Received: 22 December 2021 | Revised: 10 February 2022 | Accepted: 28 March 2022

DOI: 10.1002/hon.2995

WILEY

ORIGINAL ARTICLE

Outcomes of second allogeneic stem cell transplantation and anti-relapse strategies in patients with relapsed/refractory acute myeloid leukemia: A unicentric retrospective analysis

Evgenii Shumilov[1,2] | Justin Hasenkamp[1] | Markus Maulhardt[1] | Paolo Mazzeo[1] | Nicole Schmidt[1] | Hristo Boyadzhiev[1] | Wolfram Jung[1] | Christina Ganster[1] | Detlef Haase[1] | Raphael Koch[1] | Gerald Wulf[1]

[1]Department of Hematology and Medical Oncology, University Medicine Göttingen (UMG), Göttingen, Germany

[2]Department of Medicine A, Hematology, Oncology and Pneumology, University Hospital Münster, Münster, Germany

Correspondence

Raphael Koch, Department of Hematology and Medical Oncology, University Medicine Goettingen (UMG), Robert-Koch-Str.40, D-37075 Goettingen, Germany.
Email: raphael.koch@med.uni-goettingen.de

Funding information

Deutsche Forschungsgemeinschaft (DFG, German Research Foundation), Grant/Award Number: 413501650; Deutsche Krebshilfe, Grant/Award Number: 70113602
Open access funding enabled and organized by Projekt DEAL.

Abstract

Second allogeneic stem cell transplantation (allo-SCT2) represents a rescue option for selected patients (pts) with relapsed/refractory (r/r) acute myeloid leukemia (AML). Still, relapse rates post-allo-SCT2 remain high and effective anti-relapse strategies and predictive biomarkers remain to be defined. We here analyzed a cohort of 41 AML patients (pts) undergoing allo-SCT2 in our center. Allo-SCT2 induced a third hematologic complete remission (CR) in 37 pts, at costs of a 36% non-relapse mortality rate. Furthermore, 19 pts eventually relapsed post allo-SCT2. Addressing relapse after allo-SCT2, 14 pts (74%) underwent cell-based anti-relapse strategies, including third allogeneic transplantation (allo-SCT3; 3/14), donor lymphocyte infusions (DLIs) combined with either 5-azacytidin and venetoclax (4/14) or chemotherapeutic agents (7/14). Notably, six of seven pts (86%) who received either allo-SCT3 or a combination therapy of DLIs, 5-azacytidine and venetoclax achieved CR despite poor cytogenetics post-allo-SCT2 (e.g., *TP53*). Finally, 11 of 41 pts were alive at the last follow-up (seven CR2, three CR3, one partial remission) resulting in estimated 2- and 5-year overall survival of 35% and 25%, respectively.

KEYWORDS

anti-relapse strategies beyond allo-SCT2, donor lymphocyte infusion (DLI), relapsed/refractory acute myeloid leukemia (r/r AML), second allogeneic stem cell transplantation (allo-SCT2), venetoclax

1 | INTRODUCTION

Allogeneic stem cell transplantation (allo-SCT) is an established curative approach for both frontline therapy of high-risk acute myeloid leukemia (AML) and salvage therapy for relapsed or refractory (r/r) disease.[1] Still, up to 40% of the patients (pts) eventually relapse after first allo-SCT (allo-SCT1).[2] At present, several options are available for r/r AML following allo-SCT1: conventional chemotherapy, hypomethylating agents (HMAs), targeted agents like tyrosine kinase inhibitors or the BH3-mimetic drug venetoclax, re-boosting graft versus

Raphael Koch and Gerald Wulf contributed equally.

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leukemia effects (GvL) by tapering immunosuppression, application of donor lymphocyte infusions (DLIs) or second allo-SCT (allo-SCT2).[2–4] Of these, allo-SCT2 represents a rescue option for a distinct subset of pts with a good performance status, an available donor and intention to achieve long-term second complete remission (CR2).[5–7] So far, multiple multicentric studies documented 2- and 5-year overall survival (OS) and leukemia-free survival (LFS) post-allo-SCT2 of up to 27% and 21% for OS, and 25% and 19% for LFS, respectively.[5,8–11] Of note, the strategy of donor change for allo-SCT2 failed to provide additional survival benefit.[8,9,12] Significant non-relapse mortality (NRM) and r/r disease are the main challenges for allo-SCT2, accounting for more than 30% mortality for each category in post-allo-SCT2 follow-up.[6,13,14] For patient stratification, improved allo-SCT2 outcomes have been reported for cases with chemosensitive disease at the time of allo-SCT2 and long duration of remission after allo-SCT1.[5,11,14,15] Identifying the molecular basis and clonal architecture of the disease, though, might provide insight into clonal evolution and devolution as a driver of disease progression or cure. Consequently, comprehensive genetic profiling of AML at relapse may identify targetable alterations and open new pathways for bridging strategies toward allo-SCT.[16,17] Data on anti-relapse management after allo-SCT2, though, are scarce. Thus, we here report our experiences from a cohort of 41 AML pts undergoing allo-SCT2 in our center.

2 | MATERIALS AND METHODS

2.1 | Pts

This retrospective monocenter study included 41 consecutive adult pts (≥18 years) diagnosed initially either with de novo or secondary AML (s-AML), myelodysplastic syndrome (MDS) with excess of blasts or MDS/MPN overlap syndrome and undergoing subsequently allo-SCT1/-SCT2 between 2004 and 2020 at the University Medicine Goettingen, Germany. The inclusion criteria were as follows: (A) Pts must have relapsed following allo-SCT1. Both, hematological and cytogenetic and/or molecular relapses post-allo-SCT1 were considered. (B) At the time point of relapse post-allo-SCT1, all pts must have had criteria of AML. (C) Types of AML were de novo, secondary (s-AML) following MDS, MPN or MDS/MPN and therapy associated (t-AML). Genetic risk stratification of pts was performed according to the 2017 European LeukemiaNet (ELN) criteria for AML[18] and to the Revised International Prognostic Scoring System (IPSS-R) for MDS.[19] At relapse post-allo-SCT1, only ELN risk stratification was applied as far as all selected patients had met the criteria of AML. The primary indication to allo-SCT1 was either front-line due to adverse genetic risk or salvage following relapse/refractoriness after conventional first-line chemotherapy. Referring to selection criteria for allo-SCT2, good performance status of patient (ECOG 0-1) as well as rapid availability of new donor and particularly patient wish were considered. In context of allo-SCT2, all types of remission status, conditioning regimens and stem cell sources were included. Prophylaxis against graft-versus-host disease (GvHD) consisted of either tacrolimus or cyclosporine combined with mycophenolate mofetil and antithymocyte globulin. In case of haploidentical transplants, post-transplant cyclophosphamide was applied. All allo-SCTs were performed at the University Medicine Goettingen, except one patient who entered the program for the third allo-SCT (allo-SCT3) only. The study was conducted in compliance with the declaration of Helsinki.

