ARTICLE

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Allogeneic stem cell transplantation in second complete remission for core binding factor acute myeloid leukemia: a study from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation

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ABSTRACT

Core binding factor acute myeloid leukemia (AML) comprises two subtypes with distinct cytogenetic abnormalities of either t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22). Since long-term response to chemotherapy in these leukemias is relatively good, allogeneic hematopoietic stem cell transplantation is considered in patients who relapse and achieve second complete remission. To evaluate the outcomes of allogeneic transplantation in this indication, we studied 631 patients reported to the European Society for Blood and Marrow Transplantation Registry between the years 2000 and 2014. Leukemia-free survival probabilities at two and five years were 59.1% and 54.1%, while overall survival probabilities were 65% and 58.2%, respectively. The incidence of relapse and risk of non-relapse mortality at the same time points were 19.8% and 22.5% for relapse and 20.9% and 23.3% for non-relapse mortality, respectively. The most important adverse factors influencing leukemia-free and overall survival were: leukemia with t(8;21), presence of three or more additional chromosomal abnormalities, and Karnofsky performance score <80. Relapse risk was increased in t(8;21) leukemia and associated with additional cytogenetic abnormalities as well as reduced intensity conditioning. Measurable residual disease in molecular evaluation before transplantation was associated with increased risk of relapse and inferior leukemia-free survival.

Introduction

Core binding factor (CBF) leukemia represents up to 12% of all newly diagnosed adult acute myeloid leukemia (AML).[1] Chromosomal markers of CBF AML include t(8;21)(q22;q22) and inv(16)(p13q22) or less frequently t(16;16)(p13;q22), further described jointly as inv(16). As a result of chromosomal abnormalities, fusion transcripts *RUNX1-RUNX1T1* in t(8; 21) and *CBFB-MYH11* in inv(16) emerge. The tran-

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scripts represent molecular attributes of CBF AML and are driver mutations for leukemogenesis. They disrupt normal hematopoiesis dependent on core binding factor subunit α (*RUNX1*) and β (*CBFB*) by silencing tumor suppressor genes leading to neoplastic transformation.[2] Accompanying secondary gene mutations (mutations of *NRAS*, *KIT*, *NF1*, *FLT3*, *KRAS*, *ASXL1&2*), additional cytogenetic abnormalities, and clinical features at diagnosis (age, white blood cell and blast counts, extramedullary involvement) affect treatment outcomes, but general prognosis in CBF AML remains favorable.[3,4] Indeed, current induction chemotherapy standards lead to a complete remission (CR) rate of 87-89%, involving a high proportion of younger patients.[5,6] Repeated high or intermediate-dose cytarabine consolidation provides long-term disease control in a large proportion of patients. Conventional chemotherapy results in long-term survival in 53-64% of patients. The major reason for treatment failure in CBF AML is relapse, reported in 30-50% of patients.[7,8] Given the relatively favorable results of chemotherapy, patients with CBF leukemia are not usually candidates for allogeneic hematopoietic stem cell transplantation (HSCT) in first CR (CR1). However, CBF AML is a heterogeneous group of malignancies. Several variables, including type of CBF subunit involved, age, additional molecular or cytogenetic abnormalities, and dynamics of measurable residual disease (MRD) are known to influence the outcomes and contribute to disease recurrence.[7-11] HSCT is recognized as a standard procedure in patients who relapse and subsequently achieve CR2.[4,12] To evaluate the results of HSCT in CBF AML patients in CR2, we decided to perform a retrospective study using registry data from the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT). The EBMT is a non-profit, scientific society representing more than 600 transplant centers, mainly in Europe. Member centers are required to report all consecutive stem cell transplantations and follow ups once a year. Data are entered, managed, and maintained in a central database with internet access; each EBMT center is represented in this database. Audits are routinely performed to determine accuracy of data. Before transplantation, patients or legal guardians provide informed consent authorizing the use of their anonymized personal information for research purposes.

Methods

Patients and data selection

The study was approved by the ALWP Institutional Review Board and included all adult patients undergoing HSCT in the period from the year 2000 to 2014 reported to the EBMT. The centers were asked by survey to provide data on all patients with t(8;21) or inv(16) to verify the cytogenetic aberrations and to update the transplantation outcomes using designated clinical forms. The patients had to have *de novo* CBF AML, with classical cytogenetics confirmation of t(8;21) or inv(16) at initial diagnosis, undergoing HSCT in hematologic CR2, defined as less than 5% blasts in the bone marrow (BM) and absence of extramedullary involvement, and regardless of current peripheral blood (PB) counts (i.e. *bona fide* CR or CR with incomplete hematologic recovery). All patients received BM or PB transplantation (BMT, PBSCT) from matched sibling (MSD) or unrelated donors (UD) after myeloablative (MAC) or reduced intensity (RIC) conditioning, as defined by the EBMT criteria.[13] The variables selected to assess outcomes were: age, type of AML, white blood cell count, presence of extramedullary involvement at diagnosis, additional cytogenetic abnormalities, time from diagnosis to CR1, duration of CR1, time from diagnosis and from CR2 to transplantation, molecular remission status at transplantation, Karnofsky performance score (KPS) at transplantation, sex matching of patients and donors, cytomegalovirus (CMV) serological status of patients and donors, year of transplantation, type of the donor, source of stem cells, conditioning intensity, and *in vivo* T-cell depletion.

