Graft-*versus*-host disease and relapse/rejection-free survival after allogeneic transplantation for idiopathic severe aplastic anemia: a comprehensive analysis from the SAAWP of the EBMT

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Received: August 1, 2022.
Accepted: March 15, 2023.
Early view: March 23, 2023.

https://doi.org/10.3324/haematol.2022.281876

Abstract

Survival after allogeneic hematopoietic stem cell transplantation (allo-HSCT) for severe idiopathic aplastic anemia (SAA) has improved in recent years, approaching 75% at 5 years. However, an SAA-adapted composite endpoint, graft-*versus*-host disease (GvHD) and relapse/rejection-free survival (GRFS), may more accurately assess patient outcomes beyond survival. We analyzed GRFS to identify risk factors and specific causes of GRFS failure. Our retrospective analysis from the Severe Aplastic Anemia Working Party of the European Society for Blood and Marrow Transplantation included 479 patients with idiopathic SAA who underwent allo-HSCT in two conventional situations: i) upfront allo-HSCT from a matched related donor (MRD) (upfront cohort), and ii) allo-HSCT for relapsed or refractory SAA (rel/ref cohort). Relevant events for GRFS calculation included graft failure, grade 3-4 acute GvHD, extensive chronic GvHD, and death. In the upfront cohort (n=209), 5-year GRFS was 77%. Late allo-HSCT (i.e., >6 months after SAA diagnosis) was the main poor prognostic factor, specifically increasing the risk of death as the cause of GRFS failure (hazard ratio [HR]=4.08; 95% confidence interval [CI]: 1.41-11.83; *P*=0.010). In the rel/ref cohort (n=270), 5-year GRFS was 61%. Age was the main factor significantly increasing the risk of death (HR=1.04; 95% CI: 1.02-1.06; *P*<0.001), acute GvHD (HR=1.03; 95% CI: 1.00-1.07; *P*=0.041), and chronic GvHD (HR=1.04; 95% CI: 1.01-1.08; *P*=0.032) as the cause of GRFS failure. GRFS after upfront MRD allo-HSCT was very good, notably with early allo-HSCT, confirming that younger patients with an MRD should be transplanted immediately. GRFS was worse in cases of salvage allo-HSCT, most notably in older patients, questioning the utility of allo-HSCT earlier in the disease course.

Introduction

Due to major developments in transplantation modalities over the past 20 years (graft-*versus*-host disease [GvHD] prophylaxis, HLA typing, conditioning regimens, optimization of alternative donor transplantation), overall survival (OS) after allogeneic hematopoietic stem cell transplantation (allo-HSCT) for severe idiopathic aplastic anemia (SAA) has largely improved, approaching 80% at 5 years.[1–6] In contrast to allo-HSCT in hematological malignancies, the challenges in allo-HSCT for SAA remain the achievement

of sustained engraftment without significant clinical alloreactivity since no graft-*versus*-tumor effect is required to achieve long-term survival. Long-term follow-up studies repeatedly reported that GvHD strongly impairs quality of life and plays a pivotal role in the occurrence of late complications, including secondary cancers. Consequently, avoiding GvHD is of particular importance in allo-HSCT for SAA.[7–9] Furthermore, when considering beyond simple OS, the use of the SAA-adapted composite endpoint of GvHD and rejection-free survival (GRFS) may be a more meaningful clinical study endpoint by allowing for greater accuracy in assessing patient outcomes. Although some retrospective studies have assessed GRFS in this context, there is no published report including large numbers of patients with the goal of identifying risk factors and causes of GRFS failure.[10–12] Based on the Data Quality Initiative program of the Severe Aplastic Anemia Working Party (SAAWP) of the European Society for Blood and Marrow Transplantation (EBMT), we performed a comprehensive analysis of GRFS and causes of failure in, separately, both those previously untreated and of relapsed/refractory SAA.

Methods

Study design and selection criteria

Data were collected from the SAAWP database of the EBMT. Patients prospectively provided signed informed consent for both data collection through the ProMISe system and any subsequent *a posteriori* analyses. The study was conducted in accordance with the Declaration of Helsinki and was approved by the scientific committee of the SAAWP of the EBMT.

At the time of analysis, the Data Quality Initiative registry database included 779 patients who first underwent allo-HSCT for idiopathic SAA. We applied the following selection criteria: i) allo-HSCT between 2005 and 2016, ii) matched related donor (MRD) or unrelated donor (UD) allo-HSCT, and iii) absence of *ex vivo* graft manipulation. From there, we specifically focused our analysis on patients who had standard indications for performing allo-HSCT and thus created two cohorts: i) patients who underwent upfront allo-HSCT with an MRD (upfront cohort), and ii) patients who underwent allo-HSCT with either an MRD or a UD for post immunosuppressive therapy relapsed or refractory SAA (rel/ref cohort). A detailed patient selection flowchart is provided in the *Online Supplementary Figure S1*.

Statistical analyses

The upfront and rel/ref cohorts were analyzed separately with no aim towards comparison. Relevant events for Kaplan-Meier[13] GRFS calculation included graft failure (GF, including primary and secondary graft failure), grade 3-4 acute GvHD (aGvHD), extensive chronic GvHD (cGvHD), and death. Patients were censored in the absence of events prior to last contact. The median follow-up was estimated using the reverse Kaplan-Meier method. The log-rank test was used for univariate comparisons of stratified survival outcomes. Cumulative incidence rates for the initial causes of GRFS failure were calculated, with each event considered as competing with other GRFS causes of failure.[14] Gray's test was used for univariate comparisons.