2.2 | Genetic profiling

Description of cytogenetic and molecular-genetic methods applied within the study is given in supplemental material (Supplementary Table S1).[20,21]

2.3 | Endpoints

The primary endpoints of the study were event-free survival (EFS) and OS following allo-SCT2. Secondary endpoints included: (A) cumulative incidence of relapse mortality (RM) and NRM in the allo-SCT2-group; (B) anti-relapse treatment types and response rates following relapse post-allo-SCT2; (C) contribution of distinct clonal genetic events before and after allo-SCT2 to remission and survival results.

2.4 | Definitions

Staging and morphologic response criteria in AML were based on the 2017 ELN AML recommendations.[18] OS was defined as time from allo-SCT to death from any cause. Event-free survival was defined as the duration from allo-SCT2 to the date of refractory disease, or relapse from complete remission (CR), or CR with incomplete recovery, or death from any cause; patients not known to have any of these events were censored on the date they were last examined. Non-relapse mortality was defined as death without evidence of relapse or progression. Myeloablative conditioning (MAC) was defined as a regimen containing either a total dose of oral busulfan greater than 6.4 mg/kg busulfan i.v. or two alkylating agents (Supplementary Table S2). Regimens containing lower conditioning intensities were defined as reduced-intensity conditioning (RIC).[22] Acute and chronic GvHD was categorized according to the Consensus Conference on Acute GVHD Grading and EBMT criteria.[23,24]

2.5 | Statistics

Patient and transplantation characteristics were analyzed using descriptive statistics. Categorical variables were summarized as frequencies and percentages, and continuous variables were summarized as median and range. Probability of OS and EFS was calculated using the Kaplan-Meier method. Cumulative incidence curves were used for RM and NRM in a competing risk analysis. Statistical

analyses were performed with SPSS, version 26.0 (SPSS) and R Software for Statistical Computing and Graphics, version 3.6.2.

3 | RESULTS

3.1 | Patient characteristics at diagnosis who underwent two allo-SCTs

This study included 41 pts who had either AML (93%, 38/41), MDS with excess blasts 1 (MDS-EB 1; 5%, 2/41) or MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T; 2%, 1/41) at diagnosis and underwent subsequently two allo-SCTs for relapsed. Patient and disease characteristics at diagnosis are shown in Table 1. The median age at diagnosis was 49 years with a slight prevalence of males (24 males: 17 females). Regarding the origins of AML, de novo was the most common type (58%, 22/38) followed by s-AML (39%, 15/38) and t-AML (3%, 1/38). Of the pts with AML, five (13%) had favorable, 18 (47%) intermediate and 15 (40%) adverse ELN genetic risk. Both MDS cases referred to the high-risk prognostic category within IPSS-R. For pts with AML and favorable genetic risk group (5/38), indication for all-SCT was either relapse or refractory disease following conventional induction of first hematological remission (CR1). One patient with MDS/MPN-RS-T was transplanted due to cytogenetic evolution with emergence of 7q- and 17p-/*TP53*-deletion.

3.2 | Time from induction of first remission to relapse after allo-SCT1

Patient and disease characteristics at allo-SCT1 are shown in Table 2. Eighty-three percentage of all pts (34/41) underwent intensive induction therapy with anthracycline and cytarabine (ARAC). The remaining seven pts (17%) received either non-intensive induction with the HMA 5-azacytidine (5-AZA; 3/41) or entered allo-SCT1 without preceding therapy (4/41) in cases of MDS (2/4), MDS/MPN-RS-T (1/4) and s-AML from MDS (1/4). For details of the therapeutic modalities see Table 2. Eventually, 32 pts (78%) had front-line and nine pts (22%) salvage indication for allo-SCT1. With the median time from first diagnosis to allo-SCT1 of 4.5 months, pts underwent either myeloablative conditioning (MAC; 51%; 21/41) or reduced intensity conditioning (RIC; 49%; 20/41; Supplementary Table S2) with hematopoietic stem cells from predominantly matched donors (90%, 37/41). Following allo-SCT1, all pts included in this analysis relapsed within a median time of 11 months (range 3–77 months).

3.3 | Time from relapse after allo-SCT1 to allo-SCT2

Patient and disease characteristics at allo-SCT2 are shown in Table 2. All relapsed post-allo-SCT1 underwent immunosuppression tapering and, if available, DLIs. Intensive re-induction was a therapy of first choice for patients with hematologic r/r disease post-allo-SCT1 or molecular relapse with unfavorable cytogenetic/molecular-genetic profile and good performance status. For patients with lower blast counts and/or high HCT-CI risk non-intensive bridging (e.g., HMAs and/or DLIs) to allo-SCT2 was the preferred regimen. In details, pts underwent predominantly fludarabine/ARAC/G-CSF (FLAG) - (44%; 18/41) or anthracycline/ARAC- (24%, 10/41) based intensive reinduction. Six pts (14%) received non-intensive bridging therapy with HMAs either as monotherapy (5%, 2/6) or combined with venetoclax (7%, 3/6) or gemtuzumab ozogamicin (2%, one-sixth). Thirteen out of 41 pts (32%) received DLIs either as monotherapy (5/41, 12%) or combined with chemotherapy (8/41, 19%; Table 2). The remaining two pts (5%) proceeded directly to allo-SCT2 after relapse post-allo-SCT1. The remission status at allo-SCT2 (available for 39/41 pts) was r/r disease in 49%, CR2 in 44%, PR in 5% and SD in 2% of pts, respectively. With the median time from relapse post-allo-SCT1 to allo-SCT2 of 2.5 months, pts underwent predominantly RIC (85%), while MAC was administered to the remaining 15% of pts (Supplementary Table S2). Matched donors (88%; 36/41) were available for the majority of pts at the time of allo-SCT2. The remaining five cases had either mismatched unrelated (mMUD; 10%) or haploidentical donors (2%). At allo-SCT2, all pts received hematopoietic stem cells from a new donor.

