Minimal residual disease status predicts outcome of acute myeloid leukaemia patients undergoing T-cell replete haploidentical transplantation. An analysis from the Acute Leukaemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT)

Minimal residual disease status predicts outcome of acute myeloid leukaemia patients undergoing T-cell replete haploidentical transplantation. An analysis from the Acute Leukaemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT)

Jonathan Canaani,[1] Myriam Labopin,[2] Xiao J. Huang,[3] Fabio Ciceri,[4] Maria T. Van Lint,[5] Benedetto Bruno,[6] Stella Santarone,[7] José L. Diez-Martin,[8] Didier Blaise,[9] Patrizia Chiusolo,[10] Depei Wu,[11] Mohamad Mohty[2] and Arnon Nagler[1,2]

[1]Haematology Division, Chaim Sheba Medical Centre, Tel Aviv University, Tel Hashomer, Israel, [2]EBMT Paris study office/CEREST-TC, Hopital Saint-Antoine, Paris, France, [3]Haematology, Peking University People's Hospital, Peking, China, [4]Haematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milan, Italy, [5]Department of Haematology II, Ospedale San Martino, Genova, Italy, [6]University of Torino, Torino, Italy, [7]Bone Marrow Transplant Centre, Pescara, Italy, [8]Hospital GU Gregorio Marañon, Instituto de investigación sanitaria Gregorio Marañon, Facultad de Medicina, Universidad Complutense, Madrid, Spain, [9]Haematology Department, Institut Paoli Calmettes, Marseille, France, [10]Department of Haematology, Universita Cattolica Sacro Cuore, Rome, Italy and [11]Department of Haematology, the First Affiliated Hospital of Soochow University, Soochow, China

Summary

Assessment of minimal residual disease (MRD) is being routinely used to assess response in patients with acute myeloid leukaemia (AML). While it is well established that pre-transplant positive MRD studies predict for relapse in patients transplanted either from matched sibling donors or matched unrelated donors, it is currently unknown whether MRD has comparable prognostic value in haploidentical stem cell transplantation (haplo-SCT). To this end we performed a retrospective analysis using the Acute Leukaemia Working Party/European Society of Blood and Marrow Transplantation multicentre registry. All adult AML patients with known MRD status at transplant who underwent a first T-cell replete haplo-SCT while in remission between 2006 and 2016 were included. Two hundred and sixty-five MRD-negative and 128 MRD-positive patients were assessed. In multivariate analysis, MRD-negative patients experienced lower relapse incidence and better leukaemia-free survival (LFS) compared to MRD-positive patients. Subset analysis for MRD-positive patients revealed that patients with donors positive for cytomegalovirus experienced decreased relapse rates as well as increased survival. A 6-month landmark analysis suggests that the clinical benefit of pre-transplant MRD negativity in terms of relapse, overall survival and LFS is realized at this time point. Pre-transplant MRD status is potentially a pivotal prognosticator of outcome in AML patients undergoing T-cell replete haplo-SCT.

Keywords: acute myeloid leukaemia, haploidentical stem cell transplantation, minimal residual disease, relapse, cytomegalovirus.

Assessment of minimal residual disease (MRD) is rapidly transforming the prognostic and therapeutic landscape of a wide gamut of haematological malignancies (Beldjord *et al*, 2014; Ribera *et al*, 2014; Mailankody *et al*, 2015; Roschewski *et al*, 2015; Cheminant *et al*, 2016; Flores-Montero *et al*, 2017; Scherer *et al*, 2017). An evolving body of literature indicates that acute myeloid leukaemia (AML) is no exception to this emerging theme; indeed, it is becoming well established that the presence of MRD is significant in several key clinical settings in this disease. Previously published data from the Dutch-Belgian Haemato-Oncology Cooperative Group/Swiss Group for Clinical Cancer Research cooperative group (Terwijn *et al*, 2013), the UK National Cancer Research Institute (Freeman *et al*, 2013), the Seattle group (Chen *et al*, 2015; Othus *et al*, 2016) and others have established MRD status following initial induction treatment as a powerful independent prognosticator of patient outcome in AML. In the same vein, the importance of pre-transplantation MRD status is gaining appreciation based on several notable publications showing significantly inferior outcomes for AML patients with positive MRD studies prior to transplantation (Anthias *et al*, 2014; Walter *et al*, 2015; Ustun *et al*, 2016; Buckley *et al*, 2017; Oran *et al*, 2017), and also possibly the clinical benefit accrued by an allogeneic stem cell transplantation in AML patients with a suboptimal MRD response to initial induction chemotherapy (Balsat *et al*, 2017). With the increased use of haploidentical stem cell transplantation (haplo-SCT) in acute leukaemia patients (Bashey *et al*, 2013, 2017; Luo *et al*, 2014; Ciurea *et al*, 2015; Piemontese *et al*, 2015, 2017; Han *et al*, 2017; Lee *et al*, 2017), it is becoming increasingly imperative to determine the role pre-transplant MRD studies may assume in prognostication of patients with AML undergoing haplo-SCT. Yet there is currently a paucity of data regarding the implications of a positive MRD state prior to haploidentical stem cell transplantation. The aims of this study were to assess the potential impact of pre-transplant MRD on AML patients undergoing haplo-SCT, and to characterize in detail prognostic factors affecting the specific outcome of MRD-positive and MRD-negative patients.

Methods

Study cohort

This retrospective analysis was comprised of patients enrolled on the multicentre registry of the Acute Leukaemia Working Party (ALWP), which forms part of the European Society of Blood and Marrow Transplantation (EBMT). Adults over the age of 18 years with *de novo* AML were included in this analysis if they underwent a first T-cell replete haplo-SCT at first or second remission, and their molecular status (i.e. MRD) was available at the time of transplant. All patients provided written informed consent authorising the use of their personal information for research purposes. This study was approved by the ALWP institutional review board and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Disease risk and response classification

Cytogenetic risk was assessed according to the European LeukaemiaNet criteria (Dohner *et al*, 2010). The clinical endpoints assessed in this analysis were leukaemia-free survival (LFS), relapse incidence (RI), non-relapse mortality (NRM), acute and chronic graft-versus-host disease (GVHD), GVHD-free/relapse-free survival (GRFS), defined as events including grade 3–4 acute GVHD, severe chronic GVHD, relapse or death in the first year post-SCT (GRFS)(Ruggeri *et al*, 2016), and overall survival (OS). LFS was defined as survival with no evidence of relapse or progression. Relapse was defined as the reappearance of >5% bone marrow blasts and/or an extramedullary disease site resulting from blast cell infiltration. NRM was defined as death without evidence of relapse or progression. OS was defined as the time from haplo-SCT to death, regardless of the cause. Acute and chronic GVHD were classified and graded according to established criteria (Przepiorka *et al*, 1995; Filipovich *et al*, 2005). Intensity of conditioning was determined according to previously published EBMT criteria (Aoudjhane *et al*, 2005). Assessment of MRD was performed locally and reported by participating centres.