Multivariable competing risks analyses were performed through the multistate modeling framework to compute the predicted probabilities of the cause of GRFS failure over time.[15] Briefly, the four causes of GRFS failure (i.e., GF, aGvHD, cGvHD, and death) were set as distinct absorbing states to which patients can transit to from the initial state. The corresponding cause-specific Cox hazard models for the different causes of failure included the following transition-specific covariates: age (continuous), time from diagnosis to allo-HSCT (6-month cutoff), Cytomegalovirus (CMV) serostatus (donor negative [D-]/recipient negative [R-] *vs.* other), graft source (bone marrow [BM] *vs.* peripheral blood stem cells [PBSC]), *in vivo* T-cell depletion with ATG or alemtuzumab (yes *vs.* no), low-dose total-body irradiation (TBI) (yes *vs.* no, only for the rel/ref cohort), and donor type (MRD *vs.* UD, only for the rel/ref cohort). Based on the aforementioned multivariable models, dynamic prediction by landmarking was used to provide predicted probabilities of individual causes of GRFS failure within the 2 years immediately following selected landmark times (each month from 0 to 12 months post allo-HSCT), provided that patients are event-free at the given landmark.[16] This enables reassessment of the risks of GRFS failures and the impact of covariates over time.

Continuous variables are presented in the text as median and interquartile range (IQR), with categorical variables as percentages within the group of patients with available data. All survival estimates and hazard ratios (HR) are reported with corresponding 95% confidence intervals (CI). All *P* values were unadjusted, two-sided, and $P<0.05$ was considered significant. Statistical analyses were performed using R software 4.0.3 (survival, cmprsk and mstate package[17]). Additional details on the modeling are provided in the *Online Supplementary Figure S2*.

Results

Patient characteristics

We analyzed 479 patients, separated into two different cohorts: upfront (n=209) and rel/ref (n=270). Median time from diagnosis to allo-HSCT was 2.7 (IQR, 1.4-5.3) and 9.1 (IQR, 4.3-17.8) months in the upfront and the rel/ref co-

horts, respectively. In the upfront cohort, 188 (90%) patients were 40 years or younger and 162 (72%) underwent early allo-HSCT (i.e., within the 6 months following diagnosis). In the rel/ref cohort, 83 (31%) patients were older than 40 years and 142 (53%) received allo-HSCT from a UD. Median follow-up was 65 months (95% CI: 58-71). Patient and transplantation characteristics are detailed in Table 1.

Upfront matched related donor allogeneic hematopoietic stem cell transplantation cohort: factors influencing graft-*versus*-host disease and relapse/rejection-free survival as a composite endpoint in univariate analysis

At 5 years after allo-HSCT, OS was 88% (*Online Supplementary Figure S3*). GRFS probability at 5 years was 77% while the causes of GRFS failure were 5%, 2%, 6% and 9%, for GF, aGvHD, cGvHD, and death, respectively (Figure 1A). According to univariate analysis, age did not significantly influence 5-year GRFS (≤20 years [y] *vs.* 21-40 y *vs.* >40 y: 81% *vs.* 76% *vs.* 64%; *P*=0.114). By contrast, CMV serostatus (D-/R- *vs.* other: 85% *vs.* 74%; *P*=0.026) and particularly time from diagnosis to allo-HSCT (≤6 vs. >6 months: 82% *vs.* 61%; *P*<0.001; Figure 1B) significantly influenced GRFS. We observed that age (≤20 y *vs.* 21-40 y *vs.* >40 y: 8% *vs.* 5% *vs.* 31%; *P*<0.001) and time from diagnosis to allo-HSCT (≤6 vs. >6 months: 7% *vs.* 18%; *P*=0.005; Figure 1C, D) significantly increased the risk of death without other prior GRFS events, while only a trend was observed for CMV serostatus (D-/R- *vs.* other: 2% *vs.* 10%; *P*=0.052). *In vivo* T-cell depletion with ATG or alemtuzumab was associated with a significantly lower risk of graft failure (yes *vs.* no: 3% *vs.* 13%; *P*=0.048). No significant difference in GRFS and causes of GRFS failure was observed according to graft source. We were unable to evaluate the impact of low-dose total body irradiation (TBI) in the upfront cohort since only four patients received irradiation-based conditioning. The full table for univariate comparisons is provided in the *Online Supplementary Table S1*.

Relapsed or refractory cohort: factors influencing graft-*versus*-host disease and relapse/rejection-free survival as a composite endpoint in univariate analysis

Among the 270 patients who underwent allo-HSCT for rel/ref SAA, 5-year OS (*Online Supplementary Figure S3*) and GRFS were 73% and 61%, respectively. Cumulative incidences of initial causes of GRFS failure were 9%, 6%, 5%, and 18% for GF, aGvHD, cGvHD, and death prior to other events, respectively (Figure 2A). In this cohort, only age was significantly associated with GRFS (≤20 y *vs.* 21-40 y *vs.* >40 y: 72% *vs.* 64% *vs.* 46%; *P*=0.003; Figure 2B). The lower 5-year GRFS probability was due to a significantly higher incidence of death without a prior event in patients older than 40 years (≤20 y *vs.* 21-40 y *vs.* >40 y: 12% *vs.* 14% *vs.* 30%; *P*=0.007; Figure 2C), while other causes of GRFS failure (GF, aGvHD, and cGvHD) were not significantly different across age groups. We did not observe any difference in GRFS according to donor type (MRD *vs.* UD: 62% *vs.* 61%; *P*=0.566; Figure 2D). In addition, CMV serostatus other than D-/R- was significantly associated with a higher incidence of GF (D-/R- *vs.* other: 1% *vs.* 12%; *P*=0.021). However, this difference did not significantly influence 5-year GRFS (D-/R- *vs.* other: 69% *vs.* 59%; *P*=0.298). The use of ATG/alemtuzumab and TBI was sig-

Table 1. Patient characteristics.