3.4 | Outcomes of allo-SCT2

The outcomes of allo-SCT2 are shown in Table 2. The FU after allo-SCT2 ranged from 1 month to nearly 14 years with a median of 11 months. The incidence of GvHD post-allo-SCT2 in comparison to allo-SCT1 was lower for acute (12% vs. 34%) and higher for chronic (17% vs. 12%) GvHD, respectively.

Nineteen pts (46%) relapsed following allo-SCT2 with the median time of 6 months. At the time of the last FU, 30 pts (73%) had died. Of these, NRM was the cause of death in 16 and r/r AML in 14 pts resulting in cumulative incidence of non-relapse and RM of 36% and 37%, respectively (Figure 1A). Pts succumbed either to infection (75%, 12/16), GvHD (19%, 3/16) or veno-occlusive disease (6%, 1/16). Finally, the calculated 2- and 5-year EFS rate (Kaplan-Meier) post-alloSCT2 were 26% and 15%, respectively (Figure 1B).

3.5 | Anti-relapse strategies following relapse post-allo-SCT2

Characteristics of pts relapsed after allo-SCT2 and efficacy of anti-relapse treatment following allo-SCT2 are presented in Table 3 and Figure 1C. Of 19 relapsed pts post-allo-SCT2, 14 (74%) underwent cell-based anti-relapse strategies and five (26%) best-supportive care (BSC). Of the former group, 3 out of 14 pts (#1–3) could proceed to allo-SCT3, four pts (#4–7) received BCL-2 inhibitor venetoclax in combination with 5-AZA and DLIs, and remaining seven pts (#8–14) were treated with either DLIs only (3/6) or in combination with

TABLE 1 Clinical characteristics of the 41 patients at diagnosis in this analysis

Parameter	All patients, *n* = 41	
Gender (M/F), *n* (ratio)	24/17	1.4
Median age, years (range)	49	19–73
Disease classification		
AML, *n* (%)	*n* = 38 (93%)	
Origin of the AML	*N*	(%)
de novo AML	22	58%
s-AML	15	39%
t-AML	1	3%
WHO subtypes (2016), AML		
AML with myelodysplasia-related changes	14	36%
AML, NOS	16	42%
AML with minimal differentiation	6	16%
AML without maturation	4	10%
AML with maturation	1	3%
Acute myelomonocytic leukemia	3	7%
Acute monoblastic/monocytic leukemia	1	3%
Acute panmyelosis with myelofibrosis	1	3%
AML with recurrent genetic aberrations:	7	19%
AML with mutated *NPM1*	3	7%
AML with biallelic mutations of *CEBPA*	1	3%
AML with inv(16) (p13.1q22) or t(16;16) (p13.1;q22); *CBFB-MYH11* fusion	1	3%
AML with mutated *RUNX1*	1	3%
AML with t(9;11) (p21.3;q23.3); *MLLT3-KMT2A* fusion	1	3%
Therapy-related AML	1	3%
Genetic risk groups (ELN 2017), AML		
Favorable	5	13%
Intermediate	18	47%
Adverse	15	40%
MDS	*n* = 2 (5%)	
MDS-EB1	2	
Prognostic risk category, IPSS-R		
High risk	2	
MDS/MPN RS-T	*n* = 1 (2%)	
Molecular characterization		
Cytogenetics at diagnosis	*n* = 41 (100%)	
Normal karyotype	22	54%
Complex karyotype (≥3 aberrations)	3	8%
−7/del(7q)	3	8%
−17/abn(17p)	2	5%
inv(16) (p13.1q22) or t(16;16) (p13.1;q22); *CBFB-MYH11* fusion	1	2%

TABLE 1 (Continued)

Parameter	All patients, $n = 41$	
inv(3) (q21.3q26.2) or t(3;3) (q21.3;q26.2); *GATA2,MECOM(EVI1)*	1	2%
t(v;11q23.3); *KMT2A* rearranged	1	2%
Others	8	19%
Molecular genetics at diagnosis[a]	$n = 31$ (76%)	
FLT3-ITD/or -TKD	7	23%
NPM1	4	13%
JAK2	3	10%
ASXL1	3	10%
RUNX1	2	6%
IDH2	2	6%
CEBPA	1	3%
SF3B1	1	3%
PTPN11	1	3%
SRSF2	1	3%
DNMT3A	1	3%
SETBP1	1	3%
IDH2	1	3%
EZH2	1	3%
PTPN11	1	3%
Negative for tested mutations	11	35%

Abbreviations: AML, acute myeloid leukemia; ELN, European leukemiaNet; F, female; FAB, French-American-British classification system; IPSS-R, revised International Prognostic Scoring System; M, male; MDS, myelodysplastic syndrome; MDS-EB1, MDS with excess blasts 1; MDS/MPN RS-T, MDS/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; NOS, not otherwise specified; s-AML, secondary AML; t-AML, therapy-related AML.

[a]Twenty-eight patients tested by qPCR and/or fragment length analysis and three patients by NGS with QIASeq targeted myeloid gene panel.

conventional cytostatic agents (2/6) or radiotherapy (1/6). Donor lymphocyte infusions post-allo-SCT2 were given in all cases with curative intent and available DLIs.

3.5.1 | Allo-SCT3 and venetoclax/DLIs/5-AZA groups

Out of seven pts from these two groups, 6 (86%) could achieve third CR (CR3) as best hematologic response. The remaining patient (#5) had PR under venetoclax/DLIs/5-AZA. With a median FU of 10 months, three of seven pts (#1,4,5) were alive being either in CR3 (#1,4) or having PR (#5). The remaining 4 pts (#2,3,6,7) died either from relapse (#3,7) or NRM (#2,6). Yet, one of these pts (#2) succumbed to pneumonia being in CR3 48 months post-alloSCT3. Notably, 2 pts who achieved long-term CR3 following either allo-SCT3 (#2) or DLIs/5-AZA/Venetoclax (#4) had preceding clonal evolution at last relapse with high-risk cytogenetic and/or molecular-genetic aberrations (Table 3).

