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ORIGINAL ARTICLE

Relapse patterns and treatment strategies in patients receiving allogeneic hematopoietic stem cell transplantation for myeloid malignancies

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Abstract

Allogeneic hematopoietic stem cell transplantation (aHSCT) cures a considerable number of patients with myeloid malignancies, but relapse is the most frequent cause of death. We retrospectively studied relapse rate, kinetics, treatment, and outcome after first aHSCT in 446 patients during a 13-year period. Relapse occurred in 167 patients after a median of 4.6 months (116 hematologic (HR), 38 molecular (MR), and 13 extramedullary relapses (XR)). Median survival after relapse was 8.4 months and 2-year overall survival was 25%. Regarding survival after relapse, type (MR/HR/XR) and timepoint of relapse (</> 12 months), age (</> 50), diagnosis (MDS/AML and sAML), and remission status at transplant (CR and untreated MDS vs. refractory disease) were relevant in univariate analyses, in multivariate analyses timepoint, and type of relapse, age, and diagnosis. One hundred fifty-six patients were treated, most frequently with hypomethylating agents (HMA, $n = 109$) or intensive chemotherapy ($n = 12$). Donor lymphocyte infusion (DLI) was administered to 99 patients. Second aHSCT was performed in three patients as first and in 21 as higher salvage treatment. A complete remission (CR) was achieved in 46 patients (30%). Among CR patients, 65% had received HMA and DLI. Median survival of patients achieving CR was 105 months and 2-year overall survival was 80%. We conclude that with HMA and DLI or second aHSCT, a substantial number of patients, who relapse after aHSCT, can re-achieve remission and long-term survival. Techniques to further improve the detection of minimal residual disease are urgently needed because early treatment of MR results in significantly better survival.

Keywords Allogeneic hematopoietic stem cell transplantation · Relapse · Azacitidine · Second allogeneic hematopoietic stem cell transplantation · DLI

Introduction

Allogeneic hematopoietic stem cell transplantation (aHSCT) is a curative treatment option in patients with myeloid malignancies. Relapse is the most common cause of treatment failure after aHSCT with increasing rates in the past years (CIBMTR, 2016). Following our first report in 2007 [1], 5-azacitidine (aza) with or without donor lymphocyte infusion (DLI) has become a frequently used relapse therapy, especially for patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), with reported 2-year overall survival rates between 12 and 29% [2–5]. Other treatment options are intensive chemotherapy (IC) [6] or second aHSCT, both of which are more toxic than treatment with HMA, especially in this heavily pretreated patient population [7]. Tyrosine kinase inhibitors (TKI) targeting BCR/ABL are very useful in chronic myeloid leukemia (CML) [8, 9], and encouraging treatment results have been reported for sorafenib in patients with AML carrying a FLT3 mutation [10]. So far, standardized treatment pathways for relapse after aHSCT have not been established.

We here report on a single-center analysis of relapse patterns and treatment results of patients suffering from myeloid malignancies over a 13-year period aiming to identify factors influencing the probability of long-term survival following relapse after aHSCT.

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Methods

Study design

The study included 446 patients who received their first aHSCT for a myeloid malignancy at the Department of Hematology, Oncology and Clinical Immunology of the University Hospital Duesseldorf between February 1, 2002 and April 8, 2015. Clinical data were gathered from the original patients' charts. Follow-up data were obtained from our outpatient department or by contacting the primary care physicians. The study was approved by the local ethics committee of the Heinrich-Heine University Duesseldorf, and all patients gave informed consent on data analysis before transplant. Roughly half of the patients who received HMA ± DLI for the treatment of relapse were included in earlier publications of our group. For these patients, an extended follow-up is provided here [4, 5, 11]. Assignment to risk groups before aHSCT was done according to European leukemia net (ELN) risk groups [12] for AML and sAML, according to the revised International Prognostic Scoring System (IPSS-R) for MDS patients [13] and the chronic myelomonocytic leukemia (CMML) Prognostic Scoring System (CPSS) for CMML patients [14]. Definition of conditioning regimens regarding myeloablative (MAC) and reduced intensity (RIC) conditioning was in line with European Society for Blood and Marrow Transplantation (EBMT) criteria [15]. The choice of conditioning regimen was based on age, comorbidities, and disease risk.

Monitoring regarding hematologic and molecular disease markers was routinely performed on days 30, 60 (since 2015), day 100, day 200, and day 300 and whenever progression was suspected. With continuous improvement of methods for molecular monitoring posttransplant surveillance was intensified during recent years. Targets for molecular monitoring were individualized and included, for example, NPM1, WT1, BCR/ABL, FLT3 ITD, and FISH whenever probes were available.

Molecular relapse (MR) was defined as recurrence or increasing proportion of initial disease specific molecular markers and/or loss of complete donor chimerism in blood or bone marrow. In general, two independent markers of disease recurrence or confirmation with repeated analysis were required for diagnosis of MR. The occurrence of more than 5% bone marrow blast in line with decreasing donor chimerism was defined as hematologic relapse (HR). Extramedullary relapse (XR) was defined as histologically or cytologically proven disease recurrence outside the bone marrow. DLI was defined as the infusion of donor cells without anteceding conditioning or immunosuppression.

