haematologica
Journal of the Ferrata Storti Foundation

Epigenetic age acceleration in hematopoietic stem cell transplantation

by Margherita Ursi, Katarzyna Malgorzata Kwiatkowska, Chiara Pirazzini, Gianluca Storci, Daria Messelodi, Salvatore Nicola Bertuccio, Serena De Matteis, Francesco Iannotta, Enrica Tomassini, Marcello Roberto, Maria Naddeo, Noemi Laprovitera, Irene Salamon, Barbara Sinigaglia, Elisa Dan, Francesco De Felice, Francesco Barbato, Enrico Maffini, Sadia Falcioni, Mario Arpinati, Manuela Ferracin, Massimiliano Bonafé, Paolo Garagnani, and Francesca Bonifazi

Received: March 20, 2024.
Accepted: September 25, 2024.

Citation: Margherita Ursi, Katarzyna Malgorzata Kwiatkowska, Chiara Pirazzini, Gianluca Storci, Daria Messelodi, Salvatore Nicola Bertuccio, Serena De Matteis, Francesco Iannotta, Enrica Tomassini, Marcello Roberto, Maria Naddeo, Noemi Laprovitera, Irene Salamon, Barbara Sinigaglia, Elisa Dan, Francesco De Felice, Francesco Barbato, Enrico Maffini, Sadia Falcioni, Mario Arpinati, Manuela Ferracin, Massimiliano Bonafé, Paolo Garagnani, and Francesca Bonifazi. Epigenetic age acceleration in hematopoietic stem cell transplantation. Haematologica. 2024 Oct 3. doi: 10.3324/haematol.2024.285291 [Epub ahead of print]

Epigenetic age acceleration in hematopoietic stem cell transplantation

Margherita Ursi[1,2], Katarzyna Malgorzata Kwiatkowska[2], Chiara Pirazzini[2], Gianluca Storci[1], Daria Messelodi[1], Salvatore Nicola Bertuccio[1], Serena De Matteis[1], Francesco Iannotta[1], Enrica Tomassini[1], Marcello Roberto[2], Maria Naddeo[1], Noemi Laprovitera[1], Irene Salamon[1], Barbara Sinigaglia[1], Elisa Dan[1], Francesco De Felice[1,2], Francesco Barbato[1,2], Enrico Maffini[1], Sadia Falcioni[1], Mario Arpinati[1], Manuela Ferracin[1,2], Massimiliano Bonafè[1,2], Paolo Garagnani[1,2], Francesca Bonifazi[1,2]

[1]IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna Italy; [2]Department of Medical and surgical sciences (DIMEC) University of Bologna, Bologna, Italy;

#Correspondence: Massimiliano Bonafè
Department of Medical and surgical sciences (DIMEC) University of Bologna, Bologna, Italy; e-mail: massimiliano.bonafe@unibo.it

Acknowledgements: The authors thank AIL Bologna ODV, the Italian Association for research on leukemia, lymphoma and myeloma, for the support of the Laboratory of Immunobiology of Transplant and Cellular Therapies, IRCCS AOU di Bologna, Bologna, Italy, led by F. Bo., MD, PhD.

Running Title: Epigenetic age rate in allogeneic transplantation

Key words: Allogeneic stem cell transplantation, older recipients, aging, epigenetic age, age acceleration, graft versus host disease.

Funding: The work was funded to F.Bo. by Ricerca Corrente 2022-2024 (Code: RC-22000421), IRCCS AOU of Bologna, Italy.

Disclosures: FB: scientific advisory boards and speaker fees: Neovii, Novartis, Kite, Gilead, Pfizer, Celgene, Merck Sharp Dohme. MB: research grant from Neovii. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Contributions: Author contributions: F. Bo., P.G. and M.B: study design and study coordination. M.U., M.B., F. Bo., P.G. wrote the manuscript. P.G., C.P., K.K. made epigenetic analysis. M.U., M.R., F.D.F., F. Ba., E.M., S.F., M. A., F. Bo.: clinical assessment. G.S., D.M., N.S.B., S.D.M., E.T., M.N., N.L., I.S, B.S., E.D.: sample collection. F. Ba., M.U.: data entry. M.U., F.I., M.B., K.K.: statistical analysis. All authors have reviewed the manuscript and approved it for the submission.