3.5.2 | DLIs +/− cytostatic-/hypomethylating or radiotherapy group

Of seven pts from this group with the median FU of 5 months, only one (#8) was alive with CR3 53 months following DLIs with radiotherapy due to myeloid sarcoma after allo-SCT2. The remaining pts (#9–14) succumbed to r/r disease.

3.5.3 | Best supportive care

All pts (#15–19) receiving BSC only died from r/r disease with a median FU of 2 months.

Finally, from 19 cases relapsed beyond allo-SCT2, four pts (21%) were alive and 15 (79%) dead at the last FU with pts receiving allo-SCT3 and venetoclax/DLIs/5-AZA groups demonstrating significant survival benefits ($p < 0.0001$; Figure 1C). Referring to the survival and remission rate in the whole patient cohort undergoing allo-SCT2 at the last FU, 11 of 41 pts were alive (seven CR2, three CR3, one PR)

TABLE 2 Therapy regimens and clinical outcomes among 41 acute myeloid leukemia (AML) patients undergoing second allogeneic stem cell transplantation (allo-SCT2)

Parameter	All patients, $n = 41$	
Induction therapy		
Anthracycline/ARAC based	28	68%
Anthracycline/ARAC based followed by FLAG based regimen	6	15%
5-AZA	3	7%
No induction therapy/direct allo-SCT1[a]	4	10%
Indication to allo-SCT1		
Front-line allo-SCT1	32	78%
Salvage allo-SCT1	9	22%
Median time from first diagnosis to allo-SCT1, months (range)	4.5	(2–48)
Remission status at allo-SCT1		
CR1[b]	25	61%
PR	8	19%
SD	4	10%
Untreated disease[a]	4	10%
Conditioning allo-SCT1		
RIC	20	49%
MAC	21	51%
Donor allo-SCT1		
MUD	30	73%
MRD	7	17%
mMUD	4	10%
GvHD allo-SCT1		
aGvHD	14	34%
Grade I	9	22%
Grade II	3	7%
Grade III	2	5%
cGvHD	5	12%
Mild	4	10%
Severe	1	2%
Median time allo-SCT1 to relapse, months (range)	11	(3–77)
Reinduction of CR2 following relapse after allo-SCT1		
Intensive reinduction	28	68%
Anthracycline/ARAC based (+DLIs)	10 (3)	24% (7%)
FLAG-Ida/or -Eto (+DLIs)	18 (3)	44% (7%)
Non-intensive reinduction	6	14%
DLIs only	5	12%
5-AZA mono	2	5%
5-AZA- or decitabine combined with venetoclax (+DLIs)	3 (2)	7% (5%)
Gemtuzumab ozogamicin	1	2%

TABLE 2 (Continued)

Parameter	All patients, $n = 41$	
Direct allo-SCT2 without reinduction	2	5%
Median time from relapse post allo-SCT1 to allo-SCT2, months (range)	2.5	(1–17)
Median time allo-SCT1 to -SCT2, months (range)	15	(6–86)
Remission status at allo-SCT2 (for 39 pts available)		
r/r disease	19	49%
CR2[c]	17	44%
PR	2	5%
SD	1	2%
Conditioning allo-SCT2 (for 40 pts available)		
RIC	36	90%
MAC	4	10%
Donor allo-SCT2 (for 40 pts available)		
MUD	31	78%
MRD	4	10%
mMUD	4	10%
haploidentical	1	2%
Median follow-up after allo-SCT2, months (range)	11	(1–165)
GvHD allo-SCT2		
aGvHD	5	12%
Grade II	2	5%
Grade III	1	2%
Grade IV	2	5%
cGvHD	7	17%
Mild	3	7%
Moderate	2	5%
Severe	2	5%
Survival status following allo-SCT2 al last follow-up		
Alive	11	27%
Dead	30	73%
Causes of death		
r/r disease	13	43%
NRM	17	57%
Infection	13/17	43%
GvHD	3/17	10%
VOD	1/17	4%
Relapse incidence following allo-SCT2	19	46%
Median time from allo-SCT2 to relapse, months (range)	6	(1–116)
Remission status in alive patients following allo-SCT2 ($n = 15$)		
CR2 after allo-SCT2	7	47%
Relapse after allo-SCT2 followed by	4	27%

(Continues)

TABLE 2 (Continued)

Parameter	All patients, $n = 41$	
CR3 due to anti-relapse treatment	3	20%
SD due to anti-relapse treatment	1	7%

Abbreviations: 5-AZA, azacitidine; allo-SCT1, first allogeneic stem cell transplantation; allo-SCT2, second allogeneic stem cell transplantation; AML, acute myeloid leukemia; ARAC, cytarabine; CR1, first complete hematologic remission; CR2, second complete hematologic remission; CR3, third complete hematologic remission; DLIs, donor lymphocyte infusions; Eto, etoposide; FLAG, fludarabine, high-dose cytarabine, G-CSF; GvHD, graft versus host disease; Ida, idarubucin; MAC, myeloablative conditioning; mMUD, mismatched unrelated donor; MRD, matched related donor; MUD, matched unrelated donor; NRM, non-relapsed mortality; PR, partial remission; r/r diesase, relapsed/refractory disease; RIC, reduced intensity conditioning; SD, stable disease; VOD, veno-occlusive disease.

[a]One patient with MDS/MPN RS-T, one patient with MDS-EB1 and two patients with sAML from MDS entered allogeneic transplantation without prior induction therapy.

[b]One patient with CR2.

[c]One patient with CR3.