Statistics

All time-to-event variables were estimated by using the Kaplan-Meier method. For estimation of overall survival and survival after relapse, death from any cause was rated as an event. Surviving patients were censored at the last day of follow-up. For univariate analysis, we used the log-rank test to compare time-to-event curves from different groups and crosstabulation with chi-square test for comparison of categorical variables. A p value of <

0.05 was considered significant. After univariate analysis, variables showing a p value < 0.05, were included in a multivariate analysis with Cox-regression model, using the stepwise forward method. All data analyses were performed using SPSS 22.0 statistical software (SPSS Software GmbH, München, Germany).

Results

Patients' characteristics

A total of 446 patients received their first aHSCT for a myeloid malignancy at the Department of Hematology, Oncology and Clinical Immunology of the University Hospital Duesseldorf between February 1, 2002 and April 8, 2015. Median age at diagnosis was 51.6 years (range 15.8–71.4) and 52.7 years (range 16.6–72.2) at aHSCT. Median time from diagnosis to aHSCT was 6.1 months (range 0.4–267.9). Fifty-five percent of patients were male. The underlying diseases were de-novo AML in 216 patients (48.4%), sAML in 75 (16.8%), MDS in 102 (22.9%, including 14 CMML), and myeloproliferative neoplasms (MPN) in 53 (11.9%, 32 CML, 21 myelofibrosis (MF)). sAML evolved from MDS in 69 patients (92%) and from MPN in 6 (8%, patients' characteristics are summarized in Table 1). Cytogenetic data was available for 286 of 291 AML patients (98.3%). According to ELN risk classification at diagnosis, these included 27 (9.4%) low, 187 (65.4%) intermediate, and 72 (25.2%) high-risk patients. Patients belonging to the low-risk group were either transplanted after relapse or induction failure. According to IPSS-R cytogenetic risk group, which was available for 85 (96.6%) of 88 MDS patients, 30 (35.3%) MDS patients belonged to the very good/good, 24 (28.3%) to the intermediate, 15 (17.6%) to the high, and 16 (18.8%) patients to the very high-risk group. According to the CPSS cytogenetic risk group, which was available for 12 of 14 patients, 8 (67%) CMML patients belonged to the low and 2 each (each 16.5%) to intermediate or high-risk groups.

Regarding disease status at transplantation, 104 (23.3%) patients were untreated (74 MDS, 11 sAML, and 19 MF), 173 (38.8%) patients had active disease including partial remissions, and 169 patients (37.9%)

Table 1 Patients characteristics

Male/female in %	55/45
Age at aHSCT median (range)	52.7 years (16.6–72.2)
Time dx to aHSCT median (range)	6.1 months (0.4–267.9)
Diagnosis	*n*
de novo AML	216 (48%)
MDS	102 (23%)
MPN	53 (12%)
AML evolved from MDS	69 (15%)
AML evolved from MPN	6 (2%)
Disease status at aHSCT	
Untreated MDS and sAML	85 (19%)
Active disease including PR and untreated MF patients	192 (43%)
CR	169 (38%)
Donor type	
MSD	122 (27%)
MUD	307 (69%)
Haploidentical	10 (2%)
Other	7 (2%)
Conditioning regimen	
MAC	274 (61%)
RIC	172 (39%)
ATG containing conditioning	194 (43%)

aHSCT allogeneic hematopoietic stem cell transplantation, *dx* diagnosis, *PR* partial remission, *CR* complete remission, *MSD* matched sibling donor, *MUD* matched unrelated donor, *MAC* myeloablative conditioning, *RIC* reduced intensity conditioning

were in complete remission (CR), of those 131 in CR1 (29.3%).

Age at aHSCT changed over time; since 2010, significantly more patients over the age of 60 years received aHSCT (64.9% of the over 60-year-old patients received transplant since 2010) and the proportion of RIC increased (37.1% vs. 62.9%, $p < 0.001$ each).

Type of donors included matched sibling donors (MSD) $n = 122$ (27.4%), unrelated donors (MUD) $n = 307$ (68.8%), haploidentical donors $n = 10$ (2.2%), and mixed (i.e., cord blood and haploidentical donor) donors $n = 7$ (1.6%). Of the 307 MUD, 224 were 10 out of 10 matched and 80 mismatched (three missing information). Regarding conditioning regimens, 274 patients (61%) received myeloablative conditioning (MAC) and 172 (39%) received reduced intensity conditioning (RIC). Anti-thymocyte-globuline (ATG, Neovii) was part of the conditioning regimen in 194 patients (43%). Posttransplant cyclophosphamide was utilized in patients receiving haploidentical grafts (patients' characteristics are summarized in Table 1).

Overall survival

Median follow-up of the 209 surviving patients until December 1, 2016 was 63.2 months (range 1.5–173.1). At that time, 167 patients had relapsed and 237 patients had deceased. Median survival after aHSCT for all patients was 47.2 months (confidence interval (CI) 15.7–78.7), 1-year survival was 65.5%, 2-year survival was 56%, 5-year survival was 47.9%, and 10-year survival was 38.8%. Regarding the different diagnoses, survival time for AML patients was 47.6 months (CI 7.5–87.7), for MDS patients 99.6 months (CI 41.3–158.0), for MPN patients 21.7 months (CI 0–60.6), and 23.2 months (CI 0–59.7) for sAML patients. Median survival of patients, who received aHSCT in complete remission, was not reached; untreated MDS patients survived 99.6 months (CI 62.6–136.7), and those with active disease (excluding patients with untreated MDS) survived 12.8 months (CI 8.1–17.1, $p < 0.001$). Patients with low ELN risk had a median survival time of 85.2 months (no CI); intermediate risk patients had a median survival time of 53.6 months (CI 3.6–103.6), and high-risk patients had a median survival time of 16 months (CI 8.3–23.7, $p = 0.055$).