Statistical analysis

Cumulative incidence was used to estimate the endpoints of NRM, RI, acute GVHD and chronic GVHD to accommodate for competing risks. To study acute and chronic GVHD, we considered relapse and death to be competing events. Probabilities of OS, LFS and GRFS were calculated using the Kaplan–Meier method. Univariate analyses were done using Gray's test for cumulative incidence functions and the log rank test for OS, GRFS and LFS. A Cox proportional hazards model was used for multivariate regression. All variables differing significantly between the 2 groups (i.e. MRD^{pos} and MRD^{neg} patients) or factors associated with one outcome in univariate analysis were included in the Cox model. As planned in the protocol, multivariate analyses were also performed separately according to the MRD status at transplant. In order to test for a centre effect, we introduced a random effect or frailty for each centre into the model (Hougaard, 1995; Andersen *et al*, 1999). Results were expressed as the hazard ratio (HR) with 95% confidence interval (95% CI). All factors were tested for the proportional hazards assumption using the Grambsch-Therneau residual-based test. As we found a departure from the proportionality assumption for the impact of MRD status with time, we used a step function for estimating the variation of the coefficient associated with this variable with time. i.e., different coefficients over different time intervals post-transplant (0–3 months, 3–6 months

and more than 6 months) (Therneau *et al*, 2016). We found that the effect of MRD was significantly associated with the outcome after 6 months. We then ran a landmark analysis using a starting point of 6 months following transplant. All tests were two-sided with the type I error rate fixed at 0·05 for the determination of factors associated with time to-event outcomes. Statistical analyses were performed with SPSS 24.0 (SPSS Inc, Chicago, IL, USA) and R 3.4.0 (R Core Team (2017).

Results

Patients

A cohort consisting of 393 AML patients who underwent haplo-SCT between 2006 and 2016 with available MRD data was identified and analysed. The median age of patients was comparable between patients with pre-transplant negative MRD studies and positive MRD studies, 47 and 48 years, respectively (Table I). MRD^{neg} patients were more likely to harbour high risk baseline cytogenetic studies (15% vs. 7%; $P < 0·001$) whereas MRD^{pos} patients were more often observed to have favourable risk baseline cytogenetic studies (23% vs. 9%; $P < 0·001$). Of note, MRD^{neg} patients were more likely to be $NPM1^{wt}$ compared to their MRD^{pos} counterparts (66% vs. 46%; $P = 0·01$), however the rate of *FLT3*-internal tandem duplication (ITD) was not significantly different in both groups. There were no statistically significant differences between MRD^{neg} and MRD^{pos} patients in terms of cytomegalovirus (CMV) donor-recipient matching ($P = 0·12$), use of anti-thymocyte globulin (ATG) Ipost-transplant cyclophosphamide (PTCy) ($P = 0·7$), engraftment rate ($P = 0·5$) and conditioning intensity ($P = 0·7$). The various conditioning regimens used are outlined in Table S1.

Table I. Baseline cohort characteristics stratified per minimal residual disease status.

Variable, *n* (%)	MRD negative ($N = 265$)	MRD positive ($N = 128$)	*P*
Follow-up duration, months; median (range)	26 (1–129)	15 (1–94)	
Year of transplant, median (range)	2014 (2006–2016)	2015 (2007–2016)	0·03
Patient age, years; median (range)	47 (18–73·8)	48 (18·5–72·2)	0·7
Donor age, years; median (range)	38 (11·8–72·1)	39 (7·8–71·2)	0·33
Gender			
Male	144 (54·3)	70 (54·6)	0·9
Female	121 (45·6)	58 (45·3)	
Karnofsky performance status			
≥90	188 (83·1)	83 (77·5)	0·21
<90	38 (16·8)	24 (22·4)	
Missing	39	21	
Remission			
CR1	192 (72·4)	82 (64)	0·09
CR2	73 (27·5)	46 (36)	
ELN AML cytogenetic risk category			
Favourable	24 (9)	30 (23·4)	<0·001
Intermediate	138 (52)	63 (49·2)	
Adverse	40 (15)	9 (7)	
Missing	63 (23·7)	26 (20·3)	
NPM1 status			
$NPM1^{wt}$	79 (65·8)	29 (46)	0·01
$NPM1^{mut}$	41 (34·1)	34 (54)	
Missing	145	65	
FLT3-ITD status			
$FLT3^{wt}$	86 (58·5)	33 (48·5)	0·17
FLT3-ITD	61 (41·5)	35 (51·4)	
Missing	118	60	
Donor-recipient gender matching			
Female-Male	60 (22·7)	25 (19·5)	0·47
Other	204 (77·2)	103 (80·4)	
Missing	1	0	
CMV Donor-recipient matching			
CMV D−/R−	33 (12·6)	27 (21·7)	0·12
CMV D+/R−	13 (5%)	7 (5·6)	
CMV D−/R+	49 (18·8)	18 (14·5)	
CMV D+/R+	165 (63·4)	72 (58)	
Missing	5	4	
T-cell depletion *in-vivo*			
ATG	72 (27·2)	32 (25)	0·7
PTCy	175 (66)	89 (69·5)	
ATG+PTCy	18 (6·8)	7 (5·5)	
Stem cell source			
Bone marrow-derived graft	145 (54·7)	48 (37·5)	0·004
Peripheral blood graft	100 (37·7)	63 (49·2)	
Bone marrow and peripheral blood	20 (7·6)	17 (13·3)	
Engraftment			
Successful engraftment	249 (94·6)	120 (96)	0·57
Engraftment failure	14 (5·3)	5 (4)	
Missing	2	3	
Conditioning regimen			
Myeloablative	159 (60)	75 (59)	0·7
Reduced intensity	106 (40)	53 (41)	

AML, acute myeloid leukaemia; ATG, anti-thymocyte globulin; CMV, cytomegalovirus; CR1, first complete remission; D, donor; ELN, European LeukaemiaNet; ITD, internal tandem duplication; MRD, minimal residual disease; PTCy, post-transplant cyclophosphamide; R, recipient.

Relapse

At the 2-year mark, the rate of relapse for the cohort as a whole was 22% (95% CI: 17·6–27·1). The risk of relapse was significantly higher in the MRD^{pos} group wherein 37% of patients experienced relapse compared to 16% of patients with negative MRD studies ($P = 0·0003$). In an adjusted Cox regression model (Table S2), negative MRD status was independently associated with decreased incidence of relapse (HR = 0·31, CI 95%, 0·18–0·56; $P < 0·0001$). As shown in Table S3, this effect was confirmed also in an additional multivariate analysis conducted on the subgroup of patients in first remission (CR1), i.e., MRD^{neg} patients experienced a significantly reduced risk of relapse compared to MRD^{pos} patients (HR = 0·32, CI 95%, 0·17–0·61; $P = 0·0005$).