End points and statistical analysis

The primary end point was leukemia-free survival (LFS). Secondary end points were: overall survival (OS), relapse incidence (RI), non-relapse mortality (NRM), graft-*versus*-host disease-free and leukemia-free survival (GRFS), as well as acute and chronic graft-*versus*-host disease (aGvHD and cGvHD). LFS was defined as survival without any symptoms of disease recurrence. OS was defined as probability of survival from transplantation to the last follow up. Relapse was defined as presence of >5% blasts in the BM or extramedullary disease after transplantation. NRM was defined as mortality from any cause not related to disease recurrence and GRFS was defined as survival without leukemia, aGvHD grade III-IV or extensive cGvHD.[14] Minimal residual disease (MRD) was measured in the BM during the interval between last chemotherapy and transplantation. Real-time quantitative polymerase chain reaction (RT-qPCR) was used for *RUNX1-RUNX1T1* and *CBFB-MYH11* quantification. MRD results were reported by the centers as absent (MRDneg) or present (MRDpos) in line with their local guidelines. Acute GvHD was graded according to Glucksberg criteria.[15] Surviving patients were censored at last follow up. Probabilities of LFS, OS, and GRFS were calculated using Kaplan-Meier estimates. Cumulative incidence functions (CIF) were used to determine RI and NRM in a competing risk setting with each other. Univariate analyses were performed using Gray's test for CIF and the log-rank test for LFS and OS. For all univariate analyses, continuous variables were categorized and the median was used as cut-off point. Associations of patient and transplantation characteristics with outcomes were evaluated in multivariate analysis using Cox proportional hazards model. Multivariate models were built by using stepwise selection procedure. Results were expressed as the hazard ratio (HR) with 95% Confidence Interval (CI). All tests were two-sided. The type-1 error rate was fixed at 0.05 for determination of factors associated with time to event outcomes. Statistical analyses were performed with SPSS 24 (SPSS Inc. /IBM, Armonk, NY, USA) and R 1.3.0 (R Development Core Team, Vienna, Austria) software packages.

Results

The detailed characteristics of the 631 patients from 181 transplant centers who met the study inclusion criteria are shown in Table 1. Three hundred and sixty-six patients (58%) harbored inv(16) and 265 (42%) t(8;21). The two groups were compared for essential patient and transplant characteristics (*Online Supplementary Table S1*). The differences included: sex of the patients [with more males in the t(8;21) group], time from diagnosis to transplantation [which was longer in the t(8;21) group], and time from diagnosis to CR1 [which was also longer in the t(8;21) group]. Altogether there were 361 (57%) males and 270 (43%) females. Median age at transplantation was 41.7 years [range 18-73, interquartile range (IQR) 31.3-51.2],

Table 1. Patients' and transplant characteristics. Percentage values in parentheses refer to reported data.

Number of patients	631
Median follow up, months (range)	59.6 (0.9 - 201)
Median year of transplantation (range)	2010 (2000-2014)
Type of AML	
inv(16)	366(58%)
t(8;21)	265(42%)
Median age at transplantation, years (range; IQR)	41.7 (18 -73; 31.3-51.2)
Median CR1 duration, days (range; IQR)	318 (6-2380; 246-474)
Median time from diagnosis to transplantation, months (range; IQR)	17 (3.5-222.9; 14-22.5)
Sex	
Male	361(57.2%)
Female	270(42.8%)
Donors	
Matched siblings	264(42%)
Unrelated	367(58%)
Additional chromosomal abnormalities	
No abnormality reported	497(79%)
3 or more abnormalities	32(5%)
Abn5	2(0.3%)
Abn7	10(1.6%)
Del 9	5(0.8%)
Del X or Y	18(2.9%)
Trisomy 22	9(1.4%)
Trisomy 8	10(1.6%)
Hyperdiploidy	4(0.6%)
Hypodiploidy	7(1.1%)
Undefined/other abnormalities	34(5.39)
Molecular remission at transplantation	
Molecular CR	343(73.3%)
No molecular CR	125(26.7%)
Missing	163
Karnofsky performance score	
<80	16(2.8%)
≥80	559(97.2%)
Missing	56
Conditioning intensity	
Myeloablative	424(67.5%)
Reduced intensity	204(32.5%)
Missing	3
Source of stem cells	
Bone marrow	117(18.5%)
Peripheral blood	514(81.5%)
GvHD prophylaxis	
CsA based	584(92.6%)
Tacrolimus based	26(4%)
PTCY	6(1%)
Other	10(1.6%)
Missing	5(0.8%)
In vivo T-cell depletion	
Yes	325(51.8%)
No	302(48.2%)
Missing	4
Donor sex	
Male	369(59.4%)
Female	252(40.6%)
Missing	10
Female to male transplantation	133(21.2%)
CMV serology	
Patient CMV IgG positive	387(63%)
Donor CMV IgG positive	305(49.9%)
Engraftment	
Yes	619(98.7%)
No	8(1.3%)
Missing	4
aGvHD grade II-IV	
Yes	171(27.9%)
No	443(72.1%)
Missing	17
cGvHD	
Yes	279(46.7%)
No	318(53.3%)
Missing	34