Regarding donor type, median survival of patients receiving a stem cell graft of a MSD was 77.0 months (no CI), and patients receiving stem cell grafts from a 10 out of 10 MUD survived 51.3 months (CI 7.6–95.0, $p =$ n. s.). Survival of patients, who had mismatched donors, was worse (17.2 months, CI 7.5–26.7, $p =$ n. s.). There was no difference in survival times regarding RIC or MAC. Patients, who were

younger than 50 years at the time of transplant ($n = 184$), had a significantly longer median survival than those aged above 50 years ($n = 262$) (< 50 years 101.7 months (no CI), > 50 years 24.6 months (CI 15.7–78.8, $p = 0.001$). When looking at the elderly patients above the age of 50 years, overall survival of patients between 50 and 59 years ($n = 153$) was 24.9 months and did not differ from those above the age of 60 years (24.6 months, $p =$ n. s.).

Survival improved over time; patients transplanted until 2010 had a median survival of 24.5 months (CI 0–49.1), and patients transplanted since 2010 had a median survival time of 65.2 months (no CI, $p = 0.03$).

Relapse

Until December 1, 2016, 167 patients suffered from relapse; median time to relapse was 4.6 months (range 0.4–110). The majority of relapses occurred in the first year after aHSCT $n =$ 120 (72%), especially in the fourth month ($n = 43$). Twenty-seven (16%) patients relapsed in the second year and 12 (7%) in the third; beyond the third year, only 5% of patients developed relapse. This distribution over time was observed for AML and sAML. MDS patients relapsed significantly later (median time to relapse: AML 3.9 months, MDS 15.6 months, sAML 3.6 months (Table 2), AML vs. MDS $p = 0.004$, sAML vs MDS $p = 0.003$, (Fig. 1). Median survival after relapse was 8.4 months (CI 6.4–10.4); 1-year overall survival was 41%; 2-year survival was 25% and 5-year survival was 12%. One hundred twenty-six patients deceased after a median of 4.6 months (range 0.2–105). Forty patients were alive on the first of December 2016, three patients on treatment, and 37 in CR; one patient is lost of follow-up.

The majority of relapses were HR (116, 69%), whereas 38 patients (23%) had MR and 13 (8%) patients had XR, eight isolated and five combined with HR. Three of the patients with XR had history of extramedullary disease before aHSCT. The proportion of MR was equally distributed between AML, MDS, and sAML patients (19% vs. 16% vs. 19%). Patients with CML had more molecular relapses 8/12 (75%). The most frequent markers of MR were the detection of BCR/ABL in eight patients with MPN and the loss of complete donor chimerism in 15 patients with AML, sAML, or MDS. Further, minimal residual disease (MRD) markers that were detected were NPM1 $n = 4$, FLT3-ITD $n = 4$, MLL $n = 3$, inversion [16] $n = 1$, TET2 $n = 1$, and complex karyotypes $n = 2$. WT-1 was measured in 13 patients of non BCR/ABL positive MR and was elevated in eight cases.

Relapse frequency

In univariate analysis, neither age, sex, ELN risk group, donor type, nor conditioning regimen played a role regarding relapse frequency. The only parameter that had significant influence on relapse frequency was remission status at timepoint of aHSCT (CR and untreated MDS patients vs. refractory disease $p = 0.001$, χ^2 11.4).

Survival after relapse

Regarding the timepoint of relapse after aHSCT, we performed three analyses. Patients who relapsed before the median time to relapse—in our analyses of 4.6 months—had a median survival time of 4.6 months (CI 2.6–6.5) vs. 14.5 months (CI 7.1–21.9), when relapse occurred later ($p < 0.001$). Discriminatory power was higher, if the cutoff was set 1 year after aHSCT. Patients, who relapsed during the first year ($n = 120$) had a median survival time after relapse of 4.6 months (CI 3.3–6.0), and patients, who relapsed beyond the first year ($n = 47$) 89.3 months (0.0–179.0, $p < 0.001$, Fig. 2a). We did not gain additional information by creating three risk groups with patients, who relapsed in the first 6 months vs. 6–12 months vs. later than 12 months after aHSCT. The first two groups are not different regarding survival times (4.6 months each). There was no difference regarding the distribution of type of relapse over time; 72% of each HR and MR occurred in the first year.

