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ARTICLE

AlloHSCT for inv(3)(q21;q26)/t(3;3)(q21;q26) AML: a report from the acute leukemia working party of the European society for blood and marrow transplantation

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Abstract

Acute myeloid leukemia with inv(3)(q21;q26.2)/*t*(3;3)(q21;q26.2) (3q26 AML) is a rare disease with poor prognosis and median survival of <1 year. To evaluate allogeneic stem cell transplantation (alloHSCT) in the treatment of 3q26 AML, we studied 98 patients reported to the European Society for Blood and Marrow Transplantation between 1995 and 2013. Majority of patients were transplanted using peripheral blood, from unrelated donors and after myeloablative conditioning. Fifty-three patients were transplanted with active disease and 45 in complete remission. After a median follow-up of 47 months, 2 year leukemia-free survival (LFS), overall survival (OS), relapse incidence (RI), non-relapse mortality (NRM), and graft-versus-host disease-free, relapse-free survival (GRFS) probabilities were 20%, 26%, 64%, 16%, and 14%, respectively. Two-year LFS and OS probabilities for patients transplanted in CR vs. those transplanted in active disease were 23.8 vs. 17% (p = NS) and 34.9 vs. 18.9% (p = NS), respectively. In multivariate analysis CR was the only factor associated with a trend for better LFS ($p = 0.05$, HR 0.64) and OS ($p = 0.06$, HR 0.65). CR also significantly influenced GRFS ($p = 0.01$; HR 0.55) and NRM ($p = 0.02$; HR 0.27). The results suggest that a proportion of patients might benefit from the procedure, especially if performed in CR.

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Introduction

The outcome in AML strongly depends on cytogenetic and molecular abnormalities. In 2008 and recently revised Classification of Tumors of Haematopoietic and Lymphoid Tissues AML with inv(3)(q21;q26.2) or *t*(3;3)(q21;q26.2); *RPN1-EVI1* (3q26 AML) is listed as a separate entity [1]. Recent findings led to proposed renaming this type of leukemia as AML with inv(3)(q21;q26.2)/*t*(3;3)(q21;q26.2); *GATA2; MECOM* [2]. Patients may present initially with AML de novo or secondary, arising from myelodysplastic syndrome (MDS). Additional cytogenetic abnormalities are frequent with chromosome 7 monosomy being the most common, followed by complex karyotype. Patients with 3q26 AML usually have higher hemoglobin concentration, leukocyte, and platelet counts compared to their normal karyotype AML counterparts [3]. Multilineage dysplasia is frequent with a typical megakaryocytes as the most prominent feature [1]. The immunophenotypic profile of leukemic cells is not specific but high co-expression of myeloid markers (CD33, CD13, and CD117) with uncommitted markers (CD34, HLA-DR) is most commonly detected, which suggests evolution from early progenitor [4]. European Leukemia-Net recommendations classify 3q26 AML as high-risk disease [5]. In a large series of younger patients within United Kingdom Medical Research Council trials, 3q26 AML had the lowest chance of achieving CR among defined cytogenetic groups with 5-year survival probability of 5% and median survival of 10 months [6, 7]. 3q26 AML remains a very rare disease with frequency of about 1% of all AMLs [8]. In such instances retrospective analyses, with all their shortcomings considered, are the only way to evaluate therapeutic interventions. Several studies indicate benefit of allogeneic hematopoietic stem cell transplantation (alloHSCT) even in patients with high risk or refractory AML [9, 10]. Lack of efficient conventional therapy prompted Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT) to analyze alloHSCT outcome in 3q26 AML patients.

Subjects and methods

The analysis includes all adult 3q26 AML patients transplanted from matched related or unrelated donors between 1995 and 2013 reported to the EBMT database with adequate accuracy, who met cytogenetic WHO definition criteria. In cases of imprecise reporting of karyotype information (e.g., *t*(3;3)) the centers were asked to provide copies of cytogenetic reports from the time of diagnosis. Thus, all cytogenetic data was centrally verified. The patients could be transplanted with either bone marrow or peripheral stem cells, after any conditioning and at any stage of the disease. Complete remission before transplantation was defined as ≤5% blasts in bone marrow smears. Fifty EBMT centers participated in the study. The study was based on Minimum Essential Data-A (MED-A) and follow-up forms. Since 1990 all patients reported to the EBMT provide informed consent authorizing the use of their data for research purposes.