continued in next column

AML: acute myeloid leukemia; IQR: interquartile range; CR1: first complete remission; abn 5: abnormalities of chromosome 5; abn 7: abnormalities of chromosome 7; del 9 complete or partial deletion of chromosome 9; del X or Y, deletion of chromosome X or Y; trisomy 22: trisomy of chromosome 22; trisomy 8: trisomy of chromosome 8; CR: complete remission; GvHD: graft-*versus*-host disease; CsA: cyclosporine A; PTCY: post-transplant cyclophosphamide; CMV IgG: cytomegalovirus-specific immunoglobulin G antibody; aGvHD: acute graft-*versus*-host disease; cGvHD: chronic graft-*versus*-host disease.

and the median year of transplantation was 2010. Nearly half of the procedures were performed between the years 2010 and 2014. Additional analysis of transplantation intervals 2000-2005, 2006-2009, and 2010-2014 periods did not reveal any significant differences in outcomes. Twenty-one percent of patients had additional cytogenetic aberrations detected at diagnosis. The most frequent of them was presence of three or more abnormalities (32.5%). There was a low frequency of reports of accompanying molecular abnormalities (cKIT mutations, FLT3-ITD, NRAS mutations and KRAS mutations) which precluded subset evaluation. The most frequent available information on co-mutation pattern was FLT3-ITD, which was reported in 26 patients, with a similar distribution between the inv(16) and the t(8;21) groups (14 and 12 patients, respectively). Three hundred and forty-three (73.3%) patients were MRDneg, while 125 (26.7%) were MRDpos before transplantation. There was a trend for higher frequency of MRDpos patients in the t(8;21) compared to the inv(16) subgroup (P=0.06) (*Online Supplementary Table S1*). Further analysis showed significant differences in terms of LFS, OS, and relapse in favor of inv(16) compared to t(8;21) AML in MRDneg but not MRDpos patients (*Online Supplementary Table S2*). Engraftment was achieved in 619 (98.7%) patients.

Leukemia-free survival

The 2- and 5-year probability of LFS was 59.1% (95%CI: 55.2-63.1) and 54.1% (95%CI: 50-58.2), respectively. In univariate analysis, LFS was significantly higher for patients with inv(16) compared to patients with t(8;21) (63.8% *vs.* 52.5%, P=0.003) (Figure 1A). Presence of three or more additional cytogenetic abnormalities at diagnosis resulted in worse LFS (37.5% *vs.* 60.4%, P=0.002). For MRDpos patients, the probability of LFS was 49% compared to 61.6% for patients who were MRDneg (P=0.046) (Figure 2A). Performance status was also an important factor, with 2-year LFS probability of 59.9% for patients with KPS ≥80 *versus* 37.5% for those with KPS <80 (P=0.003). The results of the univariate analysis are provided in *Online Supplementary Table S3*. In multivariate analysis, the type of CBF AML [t (8;21) *versus* inv(16)] was an independent factor for LFS (HR=1.40, 95%CI: 1.05-1.86, P=0.022) as was presence of three or more additional cyto-

Table 2. Multivariate analysis using Cox proportional hazards model. Variables with *P*< 0.15 in univariate analysis were included in the model.