Survival times regarding relapse type were significantly different, in detail, 4.6 months (CI 1.6–7.6) for isolated XR, 4.8 months (CI 0–10.1) for combined HR and XR, 5.1 months (CI 10.1–13.7) for HR, and 47.5 months (CI 10.9–84.2) for MR ($p < 0.001$, Fig. 2b), as were survival times regarding remission status at transplant (CR 9.3 months (CI 6.1–12.5) vs. untreated MDS (89.2 months (CI 11.9–166.6) vs. refractory disease 5.1 months (CI 3.1–7.1)) (Fig. 2c). Patients younger than 50 years ($n = 70$) had a longer survival time after relapse than older patients (12.6 vs. 6.6 months, $p = 0.01$,

Table 2 Time point of relapse

	n	Time to relapse	First year	Second year	Third year	> 3 year
All	167	4.6 ms (0.4–110)	120 (72%)	27 (16%)	12 (7%)	8 (5%)
AML	82	3.9 ms (0.4–38)	66 (80%)	11 (13%)	4 (5%)	1 (1%)
MDS	31	15.6 ms (0.9–110.0)	12 (39%)	9 (29%)	7 (22%)	3 (10%)
MPN	17	5.6 ms (0.6–90.5)	12 (71%)	2 (12%)	0	3 (17%)
sAML	36	3.6 ms (0.9–61.5)	29 (81%)	5 (14%)	1 (3%)	1 (2%)

n number, *m*s months

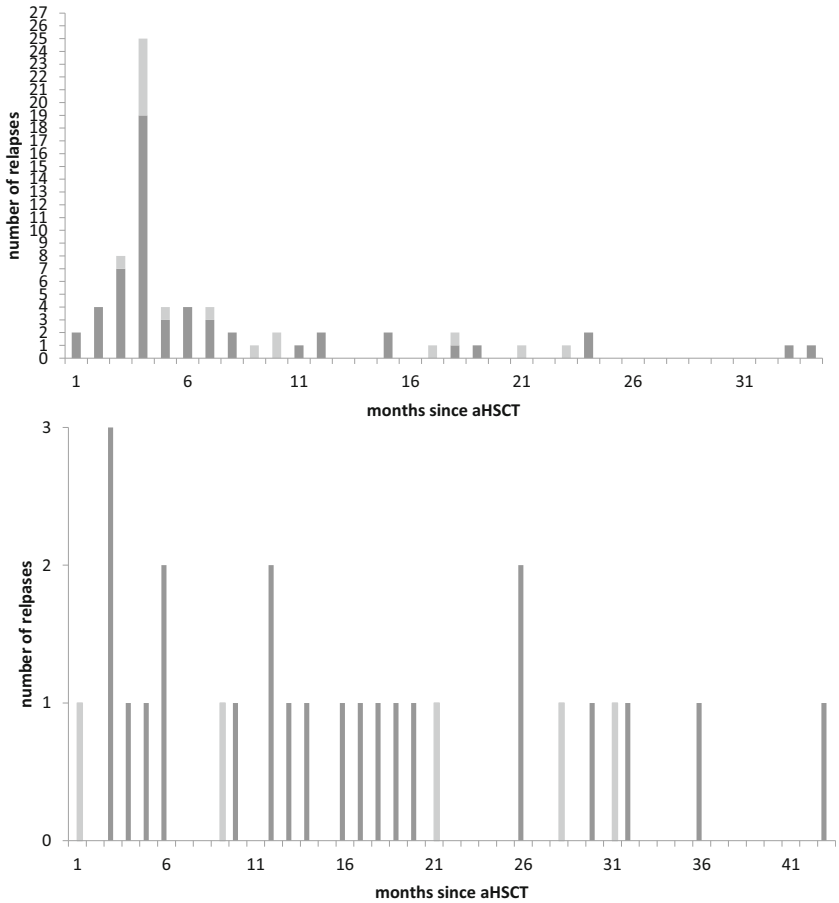

Fig. 1 Time point of relapse according to diagnosis. Three relapses in the MDS group occurred later and are not displayed, *aHSCT* allogeneic hematopoietic stem cell transplantation; light gray, molecular relapses; dark gray, hematological relapses

Fig. 2d) as had MDS patients in comparison to AML patients (83.3 vs. 6.7 months, $p < 0.001$) and to sAML (median survival 3.5 months, $p < 0.001$).

In the group that developed relapse, only 25% of patients suffered from any kind of GvHD before relapse, whereas in the group that did not relapse, 55% patients developed any type GvHD ($p < 0.001$).

No relevance was shown for ELN risk group, donor type (MSD vs. MUD), conditioning regimen (MAC vs. RIC), and dose of ATG (60 mg/KG/kg vs. less or none). In cross-tabulation comparing patients, who survived longer than 2 years after relapse versus patients with a shorter survival time, we identified age below 50 years ($p = 0.002$), MR vs HR and XR ($p = 0.002$), remission status at aHSCT ($p = 0.047$), timepoint of relapse </> 12 months ($p < 0.001$), diagnosis MDS vs. AML and sAML ($p = 0.001$), and gender male vs. female (alive are 66% of male and 34% of female patients) to be favorable for survival. Relapse frequency did not differ between genders.

In forward cox regression multivariate analysis regarding survival after relapse, the strongest discriminator was timepoint followed by type of relapse followed by age (cutoff 50 years) and diagnosis MDS vs. AML and sAML (Table 3). In multivariate analysis, remission status at the time of transplant was not an independent prognostic factor.