Statistical analyses and definitions

The endpoints of the study were 2-year leukemia-free survival (LFS), overall survival (OS), relapse incidence (RI), non-relapse mortality (NRM), and graft-versus-host disease-free, relapse-free survival (GRFS). LFS was defined as survival without disease recurrence. OS was defined as time from transplantation to death of any cause. Relapse was defined as morphologic evidence of disease recurrence in the bone marrow cytology with >5% blast cells or presence of chloroma. NRM was defined as death of other causes than relapse or progression. GRFS was defined according to Ruggeri et al.[11] as survival without symptoms of acute GvHD grade III–IV, severe chronic GvHD and progression of the disease [11]. Acute graft-versus-host disease was graded according to Glucksberg criteria [12]. Variables considered in statistical analysis were diagnosis (de novo or MDS related AML), type of chromosome 3 involvement (inv(3) or *t*(3;3)), patient age ≤ or >50 years, remission status at transplantation, presence of additional chromosome abnormalities (monosomy 7 and complex karyotype), patient and donor CMV IgG serology (positive or negative), type of donor (matched sibling donor or unrelated) and sex-matching (female to male transplant). Transplant variables included source of stem cells, intensity of conditioning and in vivo T-cell depletion. Cumulative incidence functions (CIF) were used to estimate RI and NRM in a competing risks setting, because death and relapse compete with each other. Chronic GvHD was studied as a time-dependent variable with relapse and death being the competing events. Probabilities of LFS, OS, and GRFS were calculated using the Kaplan–Meier estimates. Univariate analyses were performed using Gray's test for CIF and the log-rank test for LFS and OS. Associations of patient and graft characteristics with outcomes were evaluated in multivariate analysis, using Cox proportional hazards model. Variables associated with *p*-value <0.15 by univariate analysis were included in the final model. We also included factors known as possibly influencing the outcome, such as age or donor type. 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 22 (SPSS Inc./IBM, Armonk, NY) and R 3.2.3

Table 1 AlloHSCT for 3q26 AML: patients' and donors' characteristics

		N	%
Diagnosis	De novo AML	86	88
	MDS transformed or therapy-related AML[a]	12	12
Type of chromosome 3 abnormality	*t*(3;3)	34	35
	inv (3)	64	65
EVI1 overexpression	No	32	60
	Yes	21	40
	Missing/not done	45	
Additional chromosome 7 monosomy	No	50	51
	Yes	48	49
Complex karyotype[b]	No	62	74
	Yes	22	26
	Not available	14	
Patient sex	Male	54	55
	Female	44	45
Donor sex	Male	62	55
	Female	36	45
Female to male transplant	No	79	81
	Yes	19	19
Donor type	MSD	44	45
	UD	54	55
Source of stem cells	BM	18	18
	PB	80	82
Disease status at transplant	CR 1	43	44
	CR 2	2	2
	Active disease	*53*	*54*
	Primary refractory	37	38
	First relapse	16	16
Patient CMV serology	Negative	33	36
	Positive	60	64
	Missing	5	
Donor CMV serology	Negative	49	53
	Positive	43	47
	Missing	6	
Conditioning	*MAC*	61	62
	BuCy	22	38
	CyTBI	32	
	other	7	
	RIC	37	
	Flu-TBI	13	
	Flamsa-TBI	8	
	Flu-Mel	6	
	other	10	
GvHD prophylaxis	CsA alone	12	
	CsA + Mtx	51	
	CsA + MMF	31	
	Tacro + Siro	2	
	Tacro + MMF	2	
In vivo T-cell depletion	No	58	60
	Yes	39	40
	Missing	1	
Engraftment	No	2	2
	Yes	94	98
	Missing	2	
Acute GvHD	0–I	63	66
	II–IV	33	34
	Missing	2	
Chronic GvHD	No	75	77
	Yes	22	23
	Missing	1	

AML acute myeloid leukemia, *MDS* myelodysplastic syndrome, *MSD* matched sibling donor, *UD* unrelated donor, *BM* bone marrow, *PB* peripheral blood, *CB* cord blood, *CR* complete remission, *CMV* cytomegalovirus, *MAC* myeloablative conditioning, *Bu* busulfan, *Cy* cyclophosphamide, *TBI* total body irradiation, *RIC* reduced intensity conditioning, *Flu* fludarabine, *Flamsa* fludarabine-amsacrine-cytarabine, *Mel* melphalan, *GvHD* graft-versus-host disease, *CsA* cyclosporine A, *Mtx* methotrexate, *MMF* mycofenolate mofetil, *Tacro* tacrolimus, *Siro* sirolimus

[a] Three patients reported as secondary AML due to previous chemotherapy for a solid tumor and 9 transformed from MDS

[b] Karyotype with ≥3 abnormalities

(R Development Core Team, Vienna, Austria) software packages.

