Epigenetic Aging and Hematopoietic Cell Transplantation in Patients with Severe Aplastic Anemia

Rotana Alsaggaf, Ph.D., M.S.¹, Shilpa Katta, M.S.², Tao Wang, Ph.D.³,⁴, Belynda D. Hicks, M.S.², Bin Zhu, Ph.D.⁵, Stephen R. Spellman, M.S.³, Stephanie J. Lee, M.D., M.P.H⁶,⁷, Steve Horvath, Ph.D., Sc.D.⁸, Shahinaz M. Gadalla, M.D., Ph.D.¹

¹Clinical Genetics Branch, Division of Cancer Epidemiology & Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
²Cancer Genomics Research Laboratory, Division of Cancer Epidemiology & Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
³Center for International Blood and Marrow Transplant Research, Medical College of Wisconsin, Milwaukee, WI, USA
⁴Division of Biostatistics, Medical College of Wisconsin, Milwaukee, WI, USA
⁵Biostatistics Branch, Division of Cancer Epidemiology & Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
⁶Center for International Blood and Marrow Transplant Research, Minneapolis, MN, USA
⁷Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
⁸David Geffen School of Medicine, University of California, Los Angeles, CA, USA

Abstract

Cellular aging in hematopoietic cell transplantation (HCT) is important in the context of immune reconstitution and age-related complications. Recently, several DNA-methylation (DNAm) based biomarkers of aging known as “epigenetic clocks” have been introduced as novel tools to predict cellular age. Here, we used Cox proportional hazards models to assess the possible associations of donor pre-HCT DNAm age, and its post-HCT changes, using the recently published lifespan-associated epigenetic clock known as “DNAm-GrimAge,” with outcomes among patients with...
severe aplastic anemia (SAA). The study included 732 SAA patients from the Transplant Outcomes in Aplastic Anemia (TOAA) project, who underwent unrelated donor HCT and for whom a donor pre-HCT blood DNA sample was available; 41 also had a post-HCT sample collected at day 100. In multivariable analyses, we found similar associations for donor chronological age and pre-HCT DNAm-GrimAge with post-HCT survival (hazard ratio [HR]-per decade= 1.13, 95% confidence interval [CI]=0.99–1.28, p=0.07 and HR=1.14, 95% CI=0.99–1.28, p=0.06 respectively). In donors with 10+ years of GrimAge acceleration (i.e., deviation from expected DNAm age for chronological age), elevated risks of chronic graft vs. host disease (HR=2.4, 95% CI=1.21–4.65, p=0.01) and possibly post-HCT mortality (HR=1.79, 95% CI=0.96–3.33, p=0.07) were observed. In the subset with post-HCT samples, we observed a significant increase in DNAm-GrimAge in the first 100 days post-HCT (median change=12.5 years, range=1.4 to 26.4). Higher DNAm-GrimAge post-HCT was associated with inferior survival (HR-per year=1.11, 95% CI=1.02–1.21, p=0.01), predominantly within the first year post-HCT. This study highlights the possible role cellular aging may play in HCT outcomes.

Keywords
Severe Aplastic Anemia; Epigenetic clock; GrimAge; DNA methylation age; Survival; graft vs. host disease