Data sharing statement: This paper reports primary original data.

Trial registration: ClinicalTrials.gov Identifier: NCT03871296

Older recipients of allogeneic stem cell transplantation (HSCT) display a higher risk of non-relapse mortality (NRM)[1]. As the number of transplant procedures in the over 65-70 years old patients is increasing, physicians urgently need efficient assessment of the elderly patient's fitness. Nowadays, Sorror's comorbidity index (HCT-CI index)[2] is commonly used in clinical practice because of its extensive validation for NRM prediction as well as its easy and fast assessment. Biological age, a measure of the individual aging speed, that has been proved to be effective in predicting all-cause mortality in the general population,[3,4] might be informative of the patient's health status also in the context of HSCT. "Epigenetic clocks", based on DNA methylation assessment, have been widely applied to estimate biological age in several conditions[5]. In the setting of HSCT, epigenetic age has been described as an intrinsic property of transplanted human hematopoietic stem cells (HSC) [6] and a potential role of cellular aging into the clinical outcomes has been suggested, based on the observation that donors with accelerated epigenetic aging led to an elevated risk of graft versus host disease (GVHD)[7]. Notably, Stolzel et al. (2017) reported accelerated epigenetic aging of donor-derived HSC in eight years observation time after HSCT[8]. Moreover, a rapid shortening of telomere length in the first-year post HSCT has been reported.[9]

In the present study, we aim to test whether biological age acceleration, measured through a biological clock based on targeted DNA methylation measurement (henceforth referred to as "tDNAMet"), can provide additional insights into the biology of transplantation, and correlate with relevant clinical outcomes. tDNAmet, like other widely applied epigenetic clocks, includes both regions that are highly correlated with chronological age and regions with a weaker correlation. This allows the epigenetic clocks to pick up the characteristic features of physiological aging and, at the same time, the deviation from healthy trajectories. tDNAMet includes 6 genomic regions (ELOVL2, NHLRC1, SIRT7/MAFG, AIM2, EDARADD, TFAP2E), which harbour a total of 70 CpG sites, whose methylation level is assessed by EpiTYPER technology (Agena Bioscience, San Diego, CA, USA). The regions were selected by Gensous et al[10], who analysed the epigenetic profile of healthy subjects (ranging from 18 to 80 years old), together with accelerated- and decelerated-aging subjects (Down Syndrome and centenarians)[11,12]. In the present work, peripheral blood samples were collected from donors and patients undergoing allogeneic stem cell transplantation at the Advanced Cellular Therapies Program, IRCCS Bologna, between 2018 and 2021. Recipients' samples were collected at baseline and, then, at +30, +90, +180 and +360 days after HSCT. The study was approved by the local Ethics Committee (Comitato Etico Area Vasta Emilia-Romagna, File number: 151/2018/sper/AOUBo) and conducted according to the principles of Declaration of Helsinki on human rights. We analyzed DNA from at least 1 sample of 81 patients and 53 donors, for a total of 250 samples. The characteristics of the study population are shown in Table 1. The

epigenetic age of HSCT donors and recipients was calculated applying a mathematical model built on our cohort of 276 healthy subjects (CTR) already described by Gensous et al.[10] that were re-analyzed for the present study. This mathematical model that we applied assigns a different weight (coefficient) to the methylation value of the above mentioned 70 CpG sites (Supplementary Fig.1).