		P	HR	95% CI
LFS	t(8;21) *vs*. inv(16)	0.022	1.40	1.05-1.86
	≥3 chromosomal abnormalities *vs*. no	0.004	2.09	1.27-3.42
	Molecular MRDneg *vs*. MRDpos	0.080	0.76	0.55-1.03
	KPS ≥ 80 *vs*. < 80	0.006	0.32	0.14-0.73
OS	t(8;21) *vs*. inv(16)	0.00002	1.76	1.35-2.28
	≥3 chromosomal abnormalities *vs*. no	0.037	1.68	1.03-2.72
	KPS ≥ 80 *vs*. < 80	0.002	0.36	0.19-0.68
RI	t(8;21) *vs*. inv(16)	0.002	1.89	1.26-2.84
	≥3 chromosomal abnormalities *vs.* no	0.011	2.31	1.23-4.40
	Time from diagnosis to transplantation (>median>)	0.023	0.97	0.94-0.99
	RIC *vs*. MAC	0.017	1.64	1.09-2.47
	Molecular MRDneg *vs*. MRDpos	0.043	0.65	0.42-0.99
NRM	KPS ≥ 80 *vs*. < 80	0.001	0.29	0.14-0.59
GRFS	Molecular MRDneg *vs.* MRDpos	0.054	0.77	0.60-1.00
	≥3 chromosomal abnormalities *vs*. no	0.031	1.61	1.04-2.47
	In vivo TCD *vs*. no	0.027	0.76	0.60-0.97
	Donor CMV IgG negative *vs*. positive	0.058	0.79	0.99-1.61
aGvHD II-IV	RIC *vs*. MAC	0.011	0.64	0.45-0.90
cGvHD	*In vivo* TCD *vs*. no	<10-5	0.56	0.43-0.72
	Donor CMV IgG positive *vs*. negative	0.004	1.45	1.13-1.87
	PBSCT *vs*. BMT	0.003	1.72	1.20-2.46

LFS: leukemia-free survival; MRDneg: minimal residual disease negative; MRDpos: minimal residual disease positive; KPS: Karnofsky performance score; OS: overall survival; RI: relapse incidence; RIC: reduced intensity conditioning; MAC: myeloablative conditioning; NRM: non-relapse mortality; GRFS: graft-*versus*-host disease-free, relapse-free survival; CMV IgG: cytomegalovirus-specific immunoglobulin G antibody; TCD: T-cell depletion; aGvHD II-IV: acute graft-*versus*-host disease, grades II to IV; cGVHD: chronic graft-*versus*-host disease; PBSCT: peripheral blood stem cell transplantation; BMT: bone marrow transplantation.

genetic abnormalities (HR=2.09, 95%CI: 1.27-3.42, *P*=0.004), and KPS ≥80 (HR=0.32; 95%CI: 0.14-0.73, *P*=0.32). In multivariate analysis, MRDneg was not an independent prognostic factor for LFS (HR=0.76; 95%CI: 0.55-1.03, *P*=0.08) (Table 2).

Overall survival

Two- and 5-year OS probability for the whole group was 65% (95%CI: 61.2-68.9) and 58.2% (95%CI: 54.1-62.3), respectively. In univariate analysis, patients with t(8;21) AML had a lower probability of OS compared to those with inv(16) (57% *vs.* 70.5%, *P*=0.0003) (Figure 1B). Three or more additional cytogenetic abnormalities was associated with lower OS (49.6% *vs*. 65.9%, *P*=0.013). Performance status at transplantation influenced OS. OS of patients with KPS≥80 was 66.1% *versus* 37.5% in those with KPS<80 (*P*=0.003) (*Online Supplementary Table S3*). MRDneg was not significantly associated with OS (Figure 2B). Multivariate analysis confirmed the findings of the univariate analysis. AML with t(8; 21), additional cytogenetic abnormalities, and KPS <80 were the three independent prognostic factors for significantly worse OS with HR 1.76 (95%CI: 1.35-2.28, *P*=0.00002), HR 1.68 (95%CI: 1.03-2.72, *P*=0.037), and HR 0.36 for KPS ≥80 (95%CI: 0.19-0.68, *P*=0.002), respectively (Table 2). In multivariate analysis, MRD status was not an independent prognostic factor for OS (59.9%; 95%CI: 50.8-68.9 *vs*. 65.8%; 95%CI: 60.7-71, *P*=0.47). Age at HSCT (below or above the median) did not affect OS (66.5%; 95%CI: 61.1-71 *vs*. 63.6%; 95%CI: 58.1-69, *P*=0.39).

Relapse incidence

The risk of relapse at two and at five years was estimated at 19.8% (95%CI: 16.7-23.1) and 22.5% (95%CI: 19.2-26). In patients with t (8; 21), the risk of relapse at two years was significantly higher: 25.8% *versus* 15.6% in those with inv (16) (*P*=0.009) (Figure 1C). The risk of relapse was higher in patients with three or more additional chromosomal aberrations (34.4% *vs*. 19%, *P*=0.03). In the whole cohort, MRDneg patients had a significantly decreased risk of relapse compared to MRDpos patients (16.2% *vs*. 29.3%, *P*=0.003) (Figure 2C). In patients with CR1 shorter than the median (318 days), the risk of relapse after transplantation was higher (26.4% *vs*. 13%, *P*< 0.001). Time from diagnosis to transplantation was also significant. In patients receiving HSCT within a shorter time than the median (17 months from diagnosis), the risk of relapse was higher (26.4% *vs.* 13.1%, *P*<0.001). Conditioning intensity was also important. Patients receiving RIC experienced more leukemia relapses compared to those receiving MAC (25.9% *vs*. 17%, *P*=0.002). Finally, *in vivo* T-cell depletion led to more recurrences (22.6% *vs.* 16.7% in patients transplanted without T-cell depletion (*P*=0.02) (*Online Supplementary Table S3*). In multivariate analysis, t(8; 21) *versus* inv(16), presence of three or more additional chromosomal abnormalities, time from diagnosis to transplantation (> *vs*. ≤ median), MRDneg, and RIC were independent significant prognostic factors for relapse. The corresponding HR values for those factors were 1.89 (95%CI; 1.26-2.84, *P*=0.002), 2.31 (95%CI: 1.23-4.4, *P*=0.011), 0.97 (95%CI: 0.94-0.99, *P*=0.023), 0.65 (95%CI: 0.42-0.99, *P*=0.043), and 1.64 (95%CI: 1.09-2.47, *P*=0.017), respectively. *In vivo* T-cell depletion was not confirmed to be an independent risk factor for relapse in multivariate analysis (Table 2).