Treatment of relapse

Following first relapse, the majority of patients ($n = 156$) received at least one salvage therapy, while 11 patients received best supportive care only. Primary salvage therapies were hypomethylating agents (HMA, aza $n = 108$, decitabine $n = 1$), DLI only ($n = 14$), IC ($n = 12$), TKI targeting BCR/ABL ($n = 9$), second aHSCT ($n = 3$), sorafenib ($n = 4$), radiotherapy ($n = 4$), and surgery ($n = 1$, orchiectomy). Of the 109 HMA patients, six received sorafenib and five received lenalidomide in addition. In total, DLI were applied to 99 patients. Among patients receiving HMA, 75 (69%) received donor lymphocyte infusion in addition (Table 4). The second aHSCT was performed in 21 patients in first relapse as second- or higher line treatment.

After first-line therapy, 46 patients (29.5%) achieved CR while three were still on treatment at the time of analysis. Parameters that positively influenced the chance of achieving CR in univariate analyses were age below 50 years ($p = 0.023$), diagnosis of MDS vs. AML and sAML ($p < 0.001$), MR vs. HR or XR ($p < 0.001$), timepoint of relapse later than

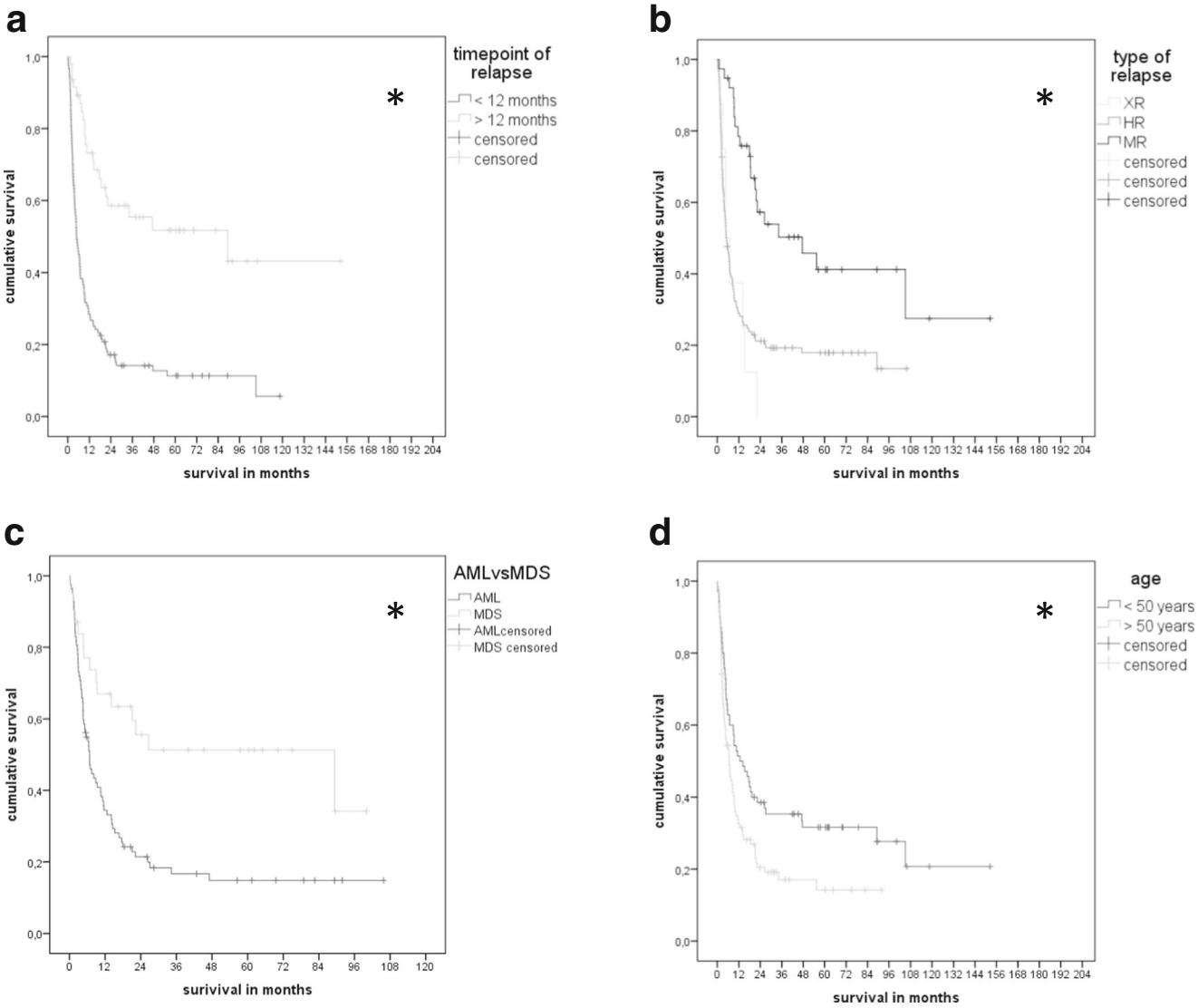

Fig. 2 Parameters influencing overall survival after relapse in multivariate analysis. *aHSCT* allogeneic hematopoietic stem cell transplantation, *XR* extramedullary relapse, *HR* hematological relapse, *MR* molecular relapse. *$p < 0.05$