The aging acceleration value (AA), *i.e.* the measure of whether individuals are aging faster or slower than their chronological age[5], was determined for each subject as a continuous variable and then categorized as a dichotomous one as follows: negative value (AA-, anti-aging profile) when the inferred biological age is inferior than the chronological one, positive value (AA+, aging profile) in the opposite case (see Supplementary figure 2 for individual trends)**.** As expected, recipients were chronologically older than donors (median age: 55 vs 29 years, Mann Whitney (MW) test, $p<0.0001$). At admission time-point, recipients had a clearcut prevalence of aging profile (AA+), while the donor profile was predominantly anti-aging (AA-), both when the AA value was considered as a dichotomous variable (% of AA+: 57.1 vs 34.0, Fisher test, p=0.059) and when AA value was expressed as a continuous variable (median AA: +3.74 vs -3.83, MW test, p=0.023, Figure 1A). The AA value of peripheral blood leukocytes was then evaluated in a longitudinal analysis, starting at the pre-infusion timepoint up to 1 year after transplant. The samples collected from patients after HSCT showed full donor chimerism (*i.e.* 100% of cells were of donor origin) in FISH or STR analysis. The median AA value increased as follows: -2.00 at day+30, +0.94 at day+90, +0.1 at day+180, +3.14 at day+360 (Kruskal-Wallis test, p=0.007, Figure 1B). At day+360, when all the recipient leukocytes are of donor origin (full donor chimerism), the median AA value was +3.14, compared to -3.83 at pretransplant (MW test, p=0.0004), *i.e.* very close to the AA value of the recipients at pre-transplant (AA: +3.14 vs +3.74, MW test, p=0.84). These data allowed us to conclude that during the first year after transplant, an acceleration of cellular aging of about 7 folds occurs to transplanted cells. Similarly, the proportion of AA+ values showed a significant trend to increase from day +30 (45.6%) up to day+360 (71.4%, Cramer's V=0.254, p=0.005, Figure 2A). Notably, such an increase was more pronounced in the GVHD subgroup (Cramer's V=0.383, p=0.021, AA+ value, donor vs recipient at day+360: 25.0% vs 78.6 %, 3.14 folds, Fisher test, p=0.009, Figure 2B) than in the no GVHD group (Cramer's V=0.246, p=0.070, AA+ value, donor 37.8% vs recipient at day+360 67.9%: 1.80 folds, Fisher test, p=0.024, Figure. 2C). Interestingly, in our cohort the GVHD donors had a slightly lower AA at baseline vs no GVHD donors and this may be part of an inherent difference between the two groups.

At variance with literature data[13], we failed to find any statistical association between acute GVHD and chronological age or AA values, neither in recipients (no GVHD vs aGVHD, median years: 53

vs 60, MW test, p=0.106; median AA value: +3.94 vs -1.76, MW test, p=0.650), nor in donors (no GVHD vs GVHD, median years 29 vs 32, MW test, p=0.763, median AA value: -5.24 vs -2.66, MW test, p=0.723). In univariate analysis we found that AA value at admission had a significant impact on overall survival (admission AA value, HR=1.021, 95%CI: 1.000-1.1042, p=0.047) while in the same cohort of patients we did not find a significantly impact of other relevant clinical variables on OS (chronological age of donor and recipient, Sorror, disease phase, intensity of conditioning regimen,

donor type, age acceleration of donor).

To our knowledge, this is the largest study investigating the epigenetic age acceleration of patients undergoing allogeneic HSCT, where we applied for the first time an original tDNAmet, which proved to be capable of capturing relevant aspects of transplant biology. This study provides at least two insights: the first one is that tDNAmet was able to get the post-transplant age acceleration already described in previous studies[8,9]. Notably, the age acceleration degree previously observed in a mean 8 years post HSCT-follow up was reached in our case set within one year[8]. Moreover, at variance with a previous report[8], we did not detect any post-transplant rejuvenation of donor's cells once transplanted in recipients. Such results suggest that tDNAmet is extremely effective in measuring cell stress in the HSCT setting. We also observed that the post HSCT age acceleration occurs to a higher extent in GVHD patients than in no-GVHD ones.