Non-relapse mortality

The 2- and 5-year incidence of NRM was 20.9% (95%CI: 17.7-24.2) and 23.3% (95%CI: 19.9-26.8), respectively. In univariate analysis, KPS <80 *versus* ≥80 was

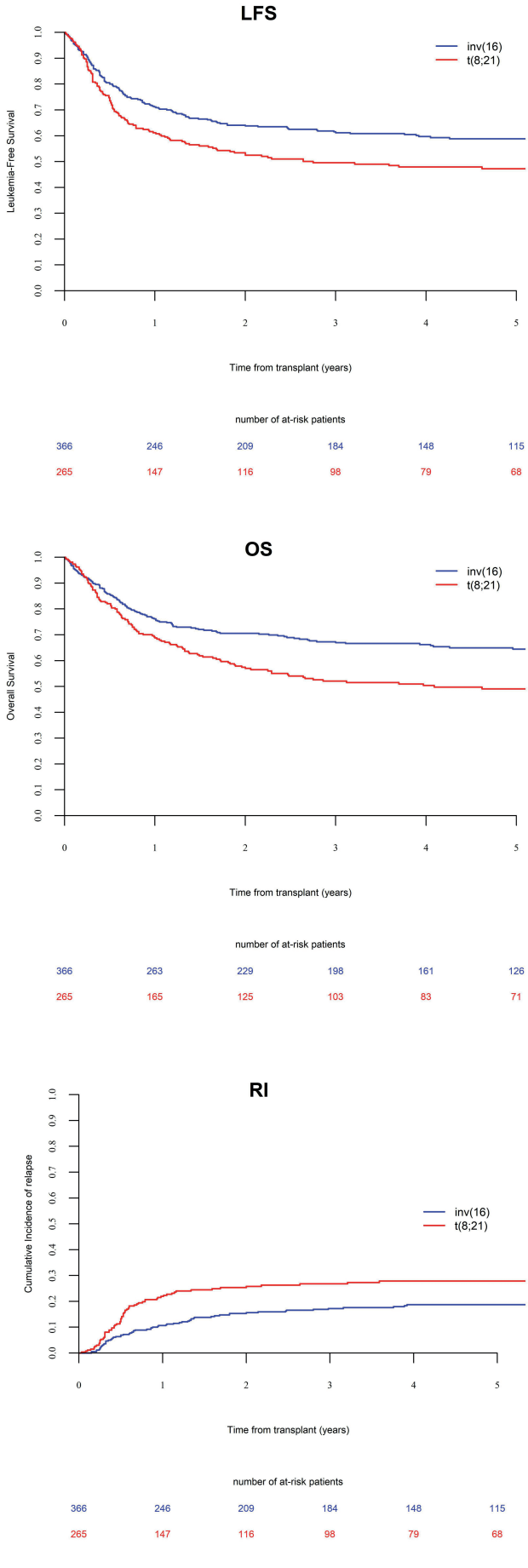

Figure 1. Leukemia-free survival (LFS), overall survival (OS), and relapse incidence (RI) in patients with core-binding factor acute myeloid leukemia (CBF AML) transplanted in second complete remission for patients with inv(16) *versus* t(8;21). 2-year probability of LFS: 63.8% (95% CI: 58.8-68.8) *vs.* 52.5% (95% CI: 46.2-58.8), *P*=0.003. 2-year probability of OS: 70.5% (95% CI: 65.8-75.3) *vs.* 57% (95% CI: 50.7-63.2), *P*=0.0003. 2-year risk of relapse: 15.6% (95% CI: 12-19.6) *vs.* 25.8% (95% CI: 20.5-31.4), *P*=0.009.

strongly associated with NRM (50% *vs.* 19.8%, *P*=0.002). Patients in whom CR1 duration was shorter than the median, or those who were transplanted at a shorter time from diagnosis than the median, experienced decreased NRM (17.1% *vs.* 25.8%, *P*=0.007 and 18% *vs.* 24%, *P*=0.01, respectively) (*Online Supplementary Table S3*). In multivariate analysis, only performance status was an independent risk factor for NRM; HR 0.29 (95%CI: 0.14-0.59, *P*=0.001) for patients with KPS ≥80 *versus* those with KPS <80 (Table 2).