	Upfront MRD allo-HSCT (N=209)		Allo-HSCT for relapsed/refractory SAA (N=270)	
	N	%	N	%
Age in years, median (range)	21	(<1-64)	27	(<1-77)
≤20	96	46	95	35
21-40	92	44	92	34
>40	21	10	83	31
Time from diagnosis to allo-HSCT in months (IQR)	2.3	(1.4-5.3)	9.1	(4.3-17.8)
≤6	162	78	91	34
>6	47	22	179	66
Donor type				
matched related	209	100	128	47
unrelated	0	0	142	53
Graft source				
BM	151	72	205	76
PBSC	58	28	65	24
Conditioning with TBI				
yes	4	2	162	60
no	199	98	108	40
missing	6	-	0	-
In vivo T-cell depletion				
Alemtuzumab	11	5	41	15
ATG/ALG	158	78	184	68
none	34	17	45	17
missing	6	-	0	-
CMV serostatus				
D-/R-	53	28	70	27
other	137	72	194	73
missing	19	-	6	-

Allo-HSCT: allogeneic hematopoietic stem cell transplant; MRD: matched sibling donor; SAA: severe aplastic anemia; IQR: interquartile range; PBSC: peripheral blood stem cell; BM: bone marrow; TBI: total body irradiation: ATG: antithymocyte globulin; ALG: antilymphocyte globulin; CMV: Cytomegalovirus; D-/R-: seronegative for both donor and recipient.

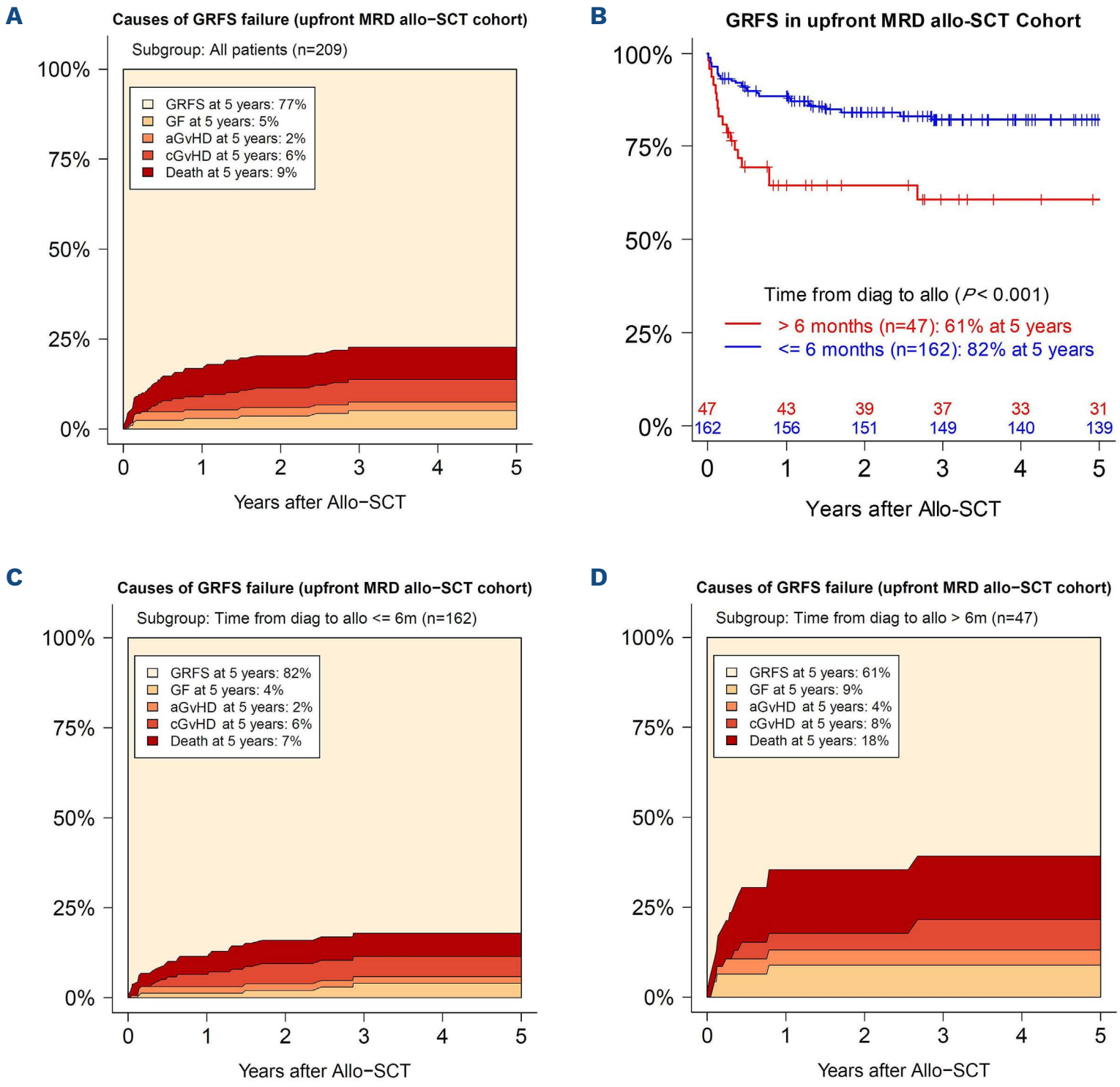

Figure 1. Univariate analysis of graft-*versus*-host disease and relapse/rejection-free survival (GRFS) and causes of GRFS failure in the upfront allogeneic hematopoietic stem cell transplantation cohort. (A) Stacked cumulative incidences of causes of GRFS failure in the entire upfront cohort (N=209). (B) Kaplan-Meier curves for GRFS according to time from diagnosis to upfront allogeneic stem cell transplantation (allo-SCT). (C) Stacked cumulative incidences of causes of GRFS failure in patients undergoing early upfront allo-HSCT (within 6 months after diagnosis, N=162). (D) Stacked cumulative incidences of causes of GRFS failure in patients undergoing late allo-HSCT (after 6 months following diagnosis, N=47). MRD: matched related donor; GF: graft failure; allo: allogeneic; aGvHD: acute graft-*versus*-host disease; cGvHD: chronic GvHD.

nificantly associated with reduced risk of aGvHD (yes *vs.* no: 4% *vs.* 16%; *P*=0.006) and GF (yes *vs.* no: 4% *vs.* 11%; *P*=0.039), respectively. No significant difference in GRFS and in causes of GRFS failure was observed according to graft source, donor type, or time from diagnosis to allo-HSCT. The full table for univariate comparison is provided in the *Online Supplementary Table S2*.

