Dynamics of epigenetic age following hematopoietic stem cell transplantation

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Supplemental literature

In addition, the utility of the *epigenetic clock* method has been demonstrated in applications surrounding mortality in a longitudinal twin study (1), ageing in semi-supercentenarians and their offspring (2), obesity (3), Down syndrome (4), HIV infection (5), Parkinson's disease (6), lifetime stress (7), and menopause (8).

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Supplemental methods

Patient data were retrieved from the database of transplanted patients at the Department of Internal Medicine I at the University Hospital Carl Gustav Carus, Dresden, Germany. All peripheral blood mononuclear cell samples (n = 187) were obtained after written informed consent according to a biobanking protocol which was approved by the Ethics Committee of the University of Dresden. Only patients with *de novo* AML in first complete remission who underwent matched sibling donor (MSD) or matched unrelated donor (MUD, HLA-allele matching 10/10), with follow-up, were

included. Furthermore, only patients who never underwent donor-lymphocyte infusion (DLI), did not experience relapse, and had a stable donor chimerism ≥ 98% the time of G-CSF induced PBSC mobilisation and from the recipient at various time points after HCT for chimerism analyses, were included. Genomic DNA from the donor was obtained at peripheral blood stem cell transplantation and from the patients at time point of chimerism analysis. Grading of acute and chronic GvHD was performed according to NIH criteria (1). A table of clinical information on the analyzed AML patients is provided in the supplemental table. Genomic DNA extraction and STR PCR were performed as reported (2). CpG methylation analysis was performed using Illumina bead chip arrays (Illumina, San Diego, USA). DNAm age was estimated using the recently published algorithm (3). All statistical analyses were performed using the R environment for statistical computing version 3.1.3 (4). Estimation of epigenetic donor-cell aging was performed using a linear mixed-effects model with random intercept to account for the dependent variance structure caused by repeated observations of the same patients. The association of the slope of DNAm aging with allogeneic HSCT parameters donor source (MUD *vs.* MRD), patient age, donor age, type of GvHD-prophylaxis, and occurrence of acute GvHD and chronic GvHD was analyzed by including the above-mentioned parameters in the linear mixed-effects model. An interaction term which allows estimation of separate DNAm age acceleration for time periods with and without chronic GvHD and tests whether both slopes are significantly different were used. Significance level for testing interactions was set to 10%. To visualize the effect DNAm age after HCT, scatter plots were generated applying a log (base 10) transformed variable ("Years after Transplantation") for whom one year was added to avoid calculating the logarithm of zero. A non-parametric group comparison test (Kruskal-Wallis test) was applied to relate grouping variables to DNAm age rejuvenation and acceleration.

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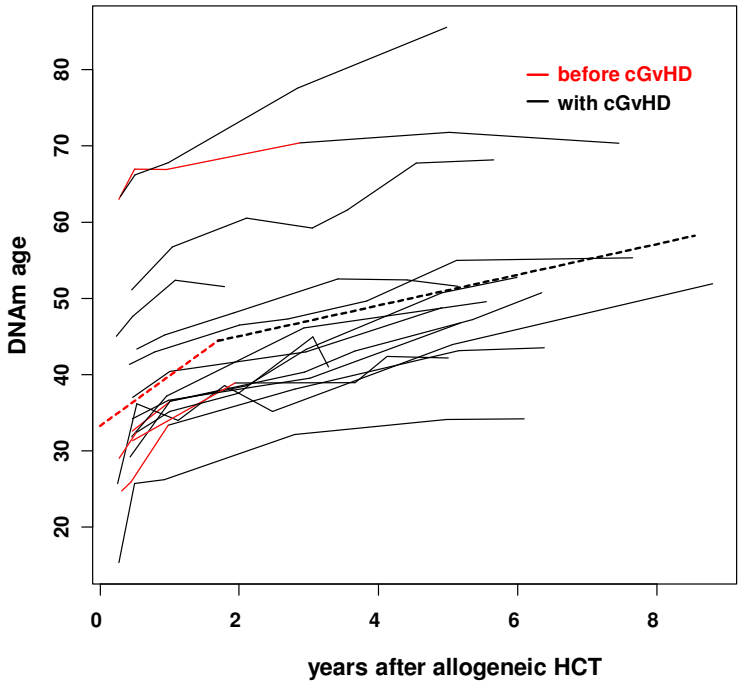
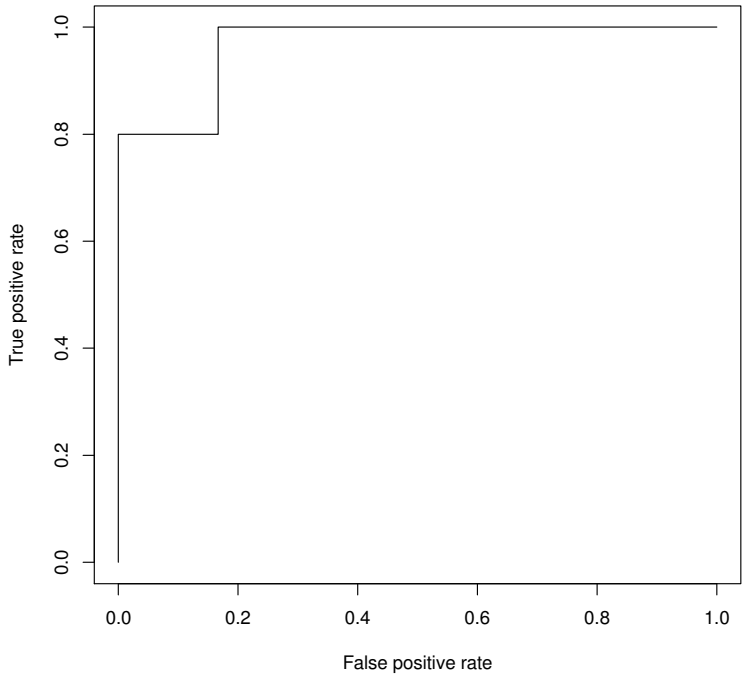
Supplemental Figure 1. DNAm age after allogeneic HCT in patients with and without cGvHD.



Supplemental Figure 1 Legend. DNAm age of patients with chronic GvHD measured from the nadir of DNAm age after allogeneic HCT. Red color indicates the time course prior to chronic GvHD whereas black color indicates the time course with chronic GVHD. Note: although there exist n = 13 patients who developed chronic GvHD in our analysis there are only n = 4 patients with chronic GvHD depicted in this figure due to limited sample availability. This is explained by the prerequisite that for such an analysis at least two time-points of DNAm analysis are necessary in order to draw a slope before the occurrence of chronic GvHD until chronic GvHD manifests. The dotted line indicates the adjusted ageing course prior to chronic GvHD (red color) and with chronic GvHD (black color).

Supplemental Figure 2. DNAm age in patients after allogeneic HCT for prediction of cGvHD.



Supplemental Figure 2 Legend. DNAm age of patients after allogeneic HCT in a ROC analysis to predict the occurrence of cGvHD.