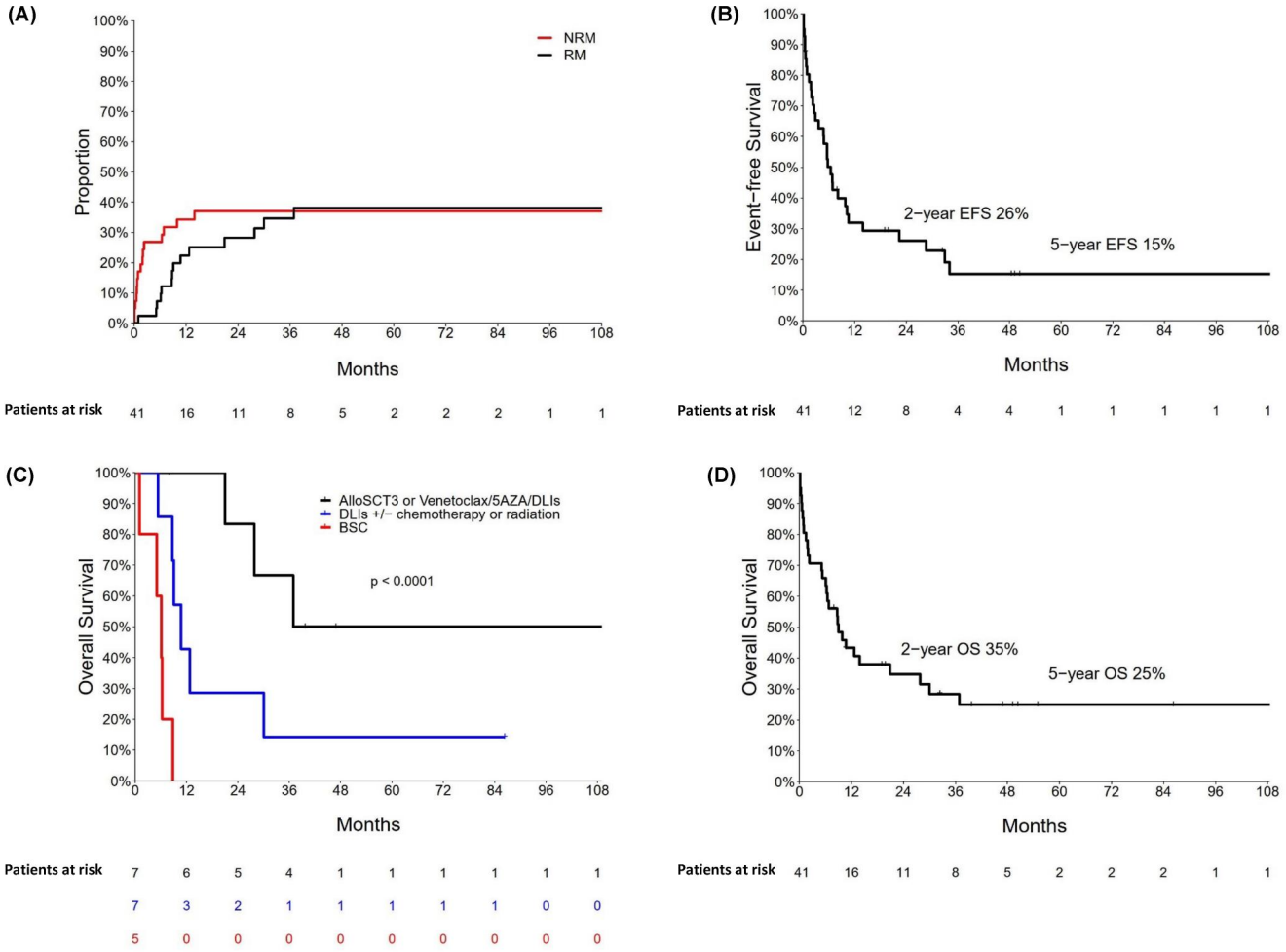

FIGURE 1 (A) Cumulative incidence of relapse and non-relapse mortality post-allo-SCT2 (B) event-free survival post-allo-SCT2 (C) overall survival in relapsed patients following second allogeneic stem cell transplantation (D) OS post-allo-SCT2 in the whole patient cohort. 5AZA, 5-azacytidine; allo-SCT2, second allogeneic stem cell transplantation; allo-SCT3, third allogeneic stem cell transplantation; BSC, best supportive care; DLIs, donor lymphocyte infusions; EFS, Event-free survival; NRM, non-relapse mortality; OS, overall survival; RM, relapse mortality

resulting in estimated 2- and 5-year OS of 35% and 25%, respectively (Figure 1D).

Notably, we identified two pts with disappearance of prognostically unfavorable genetic clones at relapse before or after allo-SCT2 who succeeded to regain a stable long-term remission afterward. The first patient was diagnosed with de novo AML presenting with a normal karyotype but mutated *FLT3*-ITD. Following intensive anthracycline/ARAC induction in combination with FLT3 inhibitor

TABLE 3 Characteristics of patients relapsed after second allogeneic stem cell transplantation (allo-SCT2) and outcomes of anti-relapse treatment following allo-SCT2

Pt. № age, sex, AML subtype, FAB	Genetic profile at initial AML diagnosis	First-line therapy	Remission status at allo-SCT1, conditioning, donor	Relapse after allo-SCT1, months	Genetic profile at relapse after allo-SCT1	Second-line therapy	Remission status at allo-SCT2, conditioning, donor	Relapse after allo-SCT2, months	Genetic profile at relapse after allo-SCT2	Anti-relapse therapy after allo-SCT2	Remission status at allo-SCT3, conditioning, donor	FU from relapse after allo-SCT2, months	Status at last FU
Allo-SCT3 following relapse after allo-SCT2													
(1) 46; F; de novo AML, M1	46, XX; *RUNX1 mt*	S-HAM followed by FLAG-Ida	CR1; BuCyATG; MUD	17	Stable: 46,XX 2/46,XY 24; *RUNX1 mt*	DLIs x4	r/r disease; FLAMSA-Mel; MUD	11	Devolution: *RUNX1* wt	No	r/r disease; ThioTreo FLU followed by HD-Cy; haploidentical donor	36	Alive in CR3
(2) 49; F; de novo AML, M0	46, XX	HAM followed by TAD-9	CR1; BuCy; MRD	31	NA	Gemtuzumab ozogamicin	NA	116	Evolution: complex karyotype	HAM	CR3; FluMel-ATG; MRD	48	Death in CR3 from pneumonia
(3) 24; M; s-AML, M4	47, XY,+8; *FLT3*-TKD	S-HAM followed by TAD-9	CR1; BuCy-ATG; MUD	50	Stable: 47, XY,+8; *FLT3*-TKD	FLAG-Eto	PR; FBM-ATG; MUD	7	NA	HAM with midostaurin followed by DLI x1	r/r disease; ThioTreo FLU followed by HD-Cy; haploidentical donor	21	Death from r/r disease following 16 months of CR3 after allo-SCT3
Venetoclax/5-Aza/DLIs following relapse after allo-SCT2													
(4) 30; M; de novo AML, M0	Complex karyotype with *TP53* deletion	DA followed by FLAG-Eto	SD; BuCy-ATG; MUD	21	Evolution: complex karyotype plus 4 new aberrations	HAM	CR2; FLAMSA-AM; MUD	22	Evolution: further new complex aberrations with *TP53* deletion	Ven/5-Aza/DLIs x5 followed by GvHD III	-	17	Alive in CR3, DC 100%, no GvHD
(5) 59; M; s-AML, M0	Complex karyotype including del (7q)/del (17p); *SF3B1 mt*, *JAK2V617F*	No induction, directly alloSCT due to MDS/MPN RARS-T	FB10-ATG; mMUD	30	Evolution: new cytogenetic aberrations; additionally *TP53 mt*, *TET2 mt*	5-AZA/Ven x1	CR2; TreoFlu-ATG; mMUD	5	Stable: *SF3B1 mt*, *TET2 mt*, *TP53 mt*; cytogenetics NA	Ven/5-Aza/DLIs x3	-	8	Alive in PR, DC 97%
(6) 68; F; s-AML, M0	46, XX	ICE	CR1; FB8-ATG; MUD	66	Evolution: + 8; *BCOR mt*, *SRSF2 mt*, *STAG2 mt*	S-HAM plus DLIs x4	r/r disease; TreoFlu-ATG; MUD	34	Evolution: three additional mutations – *STAG mt*, *CUX1 mt*, *PHF6 mt*	Ven/5-Aza/DLIs x2 followed by GvHD I	-	3	Death from infection in CR3, DC 97%

