Azacitidine, lenalidomide and donor lymphocyte infusions for relapse of myelodysplastic syndrome, acute myeloid leukemia and chronic myelomonocytic leukemia after allogeneic transplant: the Azalena-Trial

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Abstract

Azacitidine (Aza) combined with donor lymphocyte infusions (DLI) is an established treatment for relapse of myeloid malignancies after allogeneic transplantation. Based on its immunomodulatory and anti-leukemic properties we considered Lenalidomide (Lena) to act synergistically with Aza/DLI to improve outcome. We, therefore, prospectively investigated tolerability and efficacy of this combination as first salvage therapy for adults with post-transplant relapse of acute myeloid leukemia, myelodysplastic syndromes and chronic myelomonocytic leukemia. Patients were scheduled for eight cycles Aza (75 mg/m^2 day 1-7), Lena (2.5 or 5 mg, days 1-21) and up to three DLI with increasing T-cell dosages (0.5×10^6-1.5×10^7 cells/kg). Primary endpoint was safety, while secondary endpoints included response, graft-*versus*-host disease (GvHD) and overall survival (OS). Fifty patients with molecular (52%) or hematological (48%) relapse of myelodysplastic syndromes (n=24), acute myeloid leukemia (n=23) or chronic myelomonocytic leukemia (n=3) received a median of seven (range, 1-8) cycles including 14 patients with 2.5 mg and 36 with 5 mg Lena daily dosage. Concomitantly, 34 patients (68%) received at least one DLI. Overall response rate was 56% and 25 patients (50%) achieved complete remission being durable in 80%. Median OS was 21 months and 1-year OS rate 65% with no impact of type of or time to relapse and Lena dosages. Treatment was well tolerated indicated by febrile neutropenia being the only grade ≥3 non-hematologic adverse event in >10% of patients and modest acute (grade 2-4 24%) and chronic (moderate/severe 28%) GvHD incidences. In summary, Lena can be safely added to Aza/DLI without excess of GvHD and toxicity. Its significant anti-leukemic activity suggests that this combination is a novel salvage option for post-transplant relapse (*clinicaltrials gov. Identifier: NCT02472691*).

Introduction

The curative potential of allogeneic stem cell transplantation (allo-SCT) in patients with acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) is substantially impeded by the risk of relapse. As the major cause of treatment failure relapse occurs in 30% to 80% of patients[1] and only a minority of these patients achieve long-term survival with conventional treatment options such as chemotherapy, donor lymphocyte infusions (DLI) and second transplantation.[2–5] During the last years, these therapeutic options have been augmented by the hypomethylating agent Azacitidine (Aza).[6–10] Following treatment with Aza, mostly in combination with DLI, response rates ranging from 10% to 41% and 2-year survival rates ranging from 12% to 38%[7,11–13] have been reported, with patients treated at a stage of low disease burden and/or late relapse beyond 6 months after transplant having the greatest benefit.[14] Although these data clearly indicate efficacy of Aza as post-transplant salvage therapy, they also

underline the need to enhance the activity of Aza monotherapy in order to improve outcome. The immunomodulatory drug Lenalidomid (Lena) has antileukemic activity as single agent in patients who relapse after transplant.[15] Initial results in the non-transplant setting suggested synergistic acitivity of Aza and Lena in patients with high-risk MDS.[16,17] However, administration of Lena as maintenance therapy in patients, who were in remission after transplant, was associated with high rates of severe graft-*versus*-host disease (GvHD).[18,19] By contrast, Aza accelerates reconstitution of regulatory T cells after transplant,[20,21] which could explain the low incidence and severity of GvHD following the combination of Aza and DLI.[6,7,10,14] In a first phase I/II trial reported by Craddock *et al*. the combination of Aza and Lena was able to induce complete remission (CR) in 6 of 29 patients with relapsed AML and MDS after allogeneic stem cell transplantation (allo-SCT).[22] In order to expand the evidence that Aza together with Lena delivers synergistic antileukemic and immunomodulatory activity without increasing the risk of severe GvHD, we tested this combined combination incorporating also DLI into this approach in a phase II trial.