Introduction
Severe aplastic anemia (SAA) is a rare and potentially fatal bone marrow failure disorder characterized by pancytopenia and marrow hypoplasia. SAA has several causes, with the majority of cases acquired through immune-mediated defects. Allogeneic hematopoietic cell transplantation (HCT) is a curative therapy for SAA patients; yet, outcomes among patients receiving HCT from unrelated donors is still inferior to matched sibling donor HCT (3-year survival rates in patients <18 years of age=79% vs. 93%, and in patients 18+ years=69% vs. 81%, respectively). Donor selection plays a major role in reducing HCT-related morbidity and mortality, with current practices prioritizing fully human leukocyte antigen (HLA)-matched donors followed by those of younger age. Large observational studies have reported improved survival among patients receiving unrelated donor HCT from younger donors, with a recent study reporting an approximately 3% increase in 2-year survival among HCT-recipients for every decreasing decade in donor age. In SAA patients, we have previously shown a survival benefit for selecting donors with longer telomere lengths (TL), a surrogate for cellular aging, independent of donor age and HLA-matching. Cellular senescence after HCT has been suggested to contribute to the pre-mature aging phenotype (e.g. frailty, cardiovascular disease, and malignancy) observed in HCT survivors, but biological evidence is still limited. A few studies with small sample sizes (range= 11–31 participants) have shown a leukocyte TL shortening ranging between 0.3–3 kb in the first year post-HCT (reflecting decades of aging based on an expected annual TL attrition rate of 0.03–0.05kb).
In recent years, DNA-methylation (DNAm) based biomarkers of aging known as “epigenetic clocks” have been introduced as novel tools to predict cellular age. Similar to TL, epigenetic clocks have been shown to outperform chronological age in predicting all-cause mortality and have been associated with mortality from cancer or cardiovascular disease, frailty and other age-related phenotypes in the general population. Studies of epigenetic aging in HCT are limited to a recent report of 23 patients with de-novo acute myeloid leukemia showing an average DNAm aging of 2.4 years per chronological year post-HCT, with a suggested relationship with chronic graft-versus-host disease (GvHD).

To better understand cellular aging and its dynamics in the context of HCT, we explored the possible role of DNAm age in donor selection, assessed DNAm age dynamics in the first 100 days post-HCT, and tested the association of this early post-HCT change with survival among SAA patients.

**Methods**

**Data source and study population**

We used blood DNA samples and clinical data from the Transplant Outcomes in Aplastic Anemia (TOAA) study, a collaboration between the National Cancer Institute (NCI) and the Center for International Blood and Marrow Transplant Research (CIBMTR) aiming to identify molecular predictors for improving HCT outcomes in patients with SAA. The CIBMTR captures clinical and transplant outcomes data for almost all allogeneic HCT and 80% of autologous transplants in the United States. Blood samples used in TOAA were retrieved from the CIBMTR biorepository. The TOAA study included SAA donor-recipient pairs with the following eligibility criteria: 1) had an available pre-HCT blood sample from both the recipient and donor in the CIBMTR biorepository; and 2) had high resolution HLA typing. In the current study, we included 732 SAA patients for whom a sufficient DNA from the donor was available for methylation profiling; all patients included in this study received unrelated donor HCT. Analyses of post-HCT changes in DNAm age were conducted within a subset of 41 recipient-donor pairs who had DNA extracted from a blood sample collected from the recipient 100 days post-HCT. All patients and donors provided informed consent and the study was approved by the National Marrow Donor Program (NMDP) institutional review board.

**DNA methylation and epigenetic age clock**

Whole blood (68%) or peripheral blood mononuclear cell (PBMC; 32%) samples were collected, processed, and stored at −80°C or in liquid nitrogen per standard operating procedures at the CIBMTR biorepository. Samples were transferred to the National Cancer Institute’s Cancer Genomic Research Laboratory (CGR) on dry ice where we utilized QIAamp Maxi Kits (QIAGEN Inc., Valencia, CA) to extract DNA from all samples. While at CGR, all samples went through an automated and tightly regulated DNA staging procedure; DNA volume was quantified, normalized, plated, and stored in a robotic automated cold storage unit (4°C). For DNAm profiling, we used the Illumina Infinium MethylatonEPIC Bead™ array which covers more than 850,000 methylation sites across the genome. We excluded samples where >4% of probes failed detection (n=2). No sex discordance was observed. We used functional normalization to account for potential batch effects.
effects using the “minfi” R package.\textsuperscript{27} We included 48 blinded duplicate samples to assess within and across plate differences and observed a high concordance rate (Pearson’s \( r \geq 0.98 \)).