1 year after aHSCT vs. earlier ($p = 0.012$), and CR and untreated MDS vs. refractory disease at the time of transplant ($p = 0.015$). Parameters that did not influence CR were sex and ELN risk group. Patients that reached CR had a median survival time of 105 months (CI 73.5–136.6) vs. 4.6 months

Table 3 Parameters influencing overall survival after relapse in multivariate analysis

Variable	Wald	*p* value
Timepoint of relapse </> 12 months	26.051	< 0.0001
Type of relapse	23.088	< 0.0001
Age </> 50 years	8.455	0.004
Diagnosis MDS vs AML and sAML	4.061	0.044

Table 4 First-line salvage therapies

First salvage therapy	*n*	%
HMA (± DLI)	109	70
DLI only	14	9
IC (± DLI)	12	8
TKI (± DLI)	9	6
Sorafenib (± DLI)	4	2
Radiotherapy	4	2
Second aHSCT	3	2
Orchiektomie	1	1

HMA hypomethylating agents, *DLI* donor lymphocyte infusion, *IC* intensive chemotherapy, *aHSCT* allogeneic hematopoetic stem cell transplantation, *TKI* tyrosine kinase inhibitor targeting BCR/ABL

(3.3–6.0) for patients that did not reach CR ($p < 0.001$, Fig. 3a).

First salvage treatments in patients who achieved CR were aza and DLI $n = 30$, decitabine and DLI $n = 1$, aza only n= 1, DLI only n = 5, second aHSCT $n = 3$, TKI targeting BCR/ABL $n = 4$, sorafenib $n = 1$, and sorafenib and DLI $n = 1$. Patients that received IC as first salvage had a median survival time of 2.7 months (CI 0.8–4.6, Fig. 3b). Looking at the patients who had received HMA, those that additionally received DLI ($n = 75$) had a significant longer median survival than those who did not (16.7 months, CI 5.3–28.2) vs. 4.4 months (CI 3.1–5.8, $p < 0.001$). Of the 34 patients that did not receive DLI, 38% were treated with a second-line treatment in comparison to only 19% in the HMA plus DLI group ($p = 0.03$). Patients who received donor cells as first or higher treatment line alone or in combination with cytotoxic therapy had a clear survival benefit of 15.3 months (CI 8.3–22.3) vs. 2.8 months (CI 2.3–3.3, $p < 0.001$). Type of treatment changed over time with increasing use of HMA ($n = 41/82$ until 2010 vs. $n = 69/85$ since 2010, $p < 0.001$).

Of the 110 patients, who did not respond to primary salvage therapy, 41 patients were treated with a second-remission inducing therapy. Of those, 18 patients reached CR after further therapy 14 after second aHSCT, two after IC and consolidating second aHSCT, one after TKI and DLI, and one after IC and DLI.

Second allogeneic SCT

Thirty patients received second aHSCT—24 in the therapy sequence of the first relapse and six patients for the second or higher relapse. Only two patients were in CR at timepoint of the second aHSCT. Sixteen patients were in CR on day + 28 after aHSCT; one had progressive disease and four (17%) died from TRM. The initial donor was used in seven patients; and in 23 individuals, the donor was changed. MUD were used in 23 patients; two patients received grafts from haploidentical family members and five from MSD. Previous therapy for the 24 patients, who received second aHSCT for the first relapse, were none $n = 3$, HMA $n = 15$, of those 10 with DLI, DLI only $n = 1$, IC $n = 4$, and sorafenib $n = 1$. Of the 15 patients that received HMA, six patients received IC because they suffered from progression during therapy with HMA. Median time between diagnosis of first relapse and second aHSCT for first relapse was 5.4 months (range 0.59–39.16); time between diagnosis of first relapse and second aHSCT was 6.9 months (range 0.59–98.66). Median overall survival after second SCT was 12.5 months (CI 5.9–19.1); 2-year survival was 23% and 5-year survival was 10%.

Discussion

Today, relapse is the most common type of treatment failure after aHSCT for myeloid malignancies. During recent years, different treatment strategies have been described, the most important being DLI plus treatment with HMA or IC and second aHSCT. Furthermore, new targeted therapies aiming at characteristic mutations have emerged like treatment with sorafenib [16] or other FLT3 inhibitors. Still, there is an unmet medical need for defining clinical criteria that allow patient selection for one or the other treatment strategy. We here report our single-center experience on the basis of a large patient cohort with myeloid malignancies treated for relapse with a homogenous strategy based on the use of HMA and DLI.

In this study of 446 patients with myeloid disease, we demonstrate distinct relapse kinetics in AML and MDS and show that age, initial diagnosis (MDS vs. AML vs. sAML), disease