Predicted probabilities of causes of graft-*versus*-host disease and relapse/rejection-free survival failure as competing risks in multivariate model

In the upfront cohort, late allo-HSCT (>6 months) was associated with a significant increase in the risk of death as the first cause of GRFS failure (HR=4.08; 95% CI: 1.41-11.83; *P*=0.010; Table 2) and with a significantly higher risk of GF (HR=3.84; 95% CI: 1.02-14.41; *P*=0.046; Table 2). In addition, age was significantly associated with a higher risk of death as the cause of GRFS failure (HR=1.05; 95% CI: 1.01-1.09; *P*=0.011; Table 2). Furthermore, ATG/alemtuzumab reduced the risk of GF as the initial cause of GRFS failure (HR=0.24; 95% CI: 0.06-0.96; *P*=0.044; Table 2). No other covariates were found to be significantly associated with the risk of any cause of GRFS failure (Table 2).

In the rel/ref cohort, age was the major determinant of outcome. Age was significantly associated with not only the risk of death as the cause of GRFS failure (HR=1.04;

95% CI: 1.02-1.06; *P*<0.001) but also with the risk of both aGvHD (HR=1.03; 95% CI: 1.00-1.07; *P*=0.041) and cGvHD (HR=1.04; 95% CI: 1.01-1.08; *P*=0.032), without influencing the risk of GF (Table 2). In addition, CMV serostatus other than D-/R- was specifically associated with an increased risk of GF (HR=4.30; 95% CI: 1.01-18.36; *P*=0.049), without significantly influencing other causes of GRFS failure (Table 2). The use of ATG/alemtuzumab was significantly associated with a reduced risk of aGvHD as the cause of GRFS failure (HR=0.11; 95% CI: 0.03-0.41; *P*=0.011), while a trend was observed towards a reduced risk of GF using low-dose TBI (HR=0.29; 95% CI: 0.08-1.05; *P*= 0.059; Table 2). In addition, the use of a UD was associated with a higher risk of aGVHD (HR= 7.77; 95% CI: 1.54-39.23; *P*=0.013) and a trend of an increased risk of death (HR=1.89; 95% CI: 0.95-3.73; *P*=0.059) as the initial cause of GRFS failure.

Based on transition-specific HR provided by the Cox model, computing the 5-year predicted probabilities of GRFS and causes of GRFS failure with different covariate combination settings resulted in the predictions of 5-year GRFS probabilities of 86% and 64% for a 20-year-old pa-

Table 2. Multivariate Cox regression considering cause-specific covariates.

Covariates	Upfront cohort (N=209)			Rel/Ref cohort (N=270)		
	HR	95% CI	*P*	HR	95% CI	*P*
Age (continuous)						
GF	1.01	(0.96-1.07)	0.623	1.01	(0.99-1.04)	0.37
aGvHD	1.01	(0.95-1.08)	0.649	1.03	(1.00-1.07)	0.041
cGvHD	1.04	(0.99-1.09)	0.158	1.04	(1.00-1.08)	0.032
Death	1.05	(1.01-1.09)	0.011	1.04	(1.02-1.06)	<0.001
Time from diagnosis to allo-HSCT in months: >6 *vs.* ≤6 (ref)						
GF	3.84	(1.02-14.41)	0.046	1.09	(0.42-2.85)	0.857
aGvHD	2.08	(0.33-13.08)	0.433	0.53	(0.15-1.79)	0.304
cGvHD	1.99	(0.51-7.80)	0.325	0.44	(0.13-1.44)	0.174
Death	4.08	(1.41-11.83)	0.010	1.15	(0.54-2.45)	0.709
CMV serostatus: other *vs.* D-/R- (ref)						
GF	1.15	(0.23-5.74)	0.862	4.46	(1.04-19.13)	0.044
aGvHD	1.51	(0.15-14.73)	0.723	1.91	(0.46-7.96)	0.372
cGvHD	1.50	(0.31-7.22)	0.615	0.80	(0.24-2.68)	0.714
Death	3.76	(0.48-29.50)	0.207	0.83	(0.43-1.61)	0.588
Graft source: PBSC *vs.* BM (ref)						
GF	0.69	(0.13-3.63)	0.664	0.43	(0.14-1.35)	0.148
aGVHD	3.47	(0.49-24.51)	0.212	0.63	(0.17-2.37)	0.496
cGVHD	0.21	(0.02-1.76)	0.150	0.84	(0.20-3.45)	0.803
Death	1.03	(0.33-3.25)	0.955	0.63	(0.30-1.31)	0.216
In vivo T-cell depletion: yes *vs.* no (ref)						
GF	0.24	(0.06-0.96)	0.044	1.02	(0.36-2.88)	0.966
aGVHD	0.53	(0.05-5.27)	0.590	0.11	(0.03-0.41)	0.001
cGVHD	0.54	(0.13-2.18)	0.388	0.63	(0.16-2.40)	0.496
Death	0.56	(0.15-2.13)	0.393	0.81	(0.35-1.87)	0.622
Conditioning regimen with TBI: yes *vs.* no (ref)						
GF	-	-	-	0.29	(0.08-1.05)	0.059
aGvHD	-	-	-	0.43	(0.12-1.61)	0.211
cGvHD	-	-	-	1.84	(0.52-6.51)	0.343
Death	-	-	-	0.91	(0.46-1.78)	0.778
Donor type: MRD (ref) *vs.* UD						
GF	-	-	-	0.85	(0.33-2.18)	0.732
aGvHD	-	-	-	7.77	(1.5-39.23)	0.013
cGvHD	-	-	-	1.07	(0.31-3.67)	0.912
Death	-	-	-	1.89	(0.95-3.73)	0.069