(Continues)

TABLE 3 (Continued)

Pt. № age, sex, AML subtype, FAB	Genetic profile at initial AML diagnosis	First-line therapy	Remission status at allo-SCT1, conditioning, donor	Relapse after allo-SCT1, months	Genetic profile at relapse after allo-SCT1	Second-line therapy	Remission status at allo-SCT2, conditioning, donor	Relapse after allo-SCT2, months	Genetic profile at relapse after allo-SCT2	Anti-relapse therapy after allo-SCT2	Remission status at allo-SCT3, conditioning, donor	FU from relapse after allo-SCT2, months	Status at last FU
(7) 61; F; de novo AML, M0	46, XX ; *PTPN11 mt*, *PHF6 mt*	DA followed by ARA-C	CR1; FBM-ATG; MUD	9	Evolution: *RUNX1 mt* new; 46,XX 19/46,XY 3	FLAG-Eto	CR2; TreoFlu-ATG; MUD	10	Devolution: *PTPN11 wt*; persistence of *RUNX1 mut*, *PHF6 mut*	Ven/5-Aza/DLIs x5 followed by 4-AZA mono	-	11	Death in r/r following 11 months of SD
DLI +/− cytostatic/hypomethylating or radiotherapy following relapse after allo-SCT2													
(8) 45; F; de novo AML, M4	46, XX; *FLT3*-ITD high	DA	CR1; BuCy-ATG; MUD	13; hem. relapse plus CNS	Stable: 46, XX	FLAG-Ida/ radio- and intrathecal therapy	CR2; FB8-ATG; MUD	33; myeloid sarcoma, no hem. relapse	*FLT3*wt in BM	radiotherapy/ DLIs x4	-	53	Alive in CR3, DC 100%
(9) 64; M; s-AML, M0	46,XY	DA	CR1; FB-ATG; MUD	21	Stable: 46,XY	FLAG-Ida	CR2; FM-ATG; MUD	8	Evolution: new del (7q), +11	5-AZA/DLI x1	-	5	Death in r/r
(10) 36; F; de novo AML, M4	46, XX, t (11; 19) (q23; p13); *(MLL-ELL fusion)*	DA	CR1; BuCy; MRD	7	NA	DLIs x2	r/r disease; FLAMSA; MRD	28	Stable: 46, XX, t (11; 19) (q23; p13) (*MLL-ELL fusion)* 15; 46, XX 5;	FLAG-Ida/DLIs x3	-	2	Death in r/r
(11) 19; M; de novo AML, M4	46,XY, inv (16) (p13q22)	DA followed by dasatinib; *at relapse FLAG-Ida	CR2; BuCy-ATG; MRD	4	Devolution: 46,XX	FLAG-Eto	CR3; FBM; MUD	3; hem. relapse with myeloid sarcoma	Stable: 46,XX	FLAG-Eto/DLIs x2	-	2	Death in r/r
(12) 36; M; de novo AML, M1	46,XY; *NPM1 mut*	S-HAM followed by TAD-9; *at relapse FLAG	CR2; BuCy-ATG; MUD	6	Devolution: *NPM1* wt	HAM x2	r/r disease; FluMel Carm-ATG; MUD	4	Stable: 46,XX 5/ 46,XY 15; *NPM1* wt	DLIs x1	-	7	Death in r/r
(13) 50; F; t-AML, M0	Complex karyotype including del (7q)/del (17p)	DA	CR1; BuCy-ATG; MUD	11	NA	FLAG-Ida	r/r disease; TreoFlu-ATG; MUD	3	NA	DLIs x3	-	6	Death in r/r
(14) 57; M; s-AML, M0	Complex karyotype	DA	SD; FLAMSA-AMT; MUD	23	Devolution: 46, XY; del (11q), del (12p), del (20q)	DLIs x2	r/r disease; FB-ATG; MUD	5	Stable: 46,XY; del (11q), del (12p), del (20q	DLIs x3	-	4	Death in r/r

TABLE 3 (Continued)

