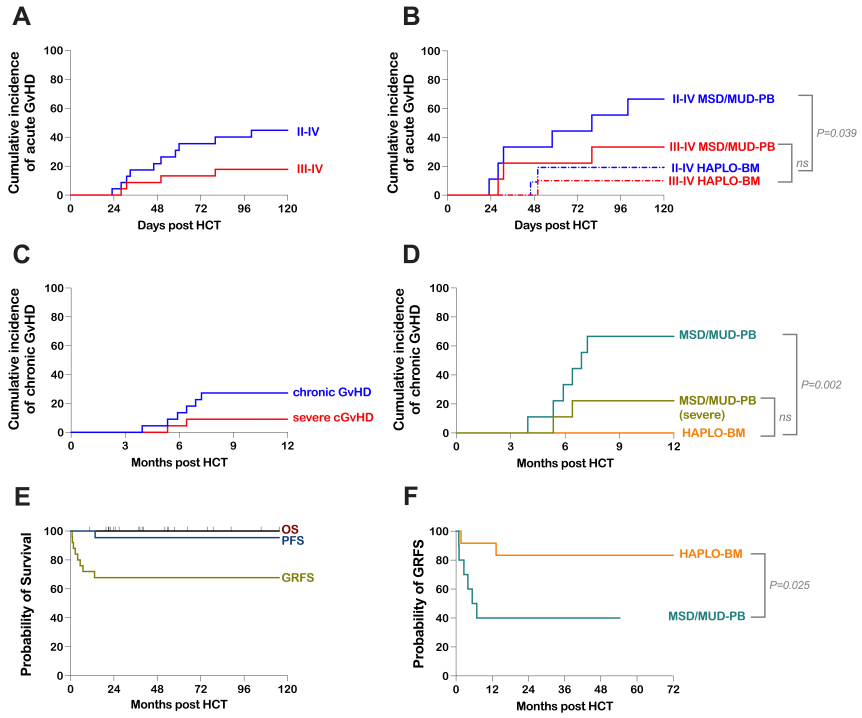

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