Non-relapse mortality

The incidence of NRM at 2 years was 22% (95% CI: 17·3–26·6). Of note, among patients with positive MRD studies, the NRM rate was not significantly increased compared to

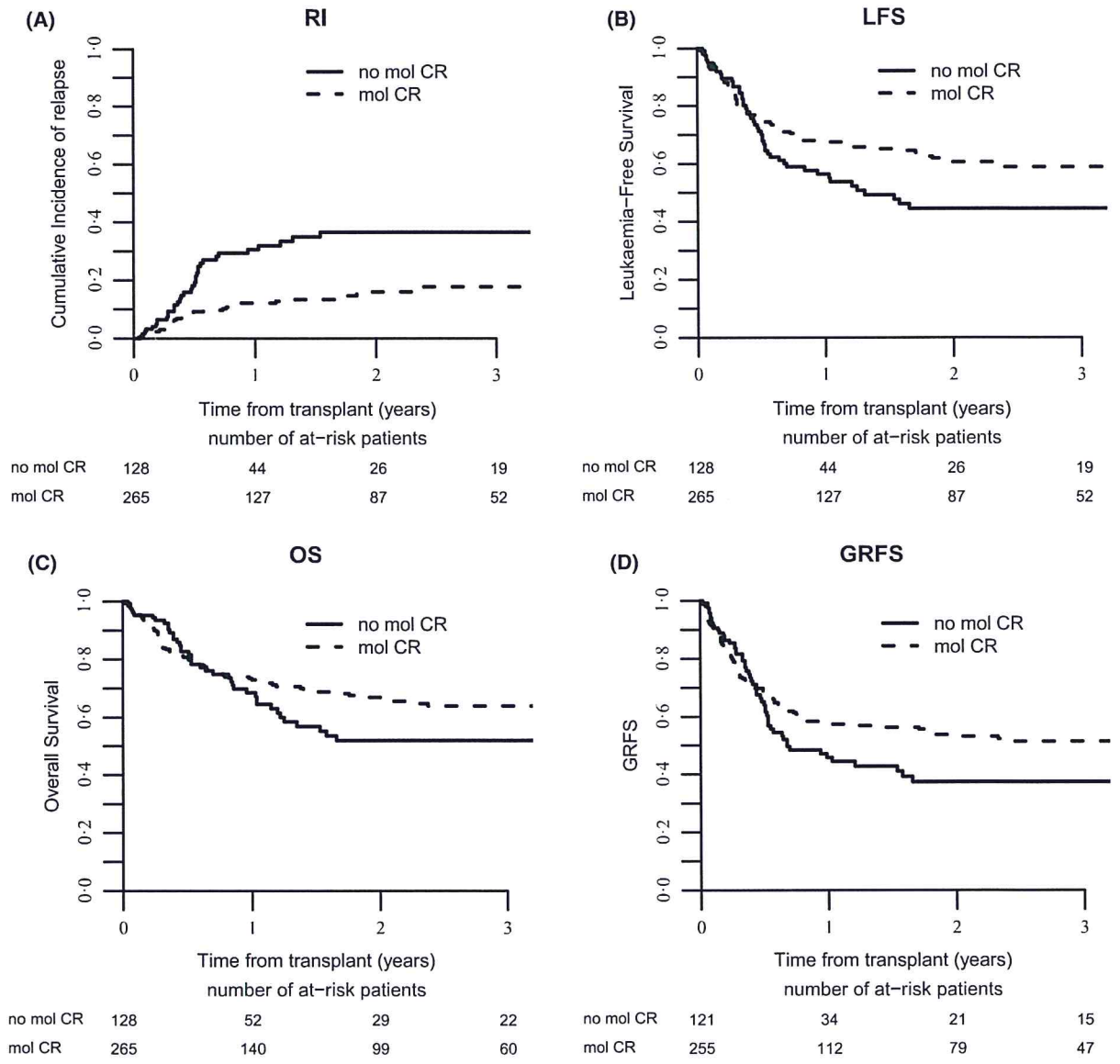

Fig 1. Outcome of acute myeloid leukaemia patients according to pre-transplant minimal residual disease status. (A) Relapse incidence (RI). (B) Leukaemia-free survival (LFS). (C) Overall Survival (OS). (D) Graft-versus-host disease free/relapse-free survival (GRFS). mol CR, complete molecular response.

MRD^{neg} patients ($P = 0{\cdot}16$). As outlined in Table S4, leukaemia, GVHD and infection accounted for most deaths in the analysed cohort.

Leukaemia-free survival and overall survival

The 2-year rate of LFS and OS in this patient cohort was 56% (95% CI: 50·1–61·6) and 63% (95% CI: 57·2–68·4), respectively. There was a trend towards inferior LFS survival in MRD^{pos} patients which did not reach statistical significance (44% vs. 60%; $P = 0{\cdot}088$). Overall survival was not significantly impacted by MRD status ($P = 0{\cdot}18$). Subsequently, a multivariate regression analysis confirmed MRD status to be independently prognostic of LFS (HR = 0·62, CI 95%, 0·41–0·93; $P = 0{\cdot}023$).

Graft-versus-host disease and graft-versus-host disease free/relapse-free survival

Grade II–IV acute GVHD was experienced by 29% (95% CI: 24–33·1) of patients in the analysed cohort whereas chronic GVHD was diagnosed in 32% (95% CI: 26·5–37·3) at 2 years of follow-up. Neither acute GVHD nor chronic GVHD rates were significantly impacted by MRD positivity. Extensive chronic GVHD was seen in 9·6% of patients and was comparable between MRD^{pos} and MRD^{neg} patients ($P = 0{\cdot}8$). The 2-year incidence of GRFS was 49% (95% CI: 43–54·6) and was also not significantly impacted by MRD status ($P = 0{\cdot}2$).

Landmark analysis at 6 months from transplantation

As shown in Fig 1, the benefit of MRD negativity was evident at circa 6 months following transplant when the curves begin to separate for RI, LFS, GRFS and OS. Thus, we performed an additional analysis at the 6-month time point which, as shown in Table II and Fig 2, confirmed that at this interval MRD positivity was significantly and independently associated with inferior rates of relapse, LFS, GRFS and OS.

Subgroup analysis of patients according to MRD status

To further characterize the impact of MRD status on outcome following haplo-SCT, we subsequently performed separate analyses for MRD^{pos} and MRD^{neg} patients. In MRD^{pos} patients, transplant at first or second remission, as well as mutations in *FLT3*-ITD and *NPM1* were found to bear no significant impact on both disease- and transplant-related outcomes. In multivariate analysis, MRD^{pos} patients with CMV^{+} donors had significantly decreased relapse rates as well as better LFS, OS and GRFS rates (Table III). In addition, MRD^{pos} patients who underwent myeloablative conditioning and those treated with PTCy experienced a lower incidence of chronic GVHD. In MRD^{neg} patients, multivariate analysis did not reveal any significant risk factor impacting on disease- or transplant-related outcomes.