Pt. № age, sex, AML subtype, FAB	Genetic profile at initial AML diagnosis	First-line therapy	Remission status at allo-SCT1, conditioning, donor	Relapse after allo-SCT1, months	Genetic profile at relapse after allo-SCT1	Second-line therapy	Remission status at allo-SCT2, conditioning, donor	Relapse after allo-SCT2, months	Genetic profile at relapse after allo-SCT2	Anti-relapse therapy after allo-SCT2	Remission status at allo-SCT3, conditioning, donor	FU from relapse after allo-SCT2, months	Status at last FU
Best supportive care following relapse after allo-SCT2													
(15) 43; M; s-AML, M7	47,XY,t (3; 3) (q21; q26),+ mar 6/46,XY 1)	5-AZA	SD; FLAMSA-AMT; MRD	19	Devolution: 46, XX; no t (3; 3)	Flu/ARA-C/Ida	CR2; BuCy; MRD	6	NA	Hydroxy-carbamid followed by BSC	-	3	Death in r/r
(16) 67; M; s-AML, M0	46,XY	S-HAM	CR1; FB-ATG; MRD	8	46,XY	5-AZA/DLIs x5	CR2; FBM-ATG; MUD	5	Evolution: 46,XY, ins (16; 8) (q1?2; q24? q1?2)6/46,XY 18/46,XX 2	BSC	-	2	Death in r/r
(17) 42; M; de novo AML, M1	46 XY, t (10; 11) (q22; q23) (*KMT2A-rearr.)*; 14, *FLT3*-ITD high	S-HAM followed by TAD-9	CR1; BuCy-ATG; MUD	10; hem. relapse plus myeloid sarcoma	Devolution: no t (10; 11), no *KMT2A*; *FLT3*-ITD high	radiotherapy/ DLIs x3 followed by FLAG-Eto	CR2; FBM-ATG; MUD	6; CNS	NA	BSC	-	2	Death in r/r
(18) 73; F; s-AML, M1	45 XY, −7, *JAK2 mut*	No induction	FB10-ATG; MUD	21	Devolution: 46 XY, no −7	DA	r/r disease; TreoFlu-ATG; MUD	6	NA	BSC	-	2	Death in r/r
(19) 42; F; de novo AML, M0	46,XX, t (9; 11) (p21; q23) 25	DA followed by FLAG-Ida	CR1; BuCy-ATG; MUD	7	Stable: 46,XX, t (9; 11)	Dec/Ven/DLI x 1 followed by FLAG-Eto	CR2; TreoFlu-ATG; MUD	1	NA	BSC	-	2	Death in r/r

Abbreviations: 5-AZA/Ven, 5-azacytidine, venetoclax; Allo-SCT1, first allogeneic stem cell transplantation; allo-SCT2, second allo-SCT; allo-SCT3, third allo-SCT; AML, acute myeloid leukemia; ARA-C, cytarabine; BSC, best supportive care; BuCyATG, busulfan, cyclophosphamide, antithymocyte globulin; CNS, central nervous system involvement; CR, complete hematologic remission; CR1, first CR; CR3, third CR; DA, daunorubicin, cytarabine; DC, donor chimerism; Dec, decitabine; del., deletion; DLIs, donor lymphocyte infusions; Eto, etoposide; F, female; FB-ATG, fludarabine, busulfan, antithymocyte globulin; FBM-ATG, fludarabine, busulfan, melphalan, antithymocyte globulin; FLAG-Ida, fludarabine, high-dose cytarabine, G-CSF, idarubicin; FLAMSA-Mel, fludarabine, amsacrine, cytarabine, busulfan, cyclophosphamide, antithymocyte globulin and melphalan; Flu/ARA-C/Ida, fludarabin, cytarabine, idarubicin; FluMelATG, fludarabin, melphalan, antithymocyte globulin; FluMelCarm, fludarabin, melphalan, carmustine; FM, fludarabin, melphalan; FU, follow-up; GvHD, graft versus host disease; HD-Cy, high-dose cyclophosphamide; hem., hematologic; ICE, idarubicin, cytarabine, etoposide; M, male; MRD, matched related donor; mt, mutation; MUD, matched unrelated donor; NA, not available; PR, partial remission; Pt., patient; r/r disease, relapsed/refractory disease; rearr., rearrangement; s-AML, secondary AML; SD, stable disease; S-HAM, sequential high-dose cytarabine and mitoxantrone; TAD-9, thioguanin, cytarabin, daunorubicin; ThioTreoFlu, thiotepa, treosulfan, fludarabine; wt, wild-type.

midostaurin, the patient proceeded to allo-SCT1 being at PR but relapsed 26 months later. At the time of relapse post-allo-SCT1, the patient presented again with a normal karyotype but disappearance of *FLT3*-ITD by fragment analysis. After salvage FLAG-based reinduction the patient underwent allo-SCT2 and remains in CR2 48 months post-allo-SCT2. In contrast, both pts (#3, 17; Table 3) who had persistence of *FLT3*-ITD/or -TKD mutations at relapse post-allo-SCT1 relapsed again following allo-SCT2.

The second patient (#1; Table 3) was diagnosed with de novo *RUNX1* mutated AML, 46 XX, and underwent front-line allo-SCT1 following salvage therapy with FLAG due to refractoriness to standard induction therapy. At relapse 17 months post-allo-SCT1, no cytogenetic aberrations were revealed by chromosome banding analysis and FISH but next-generation sequencing (NGS) confirmed the reappearance of *RUNX1* clone. Following DLIs therapy, allo-SCT2 was performed in r/r disease resulting in CR2 and followed by new relapse 11 months later. Notably, at relapse post-allo-SCT2, *RUNX1* disappeared by NGS. Subsequently, the patient underwent directly allo-SCT3 from haploidentical donor and remains in CR3 36 months post-allo-SCT3.

4 | DISCUSSION

Relapse of AML in post-allo-SCT settings remains a major clinical challenge. Although allo-SCT2 has been demonstrated to provide long-term survival in thoroughly selected AML pts (6, 7, 10, 12), up to 50% of pts relapse inevitably post-allo-SCT2. In line with previous findings, the relapse rate after allo-SCT2 compromised 46% in our analysis resulting in 2- and 5-year EFS of 26% and 15%, respectively, comparable with other studies.[5,9–11] DLIs are known to be an established anti-relapse strategy alone or in combination with cytostatic or HMAs for post-allo-SCT1 settings.[4,5,25] Yet, less is known about the success of anti-relapse strategies in r/r AML beyond allo-SCT2. In our study, cell-based anti-relapse management of r/r AML turned out to be feasible and effective even after allo-SCT2. Indeed, 74% relapsed pts post-allo-SCT2 could proceed to the cell-based anti-relapse therapy with almost 43% of them achieving CR3 as a best hematologic response and 29% being alive at the last FU. Notably, six pts with CR3 following r/r post-allo-SCT2 underwent either allo-SCT3 or venetoclax/DLIs/5-AZA therapy. So far, we identified only one report describing the application of allo-SCT3 in six AML pts who had received unrelated cord blood as a stem cell source. One of these pts could achieve long-term CR3 whereas remaining five cases relapsed within a median time of 201 days following allo-SCT3.[26] In our study, two of three allo-SCT3 were performed from haploidentical family donor resulting in ongoing long-term CR3 in one case and 16 months CR3 duration followed by relapse in the second case. The third patient underwent allo-SCT3 from MRD and succumbed to pneumonia after 48 months of CR3. Thus, allo-SCT3 can be an option for single pts with a rapidly available donor and alternative stem cell sources can be considered as well in this context.