DNAm age for this study was calculated using the most recent epigenetic clock known as “DNAm-GrimAge”, as previously described.\textsuperscript{28} Briefly, DNAm-GrimAge was developed using genome-wide DNA methylation levels and machine learned mathematical algorithms to select CpG sites most informative of lifespan (\textit{i.e.} time-to-death). This clock uses data from 1,030 unique CpG sites to produce a composite DNAm mortality risk estimator derived from the weighted average of smoking pack-years and seven lifespan-associated plasma proteins (adrenomedullin, beta-2-microglobulin, cystatin-C, growth differentiation factor 15, leptin, plasminogen activator inhibitor 1, and tissue inhibitor metalloproteinases 1). DNAm-GrimAge is presented in units of years based on a linear transformation of the mortality risk estimator. Here, we calculated donor pre-HCT DNAm-GrimAge and GrimAge acceleration, a measure that reflects deviation from expected DNAm-GrimAge based on one’s chronological age, where a positive GrimAge acceleration value indicates older biological age, while negative GrimAge acceleration indicates younger biological age compared to that expected for chronological age.\textsuperscript{28}

For the subset of patients with a post-HCT sample, we calculated the change in DNAm-GrimAge 100 days post-HCT as the difference between DNAm-GrimAge in the engrafted cells post-HCT and the donor’s pre-HCT sample.

**Statistical analysis**

We used Pearson’s correlation to evaluate the correlation between DNAm aging variables and donor or recipient chronological age. For survival analyses, we used the Kaplan-Meier estimator to calculate the survival probabilities and the exact log-rank test to compare the survival differences across groups. In multivariable analyses, we used Cox proportional hazards regression models to calculate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations between all-cause mortality post-HCT and donor chronological age, DNAm-GrimAge, or GrimAge acceleration (continuous variable or extreme agers \([ \geq 10 \text{ years}] \)), separately. The proportional hazards assumption was assessed using Schoenfeld residuals; no violations were observed. Follow-up time started at HCT and ended at the earliest of death, date of last follow-up, or end of study (August 2017). Variables included in the final models were selected by a stepwise procedure with thresholds of 0.05 for entry and retention in the model. All variables presented in Table 1, as well as methylation-imputed blood cell counts, were eligible for selection in the stepwise procedure. Final survival models were adjusted for recipient age and race, transplant year, intensity of conditioning regimen (myeloablative \textit{vs.} all others), Karnofsky performance score (KPS), HLA match (8/8 \textit{vs.} <8/8), and disease subtype (acquired or inherited).

We used the same statistical methods described above in evaluating the relationship between measures of donor DNAm-GrimAge and acute or chronic GvHD as outcomes. Models for acute GvHD were adjusted for transplant year, stem cell source, and Karnofsky performance score, and those for chronic GvHD were adjusted for recipient age, DNA source, and HLA matching.
To assess for heterogeneity by disease subtype, we conducted stratified analyses and observed no statistically significant differences of estimates across strata (Wald test p>0.05 for all models) (Supplementary Tables).

For the subset analyses where post-HCT samples were available, we used the Wilcoxon rank sum test to evaluate the statistical significance of the post-HCT change in DNAm-GrimAge. We then used stepwise multivariable Cox regression models, as described above, to evaluate the association between change in DNAm-GrimAge post-HCT [linear or in two categories: highest tertile (15+ years) vs. all others] and post-HCT all-cause mortality. This model was adjusted for conditioning regimen intensity (myeloablative vs. reduced intensity/non-myeloablative).

All tests were two-sided with statistical significance defined as p < 0.05. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and R version 3.6.0.

