Uniform conditioning regardless of donor in bone marrow transplantation for severe aplastic anemia

For nearly 40 years, the treatment of choice for young patients with severe aplastic anemia (SAA) has been matched sibling donor (MSD) bone marrow transplantation (BMT). This medical preference was related to rapid recovery of hematopoiesis, minimal complications, mitigation of clonal evolution rates, and impressive rates of overall survival.[1] Cyclophosphamide (50 mg/kg/day × 4 days) with or without ATG, has traditionally been used as conditioning before MSD BMT. Although this regimen is non-myeloablative, the immunosuppression is sufficient to allow engraftment in most cases. Avoidance of total body irradiation and busulfan has been continued for MSD BMT to reduce transplant-related complications such as mucositis, graft-*versus*-host disease (GVHD), second malignancies, and infertility. However, several conditioning regimens as well as varied GVHD approaches can be used for BMT in AA. The increasingly chosen donor is often a haploidentical, given they are more common, but a common question often arises as to how to condition and prevent GVHD in the patient with a fully matched sibling or fully matched unrelated donor. Additionally, mixed chimerism or late secondary graft failure remain obstacles to longer-term successful outcomes in BMT for SAA.[3] Survival rates following MSD allogeneic BMT have steadily improved since the 1970s largely because of improved supportive care, refined HLA-typing, and better GVHD prophylaxis.[2] However, late BMT-related complications such as chronic GVHD occur in up to one-third of patients, with many of these patients requiring long-term therapy for their GVHD. In patients under 30 years of age, the event-free survival after HLA-matched sibling BMT ranges from 70% to 90%. These reduced survival rates are predominantly due to GVHD (which steadily increases with age) and late graft failures.[3] These outcomes remain ripe for improvement.

More recent research efforts in SAA BMT have focused on transplant feasibility using mismatched donors to expand the donor pool. The most promising approach to facilitate engraftment and mitigate the risk of GVHD is the use of post-transplant cyclophosphamide (PTCy) for GVHD prophylaxis. These regimens have also augmented the total body irradiation dosing from 200 cGy to 400 cGy in 2019 to enhance engraftment as learned from sickle disease and myelodysplastic syndrome regimens. These studies demonstrated success with standardized conditioning and intensive PTCy-based GVHD prophylaxis for haploidentical donors with >90% full donor chimerism and <10% GVHD.[4,5] Given the disease rarity, there have been no comparative studies of conditioning or GVHD prophylaxis, but an important issue remains in 2023 with matched donors, especially in adult patients, where we still need to minimize any complications with BMT, namely cGVHD, mixed chimerism, relapse or clonal evolution, and other transplant-related complications. Here, we report the outcomes using the established haploidentical approach to BMT in SAA,[4,6] for patients with related or unrelated fully matched donors. The numbers are few, given haploidentical donors are more often selected, but we show both feasibility and efficacy.

We treated 11 adult patients with treatment-naïve or refractory SAA patients using matched donor marrow grafts (related and unrelated) with the Baltimore regimen as previously published[7] (Table 1). Rabbit anti-thymocyte globulin (rATG, Thymoglobulin©) dosed at 0.5 mg/kg on day -9 and 2 mg/ kg on days -8 and -7 intravenously (IV). Fludarabine was administered at 30 mg/m^2 IV daily for 5 days, from day -6 to day -2 (total dose received 150 mg/m^2). Cyclophosphamide was given at 14.5 mg/kg IV daily for 2 days from day -6 to day -5 and administered as a 1-2-hour infusion (total dose received 29 mg/kg) and total body irradiation was delivered in a single fraction of 200 cGy on day -1 until augmented this to a single fraction of 400 cGy on day -1 institutionally after first eight patients. This was an institutional change based on noted graft failures in an upfront study of haploidentical donors using the same conditioning regimen.[5] The marrow graft was infused on day 0. Granulocyte colony stimulating factor was given on day +5 at 5 μg/kg/day and continued until absolute neutrophil count was greater than 1.5x10^9/L for 3 days. Post-transplant GVHD prophylaxis included PTCy administered at 50 mg/kg/day IV on days +3 and +4, mycophenolate mofetil orally given at a dose of 15 mg/kg three times a day up to 1 gm three times a day starting on day -3 (max dose 3,000 mg/day) from day 5 through 35 and tacrolimus orally or IV was given starting day 5 to maintain a level of 10-15 ng/mL. Tacrolimus was discontinued in patients without GVHD initially on day 365 in the first ten patients and then stopped day 180 in the last patient as is now done for all haploidentical patients. There was not standardization of Cytomegalovirus prophylaxis, rather initiation of therapy when viremia present.

The median follow-up of this cohort is 53.8 (range, 6-99) months. The overall survival for 11 patients is 100% at 1, and 2 years, and 90% (95% confidence interval [CI]: 73-100) at 3 years. There was no primary graft failure. One patient died from complications of gastric adenocarcinoma diagnosed 2 years post BMT. At the time of death,

Table 1. Demographics of 11 patients and donors.