This observation suggests that biological clocks are sensitive to both cellular proliferation and leukocyte activation that likely occur during GVHD[13,14], and that in the long run can lead to the pro-inflammatory drift named inflammaging[15]. The second insight of this approach is the assessment of the biological age of both donors and recipients before HSCT. On the one hand, tDNAmet analysis shows that donors are biologically younger than their chronological age, corroborating the strict selection that donors must undergo to ensure an exceptionally good health status. On the other hand, recipients show a consistent age acceleration phenotype, likely due to the disease itself and the related treatments, that in turn correlates with overall survival. This last observation supports the effort to test in larger populations the epigenetic age acceleration at baseline in order to investigate if it can play a role as an additional tool, besides chronological age and Sorror index, to evaluate the "fitness" for transplantation of the patient, especially in the elderly.

References

1. Maffini E, Ngoya M, Galimard JE, et al. Allogeneic hematopoietic cell transplantation for patients with AML aged 70 years or older in first remission. A study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplant. 2023;58(9):1033-1041.
2. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005;106(8):2912-2919.
3. Li X, Ploner A, Wang Y, Magnusson PK, et al. Longitudinal trajectories, correlations and mortality associations of nine biological ages across 20-years follow-up. Elife. 2020;9:e51507.
4. Tian YE, Cropley V, Maier AB, Lautenschlager NT, Breakspear M, Zalesky A. Heterogeneous aging across multiple organ systems and prediction of chronic disease and mortality. Nat Med. 2023;29(5):1221-1231.
5. Horvath S. DNA methylation age of human tissues and cell types. Genome Biol. 2013;14(10):R115.
6. Søraas A, Matsuyama M, de Lima M, et al. Epigenetic age is a cell-intrinsic property in transplanted human hematopoietic cells. Aging Cell. 2019;18(2):e12897.
7. Alsaggaf R, Katta S, Wang T, et al. Epigenetic Aging and Hematopoietic Cell Transplantation in Patients With Severe Aplastic Anemia. Transplant Cell Ther. 2021;27(4):313.e1-313.e8.
8. Stölzel F, Brosch M, Horvath S, et al. Dynamics of epigenetic age following hematopoietic stem cell transplantation. Haematologica. 2017;102(8):e321-e323.
9. Rufer N, Brümmendorf TH, Chapuis B, Helg C, Lansdorp PM, Roosnek E. Accelerated telomere shortening in hematological lineages is limited to the first year following stem cell transplantation. Blood. 2001;97(2):575-577.
10. Gensous N, Sala C, Pirazzini C, Ravaioli F, et al. A Targeted Epigenetic Clock for the Prediction of Biological Age. Cells. 2022;11(24):4044.
11. Horvath S, Garagnani P, Bacalini MG, et al. Accelerated epigenetic aging in Down

syndrome. Aging Cell. 2015;14(3):491-495.

12. Horvath S, Pirazzini C, Bacalini MG, et al. Decreased epigenetic age of PBMCs from Italian semi-supercentenarians and their offspring. Aging (Albany NY). 2015;7(12):1159-1170.

13. Ernst Holler, Hildegard Greinix, and Robert Zeiser. Acute-Graft-Versus-Host Disease. In: Anna Sureda, Selim Corbacioglu, Raffaella Greco, Nicolaus Kröger, Enric Carreras, Editors. The EBMT Handbook, Hematopoietic Cell Transplantation and Cellular Therapies. 2024, Springer, pp. 385-394

14. Zeiser R, Blazar BR. Acute Graft-versus-Host Disease - Biologic Process, Prevention, and Therapy. N Engl J Med. 2017;377(22):2167-2179.

15. Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000;908:244-254.