The specific impact of covariates on the different causes of GRFS failure are provided separately for the upfront and relapsed or refractory (rel/ref) cohort. GvHD: graft-*versus*-host disease; GF: graft failure; aGvHD: acute GvHD; cGvHD: chronic GvHD; allo-HSCT: allogeneic hematopoietic stem cell transplant; ref: reference; MRD: matched sibling donor; PBSC: peripheral blood stem cell; BM: bone marrow; TBI: total body irradiation: D-/R-: seronegative for both donor and recipient; MRD: matched related donor; UD: unrelated donor.

tient who underwent early and late upfront MRD allo-HSCT, respectively (Figure 3A). This low GRFS probability after late allo-HSCT is mainly explained by the high risk of both death (13%) and GF (10%) as cause of GRFS failure, while corresponding predicted probabilities of death and GF at 5 years after early allo-HSCT were 4% and 3%, respectively. For virtual 50-year-old patients undergoing late upfront MRD allo-HSCT, the 5-year predicted probability of GRFS was 27% with a high risk of death as the first and only cause of GRFS failure (42%), while similar patients receiving early allo-HSCT reached a GRFS approaching that observed in younger patients (64%) (Figure 3A).

In the rel/ref cohort, the 5-year GRFS probabilities were 86%, 76%, and 59% after MRD allo-HSCT for patients 10, 30, and 50 years old, respectively. In cases of UD allo-HSCT the corresponding 5-year GRFS predicted probabilities were 80%, 65%, and 43%, respectively. The main cause of GRFS failure in older patients was death before any other event, with 5-year predicted probabilities of 23% and 37% for a 50-year-old patient undergoing MRD and UD allo-HSCT, respectively (Figure 3B). The complete tables of 5-year probabilities for all covariate combinations are provided in the *Online Supplementary Tables S3* and *S4* for the upfront and rel/ref cohorts, respectively.

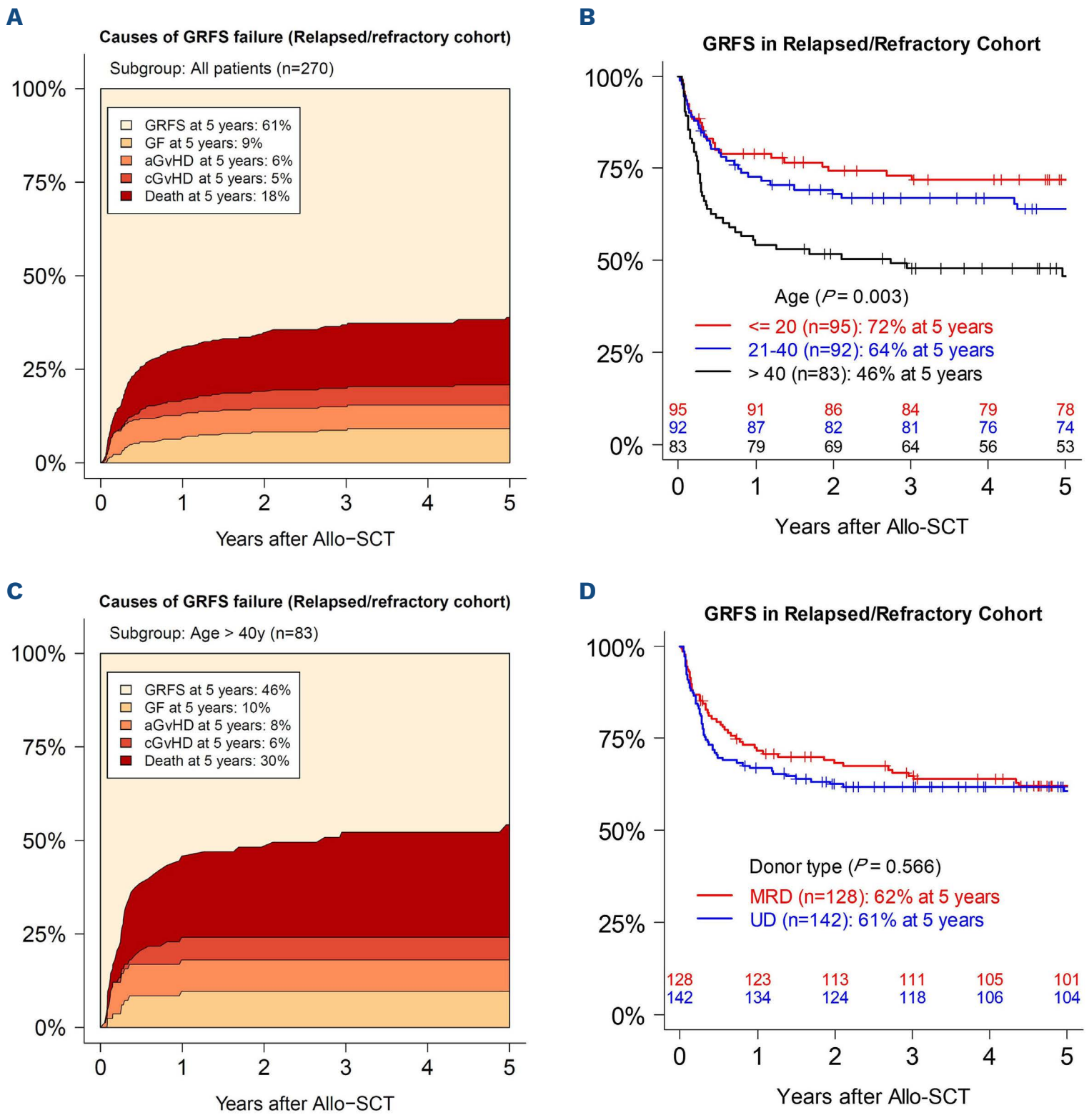

Figure 2. Univariate analysis of graft-*versus*-host disease and relapse/rejection-free survival (GRFS) and causes of GRFS failure in the relapsed or refractory allogeneic hematopoietic stem cell transplantation cohort. (A) Stacked cumulative incidences of causes of GRFS failure in the whole relapsed or refractory (rel/ref) cohort (N=270). (B) Kaplan-Meier curves for GRFS according to age group. (C) Stacked cumulative incidences of causes of GRFS failure in rel/ref patients with age >40 years old (N=83). (D) Kaplan-Meier curves for GRFS according to donor type in the rel/ref cohort. MRD: matched related donor; GF: graft failure; allo-SCT: allogeneic stem cell transplantation; aGvHD: acute graft-versus-host disease; cGvHD: chronic GvHD; UD: unrelated donor.