Graft-*versus*-host disease-free and leukemia-free survival

The 2- and 5-year probability of GRFS was 40.2% (95%CI: 36.2-44.2) and 34.6% (95% CI: 30.6-38.6), respectively. The 2-year probability of GRFS for patients with inv (16) was higher than for those with t(8; 21) (44.1% *vs.* 34.7%, *P*=0.049). Presence of three or more additional chromosomal aberrations was significantly associated with worse GRFS (20% *vs.* 41.3%, *P*=0.01). Patients who were MRDneg before transplantation had a higher probability of GRFS (42.9% *vs.* 29.2%, *P*=0.02). Similarly, those who received *in vivo* T-cell depletion had a higher GRFS (46.1% *vs.* 33.9%, *P*=0.004). Finally, there was a trend for better GRFS in patients transplanted from CMV seronegative *versus* seropositive donors (41.8% *vs.* 38.4%, *P*=0.07) (*Online Supplementary Table S3*). In multivariate analysis, factors independently associated with GRFS were three or more cytogenetic abnormalities and *in vivo* T-cell depletion (HR 1.61; 95%CI: 1.04-2.47, *P*=0.03 and HR 0.76; 95%CI: 0.6-0.97, *P*=0.027, respectively). Transplantation from CMV negative donors and MRDneg status were associated with a trend for better GRFS (HR0.79; 95%CI: 0.62-1, *P*=0.058 and HR 0.77; 95%CI: 0.6-1.0, *P*=0.054, respectively) (Table 2).

Graft-*versus*-host disease

The incidence of aGvHD grades II to IV and III-IV was 28% (95%CI: 24.5-31.6) and 9.5% (95%CI: 7.3-12), respectively. In univariate analysis, transplantation from MSD compared to UD was associated with lower incidence of grade II-IV aGvHD (24.1% *vs.* 30.8%, *P*=0.049). Grade II-IV aGvHD was higher in patients transplanted with BM *vs.* PB grafts (36% *vs.* 26.1%, *P*=0.04). MAC in comparison to RIC was associated with increased incidence of aGvHD grade II-IV (30.8% *vs.* 21.6%, *P*=0.01). *In vivo* T-cell depletion reduced grade II-IV (23.6% *vs.* 32.7%, *P*=0.01) and grade III-IV (5.7% *vs.* 13.6%, *P*=0.009) aGVHD incidence (*Online Supplementary Table S3*). In multivariate analysis, only intensity of conditioning regimen (RIC *vs.* MAC) was an independent prognostic factor for aGvHD grade II-IV: HR 0.64 (95%CI: 0.45-0.9), *P*=0.011 (Table 2).

The incidence of cGvHD at two and five years post transplant was 46.7% (95%CI: 42.5-50.8) and 48.4% (95%CI: 44-52.4), respectively. Transplantation from