Table 1: Characteristics of the population

	Total n=81
Age recipient at HSCT (years)	
Median	55
Range, min-max	18-71
Gender, n (%)	
Female	32 (39,5)
Male	49 (60,5)
HCT-CI Sorror, n (%)	
Score 0	38 (46,9)
Score 1	7 (8,7)
Score 2	10 (12,3)
Score ≥ 3	26 (32,1)
Disease, n (%)	
AL	53 (65,4)
MDS/MPN	16 (19,8)
LYM	9 (11,1)
MM	2 (2,5)
SAA	1 (1, 2)
Therapy lines before HSCT, n	
Median	2
Range, min-max	0-5
Time from diagnosis to HSCT (months)	
Median	11
Range, min-max	2-182
Status disease at HSCT, n (%)	
Early	55 (67,9)
Advanced	26 (32,1)
Donor, n (%)	
MRD	10 (12,3)
MUD	38 (46,9)
MMUD	26 (32,1)
HAPLO	7 (8,6)
Donor age (years)	
Median	29
Range, min-max	19-63
Source, n (%)	
PBSC	69 (85,2)
BM	12 (14,8)
Conditioning, n (%)	
MAC	44 (54,3)
RIC	37 (45,7)
GVHD prophylaxis, n (%)	
CSA+MTX/MMF+ATLG	70 (86,4)
FK+MTX/MMF+ATLG	4 (5)
FK+MMF+PT-CY	7 (8,6)
aGVHD, n (%)	
No	52 (64,2)
Yes (any grade)	29 (35,8)

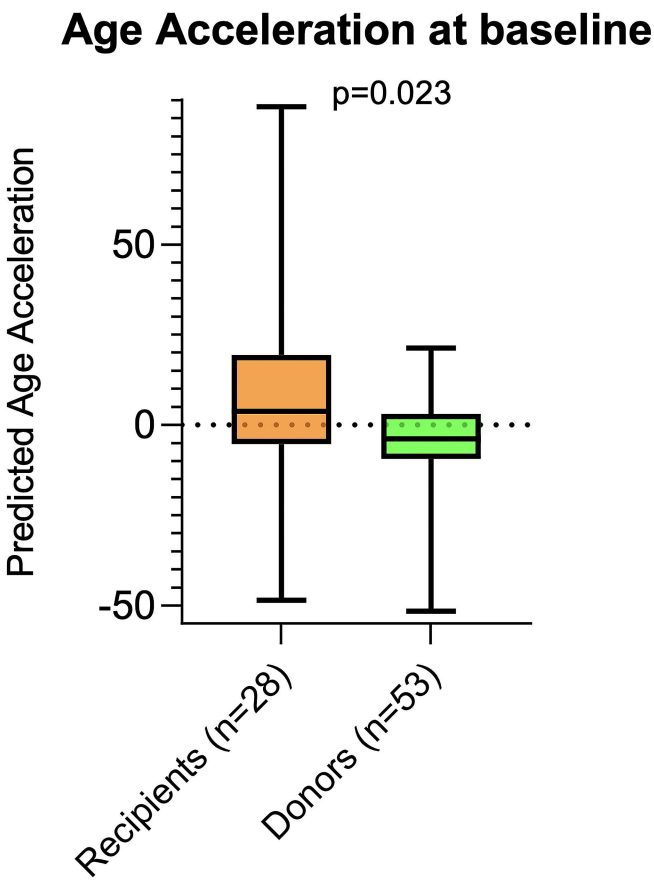
HSCT, hematopoietic stem cell transplantation; HCT-CI, Hematopoietic Cell Transplantation specific Comorbidity Index (HCT-CI); AL, Acute Leukemias; MDS/MPN, Myelodysplastic Syndromes/Myeloproliferative Neoplasms; LYM, Lymphoma; MM Multiple Myeloma; SAA Severe Aplastic Anemia; MRD, matched-related donor; MUD, matched-unrelated donor; MMUD mismatched unrelated donor; HAPLO, haploidentical donor; PBSC, peripheral blood stem cells; BM bone marrow; MAC, myeloablative conditioning; RIC reduced-intensity conditioning; CSA cyclosporine; MTX methotrexate; ATLG, anti-T lymphocyte globulin; FK, tacrolimus; MMF, mycophenolate mofetil; PT-CY, post-transplant cyclophosphamide; aGVHD, acute Graft Versus Host Disease. Sum of % might be different from 100 due to rounding