Dynamic prediction of graft-*versus*-host disease and relapse/rejection-free survival probability and causes of failure

In order to add a dynamic perspective to the risk of GRFS failure over time, GRFS probabilities and causes of failure within the next 2 years were predicted from successive landmark times (every month from 0 to 12 months post allo-HSCT) for different covariate combinations. In the upfront cohort, 30-year-old patients undergoing early allo-HSCT had a GRFS probability of 83% at 2 years after transplantation. At later landmark times (after 5 months post allo-HSCT), the risk of GRFS failure within the next 2 years was ≤10%, with ≤3% risk of death as the cause of GRFS failure (Figure 4A, B; red solid lines). A patient with the same covariates but an age of 50 years had a GRFS probability of 67% at 2 years after allo-HSCT, approaching the risks observed in younger patients at later landmark times (after 5 months post allo-HSCT the risk of GRFS failure was lower than 20% and the risk of death as the cause of failure was below 7%, Figure 4A, B; red dotted lines). By

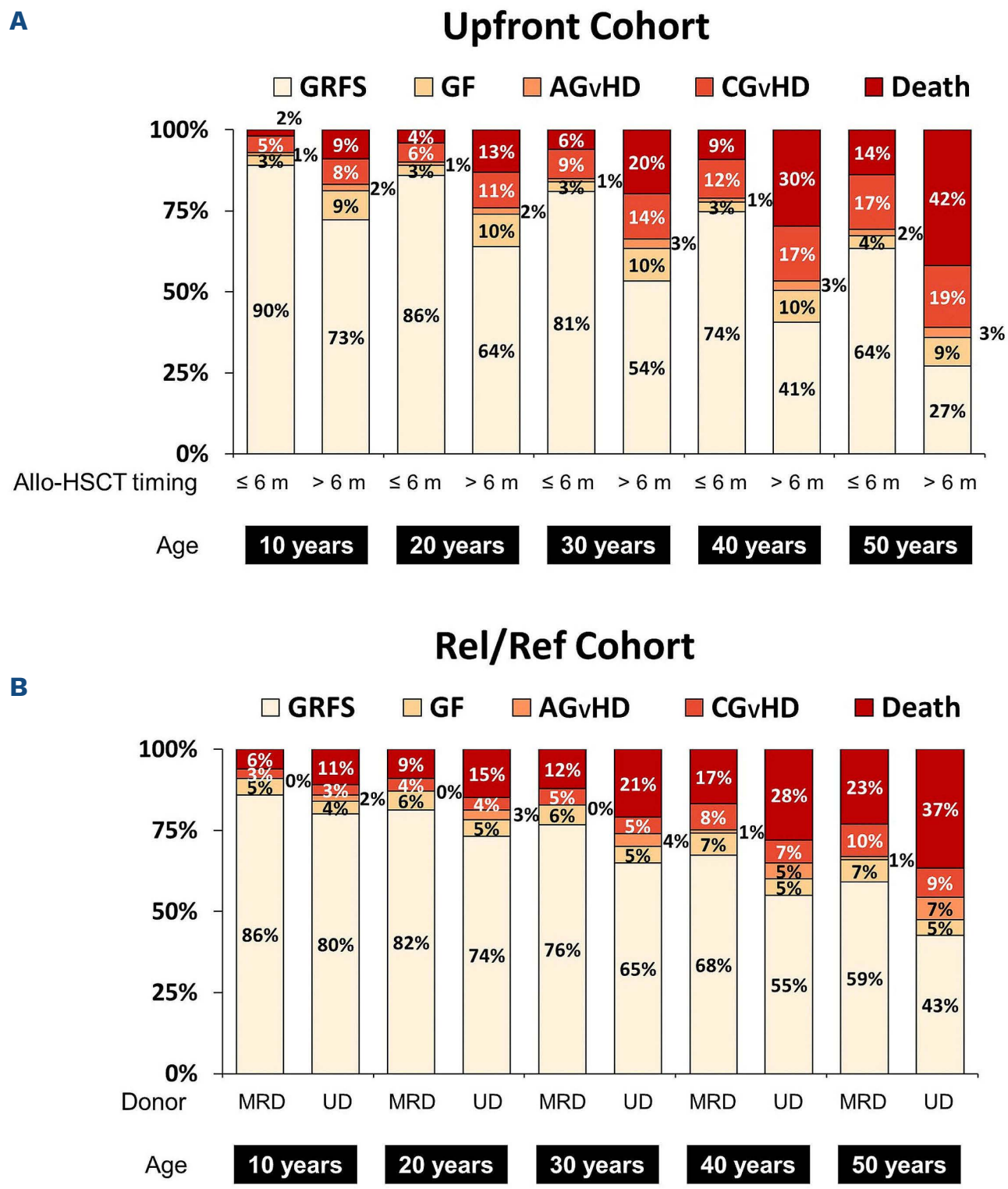

Figure 3. Five-year predicted probabilities of graft-*versus*-host disease and relapse/rejection-free survival (GRFS) and causes of GRFS failure in the upfront and relapsed or refractory cohorts. Predictions are provided by the Cox model shown in Table 2 and are given for some combinations of selected covariates: (A) age and timing of allogeneic hematopoietic stem cell transplantation (allo-HSCT) for the upfront cohort. Other covariates were arbitrarily set as follows: Cytomegalovirus (CMV): "other than sero-negative for both donor and recipient (D-/R-)"; graft source: "bone marrow [BM]"; *in vivo* T-cell depletion: "yes". (B) Age and donor type for the relapsed or refractory (rel/ref) cohort. Other covariates were arbitrarily set as follows: CMV: "other than D-/R-"; graft source: "BM"; timing of allo-HSCT: ">6 months"; total body irradiation (TBI): "yes"; *in vivo* T-cell depletion: "yes". The complete tables of 5-year predicted probabilities for all covariate combinations are provided in the *Online Supplementary Tables S3* and *S4*. MRD: matched related donor; GF: graft failure; allo: allogeneic; aGvHD: acute graft-*versus*-host disease; cGvHD: chronic GvHD; UD: unrelated donor.

contrast, late allo-HSCT (i.e., >6 months following diagnosis) remained associated with a lower probability of GRFS, most notably in older patients for whom even at later landmark times (after 5 months post allo-HSCT), the risk of GRFS failure within the next 2 years was >40%, including a 20% risk of death as the cause of failure (Figure 4A, B; blue dotted lines).