**Results**

**Patient characteristics**

Median age at transplant for SAA patients in this study was 19.6 years (range <1–77 years). SAA patients had a nearly equal sex distribution (55% male), and 58% received a transplant from donors of the same sex. Both donors and recipients were mostly white (71% and 80%, respectively). The majority of patients had acquired SAA (89%), a KPS of 90 or higher, an 8/8 high resolution HLA matched donor (61%) and received a bone marrow graft (82%). Forty percent of the patients in this study received a myeloablative conditioning regimen pre-HCT (~60% of which occurred before 2006). Of the 11% with inherited marrow failure, the majority were Fanconi anemia patients, who received HCT between 1989–2010, and 51% of whom received Fludarabine-based conditioning regimen. The most commonly used GvHD prophylaxis regimens were cyclosporin A (CSA) and methotrexate (35%) followed by tacrolimus and methotrexate (24%), with 54% of patients subsequently developing acute or chronic GvHD. Data on the use of antithymocyte globulin (ATG) were available for 482 patients, of whom 332 received ATG. More than half of the patients were alive at the last date of follow-up (57%) with a median follow-up time in survivors of 5.7 years. Table 1 summarizes demographic, clinical, and HCT characteristics of the donor-recipient pairs included in this study.

**Donor Pre-HCT DNAm-GrimAge and GrimAge acceleration in relation to post-HCT survival**

Donor chronological age was highly correlated with DNAm-GrimAge pre-HCT (r=0.85, p< 0.001) (Figure 1A), but not with GrimAge acceleration (r= −0.03, p=0.44). Of interest, GrimAge acceleration in donors showed only a small negative deviation from expected for chronological age (median= −0.5 years [range −10 to 16 years]).

In multivariable analyses, we observed similar associations for donor chronological age and pre-HCT DNAm-GrimAge with post-HCT survival (for every increasing decade, HR=1.13, 95%CI=0.99 to 1.28, p=0.07 and HR=1.14, 95% CI=0.99 to 1.32, p=0.06, respectively). Results from donor pre-HCT GrimAge acceleration showed no statistically significant linear
relationship with post-HCT survival (HR per decade=1.10, 95% CI=0.81–1.49, p=0.54); Further, the data suggested that those receiving HCT from donors with extreme GrimAge acceleration (10+ years older than expected; n=22) may be at higher risk of post-HCT mortality (HR=1.79, 95% CI=0.96–3.33, p=0.07), though results were not statistically significant (Table 2 and Figure 1B).

**Donor Pre-HCT DNAm-GrimAge and GrimAge acceleration in relation to risk of acute and chronic GvHD**

Neither donor pre-HCT DNAm-GrimAge nor GrimAge acceleration were associated with risk of acute GvHD (HR per decade=1.10, 95% CI=0.92 to 1.32, p=0.29 and HR=1.04, 95% CI=0.72 to 1.51, p=0.84, respectively). In contrast, higher DNAm-GrimAge was associated with excess risk of chronic GvHD (HR per decade=1.17, 95% CI=1.01 to 1.35, p=0.03). A higher risk of chronic GvHD was also noted in patients receiving HCT from donors with extreme GrimAge acceleration compared with all others (HR=2.4, 95% CI=1.21 to 4.65; p=0.01) (Table 3 and Figure 1C). Further adjusting for methylation predicted donor blood cell counts in all models did not change our findings.

**Post-HCT change in DNAm GrimAge and its relationship with post-HCT survival**

Post-HCT DNAm-GrimAge measured at day 100 was correlated with donor chronological age (r=0.79, p< 0.001) and pre-HCT donor DNAm-GrimAge (r=0.83, p< 0.001), but not correlated with recipient age (r=0.12, p=0.44). We observed a significant increase in DNAm-GrimAge at day 100 post-HCT, but wide variation existed (median change=12.5 years, range=1.4 to 26.4) (Figure 2A). Higher DNAm-GrimAge post-HCT was associated with inferior survival (HR-per year=1.11, 95% CI= 1.02 to 1.21, p=0.01). Patients in the highest tertile of post-HCT DNAm-GrimAge increase (15+ years; n=15) showed a five-fold excess risk of death compared with those who aged <15 years (HR=4.91, 95%CI=2.01 to 11.98, p<0.001), with 75% of deaths among those who aged 15+ years occurring in the first year post-HCT (Figure 2B). Our conclusions remained unchanged after controlling for post-HCT methylation predicted blood cell counts (p=0.047 and p<0.001, respectively).