Results

The study group consisted of 98 patients transplanted between 1995 and 2013. The median year of transplantation was 2007. Table 1 presents essential characteristics of the cohort. The median age of patients was 44 years (range 18–76; IQR 35–54) and more than half of them were male. AlloHSCT was performed at median of 159 days from primary diagnosis (range 46–770; IQR 115–204). In 65% of patients inv(3) was identified, while 35% showed *t*(3;3) mutation. *EVI1* overexpression was confirmed only in 21 patients. The type of chromosome 3 abnormalities, the presence of additional chromosomal aberrations and AML evolving from MDS were seen with comparable frequencies among patients transplanted in remission or active disease. Chromosome 7 monosomy was the most frequent additional mutation and was reported in 49% of the patients. In addition complex karyotype was found in 22 out of 84 patients with available information. At transplantation 54% of the patients had active disease: 38% of patients were

primary refractory and 16% were in first relapse. Myeloablative conditioning (MAC) was used in 62% with cyclophosphamide/total body irradiation being the most frequently applied regimen. Reduced intensity conditioning (RIC) incorporated different protocols based mostly on fludarabine. Grafts from unrelated donors constituted 55% of the transplants and peripheral blood was the main source of stem cells. Graft-versus-host disease prophylaxis was based on cyclosporine in almost all patients. T-cell depletion in vivo with antitymocyte globulin was performed in 36 and with anti-CD52 antibodies in three patients transplanted from unrelated donors. Engraftment was achieved in 98% of patients with available data, at a median of 16 days (range 9–74). For two patients the data were missing and two patient did not engraft. Grade II–IV aGvHD was reported for 34% and cGvHD for 23% of patients. The median follow-up for surviving patients was 47 months (range 14–172). Of 53 patients transplanted while not in remission, 35 (66%) achieved CR after alloHSCT. During the follow-up 81 (83%) patients died. Disease relapse or progression was the primary cause of death in 51 cases, followed by infection in 18 and GvHD in 5 cases. The year of transplantation (before or after 2007) did not influence the endpoints, although a trend toward less NRM was seen in patients transplanted after 2007 (11.7 vs. 22.2%, $p = 0.08$). Fourteen of relapsing patients received donor lymphocyte infusions (DLI) but only one patient was reported alive at 82 days post DLI. Twelve patients died due to original disease and one due to infection. Additionally 12 patients underwent second alloHSCT in an attempt to treat relapse. Of those, only one patient was alive at 215 days, while others died at a median time of 166 days (range 9–1375) after second transplant due to AML (7), infection (3), or hemorrhage (1).

Leukemia-free survival

Probability of 2-year LFS in the whole cohort was 20% (95% CI, 11–24%). In univariate analysis no factors were associated with improved LFS (Table 2). In multivariate analysis a trend toward better LFS was seen for patients transplanted in CR vs. those not in remission, with $p = 0.05$ and hazard ratio (HR) 0.64 (Table 3), (Fig. 1a). Patients transplanted in CR had 24% probability of LFS at 2 years. For those not in remission LFS probability was estimated at 17%. Other analysed variables such as cGvHD (HR = 1.09 (0.56–2.1); $p = 0.8$) or intensity of conditioning, were not found to have impact on LFS.

Overall survival

Probability of 2-year OS was 26% (95% CI, 17–35%) (Fig. 1b). None of the analyzed factors in univariate analysis influenced OS (Table 2), although CR before transplantation was associated with a trend for better OS in multivariate analysis ($p = 0.06$; HR 0.65) (Table 3). For patients transplanted in CR vs. those not in CR, 2-year probability of OS was calculated at 35% vs. 19%, respectively.

Relapse incidence

In patients who relapsed, the median time to disease recurrence was 4 months (range 0.4–32). Two-year risk of relapse was estimated at 64% (95% CI, 53–72%) (Fig. 1c). In univariate analysis it was significantly higher for patients with additional chromosome 7 monosomy ($p = 0.04$), but this finding was not confirmed in multivariate analysis as an independent risk factor (Tables 2 and 3). Chronic GvHD did not influence the risk of relapse (HR = 0.65 (0.28–1.49); $p = 0.31$). Multivariate analysis showed a trend toward decreased risk of relapse for transplants from unrelated donors ($p = 0.09$; HR = 0.63 (0.37–1.07)) (Table 3).

Non-relapse mortality

The risk of 2-year NRM was 16% (95% CI, 10–24%) and was lower for patients transplanted in CR. Thus, a trend for decreased NRM in patients transplanted in CR was noted in univariate (9% vs. 23%; $p = 0.03$) and it was confirmed in multivariate analysis ($p = 0.02$; HR 0.27). In addition, chromosome 7 monosomy was found to be significantly associated with decreased NRM risk (7.8 vs. 25.6%; $p = 0.02$ in univariate and $p = 0.05$; HR 0.35 in multivariate analyses) (Tables 2 and 3).