In the rel/ref cohort, similar analyses showed that age was the major determinant of outcome, with persistent risk of GRFS failure and high risk of death over time, no matter the donor type (Figure 4C, D). The full tables of dynamic prediction of GRFS and GRFS failures for all covariate combinations are provided in the *Online Supplementary Tables S5* and *S6* for the upfront and rel/ref cohorts, respectively.

Discussion

In patients with idiopathic SAA, long-term survival can be achieved with both immunosuppressive therapy (IST) and

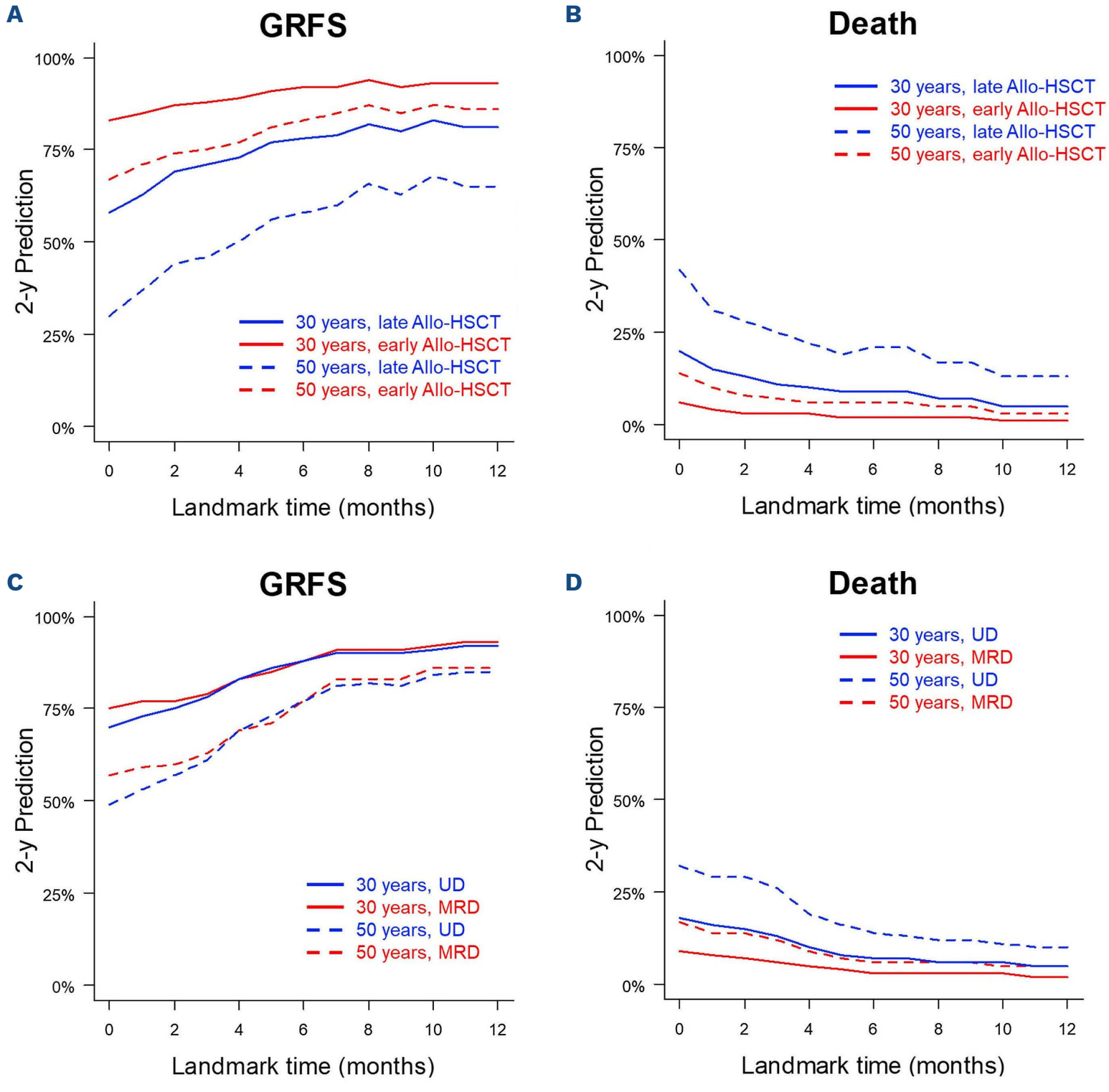

Figure 4. Dynamic prediction of outcome in the upfront and relapsed or refractory cohorts. Curves showing the probabilities of graft-*versus*-host (GvHD) disease and relapse/rejection-free survival (GRFS) (A, C) or death as first cause of GRFS (B, D) within the next 2 years according to landmark times (from 0 to 12 months after allogeneic hematopoietic stem cell transplantation [allo-HSCT]). Predictions are shown for different relevant covariate combinations from the Cox model. (A, B) Age and timing of allo-HSCT in the upfront cohort. Other covariates were arbitrarily set as follows: Cytomegalovirus (CMV): "other than sero-negative for both donor and recipient (D-/R-)"; graft source: "bone marrow [BM]"; *in vivo* T-cell depletion: "yes". (C and D) Age and donor type in the relapsed or refractory (rel/ref) cohort. Other covariates were arbitrarily set as follows: CMV: "other than D-/R-"; graft source: BM"; timing of allo-HSCT: ">6 months"; total body irradiation (TBI): "yes"; *in vivo* T-cell depletion: "yes". The full tables of dynamic predictions of GRFS and GRFS failures for all covariate combinations are provided in the *Online Supplementary Tables S5* and *S6*. UD: unrelated donor; MRD: matched related donor.