Meanwhile, there is growing evidence for venetoclax-based therapy as a meaningful option in r/r AML after allo-SCT1.[27–30] To our knowledge, we report for the first time the efficacy of venetoclax containing therapy post-allo-SCT2. Of note, previous studies documented hematological response on venetoclax even among r/r AML pts with adverse genetics, for example, mutated *TP53*, *RUNX1* or poor risk karyotype aberrations.[31,32] Accordingly, three pts receiving venetoclax/DLIs/5-AZA after failure of allo-SCT2 in our study responded with CR3 (2/3) and PR (1/3) despite genetic evolution within the course of AML. Of two pts with CR3, the first patient presented at diagnosis with a complex karyotype including chromosome 17p loss (the region of the *TP53* gene) and demonstrated emergence of new cytogenetic aberrations at each relapse subsequently preceding ongoing CR3 after five cycles of venetoclax/DLIs/5-AZA. The second patient had a normal karyotype initially but showed both cytogenetic (trisomy 8) and molecular-genetic evolution (emergence of mutations of *BCOR*, *SRSF2*, *STAG2*, *CUX1*, *PHF6*) following relapses as well. In the third patient with PR, the response could be achieved despite clonal evolution including mutated *TP53* emerging in relapse post-allo-SCT1. Finally, one patient undergoing allo-SCT3 had cytogenetic evolution at last relapse with emergence of a complex karyotype but could also achieve long-term CR3 afterward. Accordingly, we can postulate that even pts with adverse genetics post-allo-SCT2 can benefit from cell-based anti-relapse treatment. Particularly, introduction of venetoclax showed promising results even in refractory cases post-allo-SCT2. Following the application of cell-based anti-relapse treatments, the 2- and 5-year OS post-allo-SCT2 in our study compromised 35% and 25%, respectively, and was higher in comparison to several other studies.[5,8–10]

Acknowledging the high risk of NRM post-allo-SCT2 due to prior therapies as well as r/r AML itself, RIC was the preferred option for allo-SCT2 and applied in 90% of our pts. Indeed, Gilleece et al recently reported on equivalent OS and LFS rates post-allo-SCT2 among 1879 AML pts undergoing either MAC (54%) or RIC (46%) within allo-SCT2. Of note, NRM was significantly worse for ≥50 years patients in the group of MAC (27% vs. 19% for RIC).[33]

cGVHD is known to be another important prognostic factor for NRM and survival post-allo-SCT2. Although the incidence of cGvHD within our study (17%) was lower in comparison to other multicentral studies,[5,8,9] three of seven pts with cGvHD (43%) post-allo-SCT2 succumbed to cGvHD.

Since relapse of AML remains a major clinical challenge, comprehensive molecular genetic diagnostics during FU and at relapse has become an integrative part of clinical decision making.[17,34–36] The predominant part of our pts was diagnosed and treated preceding the genomic revolution era by NGS without a reference to the somatic mutations discovered recently. However, we could identify two pts with disappearance of *FLT3*-ITD and *RUNX1* clones at relapse after allo-SCT1 and -SCT2, respectively, who regained long-term remissions following anti-relapse therapy.

The molecular genetic characterization of the clonal composition will have growing therapeutic relevance in pre- and post-allo-SCT2 settings, as a number of molecularly directed treatment options (e.g., FLT3, IDH1/2 inhibitors) have recently become available.[37–40] Thus, comprehensive monitoring of molecular MRD will gain increasing importance in this scenario.

5 | CONCLUSIONS

In summary, second allo-SCT represents a meaningful approach for selected r/r AML pts. Still, allo-SCT2 is challenged by high rates of NRM and relapses. To address relapses, molecular profiling might guide and monitor the course of treatment and multilayered therapeutic strategies including cellular therapies, HMAs and targeted therapeutics may have the potential to improve outcomes.

ACKNOWLEDGMENTS

The authors wish to thank the stem cell coordinating team and the members of the stem cell processing unit as well as all staff members involved in the care of the patients reported in this study. Evgenii Shumilov is funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – 413501650; Raphael Koch receives funding from Deutsche Krebshilfe (Max-Eder grant 70113602).

Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST

The authors report no relevant conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Evgenii Shumilov, Raphael Koch and Gerald Wulf. Methodology: Evgenii Shumilov, Justin Hasenkamp, Detlef Haase, Raphael Koch and Gerald Wulf. Software: Evgenii Shumilov, Markus Maulhardt, Paolo Mazzeo and Nicole Schmidt. Validation: Evgenii Shumilov, Justin Hasenkamp, Hristo Boyadzhiev, Wolfram Jung, Christina Ganster. Investigation: Evgenii Shumilov, Markus Maulhardt and Paolo Mazzeo. Data curation: Justin Hasenkamp, Nicole Schmidt, Hristo Boyadzhiev, Wolfram Jung, Christina Ganster, Detlef Haase, Raphael Koch and Gerald Wulf. Writing-original draft preparation: Evgenii Shumilov, Raphael Koch and Gerald Wulf. Writing-review and editing: Justin Hasenkamp, Paolo Mazzeo, Detlef Haase, Raphael Koch and Gerald Wulf. Supervision: Detlef Haase, Raphael Koch and Gerald Wulf. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author.

ORCID

Evgenii Shumilov https://orcid.org/0000-0003-0178-1729
Raphael Koch https://orcid.org/0000-0002-2018-5685

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/hon.2995.

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How to cite this article: Shumilov E, Hasenkamp J, Maulhardt M, et al. Outcomes of second allogeneic stem cell transplantation and anti-relapse strategies in patients with relapsed/refractory acute myeloid leukemia: a unicentric retrospective analysis. *Hematol Oncol*. 2022;40(4):763-776. https://doi.org/10.1002/hon.2995