Table II. Landmark analysis at 6 months

	RI			NRM			LFS			OS			GRFS		
	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*
MRD negative vs. positive	0·25	0·1–0·63	0·003	0·41	0·09–1·83	0·24	0·3	0·14–0·61	0·001	0·32	0·16–0·65	0·001	0·45	0·22–0·88	0·02
Increasing patient age	1·05	0·76–1·45	0·72	1·27	0·74–2·16	0·37	1·12	0·86–1·45	0·38	1·29	1–1·66	0·05	1·02	0·8–1·31	0·84
CR2 vs. CR1	0·83	0·31–2·17	0·7	0·63	0·14–2·71	0·53	0·90	0·43–1·86	0·77	1·44	0·71–2·93	0·3	0·97	0·49–1·91	0·9
PTCy vs. ATG	0·77	0·28–2·08	0·6	0·43	0·08–2·27	0·32	0·72	0·34–1·53	0·40	0·85	0·37–1·91	0·69	0·9	0·44–1·87	0·79
RIC vs MAC	3·13	1·12–8·68	0·028	0·65	0·13–3·3	0·61	1·95	0·93–4·07	0·07	1·61	0·78–3·29	0·19	1·79	0·9–3·57	0·09

ATG, anti-thymocyte globulin; CI, 95% confidence interval; CR1/2, first/second complete remission; GRFS, graft versus host disease-free/relapse-free survival; HR, hazard ratio; LFS, leukaemia-free survival; MAC, myeloablative conditioning; MRD, minimal residual disease; NRM, non-relapse mortality; OS, overall survival; PTCy, post-transplant cyclophosphamide; RI, relapse incidence; RIC, reduced intensity conditioning.

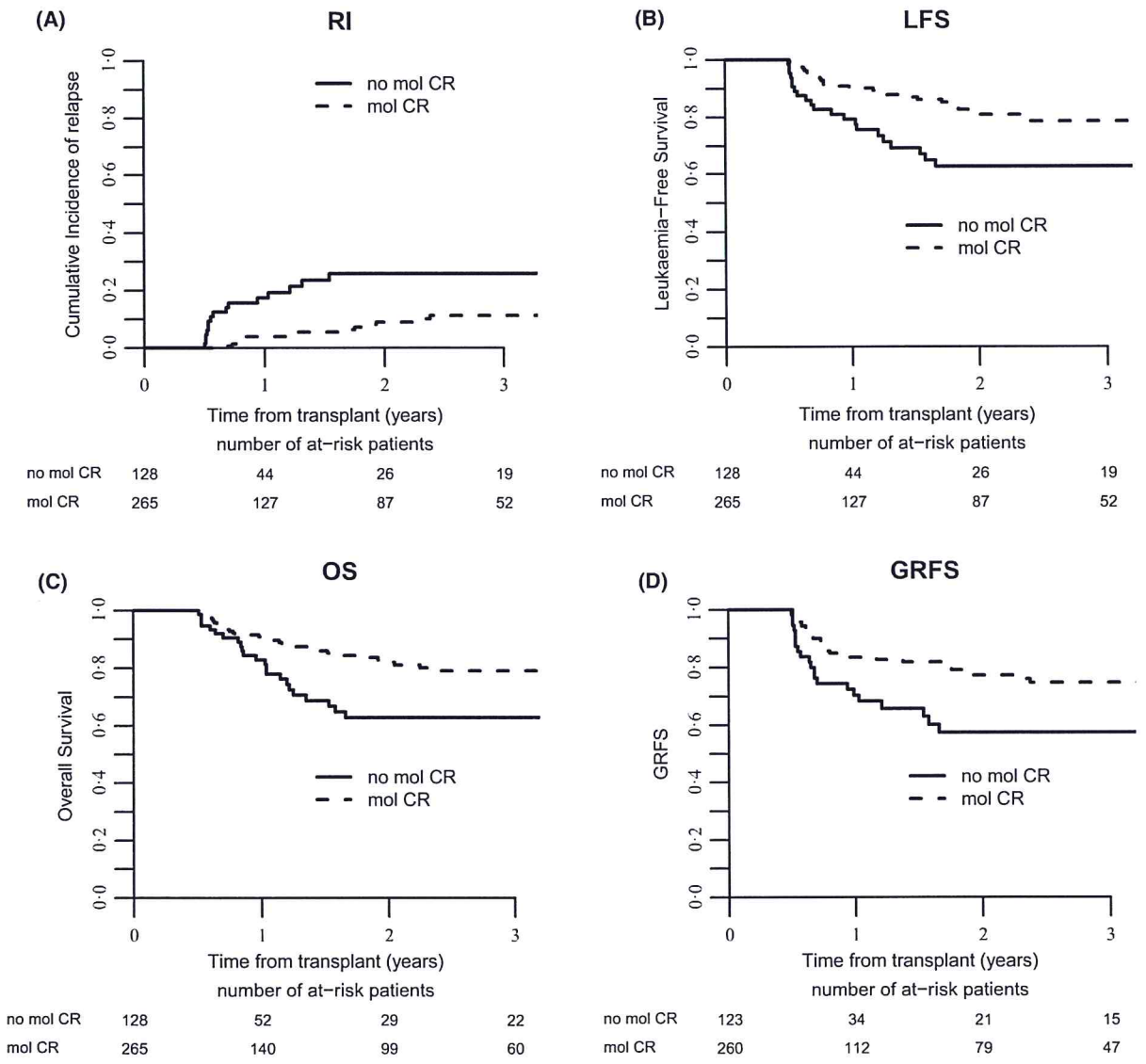

Fig 2. Six-month landmark analysis of patient outcome according to minimal residual disease status. (A) Relapse incidence (RI). (B) Leukaemia-free survival (LFS). (C) Overall Survival (OS). (D) Graft-versus-host disease free/relapse-free survival (GRFS). mol CR, complete molecular response.

Discussion

Incorporation of MRD platforms into the diagnostic and therapeutic management paradigm of patients with AML is proving to be a transformative approach in the risk stratification of patients with this clinically challenging disease. In this analysis we extend on the established MRD experience in related and unrelated donors (Freeman *et al*, 2013; Terwijn *et al*, 2013; Chen *et al*, 2015; Othus *et al*, 2016) and show that, in haplo-SCT pre-transplant, MRD status also holds a significant prognostic role.