Figure Legends:

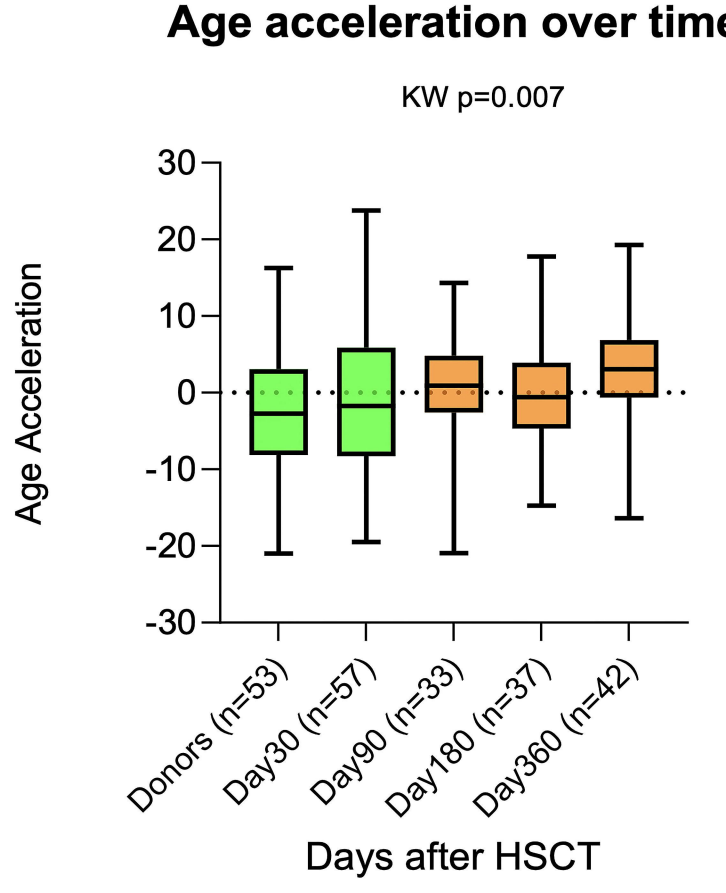
Fig.1 Age acceleration (AA) throughout the first year: Age acceleration (AA) expressed as a continuous variable in recipients vs donors at baseline (A) and in recipients (donors' cells) throughout the first year post transplant (B), Mann-Whitney and Cramer V test, respectively.