Graft-versus-host disease-free, relapse-free survival

Two-year probability of GRFS was 14% (95% CI, 7–21%) (Fig. 1d). The only factor associated with higher probability of GRFS in our study was transplantation in CR, 18 vs. 11% at 2 years, which was confirmed in both univariate and multivariate analyses ($p = 0.02$ and $p = 0.01$; HR 0.55, respectively) (Tables 2 and 3).

Patients transplanted in remission

A separate analysis (data not shown) was performed for 45 patients transplanted in CR. In univariate analysis age >50 years was found to negatively affect LFS, OS, RI, and GRFS (p-values 0.02; 0.03; 0.03, and 0.03 respectively). In multivariate analysis the age retained its significance for LFS ($p = 0.048$, HR 2.0) and RI ($p = 0.035$, HR 2.3). Intensity of conditioning significantly influenced OS in univariate analysis. Two-year OS probability with MAC was estimated at 41.5% and RIC at 19% ($p = 0.036$);

Table 2 AlloHSCT for 3q26 AML: univariate analysis of prognostic factors. Two-year estimates for each variable category are provided

Variables		LFS (95% CI)	OS (95% CI)	RI (95% CI)	NRM (95% CI)	GRFS (95% CI)
Diagnosis	De novo	17.4% (9.3–25.4)	23.1% (14.2–32)	64% (52.8–73.3)	18.6% (11.2–27.5)	11.6% (4.9–18.4)
	Secondary	41.7% (13.8–69.6)	50% (21.7–78.3)	58.3% (24.7–81.2)	0% (0–0)	33.3% (6.7–60)
P-value		0.19503	0.11816	0.86615	0.32361	0.21069
Type of chromosomal	inv (3)	16.7% (7.5–26)	24.7% (14–35.3)	64.4% (51–75)	18.9% (10.3–29.4)	12.2% (4–20.3)
Abnormality	*t*(3;3)	26.5% (11.6–41.3)	28.9% (13.5–44.3)	61.8% (42.8–76)	11.8% (3.6–25.3)	17.6% (4.8–30.5)
P-value		0.24362	0.13734	0.91808	0.23957	0.43238
Age at transplant	≤50	19.9% (9.7–30)	24.7% (13.7–35.7)	60.1% (46.3–71.5)	20% (10.9–31.1)	13.3% (4.7–21.9)
	>50	20.7% (7.7–33.7)	28.5% (14–43)	68.7% (50.7–81.3)	10.5% (3.3–22.8)	15.4% (3.7–27)
P-value		0.6529	0.58007	0.34052	0.6078	0.77677
Remission status	Active disease	17% (6.9–27.1)	18.9% (8.3–29.4)	60.4% (45.6–72.3)	22.6% (12.3–34.8)	11.3% (2.8–19.9)
At transplant	CR	23.8% (11.2–36.5)	34.9% (20.8–49)	67.3% (50.8–79.3)	8.9% (2.8–19.5)	17.5% (6.3–28.7)
P-value		0.11692	0.13045	0.77255	0.039317	0.019438
Monosomy 7	No	21.8% (10.3–33.3)	25.2% (12.9–37.4)	54% (39–66.8)	24.2% (13.2–36.9)	18% (7.4–28.6)
	Yes	18.8% (7.7–29.8)	27.1% (14.5–39.7)	72.9% (57.5–83.5)	8.3% (2.6–18.5)	10.4% (1.8–19.1)