**Discussion**

In this study of 732 SAA patients who underwent unrelated donor HCT, we found no added advantage for using pre-HCT donor DNAm-GrimAge or its acceleration over chronological age in survival prediction, except perhaps for donors with extreme age acceleration. SAA patients who received HCT from donors with extreme age acceleration were at high risk of developing chronic GvHD. An increase in post-HCT DNAm-GrimAge was observed in all evaluated 41 patients (range=1.4 to 26.4 years). This post-HCT aging at day 100 was associated with an 11% higher risk of death for every increasing year. Patients with 15+ years of DNAm-GrimAge increase after HCT had an approximately 5-fold increase in mortality compared with those who aged less than 15 years; this excess risk was most significant in the first year post-HCT.

The similar associations for donor DNAm-GrimAge and chronological age with post-HCT survival observed in the current study are in contrast to our previous finding of an
association between survival and donor TL (another marker for cellular aging). The relationship between epigenetic age and TL is not well understood; we found a modest correlation between TL and DNAm-GrimAge in a subset of 635 donors for whom TL was available (Pearson’s r = −0.27, p<0.001). Weak correlations between TL and other epigenetic clocks have been recently published.29,30 This suggests that the two measures likely capture different aspect of cellular aging. Surprisingly, we did not observe a significant association between post-HCT mortality and donor GrimAge acceleration, however, the elevated mortality risk noted in extreme agers (≥10 years; HR=1.79, p=0.07), albeit not statistically significant, warrants further investigation. It is important to note that GrimAge acceleration in donors was very limited (median=−0.5 years; only 3% had extreme GrimAge acceleration of 10 or more years). This supports the effectiveness of the current donor pre-screening strategy in selecting healthy individuals for peripheral blood stem cells or marrow donation.

We found that extreme GrimAge acceleration in donors was associated with patient risk of chronic GvHD. The relationship between cellular senescence and chronic GvHD has been suggested but evidence is still limited.31 A study of HCT long-term survivors showed shorter TL in those with chronic GvHD.32 Validation and follow-up on our findings in studies with a wider range of donor GrimAge acceleration as well as for other HCT indications may provide more insight into the utility of donor epigenetic age acceleration in donor selection.

In analysis focusing on DNAm-GrimAge change 100 days post-HCT, epigenetic aging was observed in all patients, ranging between 1.4 and 26.4 years. A previous study of 23 AML HCT recipients reported a drop in epigenetic age (using the Horvath pan-tissue clock) early post-HCT (up to day 178 post-HCT), followed by a significant increase of epigenetic age thereafter.26 Our results do not necessarily conflict with the previous finding, as we used a different epigenetic clock. The GrimAge clock used in the current study was based on life-span prediction (time to death) while the pan-tissue clock used in the previous study was purely based on age prediction.33 To investigate that, we assessed the change in epigenetic age using the pan-tissue clock and observed a similar decrease in post-HCT DNAm age on average (mean=−5.07 years), however, variation was noted (range=−15.2 to 3.4 years). Post-HCT cellular aging was first described in studies showing accelerated telomere attrition within the first year post-HCT.34,35 Telomere attrition post-HCT is known to be a consequence of the replicative stress caused by hematopoietic reconstitution, but the cause behind post-HCT epigenetic aging is expected to be more complex since it reflects dynamic changes throughout the genome.36 The small number of patients with a post-HCT sample limited our ability to identify variables predictive of post-HCT cellular aging. It is biologically plausible that cytotoxic effects of myeloablative conditioning may have a role in post-HCT cellular aging, though, a previous study showed similar TL attrition in patients receiving myeloablative or non-myeloablative conditioning.26 A larger study is needed to answer this question.