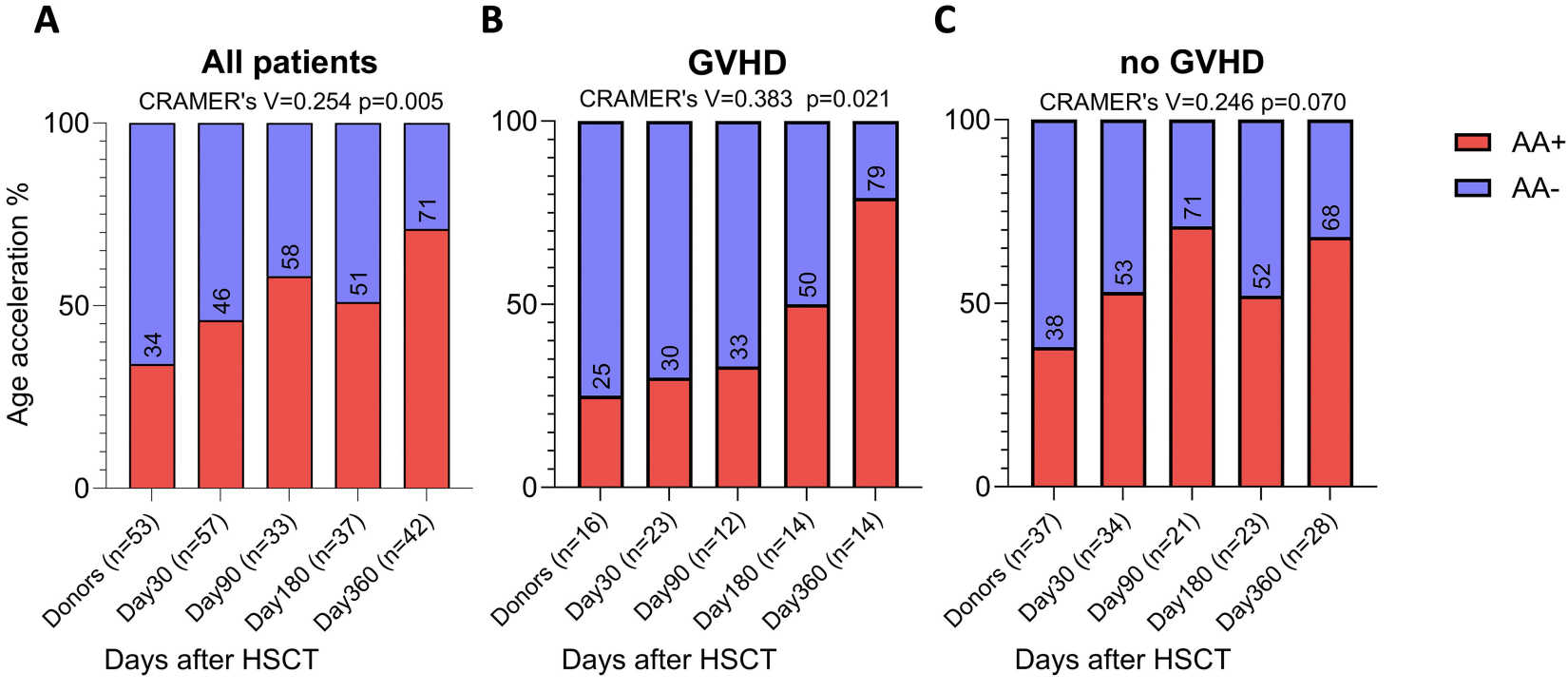
Fig.2 Age Acceleration (AA) and Acute Graft versus Host Disease (GVHD): Age acceleration (AA) expressed as percentage throughout the transplantation time in all patients (A), in those who developed acute GVHD (B) or did not develop acute GVHD (C)