Patient characteristics	**Total N=11**
Sex, N (%)	
Female	5 (45)
Male	6 (55)
Self-identified as minority, N (%)	3 (27)
Age in years	
Mean (SD)	32 (12)
Median (range)	25 (24-38)
Very severe aplastic anemia diagnosis (absolute neutrophil <0.2x10^9/L), N (%)	6 (55)
Severe aplastic anemia diagnosis (absolute neutrophil <0.5x10^9/L), N (%)	5 (45)
Treatment-naïve, N (%)	5 (45)
Refractory, N (%)	6 (55)
Clonality at baseline (PNH clone or molecular data including karyotype), N (%)	11 (100)
Total body irradiation dose, N (%)	
200 cGY	4 (36)
400 cGy	7 (64)
Donor characteristics	**Total N=11**
Age in years	
Mean (SD)	29 (6)
Median (range)	29 (28-32)
Relationship, N (%)	
Sibling	4 (36)
Unrelated 10/10	4 (36)
Unrelated 9/10	3 (27)
Sex, N (%)	
Female	2 (18)
Male	9 (82)
Reason for use of non-haploidentical related donor, N (%)	
Donor specific antibodies to related donors	0
No available related donors (i.e., adopted, parent with illness, childlessness)	7 (64)
Patient above age 25 years with available matched sibling	4 (36)

SD: standard deviation; PNH: paroxysmal nocturnal hemoglobinuria.

he was transfusion independent without GVHD and had >80% donor chimerism in the whole blood. This mixed chimerism was considered secondary graft loss after adenovirus infection but did not result in relapse of his SAA. The median CD34$^+$ cell count of all grafts was 3.86x10^6/kg recipient ideal body weight (range, 1.90-8.30x10^6). The median time to neutrophil recovery was 17 (range, 14-41) days. The day 28 cumulative incidence of neutrophil recovery was 91% (95% CI: 69-100). The median time to platelet recovery was 26 days with 91% transfusion independence by day 100. The median time to red cell recovery was 31 days with 91% transfusion independence by day 100. This is quite consistent with published timelines from other donor sources.[7] Ten of the 11 patients had sustained >95% donor chimerism in both whole blood and CD3 compartments through 1 year. Nine (81%) patients experienced infections post-transplant. Of a total of 14 infection events, 11 were grade 2 and three were grade 3. Two of the documented grade 3 infections occurred in the patient with secondary graft failure from adenovirus. Two patients experienced Cytomegalovirus reactivation evidenced by viremia treated to undetectable by day 100, and no patients had Epstein-Barr virus. The cumulative incidence of grade 2-4 acute GVHD at day 100 was 9% (95% CI: not applicable [NA]-27) while the cumulative incidence of chronic GVHD at 2 years is 19% (95% CI: NA-45) and none was beyond score 2 skin and mouth and lacked any other organ scoring. The two patients with chronic GVHD were off all treatment by months 13 and 17 post BMT, respectively. As all other patients are beyond 6 months follow-up, they are all off immunosuppression. We followed patients clinically for early events so lack longer-term data on fertility, other secondary malignancies, and extended chimerism data. The role of the BMT for the patient that died of a solid malignancy was less clear but could have contributed. This patient had an unrevealing work-up for inherited causes of disease as there was the presence of a paroxysmal nocturnal hemoglobinuria clone, his telomere length in the lymphocytes was at the tenth percentile when white blood cell count was very low, and his next-generation sequencing did not identify targeted mutations. He had a history of significant gastroesophageal reflux since infancy and esophageal evaluations akin to Barrett's disease so it is possible this contributed to his malignancy risk.

The choice to use a matched donor in each of these pa-

tients was related to a lack of suitable haploidentical donor (Table 1). All patients were believed to have acquired disease. Anecdotally, another refractory acquired SAA patient was treated at our institution with this identical platform using an 8/10 unrelated donor peripheral blood graft, given very high levels of donor-specific antibodies to all available related donors, and this 55 years SAA patient also had full engraftment and no GVHD and is doing well at 3 years post transplant. Thus, this platform may even be further extended to mismatched unrelated donors and a peripheral blood stem cell source. However, augmentation to 400 cGY was first used in SAA in the treatment-naïve haploidentical setting[5] to ensure engraftment and 200 cGy in the matched donor setting may be sufficient to allow engraftment and preserve fertility, as has been seen in the refractory setting.[4]

Given that more than 40% of patients initially treated with immunosuppression will ultimately need a transplant[8] due to relapse or clonal evolution, the use of BMT as the therapeutic approach should be consistent. There is merit in a uniform condition approach in all patients, regardless of the marrow donor's relationship or matched status, to maintain clinical expertise and consistent outcomes. Experience[9] and consistency of a regimen as described here will allow uniform management and follow-up for relevant clinical outcomes. Upfront use of this regimen was suggested by the BMT CTN State of the Science Symposium 2021 as the next needed innovation in the non-malignant field,[10] and a trial using this exact approach for both haploidentical and unrelated donors is anticipated later in 2024. In summary, the Hopkins group uses ATG, fludarabine, cyclophosphamide, and PTCy always with 400 cGy in the upfront and refractory settings as well as with mismatched and matched donors.[7] Rarely do we use immunosuppressive therapy for SAA patients anymore, given this platform has durability, no early transplant-related mortality, faster hematopoietic recovery, low rates of acute and chronic GvHD, and higher treatment-free remissions. Additional investigations will provide valuable data to determine the effects on fertility and secondary malignancies as well as relevance of noted differences in financial cost in comparison to IST.[11] The forthcoming multicenter BMT CTN 2207 study may establish this intensive GVHD prophylaxis and augmented conditioning as the standard in all SAA bone marrow transplantation, regardless of donor.

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Contributions

AED, RJJ and RAB performed research. MZ performed data analysis. AED wrote the manuscript and supervised the study. All authors reviewed, edited, and approved the manuscript.

Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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