Our data indicate that patients with positive MRD studies prior to haplo-SCT experience significantly increased relapse rates compared to MRD^{neg} patients. Moreover, we establish pre-transplant MRD status to be an independent predictor of LFS whereby MRD^{neg} patients have clearly superior rates of achievement of LFS compared to their MRD^{pos} counterparts. These findings are not surprising given the bulk of evidence showing our findings to be comparable with results in AML patients undergoing transplantation from matched sibling donors (MSD) as well as from matched unrelated donors (MURD). Indeed, in a recent study from the MD Anderson Cancer Center examining the outcomes of 152 transplanted AML patients, consisting mostly of MSD and MURD, pre-transplant MRD was shown to be a potent predictor of both LFS and OS. For patients with positive MRD studies the 1-

Table III. Multivariate subgroup analysis according to MRD status

	RI			NRM			LFS			OS			GRFS			Grade II–IV acute GVHD			Chronic GVHD		
	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*	HR	CI	*P*
MRD^{pos}																					
Increasing patient age	1·0	0·75–1·39	0·88	1·11	0·67–1·82	0·68	1·02	0·78–1·33	0·85	1·26	0·92–1·71	0·13	1·03	0·8–1·32	0·8	1·04	0·78–1·37	0·77	1·07	0·73–1·57	0·7
CR2 vs. CR1	0·6	0·28–1·29	0·19	1·29	0·35–4·67	0·69	0·72	0·37–1·41	0·35	0·86	0·37–1·98	0·72	0·6	0·3–1·17	0·13	1·38	0·61–3·11	0·43	1·01	0·37–2·8	0·97
Donor CMV^+	0·3	0·15–0·65	0·001	0·46	0·13–1·57	0·21	0·36	0·19–0·68	0·001	0·37	0·17–0·8	0·012	0·53	0·29–0·95	0·03	1·61	0·69–3·75	0·3	1·69	0·59–4·89	0·32
PTCy vs. ATG	2·0	0·78–5·31	0·14	0·77	0·15–3·92	0·75	1·60	0·7–3·68	0·26	1·59	0·51–4·96	0·41	1·17	0·51–2·71	0·7	1·11	0·4–3·07	0·83	0·34	0·13–0·89	0·02
MAC vs. RIC	0·6	0·23–1·34	0·19	0·99	0·25–3·98	0·99	0·62	0·29–1·32	0·22	0·90	0·39–2·1	0·82	0·42	0·2–0·88	0·02	0·83	0·35–1·95	0·67	0·23	0·08–0·69	0·01
MRD^{neg}																					
Increasing patient age	0·8	0·64–1·11	0·23	1·16	0·92–1·46	0·19	1·02	0·86–1·21	0·74	1·13	0·94–1·35	0·18	0·9	0·77–1·05	0·2	0·97	0·8–1·18	0·77	0·9	0·75–1·08	0·26
CR2 vs. CR1	0·9	0·36–2·01	0·73	0·84	0·43–1·63	0·61	0·86	0·51–1·44	0·57	1·11	0·65–1·88	0·68	0·9	0·57–1·42	0·65	1·02	0·59–1·76	0·92	0·9	0·5–1·63	0·8
Donor CMV^+	0·7	0·34–1·48	0·36	1·05	0·57–1·92	0·86	0·84	0·53–1·32	0·45	0·76	0·47–1·23	0·26	0·69	0·46–1·06	0·09	0·72	0·42–1·21	0·22	0·66	0·39–1·11	0·11
PTCy vs. ATG	1·2	0·51–2·95	0·63	0·97	0·52–1·81	0·94	1·07	0·66–1·72	0·77	0·96	0·58–1·59	0·88	1·08	0·68–1·69	0·73	1·32	0·67–2·6	0·4	1·08	0·62–1·87	0·76
MAC vs. RIC	1·3	0·52–3	0·6	0·74	0·4–1·36	0·33	0·90	0·56–1·46	0·69	0·94	0·57–1·56	0·82	0·86	0·55–1·34	0·52	1·009	0·56–1·8	0·97	1·16	0·65–2·06	0·59

ATG, anti-thymocyte globulin; CI, 95% confidence interval; CMV, cytomegalovirus; CR1/2, first/second complete remission; GRFS, GVHD-free/relapse-free survival; GVHD, graft versus host disease; HR, hazard ratio; LFS, leukaemia-free survival; MAC, myeloablative conditioning; MRD, minimal residual disease; NRM, non-relapse mortality; OS, overall survival; PTCy, post-transplant cyclophosphamide; RI, relapse incidence; RIC, reduced intensity conditioning.

year relapse rate was 32% compared with 14% for MRD^{neg} patients (Oran *et al*, 2017). Furthermore, the predictive value of MRD extends into patients conditioned both with myeloablative as well as reduced intensity conditioning, as shown by reports from the UK (Anthias *et al*, 2014) and the US (Walter *et al*, 2015), although we note some data suggesting that myeloablative conditioning may be superior in the setting of pre-transplant MRD-positive disease(Ustun *et al*, 2016). Furthermore, a recently published meta-analysis of 19 studies lays further credence to the concept of pre-transplant MRD by showing pre-transplant MRD status to be independently associated with inferior LFS and disease relapse, irrespective of detection method (Buckley *et al*, 2017). Finally, pre-transplant MRD status may be pivotal in determination of post-induction therapy intensity, as suggested by the results of the ALFA-0702 trial, in *NPM1* mutated AML patients, which showed that the clinical benefit of allogeneic transplantation was only seen in patients who had detectable peripheral blood MRD levels following initial induction therapy as opposed to those who had undetectable MRD levels (Balsat *et al*, 2017). Interestingly, in MRD^{pos} patients, harbouring either a *FLT3*-ITD or *NPM1* mutation did not affect relapse or LFS rates. These findings concur with the MD Anderson experience who also found that in patients with pre-transplant MRD-positive studies, RI was not significantly different between *FLT3*-ITD mutated patients and $FLT3^{wt}$ patients (Oran *et al*, 2017). Similar findings were published by Chen *et al* (2015) who observed that consideration of MRD status rendered *FLT3*-ITD and *NPM1* mutational status as much weaker determinants of relapse. Thus, the marked deleterious prognostic influence imparted by a positive pre-transplant MRD study seems to outweigh the prognostic effect of either *FLT3*-ITD or *NPM1*.