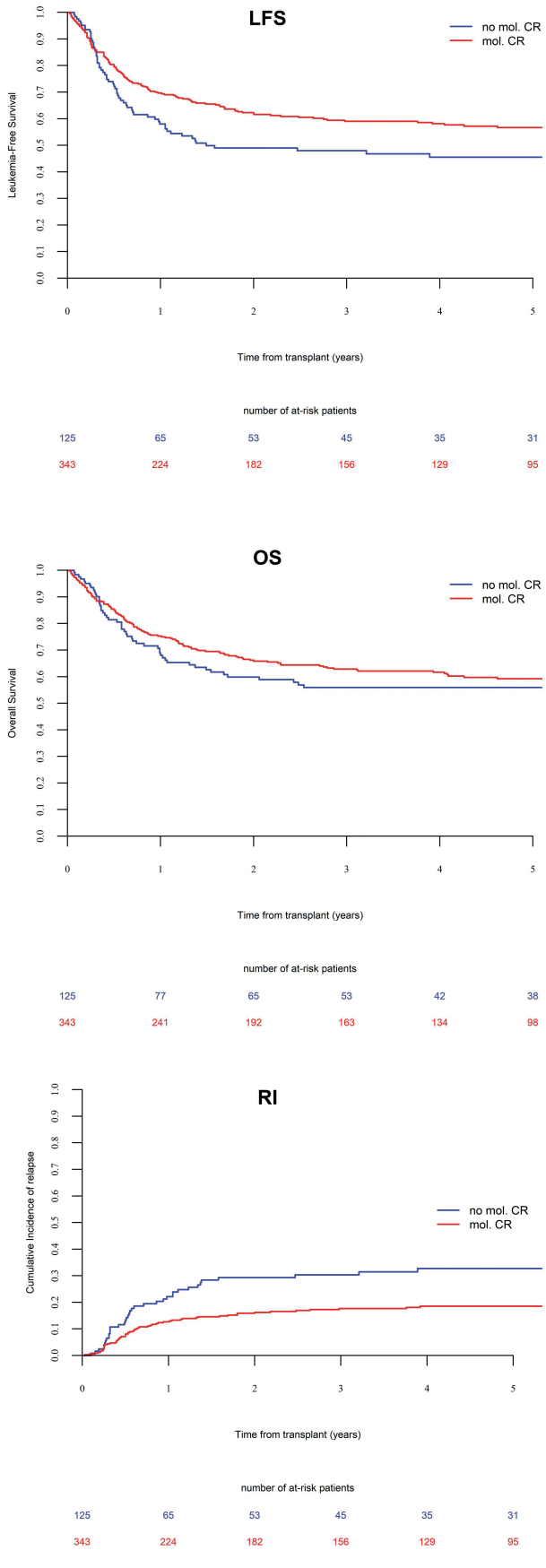

Figure 2. Leukemia-free survival (LFS), overall survival (OS), and relapse incidence (RI) in patients with core-binding factor acute myeloid leukemia in patients without *versus* with molecular remission pre-transplant. 2-year probability of LFS: 49% (95%CI: 39.8-58.2) *vs.* 61.6% (95%CI: 56.3-66.9), *P*=0.046. 2-year probability of OS: 59.9% (95%CI: 50.8-68.9) *vs.* 65.8% (95%CI: 60.7-71), *P*=0.47. 2-year risk of relapse: 29.3% (95%CI: 21.2-37.8) *vs.* 16.2% (95%CI: 12.4-20.4), *P*=0.003.

Table 3. Mortality during follow up.

Causes of death	Number
Total	257
Original disease	83
Infection	62
Graft-*versus*-host disease	59
Other related to transplantation	21
Interstitial pneumonitis	9
Sinusoidal obstruction syndrome	5
Hemorrhage	4
Second malignancy	4
Cardiac toxicity	2
Missing	8

female *versus* male donors was associated with increased risk of cGvHD (52.1% *vs.* 43.4%, *P*=0.01); the same was true for female to male transplantations *versus* other combinations (55.2% *vs.* 44.5%, *P*=0.03). Transplantation from CMV positive *versus* CMV negative donors also correlated with increased risk of cGvHD (53.2% *vs.* 40.5%, *P*=0.002). BM *versus* PB grafts resulted in lower incidence of cGVHD (37.1% *vs.* 49.1%, *P*=0.04). *In vivo* T-cell depletion decreased risk of cGVHD (37.7% *vs.* 55.9%, *P*<0.001) (*Online Supplementary Table S3*). In multivariate analysis, *in vivo* T-cell depletion was an independent factor for decreased risk of cGvHD (HR=0.56; 95%CI: 0.43-0.72, *P*<0.001), while PBSCT and CMV donor seropositivity were associated with increased risk of cGVHD (HR=1.72; 95%CI; 1.2-2.46, *P*=0.003 and HR=1.45; 95%CI: 1.13-1.87, *P*=0.004, respectively) (Table 2).

Mortality

During follow up, 257 of 631 patients died. The main causes of death were recurrence of the original disease, infection, and GvHD (Table 3).

Discussion

This retrospective analysis of HSCT in CBF AML in second hematologic CR was based on a large number of patients reported to the EBMT. Chemotherapy alone after relapse in patients with favorable risk AML is able to produce 5-year survival in 42-44% of patients.[16,17] Allogeneic HSCT is recommended by leading organizations in Europe and the USA as consolidation treatment for AML patients achieving CR2.[18] In our study, the results of transplantation in terms of OS and LFS were a little worse than those described previously for patients with CBF AML transplanted in CR1 and comparable with published outcomes of HSCT performed in CR2.[19,20] Similarly to those

studies, in our group, patients with inv(16) had a higher probability of LFS, OS, and a lower risk of relapse than those with t(8;21). Interestingly, these end points reported in most papers for patients treated with chemotherapy alone are not usually different for inv(16) and t(8;21) AML. On the other hand, the MD Anderson study, for example, pointed out that patients diagnosed with t(8;21) have a worse prognosis than those with inv(16).[5]