P-value		0.81291	0.91273	0.040942	0.039295	0.33367
Complex caryotype	No	24.1% (13.4–34.8)	30.5% (19–42)	64.6% (51.1–75.3)	11.3% (4.9–20.6)	16.1% (7–25.3)
	Yes	13.6% (0–28)	17% (0.8–33.3)	63.6% (38.7–80.6)	22.7% (7.6–42.6)	8.7% (0–20.2)
P-value		0.24058	0.29048	0.91835	0.30015	0.18218
EVI1 overexpression	No	6.3% (0–14.6)	12.5% (1–24)	81.2% (61.5–91.5)	12.5% (3.5–27.6)	3.1% (0–9.2)
	Yes	27.8% (8.3–47.3)	38.1% (17.3–58.9)	57.9% (32.8–76.5)	14.3% (3.4–32.8)	14.3% (0–29.3)
P-value		0.15133	0.12536	0.18071	0.8244	0.31941
Type of donor	MSD	13.3% (3.1–23.4)	19.1% (7.1–31.2)	70.5% (54.1–81.9)	16.3% (6.9–29.2)	11.4% (2–20.7)
	UD	25.9% (14.2–37.6)	31.5% (19.1–43.9)	57.4% (42.9–69.5)	16.7% (8.1–27.8)	16.7% (6.7–26.6)
P-value		0.29813	0.4735	0.21282	0.66097	0.96696
Female to male transplant	No	20% (11.1–28.8)	26.1% (16.3–35.9)	62.3% (50.4–72.1)	17.8% (10.2–27.1)	13.8% (6.2–21.5)
	Yes	21.1% (2.7–39.4)	26.3% (6.5–46.1)	68.4% (40.8–85.2)	10.5% (1.6–29.4)	15.8% (0–32.2)
P-value		0.99886	0.80753	0.43738	0.30529	0.95591
Source of stem cells	BM	16.7% (0–33.9)	22.2% (3–41.4)	55.6% (28.7–75.8)	27.8% (9.4–49.9)	11.1% (0–25.6)
	PB	20.9% (11.9–29.9)	27.2% (17.3–37)	65.3% (53.5–74.8)	13.8% (2.6–34.2)	14.8% (7–22.6)
P-value		0.92335	0.91784	0.29276	0.22806	0.82942
Patient CMV serology	Negative	21.2% (7.3–35.2)	24.2% (9.6–38.9)	54.5% (35.7–70)	24.2% (11.1–40.1)	12.1% (1–23.3)
	Positive	19.4% (9.2–29.6)	26.1% (14.8–37.3)	67.2% (53.3–77.8)	13.4% (6.2–23.5)	14.7% (5.6–23.7)
P-value		0.9929	0.61219	0.26056	0.11074	0.66832
Donor CMV serology	Negative	22.4% (10.8–34.1)	28.6% (15.9–41.2)	59.2% (43.8–71.6)	18.4% (8.9–30.5)	12.2% (3.1–21.4)
	Positive	17.9% (6.2–29.7)	25% (11.9–38.1)	65.8% (48.7–78.4)	16.3% (7–28.9)	16.3% (5.2–27.3)
P-value		0.45188	0.63476	0.44909	0.72426	0.83082
Conditioning regimen	MAC	21.3% (11–31.6)	31.1% (19.5–42.8)	59% (45.4–70.3)	19.7% (10.7–30.6)	13.1% (4.6–21.6)
	RIC	24% (10.1–37.9)	29.5% (14.7–44.3)	65.2% (46.9–78.5)	10.8% (3.3–23.5)	18.5% (5.9–31.2)
P-value		0.72716	0.61753	0.29764	0.63309	0.75004
In vivo T cell	No	17.1% (7.3–26.8)	21.6% (10.8–32.4)	67.2% (53.3–77.9)	15.7% (7.6–26.5)	10.3% (2.5–18.2)
Depletion	Yes	25.6% (11.9–39.3)	33.3% (18.5–48.1)	56.4% (39.1–70.5)	17.9% (7.8–31.5)	20.5% (7.8–33.2)
P-value		0.38146	0.40773	0.15999	0.40626	0.3542