As haplo-SCT gains in acceptance, it is becoming increasingly important to determine whether pre-transplant MRD assessment is clinically impactful also with this transplant modality. Our analysis reaffirms the significant impact of pre-transplant MRD on relapse and LFS. Moreover, the six-month landmark analysis reveals that the full magnitude of clinical benefit of an MRD-negative state, in terms of OS, is realized at this time point. Interestingly, these results contrast with those of a previously published analysis looking at the outcomes of 240 AML patients undergoing haplo-SCT and which did not find pre-transplant positive MRD studies to be associated with inferior clinical outcomes (Chang *et al*, 2017). The divergence in results may be possibly accounted by the different patient milieu analysed in both cohorts where ours consisted of significantly older patients (median age of 47 years compared to 27 years), different disease characteristics (our analysis included only patients with *de novo* AML), conditioning intensity [Chang *et al* (2017) used solely myeloablative conditioning], and differences in GVHD prophylaxis (i.e. ATG, PTCy). As stated earlier, a survival advantage for MRD^{neg} patients was seen only at the 6-month mark, a fact that could be possibly accounted for by a smaller effect in haplo-transplants compared to what was seen in similar analyses in matched donor transplants. Alternatively, our sample size or heterogeneity of platforms used for MRD detection could be additional possible reasons. A definitive answer to this question would be optimally addressed by future prospective studies in this patient population.

This analysis was limited by the retrospective nature of this study, which did not allow for specific assessment of the various MRD platforms used by the participating centres, as well as their specific sensitivity thresholds. As our cohort was comprised solely of transplanted patients we acknowledge the inherent selection bias which needs to be taken into consideration when interpreting of our findings. Future studies focusing on specific MRD modalities will help in further delineating the prognostic role of MRD in haplo-SCT.

Collectively, our data further support the emerging role of pre-transplant MRD in risk stratification of AML patients and indicate that MRD status is potentially a pivotal determinant of outcome in AML patients undergoing haplo-SCT. It is hoped and anticipated that pre-emptive and maintenance therapy with existing therapies, such as hypomethylating agents (Sockel *et al*, 2011; Platzbecker *et al*, 2012), FLT3 inhibitors (Battipaglia *et al*, 2017; Sandmaier *et al*, 2018) and immunotherapy (Di Grazia *et al*, 2016), as well as the introduction of novel agents will reduce relapse rates and improve outcomes in this high risk patient population.

References

Andersen, P.K., Klein, J.P. & Zhang, M.J. (1999) Testing for centre effects in multi-centre survival studies: a Monte Carlo comparison of fixed and random effects tests. *Statistics in Medicine*, **18**, 1489–1500.

Anthias, C., Dignan, F.L., Morilla, R., Morilla, A., Ethell, M.E., Potter, M.N. & Shaw, B.E. (2014) Pre-transplant MRD predicts outcome following reduced-intensity and myeloablative allogeneic hemopoietic SCT in AML. *Bone Marrow Transplantation*, **49**, 679–683.

Aoudjhane, M., Labopin, M., Gorin, N.C., Shimoni, A., Ruutu, T., Kolb, H.J., Frassoni, F., Boiron, J.M., Yin, J.L., Finke, J., Shouten, H., Blaise, D., Falda, M., Fauser, A.A., Esteve, J., Polge, E., Slavin, S., Niederwieser, D., Nagler, A. & Rocha, V.; Acute Leukemia Working Party of the European group for Blood and Marrow Transplantation. (2005) Comparative outcome of reduced intensity and myeloablative conditioning regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukaemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT). *Leukemia*, **19**, 2304–2312.

Balsat, M., Renneville, A., Thomas, X., de Botton, S., Caillot, D., Marceau, A., Lemasle, E., Marolleau, J.P., Nibourel, O., Berthon, C., Raffoux, E., Pigneux, A., Rodriguez, C., Vey, N., Cayuela, J.M., Hayette, S., Braun, T., Coude, M.M., Terre, C., Celli-Lebras, K., Dombret, H., Preudhomme, C. & Boissel, N. (2017) Postinduction minimal residual disease predicts outcome and benefit from allogeneic stem cell transplantation in acute myeloid leukemia With NPM1 Mutation: a Study by the Acute Leukemia French Association Group. *Journal of Clinical Oncology*, **35**, 185–193.

Bashey, A., Zhang, X., Sizemore, C.A., Manion, K., Brown, S., Holland, H.K., Morris, L.E. & Solomon, S.R. (2013) T-cell-replete HLA-haploidentical hematopoietic transplantation for hematologic malignancies using post-transplantation cyclophosphamide results in outcomes equivalent to those of contemporaneous HLA-matched related and unrelated donor transplantation. *Journal of Clinical Oncology*, **31**, 1310–1316.

Bashey, A., Zhang, M.J., McCurdy, S.R., St Martin, A., Argall, T., Anasetti, C., Ciurea, S.O., Fasan, O., Gaballa, S., Hamadani, M., Munshi, P., Al Malki, M.M., Nakamura, R., O'Donnell, P.V., Perales, M.A., Raj, K., Romee, R., Rowley, S., Rocha, V., Salit, R.B., Solh, M., Soiffer, R.J., Fuchs, E.J. & Eapen, M. (2017) Mobilized peripheral blood stem cells versus unstimulated bone marrow as a graft source for T-cell-replete haploidentical donor transplantation using post-transplant cyclophosphamide. *Journal of Clinical Oncology*, **35**, 3002–3009.

Battipaglia, G., Ruggeri, A., Massoud, R., El Cheikh, J., Jestin, M., Antar, A., Ahmed, S.O., Rasheed, W., Shaheen, M., Belhocine, R., Brissot, E., Dulery, R., Eder, S., Giannotti, F., Isnard, F., Lapusan, S., Rubio, M.T., Vekhoff, A., Aljurf, M., Legrand, O., Mohty, M. & Bazarbachi, A. (2017) Efficacy and feasibility of sorafenib as a maintenance agent after allogeneic hematopoietic stem cell transplantation for Fms-like tyrosine kinase 3-mutated acute myeloid leukemia. *Cancer*, **123**, 2867–2874.

Beldjord, K., Chevret, S., Asnafi, V., Huguet, F., Boulland, M.L., Leguay, T., Thomas, X., Cayuela, J.M., Grardel, N., Chalandon, Y., Boissel, N., Schaefer, B., Delabesse, E., Cave, H., Chevallier, P., Buzyn, A., Fest, T., Reman, O., Vernant, J.P., Lheritier, V., Bene, M.C., Lafage, M., Macintyre, E., Ifrah, N. & Dombret, H.; Group for Research on Adult Acute Lymphoblastic Leukemia. (2014) Oncogenetics and minimal residual disease are independent outcome predictors in adult patients with acute lymphoblastic leukemia. *Blood*, **123**, 3739–3749.

Buckley, S.A., Wood, B.L., Othus, M., Hourigan, C.S., Ustun, C., Linden, M.A., DeFor, T.E., Malagola, M., Anthias, C., Valkova, V., Kanakry, C.G., Gruhn, B., Buccisano, F., Devine, B. & Walter, R.B. (2017) Minimal residual disease prior to allogeneic hematopoietic cell transplantation in acute myeloid leukemia: a meta-analysis. *Haematologica*, **102**, 865–873.