A

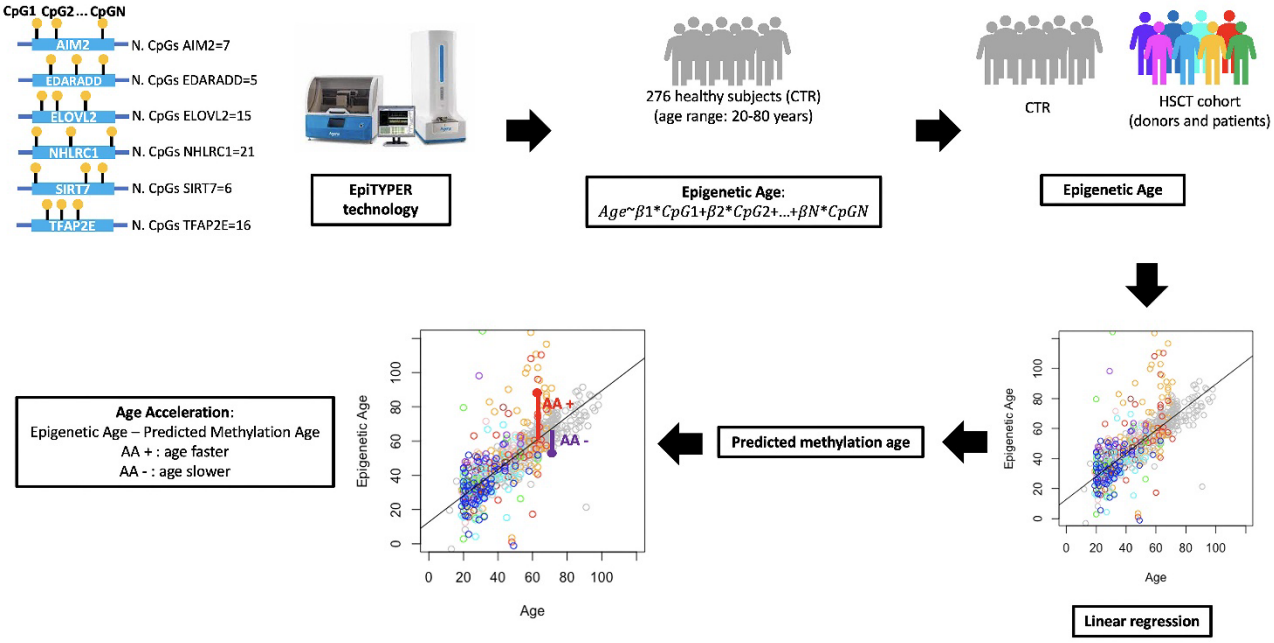

Age Acceleration at baseline
p=0.023
Predicted Age Acceleration
50
0
-50
Recipients (n=28)
Donors (n=53)

B


Age acceleration over time
KW p=0.007
Age Acceleration
30
20
10
0
-10
-20
-30
Donors (n=53)
Day30 (n=57)
Day90 (n=33)
Day180 (n=37)
Day360 (n=42)
Days after HSCT


A
All patients
CRAMER's V=0.254 p=0.005
Age acceleration %
100
50
0
34
46
58
51
71
Donors (n=53)
Day30 (n=57)
Day90 (n=33)
Day180 (n=37)
Day360 (n=42)
Days after HSCT
B
GVHD
CRAMER's V=0.383 p=0.021
100
50
0
25
30
33
50
79
Donors (n=16)
Day30 (n=23)
Day90 (n=12)
Day180 (n=14)
Day360 (n=14)
Days after HSCT
C
no GVHD
CRAMER's V=0.246 p=0.070
100
50
0
38
53
71
52
68
Donors (n=37)
Day30 (n=34)
Day90 (n=21)
Day180 (n=23)
Day360 (n=28)
Days after HSCT
AA+
AA-

Supplementary Fig.1 Description of the method applied to estimate epigenetic age acceleration. tDNAMet is a targeted DNA methylation clock that includes 6 genomic regions (AIM2, EDARADD, ELOVL2, NHLRC1, SIRT7 and TFAP2E) identified by Gensous et al. by analyzing healthy subjects with a wide age range (20-80 years), accelerated- and decelerated-aging subjects (Down Syndrome and centenarians). Each selected region contains several CpG sites (total number: 70 CpGs) whose methylation level is assessed by EpiTYPER technology. We used our data previously generated on a cohort of 276 healthy subjects (CTR) and already described in Gensous et al. to generate a model to estimate Epigenetic Age. We applied the model to controls (CTR) and to the HSCT cohort to get Epigenetic Age for all of the subjects (controls, donors and patients). Then, we performed a linear regression analysis between chronological and epigenetic age to get the Predicted methylation age that we used to estimate Age Acceleration (AA = Epigenetic Age – Predicted Methylation Age).



Supplementary Fig. 2 Trajectory of individual patient change of AA over time.
2A Subjects who remained AA-
2B Subjects who shifted from AA- to AA+
2C Subjects who remained AA+
2D Subjects who shifted from AA+ to AA-