LFS leukemia-free survival, *OS* overall survival, *RI* relapse incidence, *NRM* non-relapse mortality, *GRFS* graft-versus-host disease-free, relapse-free survival, *aGvHD* acute graft-versus-host disease, *cGvHD* chronic graft-versus-host disease, *CR* complete remission, *MSD* matched sibling donor, *UD* unrelated donor, *BM* bone marrow, *PB* peripheral blood, *CMV* cytomegalovirus, *MAC* myeloablative conditioning, *RIC* reduced intensity conditioning

Table 3 AlloHSCT for 3q26 AML: multivariate analysis for all patients

		p-value	HR	95% CI	
				Lower	Upper
LFS	Age >50y	0.36	1.24	0.79	1.95
	inv(3) vs. *t*(3;3)	0.22	1.35	0.84	2.18
	Monosomy 7	0.82	1.05	0.67	1.65
	CR vs. active disease	0.05	0.64	0.41	1.00
	UD vs. MSD	0.13	0.70	0.44	1.12
OS	Age >50y	0.31	1.27	0.80	2.02
	inv(3) vs. *t*(3;3)	0.11	1.47	0.92	2.37
	Monosomy 7	0.87	1.04	0.66	1.63
	CR vs. active disease	0.06	0.65	0.41	1.02
	UD vs. MSD	0.24	0.76	0.48	1.21
RELAPSE	Age >50y	0.31	1.30	0.78	2.17
	inv(3) vs. *t*(3;3)	0.67	1.12	0.66	1.90
	Monosomy 7	0.18	1.42	0.85	2.37
	CR vs. active disease	0.36	0.79	0.47	1.31
	UD vs. MSD	0.09	0.63	0.37	1.07
NRM	Age >50y	0.87	1.09	0.40	2.98
	inv(3) vs. *t*(3;3)	0.08	2.73	0.88	8.50
	Monosomy 7	0.05	0.35	0.12	1.02
	CR vs. active disease	0.02	0.27	0.09	0.85
	UD vs. MSD	0.98	1.01	0.37	2.76
GRFS	Age >50y	0.83	0.95	0.61	1.49
	inv(3) vs. *t*(3;3)	0.43	1.21	0.76	1.92
	Monosomy 7	0.35	1.23	0.79	1.91
	CR vs. active disease	0.01	0.55	0.35	0.87
	UD vs. MSD	0.90	1.04	0.61	1.76
	In vivo TD	0.18	0.69	0.40	1.19

LFS leukemia-free survival, *OS* overall survival, *RI* relapse incidence, *NRM* non-relapse mortality, *GRFS* graft-versus-host disease-free, relapse-free survival, *aGvHD* acute graft-versus-host disease, *cGvHD* chronic graft-versus-host disease, *CR* complete remission, *MSD* matched sibling donor, *UD* unrelated donor, *TD* T-cell depletion

however, the significance was not confirmed in multivariate analysis ($p = 0.2$; HR 1.82). The best results were seen in individuals aged <50 who received MAC ($n = 19$). In this small subgroup of patients probabilities of 2-year LFS, OS, RI, NRM, and GRFS were 42.1% (95% CI: 19.9–64.3), 46.3% (95% CI: 23.5–69.2), 47.4% (95% CI: 23.5–68), 10.5% (95% CI: 1.7–29) and 31.6% (95% CI: 10.7–52.5), respectively (Table 4).

Discussion

AML with *t*(3;3)(q21;q26)/inv(3)(q21;q26) is a well characterized entity with a very poor outcome, demonstrated by the virtual absence of long-term survivors after conventional AML-type chemotherapy. In an effort to evaluate the added benefit of alloHSCT in patients diagnosed with acute myeloid leukemia with 3q26 AML we performed this registry-based study. The analysis showed limited capability of efficient long-term disease control with alloHSCT, which was obtained only in approximately one quarter of the patients. The failure was due mainly to a very high rate of relapse or progression after transplant. Nonetheless, alloHSCT arises currently as the only strategy that provides durable response in a subset of 3q26 AML patients. Remission status at transplantation was the only factor with a moderately positive effect on transplant outcome, probably identifying a subgroup of patients with a more chemosensitive disease and lower tumor burden. Other transplant-related variables, such as age, conditioning intensity, T-cell depletion strategies or donor type, did not influence outcome in the whole group. However in patients transplanted in CR, age ≤50 years and MAC were beneficial. On the other hand the limited population size of the study and the fact that minority of patients were in CR at transplantion preclude identification of the best transplantation strategy in this setting. The first important finding of our analysis was that only 46% of patients were in first or second complete remission and, therefore, achieved an optimal response status at time of transplant. This confirms the difficulty in obtaining and maintaining remission in 3q26 AML patients with currently used standard chemotherapy regimens [7]. Thus, the majority of the patients had active disease and the transplantation was performed as a salvage procedure. Nevertheless the findings of our study confirm the possibility of obtaining long-term disease control with allogeneic transplantation in a subset of 3q26 AML patients, with a 2-year LFS and OS of 20% and 26%, respectively. These outcome results are comparable to those achieved in very high-risk AML populations, such as patients with determined highly adverse genetic categories like monosomal karyotype or TP53 mutated AML and patients with primary refractory disease or refractory relapse [13, 14]. In those studies, remission status at transplantation was also a key prognostic factor, as confirmed in the present study, being the only variable associated with better LFS and OS. Therefore improvement of pre-transplant treatment to obtain a deeper response on one hand and developing better strategies to maintain response post-transplant on the other, seem indispensable in order to increase probability of long-term success after alloHSCT. Epigenetic modification with azacitidine, although demonstrating positive results with improved treatment outcome as compared to standard chemotherapy in specific high-risk AML subsets, has a limited role in 3q26 AML [15]. Recent research threw light on the biology of 3q26 AML and revealed that the chromosomal abnormalities that define the disease result in *EVI1* (or *MECOM*) overexpression due to *GATA2* enhancer