Chang, Y.J., Wang, Y., Liu, Y.R., Xu, L.P., Zhang, X.H., Chen, H., Chen, Y.H., Wang, F.R., Han, W., Sun, Y.Q., Yan, C.H., Tang, F.F., Mo, X.D., Liu, K.Y. & Huang, X.J. (2017) Haploidentical allograft is superior to matched sibling donor allograft in eradicating pre-transplantation minimal residual disease of AML patients as determined by multiparameter flow cytometry: a retrospective and prospective analysis. *Journal of Hematology & Oncology*, **10**, 134.

Cheminant, M., Derrieux, C., Touzart, A., Schmit, S., Grenier, A., Trinquand, A., Delfau-Larue, M.H., Lhermitte, L., Thieblemont, C., Ribrag, V., Cheze, S., Sanhes, L., Jardin, F., Lefrere, F., Delarue, R., Hoster, E., Dreyling, M., Asnafi, V., Hermine, O. & Macintyre, E. (2016) Minimal residual disease monitoring by 8-color flow cytometry in mantle cell lymphoma: an EU-MCL and LYSA study. *Haematologica*, **101**, 336–345.

Chen, X., Xie, H., Wood, B.L., Walter, R.B., Pagel, J.M., Becker, P.S., Sandhu, V.K., Abkowitz, J.L., Appelbaum, F.R. & Estey, E.H. (2015) Relation of clinical response and minimal residual disease and their prognostic impact on outcome in acute myeloid leukemia. *Journal of Clinical Oncology*, **33**, 1258–1264.

Ciurea, S.O., Zhang, M.J., Bacigalupo, A.A., Bashey, A., Appelbaum, F.R., Aljitawi, O.S., Armand, P., Antin, J.H., Chen, J., Devine, S.M., Fowler, D.H., Luznik, L., Nakamura, R., O'Donnell, P.V., Perales, M.A., Pingali, S.R., Porter, D.L., Riches, M.R., Ringden, O.T., Rocha, V., Vij, R., Weisdorf, D.J., Champlin, R.E., Horowitz, M.M., Fuchs, E.J. & Eapen, M. (2015) Haploidentical transplant with posttransplant cyclophosphamide vs matched unrelated donor transplant for acute myeloid leukemia. *Blood*, **126**, 1033–1040.

Di Grazia, C., Pozzi, S., Geroldi, S., Grasso, R., Miglino, M., Colombo, N., Tedone, E., Luchetti, S., Lamparelli, T., Gualandi, F., Ibatici, A., Bregante, S., Van Lint, M.T., Raiola, A.M., Dominietto, A., Varaldo, R., Galaverna, F., Ghiso, A., Sica, S. & Bacigalupo, A. (2016) Wilms tumor 1 expression and pre-emptive immunotherapy in patients with acute myeloid leukemia undergoing an allogeneic hemopoietic stem cell transplantation. *Biology of Blood and Marrow Transplantation*, **22**, 1242–1246.

Dohner, H., Estey, E.H., Amadori, S., Appelbaum, F.R., Buchner, T., Burnett, A.K., Dombret, H., Fenaux, P., Grimwade, D., Larson, R.A., Lo-Coco, F., Naoe, T., Niederwieser, D., Ossenkoppele, G.J., Sanz, M.A., Sierra, J., Tallman, M.S., Lowenberg, B., Bloomfield, C.D. & European, L. (2010) Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*, **115**, 453–474.

Filipovich, A.H., Weisdorf, D., Pavletic, S., Socie, G., Wingard, J.R., Lee, S.J., Martin, P., Chien, J., Przepiorka, D., Couriel, D., Cowen, E.W., Dinndorf, P., Farrell, A., Hartzman, R., Henslee-Downey, J., Jacobsohn, D., McDonald, G., Mittleman, B., Rizzo, J.D., Robinson, M., Schubert, M., Schultz, K., Shulman, H., Turner, M., Vogelsang, G. & Flowers, M.E. (2005) National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biology of Blood and Marrow Transplantation*, **11**, 945–956.

Flores-Montero, J., Sanoja-Flores, L., Paiva, B., Puig, N., Garcia-Sanchez, O., Bottcher, S., van der Velden, V.H.J., Perez-Moran, J.J., Vidriales, M.B., Garcia-Sanz, R., Jimenez, C., Gonzalez, M., Martinez-Lopez, J., Corral-Mateos, A., Grigore, G.E., Fluxa, R., Pontes, R., Caetano, J., Sedek, L., Del Canizo, M.C., Blade, J., Lahuerta, J.J., Aguilar, C., Barez, A., Garcia-Mateo, A., Labrador, J., Leoz, P., Aguilera-Sanz, C., San-Miguel, J., Mateos, M.V., Durie, B., van Dongen, J.J.M. & Orfao, A. (2017) Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. *Leukemia*, **31**, 2094–2103.

Freeman, S.D., Virgo, P., Couzens, S., Grimwade, D., Russell, N., Hills, R.K. & Burnett, A.K. (2013) Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. *Journal of Clinical Oncology*, **31**, 4123–4131.

Han, L.J., Wang, Y., Fan, Z.P., Huang, F., Zhou, J., Fu, Y.W., Qu, H., Xuan, L., Xu, N., Ye, J.Y.,

Bian, Z.L., Song, Y.P., Huang, X.J. & Liu, Q.F. (2017) Haploidentical transplantation compared with matched sibling and unrelated donor transplantation for adults with standard-risk acute lymphoblastic leukaemia in first complete remission. *British Journal of Haematology*, **179**, 120–130.

Hougaard, P. (1995) Frailty models for survival data. *Lifetime Data Analysis*, **1**, 255–273.

Lee, C.J., Savani, B.N., Mohty, M., Labopin, M., Ruggeri, A., Schmid, C., Baron, F., Esteve, J., Gorin, N.C., Giebel, S., Ciceri, F. & Nagler, A. (2017) Haploidentical hematopoietic cell transplantation for adult acute myeloid leukemia: a position statement from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. *Haematologica*, **102**, 1810–1822.

Luo, Y., Xiao, H., Lai, X., Shi, J., Tan, Y., He, J., Xie, W., Zheng, W., Zhu, Y., Ye, X., Yu, X., Cai, Z., Lin, M. & Huang, H. (2014) T-cell-replete haploidentical HSCT with low-dose anti-T-lymphocyte globulin compared with matched sibling HSCT and unrelated HSCT. *Blood*, **124**, 2735–2743.

Mailankody, S., Korde, N., Lesokhin, A.M., Lendvai, N., Hassoun, H., Stetler-Stevenson, M. & Landgren, O. (2015) Minimal residual disease in multiple myeloma: bringing the bench to the bedside. *Nature Reviews. Clinical Oncology*, **12**, 286–295.