allo-HSCT. Improvements in supportive care and IST modalities, such as horse ATG and the recent addition of eltrombopag, have now increased OS after IST to approximately 90% at 2 years. However, a third of patients have no response at 6 months, and 10% to 20% of responsive patients will relapse within 2 years after IST, thus becoming candidates for allo-HSCT.[18,19] Allo-HSCT has advantages over IST regarding better remission rates and duration, as well as the prevention of clonal evolution but is limited by higher morbidity and the availability of a suitable donor. Thus, first-line treatment algorithms usually consider upfront allo-HSCT as the standard of care only in younger patients (<40-50 years) with an available MRD.[1,20,21] However, improvements in transplantation procedures (HLA typing, conditioning regimens, GvHD prophylaxis, alternative donors) have significantly improved OS to nearly 80% at 5 years. Thus, a SAA-adapted GRFS composite endpoint may be more accurate to assess post-transplantation outcomes.

Our study is the first large report evaluating GRFS and its risk factors. In addition, we performed a comprehensive analysis to uncover the effects of covariates on the different causes of GRFS failure. We focused our analyses on two different patient cohorts, where patients were selected because they had received allo-HSCT with the most common indications: upfront allo-HSCT from a MRD (upfront cohort) and allo-HSCT for relapse and/or refractory SAA from a MRD or a UD (rel/ref cohort). By using this method we reduce the impact of confounding factors and their interactions, making it a point of strength in our study.

In the upfront cohort, 5-year GRFS was 77%, which further supports the use of allo-HSCT especially in younger patients. In this situation, our results indicated that the time between diagnosis and allo-HSCT was the most critical predictive factor and confirms that transplantation should occur immediately in younger patients if an MRD is available. If an MRD is not rapidly available, conservative or prompt upfront allo-HSCT from an alternative donor is currently under debate even though recent reports have disclosed promising outcomes in this situation.[22–25] Furthermore, the recent results of IST plus eltrombopag in previously untreated SAA also appear encouraging and must be taken into account when considering upfront transplant with an alternative donor (especially in adults).[19] Interestingly, although age was still associated with poor outcomes, 5-year GRFS in patients older than 40 years was promising (64%) after upfront MRD allo-HSCT. This observation, although limited by the low number of older patients in the upfront cohort, suggests that some older patients may benefit from upfront MRD allo-HSCT. Indeed, a remission rate of only 47% was reported after conventional IST for older patients with SAA (32% CR + 15% PR)[26] and thus many patients will still require salvage therapy for relapse or refractory SAA, a situation that is associated with a worse outcome. Furthermore, in patients over 40 years old with rel/ref SAA, we observed a 5-year GRFS of only 46%, notably due to a high risk of death as the cause of GRFS failure (30%) no matter the donor type. Rather than just performing a basic analysis of GRFS, we also dynamically evaluated the risks of GRFS failure over time. Although older patients undergoing upfront MRD allo-HSCT initially have a higher risk of death, their risk rapidly approaches that of younger patients a few months after Allo-HSCT, most notably in cases of early transplantation. By contrast, patients in the rel/ref cohort continue to experience GRFS failure over time, even at late landmark times. Our analysis, described for the first time in SAA, adds a dynamic point of view to the impact of risk factors.

Initially, it is recommended to treat patients older than 40 years with frontline IST[1,20,21] since the debate is still ongoing concerning the use of upfront MRD allo-HSCT in this situation, notably when considering that age >40 years is also associated with IST failure.[19] Different studies have demonstrated the feasibility of allo-HSCT in older patients, but did not specifically analyze outcomes after upfront allo-HSCT.[11,27,28] As such, the recommendations from the Fred Hutchinson Cancer Research Center suggest that allo-HSCT should be the first curative option for SAA in fit patients until 70 years of age, no matter the donor type.[29] The recommendation may be further supported by a recent development of a conditioning regimen using both ATG and post-transplantation cyclophosphamide with a haploidentical and unrelated donor in treatment naive and refractory SAA patients.[25,30] However, prospective evaluations are necessary to determine whether IST or allo-HSCT will result in better long-term outcomes in older SAA patients. Therefore, the identification and validation of predictive biomarkers of frontline IST failure may help in the decision-making algorithm.

We acknowledge that our model is incomplete, both lacking an assessment of comorbidity and neglecting post-transplantation time-dependent covariates like hematological recovery, organ dysfunction, and/or infections. However, data such as these are not routinely collected in the DaVita quality index DQI (data qualitative initiative) which makes it impossible to create a more complex model. In addition, an external validation cohort would have been useful in confirming our findings.

We conclude that GRFS significantly increases after upfront MRD allo-HSCT, though this can be strongly influenced by the delay between diagnosis and transplantation. Our results not only confirm that younger patients should undergo upfront MRD allo-HSCT without delay, but also suggest the potential benefit of the same strategy in certain patients >40 years old, most notably in the presence of a rapidly available MRD. In the poor prognostic setting of rel/ref SAA, GRFS is obviously worse due to an increased risk of death, with donor type having a marginal effect. In this situation, advanced age is the

major poor prognostic factor for GRFS failure, calling into question the utility of allo-HSCT earlier in the disease course.

Disclosures
No conflicts of interest to disclose.

Contributions
RD and DJE performed statistical analyses. RD, DJE, GS and RP analyzed the results and wrote the manuscript. RP supervised the study. All authors included patients, read, edited and approved the manuscript

Acknowledgments
We would like to thank patients and their family and Anne Lippinkhof and Paul Bosman as SAAWP study coordinators.

Data-sharing statement
Data are available upon specific request to the SAAWP of the EBMT.

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