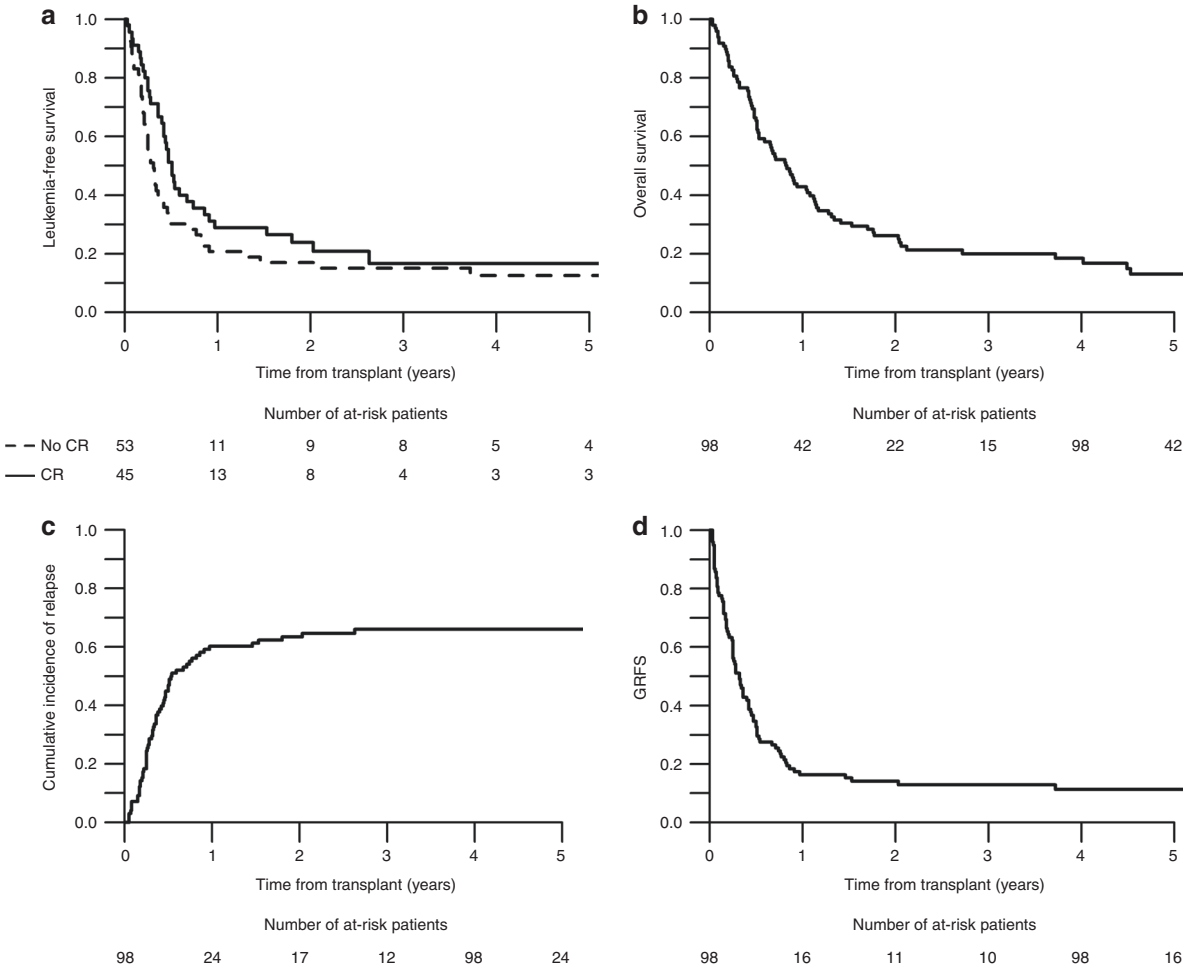

Fig. 1 AlloHSCT for 3q26 AML: **a** leukemia-free survival; **b** overall survival; **c** cumulative incidence of relapse; **d** graft-versus-host-free, relapse-free survival

Table 4 AlloHSCT for 3q26 AML: 2-year outcome probabilities for patients aged <50 transplanted in complete remission with myeloablative conditioning ($n = 19$)

LFS	42.1% (95% CI: 19.9–64.3)
OS	46.3% (95% CI: 23.5–69.2)
RI	47.4% (95% CI: 23.5–68)
NRM	10.5% (95% CI: 1.7–29)
GRFS	31.6% (95% CI: 10.7–52.5)