Methods

Eligibility

As a prospective, open-label, phase-II single-arm, multicenter study the AZALENA trial (*EudraCT 2013-001153-27*) aimed to assess the safety and efficacy of Lena (investigational medical product) in combination with Aza and DLI (standard of care) as first salvage therapy for relapsed MDS, chronic myelomonocytic leukemia (CMML) or AML after first allo-SCT. Fifty adult patients with first molecular or hematological relapse as defined in detail in the *Online Supplementary Appendix* were included.

According to the license status of Aza, only AML patients with a bone marrow (BM) blast count ≤29% were initially eligible. After extended marketing authorization in 2017, inclusion was expanded to all *FLT3* and *IDH2* wild-type AML patients independent from blast count acknowledging the option for targeted therapies such as Gilteritinib or Enasidenib. The absence of active GvHD treated with systemic immunosuppression within 4 weeks before inclusion and availability of DLI (donor still contactable, no cord blood as stem cell source) were indispensable prerequisites. Exclusion criteria included any bridging therapy between diagnosis of relapse and start of study treatment, uncontrolled infections, as well as renal and hepatic impairment (for details see the *Online Supplementary Appendix*).

The Heinrich Heine University of Duesseldorf was the sponsor of the study, which was approved by the Ethics Committees of the Heinrich Heine University (approval number: MC-LKP-738) and the five other sites.

Study design and treatment

After inclusion, patients were scheduled to receive up to eight cycles Aza (Vidaza, Celgene Corporation, Summit, NJ, USA) at a dose of 75 mg/m^2/day subcutaneously on days 1-7 repeated every 28 days. In the absence of active GvHD DLI were envisaged after cycle 4, 6 and 8 at a dose of 0.5-1x10^6 CD3$^+$ cells/kg (1st DLI), 1-5x10^6 CD3$^+$ cells/kg (2nd DLI) and 5-15x10^6 CD3$^+$ cells/kg (3rd DLI). Additional DLI were permitted according to the individual decision of the treating physician, but only beyond cycle 4.

Lena was given concomitantly starting from cycle 1 on days 1-21 followed by a 7-day break every 28 days for a maximum of eight cycles. Acknowledging the potential risk of GvHD induction, the study incorporated a dose escalating schedule for Lena and two safety interim analyses. The first interim analysis was planned, as soon as ten patients had been treated with Lena (2.5 mg/day) and the tenth patient had either completed four cycles or had discontinued treatment. If the criteria to stop or modify study treatment (= dose limiting toxicity [DLT], defined in the *Online Supplementary Appendix*) were observed in this cohort, the study would have been closed. If these criteria were not met, the next ten patients would have been treated with 5 mg/day Lena followed by a second interim analysis. In case DLT criteria occurred in these ten patients, the remaining patients would have been treated with 2.5 mg/day, while in the absence of DLT 5 mg/day would have been the dosage for the remaining patients (*Online Supplementary Figure S1*). Independent from dose level and DLT, Lena had to be stopped in case of acute GvHD grade ≥2.

As Aza was given in-label, treatment was allowed to be continued beyond eight cycles, and additional DLI were allowed based on an individual decision of the treating physician, but only after cycle 4.

Study endpoints

Primary endpoint was safety defined by incidence and severity of AE, which were assessed according National Cancer Institute Common Terminology Criteria for AE v4.0. Secondary endpoints included response as defined by the European Leukemia Net 2017 and International Working Group criteria,[23,24] time to and duration of response, restoration of complete donor chimerism (DC) as determined and classified by the local standard methodology and overall survival (OS) as calculated from treatment onset. Secondary safety parameters consisted of incidence, course and severity of acute and chronic GvHD reported according to established criteria[25,26] as well as the number of hospitalizations. Patients were followed until death or data lock (April 12, 2021).

Immune evaluation

Peripheral blood (PB) lymphocyte subsets including acti-

vation status and exhaustion markers were serially monitored in a subgroup of 11 patients as described in detail in the *Online Supplementary Appendix*.

Statistical analyses

Details on statistical analyses as well as are available in the *Online Supplementary Appendix*.