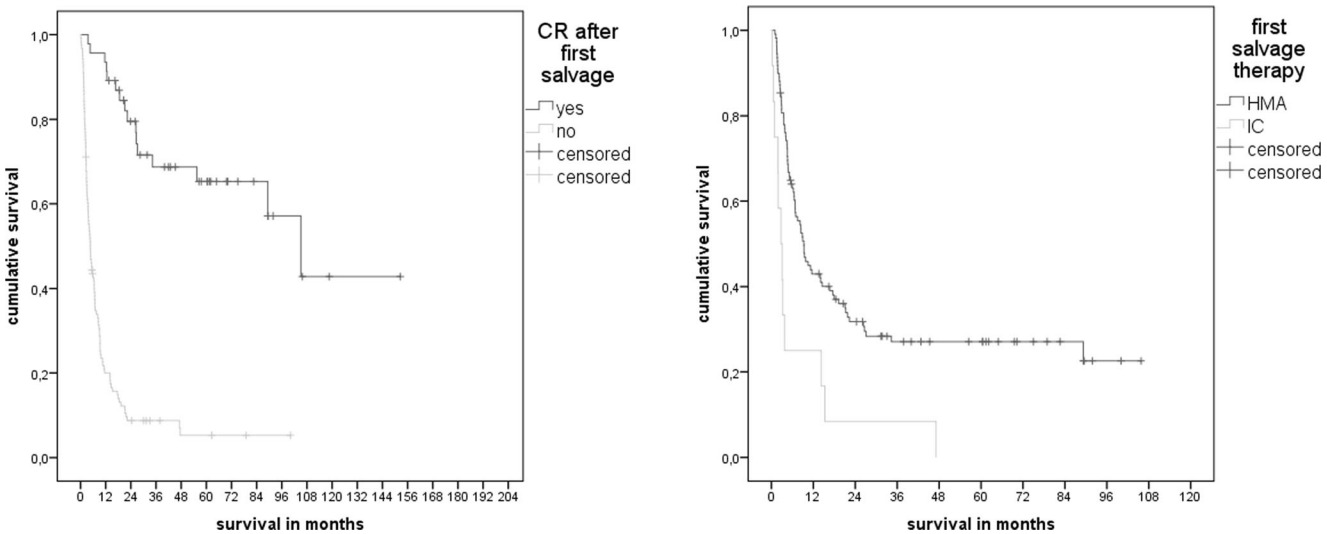

Fig. 3 Overall survival after relapse according to first salvage therapy and response. *CR* complete remission, *HMA* hypomethylating agents, *IC* intensive chemotherapy, *aza* 5-azacitidine, *DLI* donor lymphocyte infusion. *$p < 0.05$

status at transplant, type of relapse, and duration of remission after aHSCT were the major factors determining the patients prognosis after relapse. Some factors are fateful, like age and diagnosis, but some can be influenced by the physician, most importantly treatment before transplant and early detection of MR. Interestingly, independent from ELN or IPSS-R- risk groups, patients with MDS who were treated upfront without remission induction before transplant had similar chance to achieve a second remission following relapse after transplant like patients who were in CR at the time of transplant. In contrast, patients with refractory disease at the time of transplant were unlikely to achieve remission after relapse. This argues for a "transplant as early as possible" strategy in patients with adverse prognostic factors that predispose them for failure with current conventional treatment strategies like complex karyotype and/or p53 mutations.

In addition, our analysis shows that early detection and treatment of MR improves the chance to achieve CR and long-term survival when compared to overt hematologic or extramedullary relapse. Consequently, we emphasize the urgent need for frequent MRD-monitoring using highly sensitive molecular detection techniques. These data are in line with previous reports from our center and others [2–4]. Further improvement of MRD-monitoring utilizing a combination of broadly applicable, sensitive, and specific markers may also improve treatment results in patients relapsing after aHSCT [17].

Duration of remission after transplant was an additional very important factor for survival time after relapse; as identified in most studies irrespective of reported treatment [2, 4, 6, 18, 19], patients who relapsed later than the median time to relapse of 4.6 months in our cohort had a significantly longer survival time, even more clear was the difference when choosing 1 year as cutoff. This finding stresses the need for experimental treatment in high-risk patients who are at risk for early relapse. Craddock et al. have analyzed molecular alterations that put patients at high-risk for early relapse and identified for example FLT3-ITD mutations as one important AML subgroup [20]. We also show in our analysis that AML-relapse occurs earlier than MDS-relapse with a peak during the first 6 months after transplant. This argues for clinical studies testing maintenance or consolidation therapy especially in patients with high-risk for early relapse. Maintenance therapy should ideally carry only moderate risks for severe side effects because the dilemma of overtreating some potentially cured patients has not been solved yet. Nevertheless, currently maintenance strategies with tyrosine kinase inhibitors, like midostaurine, sorafenib, or gilteritinib, and the histondeacetylase inhibitor panabinostat are subjects of clinical studies for high-risk AML and MDS patients [21–24].

Further, there is some data on prophylactic aza as well as aza for consolidation or maintenance therapy after aHSCT. A significant problem with HMA as maintenance treatment after transplant is a high drop off rate since HMA carry a substantial hematologic toxicity, especially when treatment has to start early after transplant to avoid relapses during the first 6 months [25, 26].

Whenever possible, we tried to treat patients with relapse of AML and MDS after transplant with aza and DLI. The success of this treatment strategy largely depends on disease dynamics at relapse and the availability of DLI. We scheduled our patients to receive up to eight cycles aza (75 mg/m^2 day 1–7) followed by DLI (from 1 to 5 × 10^6 to 1–5 × 10^8 CD3 + cells/kg) after every second aza cycle [5]. In the presence of FLT3 mutations, sorafenib was given in addition to aza/DLI [10].

Among our patients, median survival with aza and DLI was 9.3 months in comparison to 2.7 months with IC ($p = 0.001$).