LFS leukemia-free survival, *OS* overall survival, *RI* relapse incidence, *NRM* non-relapse mortality, *GRFS* graft-versus-host disease-free, relapse-free survival

reposition from 3q21 to *EVI1* locus at 3q26.2 and in *GATA2* haploinsufficiency at its original locus [16, 17]. Based on those findings, early in vitro experiments give some hope for controlling the disease in the future by silencing *EVI1* overexpression, which can be obtained by disrupting superenhancers like *GATA2* or targeting the essential interaction of PLDLS domain of EVI1 with C-terminal DNA binding protein [16, 18]. The molecular analysis in our group of patients was not possible as only in 21 cases *EVI1* overexpression was reported and in majority of patients it was not evaluated. Paucity of molecular data is a typical shortcoming of large databases and only prospective clinical trials are able to overcome this obstacle. Potential relevance of developing effective post-transplant intervention for a sustained response is reflected in the observation that 66% of patients allografted active disease achieved a morphologic CR after alloHSCT; however, 2-year LFS and OS was only 17% in this refractory patient population. Targeting CD33 with conjugated antiCD33 antibody gemtuzumab ozogamycin (GO), highly expressed in 3q26 AML, might be an effective post-transplant intervention in this entity. As recently reported, 14 pediatric patients with CD33+ AML treated with low-dose GO post-transplant, showed promising results with 1-year OS of 78% [19]. Several studies indicate a beneficial effect of chronic and mild forms of acute GvHD on LFS and relapse incidence in AML patients [20, 21]. In this study however, cGvHD was

not found to exert positive impact on LFS or RI. Of note, the median time to relapse in our study was 4 months, which is probably too short for graft-versus-leukemia (GvL) effect to develop. In multivariate analysis, we observed a trend for lower relapse risk in patients receiving grafts from unrelated donors, indicating possible stronger GvL effect after unrelated transplants, although the magnitude of this effect in this setting is uncertain. Our observation is in line with findings of a recent ALWP study of AML patients transplanted in first relapse. The use of unrelated donors in those very high-risk patients was associated with decreased risk of subsequent relapse post-transplant, as compared to matched sibling donors [22]. Recent publications of the ALWP of the EBMT suggests the potential role of prophylactic or pre-emptive donor lymphocyte infusions aimed to increase the GvL effect and prevent relapse in high-risk AML patients, which could be of interest also in 3q26 AML patients [23]. Therapeutic use of DLI in relapsing patients in our cohort unfortunately proved ineffective. In another publication by the ALWP an enhanced GvL effect was observed with the use of peripheral blood as compared to bone marrow grafts in patients undergoing alloHSCT with RIC [24]. However, stem-cell source did not show any correlation with RI and LFS in the current analysis. As for the type of the conditioning, preliminary results of a randomized trial by the Blood and Marrow Clinical Trials Network comparing MAC and RIC in patients with AML indicated that myeloablative regimens are more effective, leading to substantially decreased relapse incidence and improved LFS and OS after alloHSCT [25]. However data on the role of conditioning intensity in AML patients with very high-risk cytogenetic categories such as monosomal karyotype acute myeloid leukemia (MK-AML) are still conflicting. In a recent ALWP–EBMT analysis, relapse rate after alloHSCT in MK-AML was not significantly influenced by the type of conditioning [26]. On the other hand, in a study undertaken by the CIBMTR, MAC alloHSCT in patients aged 41 to 60 years performed in MK-AML in first complete remission led to a decreased risk of relapse [27]. In the present study, conditioning intensity did not show impact on disease control in the whole group. Nevertheless in patients transplanted in CR, 2-year OS probability for those who received MAC vs. RIC was 41.5 vs. 19% ($p = 0.036$) in univariate analysis, which may support the use of intensive conditioning. Only two factors were associated with decreased risk of NRM in multivariate analysis, namely CR at transplantation and presence of chromosome 7 monosomy. Remission status is a recognized factor for reduced NRM and was first proposed by Gratwohl for transplantation-risk assessment in patients with chronic myeloid leukemia [28]. The interaction between additional monosomy 7 and NRM in this study must be interpreted very cautiously, since it may be due to small study population and low number of NRM events, competitive with high relapse incidence. Moreover, an additional deleterious effect of monosomy 7 in this entity was detected in a previously published analysis of 103 newly diagnosed patients with 3q26 AML or MDS [29]. In our study higher RI seen in patients with monosomy 7 in the univariate analysis was not confirmed in multivariate, as the present study is probably underpowered to identify this effect.

Current study demonstrates that long-term disease control is possible with alloHSCT in a subgroup of patients. Based on our findings no definite recommendations can be made, but better results may be expected with transplantation in CR, in younger patients, and probably with the use of MAC in patients transplanted in CR. High risk of progression after alloHSCT and the impact of disease status at transplantation underline the urgent need to develop novel, more effective pre- and post-transplant treatment strategies for these patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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