Higher post-HCT DNAm-GrimAge was associated with increased mortality, predominately within the first year post-HCT among patients with the highest tertile of epigenetic age increase (15+ years). Death from infection or graft failure were more frequent in those patients than others, indicating a possible role for DNAm aging in proper immune function,
however we did not have a sufficient sample size to conduct cause of death-specific analyses. We also were not able to rule out possible confounding by infused stem cell dose because of lack of information. Of interest, DNA-GrimAge has been shown to capture age-related changes in blood cell composition indicative of immunosenescence. Because immune reconstitution might vary between patients early post-HCT, it is possible that this may play a role in the observed post-HCT DNA-GrimAge changes, however, adjusting for donor or post-HCT methylation predicted blood cell counts did not change our findings. Larger studies with serial post-HCT samples are warranted to better describe the trajectory of post-HCT cellular aging and identify factors associated with its dynamics; of particular interest are modifiable factors related to donor selection and HCT procedure, therapeutics, or lifestyle interventions that can reverse this phenomenon. Resetting cellular age through epigenetic reprogramming is a growing area of research (reviewed by Kane and Sinclair). Of note, rapamycin treatment slowed epigenetic aging in mice, and growth hormone administration promoted thymic regeneration and reversed the epigenetic clock in a recent small human trial.

Strengths of this study included the relatively large sample size, prospective analysis to eliminate the potential for reverse causation, and the availability of detailed high-quality clinical information and biological samples. Nonetheless, the study had some limitations including the small number of patients with post-HCT samples; validation of our findings in a larger cohort is needed. Our study only included data from unrelated donors, so our results may not be applicable to patients receiving HCT from related donors. In addition, our study spanned a large number of years with transplants dating as far back as 1989; as preparative regimens pre-HCT and other transplant-related factors have changed over the years, this may limit the applicability of our results to current HCT practices. However, it is also important to note that our models considered transplant-related factors as well as transplant year in all analyses and our reported results are independent of those factors. Our findings warrant further studies in other HCT indications, particularly malignant diseases. Future studies to better understand the dynamics of epigenetic aging post-HCT may aid in identifying patients who may need closer follow-up and perhaps will give us an opportunity to test if suggested epigenetic age modifying therapeutics such as sirolimus (frequently used in HCT setting) may have the potential of reversing or slowing post-HCT pre-mature aging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Extreme epigenetic age acceleration in donors was associated with chronic GvHD
- Significant epigenetic aging was noted in the first 100 days post-HCT
- Epigenetic aging post-HCT was associated with inferior survival in SAA patients
Figure 1. (a).
Donor DNA-methylation (DNAm) GrimAge before hematopoietic stem cell transplantation (HCT) and its association with post-HCT outcomes in patients with severe aplastic anemia (a) Correlation and agreement between donor chronological age and DNAm-GrimAge pre-HCT
Figure 1. (b).
Donor DNA-methylation (DNAm) GrimAge before hematopoietic stem cell transplantation (HCT) and its association with post-HCT outcomes in patients with severe aplastic anemia (b) Kaplan Meier plot for post-HCT patient survival by donor GrimAge acceleration pre-HCT
Figure 1. (c).
Donor DNA-methylation (DNAm) GrimAge before hematopoietic stem cell transplantation (HCT) and its association with post-HCT outcomes in patients with severe aplastic anemia (c) Cumulative incidence curve for patient risk of chronic graft vs. host disease by donor GrimAge acceleration pre-HCT
Figure 2. (a).
Change in DNA methylation (DNAm)-GrimAge 100 days post-hematopoietic stem cell transplant among 41 severe aplastic anemia patients and its association with post-HCT survival
(a) Change in DNAm-GrimAge 100 days post-HCT
Figure 2. (b).
Change in DNAmethylation (DNAm)-GrimAge 100 days post-hematopoietic stem cell transplant among 41 severe aplastic anemia patients and its association with post-HCT survival
(b) Kaplan Meier plot for survival post-HCT by post-HCT change in DNAm-GrimAge
Table 1.
Characteristics of the 732 severe aplastic anemia donor-recipient pairs included in this study before hematopoietic stem cell transplantation (HCT)