It is believed that donor cells are crucial for long-term survival; however, our data cannot proof this statement since all our patients were scheduled to receive DLI and those who did not receive DLI died too early after relapse. To finally answer the question on the role of donor cells within a combined modality treatment strategy, this intervention has to be tested in a randomized trial, which we think is unethical.

IC as first salvage treatment for relapse after transplant was given only to patients with fulminant hematologic relapse with progressive leucocytosis. The 12 patients, who initially received IC had more advanced disease and mostly early relapses.

In our experience, aza/DLI is associated with acceptable side effects and feasible as an outpatient approach for most patients. We and others have demonstrated that this therapy is particularly successful in patients with MDS. In our patient cohort, survival for MDS patients receiving aza/DLI was 89.3 months [2, 4]. A recently published study by the Chronic Malignancies Working Party of the EBMT analyzed the outcome of MDS and sAML after HR in patients not receiving HMA but cellular therapy either DLI or second aHSCT or chemotherapy and reported a disappointing median survival of only 4.7 months [27]. Therefore, a dual treatment approach using cytotoxic treatment with HMA on the one and cellular therapy on the other hand seems to carry the greatest potential to induce durable remissions in patients with MDS who relapse after aHSCT. Under current investigation are combination therapies using, for example, lenalidomide in addition to aza and DLI to enhance the GvL effect (EudraCT 2013–001153-27). As a conclusion from our data, we identified older patients with AML and early hematologic relapse as a group unlikely to respond to current salvage treatment. However, even in this unfavorable group, there were individual patients who were able to achieve CR and long-term remission. Patients who did not respond to first salvage treatment and did not qualify for a second transplant had a dismal outcome and should be offered palliative therapy only.

In Fig. 4, our treatment algorithm for MDS and AML is displayed. If a targeted therapy strategy is available, i.e., TKIs

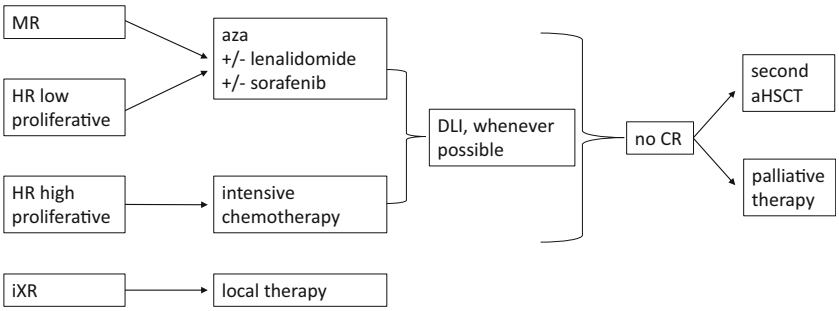

Fig. 4 Treatment algorithm in AML/MDS relapse after aHSCT. *MR* molecular relapse, *HR* hematologic relapse, *iXR* isolated extramedullary relapse, *aza* 5-azacitidine, *DLI* donor lymphocyte infusion, *CR* complete remission, *aHSCT* allogeneic hematopoetic stem cell transplantation

for BCR/ABL positive CML or FLT3-mutated AML, we would favor targeted therapy in combination with DLI. Median survival of the nine patients with CML who received a TKI for the treatment of relapse after transplant was 18.6 months.

It is an open question if patients who achieve a CR after treatment with IC or HMA with or without DLI should receive a second aHSCT. To our knowledge, there is no randomized study on this issue so far. We did not perform a second aHSCT if patients achieved CR with aza/DLI or DLI and IC. However, we tried to induce some kind of GvHD with repeated administration of DLI even in patients with CR. A second aHSCT was applied only in patients failing aza/IC/DLI combinations. Interestingly, in our small cohort of patients receiving second aHSCT, the vast majority of patients was not in CR at second transplant, and aHSCT was not the first treatment approach, but still there are long-term survivors. We therefore conclude that CR of the underlying disease at the timepoint of second aHSCT is not an absolute requirement for long-term survival. Among our patients, 2-year survival is 23% and 5-year survival is 10%, which is comparable to the data of Christopeit et al., who showed a 2-year survival of 25%, for a multicenter patient cohort with a CR rate at second transplant of 66% [28]. Schmid et al. reported a 2-year survival rate of 17% for MDS and sAML patients, who were mostly not in CR (90%) at second transplant [27].

In summary, most relapses of myeloid malignancies occur within the first 3 years after transplant, but relapse patterns are different in AML and MDS. Molecular relapse has a better prognosis than hematologic and extramedullary relapse arguing for intensified methods for MRD detection. Patients, who achieve a second CR with HMA or TKIs in combination with DLI have a reasonable chance for long-term survival. Second aHSCT is a valuable option for patients not achieving CR or long-term remission with non-transplant salvage therapy and DLI. New approaches including the use of emerging targeted therapies or agents interfering with immunologic tolerance may be able to further improve treatment results in patients relapsing after aHSCT [29].

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Compliance with ethical standards

Informed consent Informed consent was obtained from all patients for being included in the study.

Conflict of interest Esther Schuler and Christina Rautenberg received travel support by Celgene, Roland Fenk, Thomas Schroeder, and Guido Kobbe received research funding, lecture fees, and travel support by Celgene. Ulrich Germing received research funding and lecture fees by Celgene.

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