Response to chemotherapy with clearance of *RUNX1-RUNX1T1* and *CBFB-MYH11* evaluated with RT-qPCR, as well as additional molecular aberrations detected at diagnosis, but not type of CBF AML *per se*, are most frequently emphasized as the factors determining outcome in chemotherapy-treated patients.[9,21,22] Presence of MRD assessed with flow cytometry in AML before transplantation is a recognized risk factor for inferior outcome.[23] Molecular evaluation of MRD in CBF AML before transplantation has not been extensively studied to date. In our cohort, MRDneg patients had a significantly decreased risk of relapse compared with MRDpos patients (HR=0.65, *P*=0.043); this translated into a trend for improved LFS (HR=0.76, *P*=0.08) and GRFS (HR=0.77, *P*=0.054) but showed no significant influence on OS (*P*=0.47). Data analysis revealed that MRDpos patients more frequently received donor lymphocyte infusions or subsequent transplants after relapse than MRDneg patients. Those therapeutic interventions, and probably lack of statistical power, may explain why we did not find a significant difference in OS in favor of MRDneg patients. The results of our study indicate that even patients who are MDRpos can expect survival advantage from transplantation compared to those who are treated with chemotherapy alone.[9] A recent paper showed that evaluation of *RUNX1*-RUNX1T1 was useful to predict relapse not only before but also after HSCT.[24] It should be emphasized that the kinetics of relapse in inv(16) and t(8;21) patients differ, and the latter group requires more frequent molecular testing.[25]

According to the 2017 European Leukemia Net and National Comprehensive Cancer Network guidelines, additional cytogenetic aberrations in CBF AML do not modify disease risk.[4,26] In our study group, the presence of concurrent three or more chromosomal abnormalities had a marked deleterious effect on relapse (HR=2.31, *P*=0.011), LFS (HR=2.09, *P*=0.004), and even OS (HR=1.68, *P*=0.037) after HSCT. Indeed, earlier reports documented worse outcomes in newly diagnosed CBF AML patients with three or more cytogenetic abnormalities.[5] This finding may indicate a more complex clonal evolution, and could support the adoption of anticipated measures to avoid relapse, such as indication of transplantation in first remission.

Not surprisingly, in our study, performance status was a strong independent risk factor for NRM, LFS, and OS. Thus, patients with KPS ≥80 had decreased NRM and improved LFS and OS, which was similar to the findings of previous studies.[27]

The intensity of conditioning regimen in the current analysis favored MAC over RIC in terms of relapse. Comparable findings were described in a recent EBMT ALWP study in patients transplanted for secondary AML with additional benefit of higher probability of LFS and OS in individuals receiving MAC.[28] The results of an American phase III prospective randomized trial of MAC *versus* RIC in AML and myelodysplastic syndrome patients published in 2017 also revealed statistically higher relapse rates and worse LFS with a trend for decreased OS after RIC.[29] In contrast, in a German randomized study including AML patients published a few years earlier, RIC and MAC yielded identical results for both types of conditioning, even in terms of disease recurrence.[30] In our cohort, conditioning intensity had no significant impact on LFS or OS. In the German trial, MAC was also a predictor for aGvHD. Similarly, in our study, MAC was the only independent risk factor for clinically significant, grade II-IV aGvHD. The same correlation had been described previously, and is supported by the concept of a more pronounced inflammatory reaction after MAC.[31]

Independent factors influencing cGvHD in our study were: *in vivo* TCD, the use of PBSC *versus* BM, and transplantation from CMV seropositive donors; these findings are in agreement with previous literature.[32,33] Only recently, possible mechanisms linking CMV immunity and cGvHD were studied in HSCT recipients. In patients with cGvHD, a higher proportion of donor-origin high-affinity CMV-specific cytotoxic T lymphocytes was demonstrated.[34] The composite end point described as GRFS represents the most desirable outcome of HSCT. In our study, 2- and 5-year probabilities of GRFS were 40.2% and 34.6%, respectively. Recently, a large analysis of 5,059 AML patients from the EBMT database defined transplantation from unrelated donors, PB stem cell transplants, and unfavorable cytogenetics as prognostic factors for worse GRFS. In contrast, *in vivo* TCD was associated with better results and was the main beneficial factor for GRFS.[35] In our cohort, type of donor and source of stem cells did not have a significant impact on GRFS, which may be due to a considerably smaller study sample. Adverse cytogenetics decreased, while *in vivo* TCD increased the probability of GRFS in our patients, which is in line with the results of the above-mentioned study.

Our registry-based, retrospective study has various well-known limitations. For example, due to low reporting, we were not able to investigate the prognostic impact of additional genetic co-mutations frequently observed in CBF-AML, such as mutations in signaling pathways KIT, NRAS, KRAS and FLT3.[36]

The most important findings of our study show that HSCT in CBF AML in CR2 was able to cure a large proportion of patients, with 2-year and 5-year OS 65% and 58.2%, respectively. The survival of patients with inv(16) was better than those with t(8;21); an observation which confirms a substantial underlying difference between the two CBF AML subtypes also in the transplant setting. Based on our results, CBF AML patients should receive MAC rather than RIC, if eligible. Although patients who were MRDneg had lower risk of relapse and higher probability of survival without recurrence of leukemia, a significant proportion of MRDpos patients obtained durable response following HSCT. In view of our study, lack of MRD clearance should not be considered a contraindication for allogeneic transplantation.

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