| Characteristics                                      | All (n=732) | Post-HCT sample available (n=41) |
|-------------------------------------------------------|-------------|----------------------------------|
| Age variables at HCT (years)                          |             |                                  |
| Recipient chronological age                           | Median      | Range                            |
|                                                       | 19.6        | <1 to 77                         |
|                                                       | Median      | Range                            |
|                                                       | 18.4        | <1 to 46                         |
| Donor chronological age                               | 33.6        | 19 to 61                         |
|                                                       | 34.0        | 21 to 50                         |
| Donor GrimAge                                         | 36.2        | 18 to 64                         |
|                                                       | 33.1        | 21 to 51                         |
| Donor AgeAccelGrim                                    | −0.5        | −10 to 16                        |
|                                                       | −4.9        | −9 to 7                          |
| Donor sex                                             | N           | %                                |
| Male                                                  | 488         | 67%                              |
|                                                       | 30          | 73%                              |
| Female                                                | 244         | 33%                              |
|                                                       | 11          | 27%                              |
| Recipient sex                                         |             |                                  |
| Male                                                  | 405         | 55%                              |
|                                                       | 24          | 59%                              |
| Female                                                | 327         | 45%                              |
|                                                       | 17          | 41%                              |
| Donor/recipient sex match                             |             |                                  |
| Donor male/recipient male                             | 291         | 40%                              |
|                                                       | 20          | 49%                              |
| Donor male/recipient female                           | 196         | 27%                              |
|                                                       | 10          | 24%                              |
| Donor female/recipient male                           | 115         | 16%                              |
|                                                       | 4           | 10%                              |
| Donor female/recipient female                         | 130         | 18%                              |
|                                                       | 7           | 17%                              |
| Donor race                                            |             |                                  |
| White                                                 | 521         | 71%                              |
|                                                       | 33          | 80%                              |
| Other                                                 | 138         | 19%                              |
|                                                       | 7           | 17%                              |
| Unknown/missing                                       | 73          | 10%                              |
|                                                       | 1           | 2%                               |
| Recipient race                                        |             |                                  |
| White                                                 | 584         | 80%                              |
|                                                       | 33          | 80%                              |
| Other                                                 | 146         | 20%                              |
|                                                       | 8           | 20%                              |
| Unknown/missing                                       | 2           | 0%                               |
|                                                       | 0           | 0%                               |
| Indication for transplant                             |             |                                  |
| Acquired severe aplastic anemia                       | 650         | 89%                              |
|                                                       | 35          | 85%                              |
| Inherited marrow failure                              | 82          | 11%                              |
|                                                       | 6           | 15%                              |
| CMV matching                                          |             |                                  |
| Donor −/recipient −                                   | 215         | 29%                              |
|                                                       | 10          | 24%                              |
| Donor −/recipient +                                  | 214         | 29%                              |
|                                                       | 15          | 37%                              |
| Donor +/recipient −                                  | 97          | 13%                              |
|                                                       | 8           | 20%                              |
| Donor +/recipient +                                  | 175         | 24%                              |
|                                                       | 8           | 20%                              |
| Unknown/missing                                       | 31          | 4%                               |
|                                                       | 0           | 0%                               |
| Conditioning regimen intensity                        |             |                                  |
| Myeloablative*                                       | 293         | 40%                              |
|                                                       | 35          | 85%                              |
| Reduced intensity conditioning                        | 164         | 22%                              |
|                                                       | 5           | 12%                              |
| Non-myeloablative                                    | 147         | 20%                              |
|                                                       | 0           | 0%                               |
| Other                                                 | 128         | 17%                              |
|                                                       | 1           | 2%                               |
### Characteristics