Oran, B., Jorgensen, J.L., Marin, D., Wang, S., Ahmed, S., Alousi, A.M., Andersson, B.S., Bashir, Q., Bassett, R., Lyons, G., Chen, J., Rezvani, K., Popat, U., Kebriaei, P., Patel, K., Rondon, G., Shpall, E.J. & Champlin, R.E. (2017) Pre-transplantation minimal residual disease with cytogenetic and molecular diagnostic features improves risk stratification in acute myeloid leukemia. *Haematologica*, **102**, 110–117.

Othus, M., Wood, B.L., Stirewalt, D.L., Estey, E.H., Petersdorf, S.H., Appelbaum, F.R., Erba, H.P. & Walter, R.B. (2016) Effect of measurable ('minimal') residual disease (MRD) information on prediction of relapse and survival in adult acute myeloid leukemia. *Leukemia*, **30**, 2080–2083.

Piemontese, S., Ciceri, F., Labopin, M., Bacigalupo, A., Huang, H., Santarone, S., Gorin, N.C., Koc, Y., Wu, D., Beelen, D., Tischer, J., Ehninger, G., Arcese, W., Nagler, A. & Mohty, M.; Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. (2015) A survey on unmanipulated haploidentical hematopoietic stem cell transplantation in adults with acute leukemia. *Leukemia*, **29**, 1069–1075.

Piemontese, S., Ciceri, F., Labopin, M., Arcese, W., Kyrcz-Krzemien, S., Santarone, S., Huang, H., Beelen, D., Gorin, N.C., Craddock, C., Gulbas, Z., Bacigalupo, A., Mohty, M. & Nagler, A.; Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. (2017) A comparison between allogeneic stem cell transplantation from unmanipulated haploidentical and unrelated donors in acute leukemia. *Journal of Hematology & Oncology*, **10**, 24.

Platzbecker, U., Wermke, M., Radke, J., Oelschlaegel, U., Seltmann, F., Kiani, A., Klut, I.M., Knoth, H., Rollig, C., Schetelig, J., Mohr, B., Graehlert, X., Ehninger, G., Bornhauser, M. & Thiede, C. (2012) Azacitidine for treatment of imminent relapse in MDS or AML patients after allogeneic HSCT: results of the RELAZA trial. *Leukemia*, **26**, 381–389.

Przepiorka, D., Weisdorf, D., Martin, P., Klingemann, H.G., Beatty, P., Hows, J. & Thomas, E.D. (1995) 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplantation*, **15**, 825–828.

R Core Team (2017). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/).

Ribera, J.M., Oriol, A., Morgades, M., Montesinos, P., Sarra, J., Gonzalez-Campos, J., Brunet, S., Tormo, M., Fernandez-Abellan, P., Guardia, R., Bernal, M.T., Esteve, J., Barba, P., Moreno, M.J., Bermudez, A., Cladera, A., Escoda, L., Garcia-Boyero, R., Del Potro, E., Bergua, J., Amigo, M.L., Grande, C., Rabunal, M.J., Hernandez-Rivas, J.M. & Feliu, E. (2014) Treatment of high-risk Philadelphia chromosome-negative acute lymphoblastic leukemia in adolescents and adults according to early cytologic response and minimal residual disease after consolidation assessed by flow cytometry: final results of the PETHEMA ALL-AR-03 trial. *Journal of Clinical Oncology*, **32**, 1595–1604.

Roschewski, M., Dunleavy, K., Pittaluga, S., Moorhead, M., Pepin, F., Kong, K., Shovlin, M., Jaffe, E.S., Staudt, L.M., Lai, C., Steinberg, S.M., Chen, C.C., Zheng, J., Willis, T.D., Faham, M. & Wilson, W.H. (2015) Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *Lancet Oncology*, **16**, 541–549.

Ruggeri, A., Labopin, M., Ciceri, F., Mohty, M. & Nagler, A. (2016) Definition of GvHD-free, relapse-free survival for registry-based studies: an ALWP-EBMT analysis on patients with AML in remission. *Bone Marrow Transplantation*, **51**, 610–611.

Sandmaier, B.M., Khaled, S., Oran, B., Gammon, G., Trone, D. & Frankfurt, O. (2018) Results of a phase 1 study of quizartinib as maintenance therapy in subjects with acute myeloid leukemia in remission following allogeneic hematopoietic stem cell transplant. *American Journal of Hematology*, **93**, 222–231.

Scherer, F., Kurtz, D.M., Diehn, M. & Alizadeh, A.A. (2017) High-throughput sequencing for noninvasive disease detection in hematologic malignancies. *Blood*, **130**, 440–452.

Sockel, K., Wermke, M., Radke, J., Kiani, A., Schaich, M., Bornhauser, M., Ehninger, G., Thiede, C. & Platzbecker, U. (2011) Minimal residual disease-directed preemptive treatment with azacitidine in patients with NPM1-mutant acute myeloid leukemia and molecular relapse. *Haematologica*, **96**, 1568–1570.

Terwijn, M., van Putten, W.L., Kelder, A., van der Velden, V.H., Brooimans, R.A., Pabst, T., Maertens, J., Boeckx, N., de Greef, G.E., Valk, P.J., Preijers, F.W., Huijgens, P.C., Drager, A.M., Schanz, U., Jongen-Lavrecic, M., Biemond, B.J., Passweg, J.R., van Gelder, M., Wijermans, P., Graux, C., Bargetzi, M., Legdeur, M.C., Kuball, J., de Weerdt, O., Chalandon, Y., Hess, U., Verdonck, L.F., Gratama, J.W., Oussoren, Y.J., Scholten, W.J., Slomp, J., Snel, A.N., Vekemans, M.C., Lowenberg, B., Ossenkoppele, G.J. & Schuurhuis, G.J. (2013) High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from the HOVON/SAKK AML 42A study. *Journal of Clinical Oncology*, **31**, 3889–3897.

Therneau, T., Crowson, C. & Atkinson, E. (2016) Using time dependent covariates and time dependent coefficients in the Cox model. *Mayo Clinic*. Available at: https://cran.r-project.org/web/packages/survival/vignettes/timedep.pdf

Ustun, C., Courville, E.L., DeFor, T., Dolan, M., Randall, N., Yohe, S., Bejanyan, N., Warlick, E., Brunstein, C., Weisdorf, D.J. & Linden, M.A. (2016) Myeloablative, but not reduced-intensity, conditioning overcomes the negative effect of flow-cytometric evidence of leukemia in acute myeloid leukemia. *Biology of Blood and Marrow Transplantation*, **22**, 669–675.

Walter, R.B., Gyurkocza, B., Storer, B.E., Godwin, C.D., Pagel, J.M., Buckley, S.A., Sorror, M.L., Wood, B.L., Storb, R., Appelbaum, F.R. & Sandmaier, B.M. (2015) Comparison of minimal residual disease as outcome predictor for AML patients in first complete remission undergoing myeloablative or nonmyeloablative allogeneic hematopoietic cell transplantation. *Leukemia*, **29**, 137–144.