| Characteristics                       | All (n=732) | Post-HCT sample available (n=41) |
|--------------------------------------|-------------|----------------------------------|
|                                     |             |                                  |
| **GvHD prophylaxis**                 |             |                                  |
| Tacrolimus-based                     | 280         | 3                                |
|                                     | 38%         | 7%                               |
| Cyclosporine A-based                 | 327         | 28                               |
|                                     | 45%         | 68%                              |
| Other                                | 118         | 10                               |
|                                     | 16%         | 24%                              |
| No prophylaxis                       | 7           | 0                                |
|                                     | 1%          | 0%                               |
| **Stem cell source**                 |             |                                  |
| Bone marrow                          | 599         | 40                               |
|                                     | 82%         | 98%                              |
| Peripheral blood stem cells          | 132         | 0                                |
|                                     | 18%         | 0%                               |
| Other                                | 1           | 1                                |
|                                     | 0%          | 2%                               |
| **Karnofsky performance score**      |             |                                  |
| <90                                  | 164         | 9                                |
|                                     | 22%         | 22%                              |
| ≥90                                  | 514         | 32                               |
|                                     | 70%         | 78%                              |
| Unknown/missing                      | 54          | 0                                |
|                                     | 7%          | 0%                               |
| **Number of high-resolution HLA match out of 8** | | |
| 8/8                                  | 447         | 20                               |
|                                     | 61%         | 49%                              |
| 7/8                                  | 163         | 10                               |
|                                     | 22%         | 24%                              |
| ≤6/8                                 | 101         | 11                               |
|                                     | 14%         | 27%                              |
| Unknown/missing                      | 41          | 0                                |
|                                     | 3%          | 0%                               |
| **Year of transplant**               |             |                                  |
| 1989–1996                            | 78          | 18                               |
|                                     | 11%         | 44%                              |
| 1997–1999                            | 77          | 23                               |
|                                     | 11%         | 56%                              |
| 2000–2005                            | 165         | 0                                |
|                                     | 23%         | 0%                               |
| 2006–2010                            | 260         | 0                                |
|                                     | 36%         | 0%                               |
| 2011–2014                            | 152         | 0                                |
|                                     | 21%         | 0%                               |

CMV= Cytomegalovirus; GvHD= Graft vs. host disease; HLA= human leukocyte antigen

* The most common myeloablative regimen used was total body irradiation/cyclophosphamide in the full cohort and in those included in the subset analysis.
Table 2.

Hazard ratios of the associations between donor aging variables before hematopoietic stem cell transplant (HCT) and all-cause mortality post-HCT in severe aplastic anemia patients

| Donor aging variable at HCT | Hazard Ratio* | 95% Confidence Interval | p-value |
|-----------------------------|---------------|-------------------------|---------|
| Chronological age           | 1.13          | 0.99 - 1.28             | 0.07    |
| DNAm-GrimAge                | 1.14          | 0.99 - 1.32             | 0.06    |
| GrimAge acceleration        | 1.10          | 0.81 - 1.49             | 0.54    |
| < 10 years                  | 1.79          | 0.96 - 3.33             | 0.07    |

* Hazard ratios per decade for continuous age variables; models were adjusted for recipient age and race, transplant year, conditioning regimen, Karnofsky performance score, HLA match, and disease subtype
Table 3.
Hazard ratios of the associations between donor aging variables before hematopoietic stem cell transplant (HCT) and chronic or acute graft vs. host disease (GvHD) in severe aplastic anemia patients

| Donor aging variable at HCT | Chronic GvHD |  | Acute GvHD |  |
|----------------------------|-------------|-----------------|-------------|-----------------|
|                            | Hazard Ratio | 95% Confidence Interval | p-value | Hazard Ratio | 95% Confidence Interval | p-value |
| Chronological age          | 1.13        | 0.99 - 1.28      | 0.06      | 1.10          | 0.94 - 1.30            | 0.25    |
| DNAm-GrimAge               | 1.17        | 1.01 - 1.35      | 0.03      | 1.10          | 0.92 - 1.32            | 0.29    |
| Age/AccelGrim              | 1.24        | 0.88 - 1.75      | 0.21      | 1.04          | 0.72 - 1.51            | 0.84    |
| < 10 years                 | Reference   |                 |           | Reference      |                         |         |
| ≥ 10 years                 | 2.38        | 1.21 - 4.65      | V 0.01    | 1.47          | 0.70 - 3.06            | 0.31    |

* Hazard ratios per decade for continuous age variables; models adjusted for recipient age, DNA source, and HLA matching

† Hazard ratios per decade for continuous age variables; models adjusted for transplant year, stem cell source, and Karnofsky performance score