Results

Patients characteristics

Fifty patients with AML (n=23, 46%), MDS (n=24, 48%) or CMML (n=3, 6%), who relapsed in median 233 days (range, 61-2,659 days) after transplant were recruited between June 2015 and August 2018. Of these, 26 patients (52%) experienced molecular relapse (median BM blast count 3%; range, 0-4%), while 24 patients (48%) suffered from hematological relapse (median BM blast count 18%; range, 0-70%; *P*<0.0001) with no statistical difference regarding time to relapse (255 *vs*. 188 days; *P*=0.74) and BM chimerism (85% *vs*. 63%; *P*=0.149). In one MDS patient hematological relapse was diagnosed on the basis of recurrent sign of dysplasia and cytogenetic features accompanied by a drop of donor chimerism. Molecular relapse was detected by reoccurrence of disease-specific markers in 21 patients (molecular n=8, cytogenetic n=6, combined molecular and cytogenetic n=7) with associated decrease of DC in 20, while isolated loss of complete DC was indicative for molecular relapse in the remaining five patients. Median follow-up of all patients was 20 months (range, 1-23 months). Detailed information on patient, transplant and relapse characteristics are given in Tables 1 and 2.

Treatment

The combination of Aza, Len and DLI was commenced as first treatment of relapse in median 14 days (range, 1-52 days) after diagnosis of relapse. There was no dropout of patients occurring between study inclusion and envisaged start of study treatment. Overall, 275 treatment cycles were administered to the 50 patients corresponding to a median of seven cycles (range, 1-8 cycles) per patient (*Online Supplementary Figure S2*). Lena was given concomitantly with Aza in 246 treatment cycles (89%), corresponding to a median of five cycles (range, 1-8 cycles) per patient. In the remaining 29 treatment cycles (11%) Lena as study medication was omitted in five individual patients due to hematoxicity (n=1), non-hematologic AE (n=1) and acute (n=2) or chronic GvHD (n=1).

According to the study design 14 patients (28%) received Lena at a daily starting dosage of 2.5 mg. Since no DLT requiring premature stop or dose modifications were observed in two interim analyses, the remaining 36 patients (72%) were treated with a daily starting dosage of 5 mg Lena. There were no differences between the two dose levels in number of Lena cycles per patient and length of treatment cycles (*data not shown*).

A total of 101 DLI were administered to 34 patients (68%) corresponding to a median of three DLI (range, 1-11 DLI) and a median of $6.75x10^6$/kg $CD3^+$ cells (range, 0.5- $336.7x10^6$/kg) per patient. Reasons to omit DLI in the remaining 16 patients were disease progression (n=13), GvHD (n=2) and unavailability of the donor (n=1). In those 16 patients, who did not receive DLI, the median number of cycles of Aza + Lena was two (range, 1-8).

Safety and toxicity

At study entry, 42% of patients exhibited at least one cytopenia grade >2 with grade 3/4 neutropenia and thrombopenia being already present in 30% and 38% of patients respectively, while no patient had grade 3/4 anemia. During the study, 275 treatment cycles were administered. Table 3 indicates that grade 3/4 neutropenia, anemia and thrombopenia occurred during 76%, 15% and 46% of treatment cycles, while renal and liver dysfunctions were uncommon. A total of 305 non-hematological AE were considered to be drug-related and the only treatment-related non-hematologic toxicities occurring in more than 10% of patients at grade 3 or greater was febrile neutropenia (12%) (Table 4). Overall, 19 patients (38%) had to be hospitalized at least once during treatment. Three patients (6%) developed a second primary malignancy (squamous cell carcinoma, basal cell carcinoma and vulvar carcinoma) during (n=1) or after study treatment (n=2).

Clinical response and overall survival

During the 8-month treatment period, 25 patients (50%) achieved CR and three patients (6%) achieved partial remission, resulting in an overall response rate of 56%. CR was achieved in 14 patients treated at the stage of molecular relapse and 11 patients treated at the stage of hematological relapse, respectively. Achievement of CR was accompanied by restoration of complete donor chimerism in 21 patients (84%) and disappearance of molecular/cytogenetic markers in all but one patient with trackable markers (*Online Supplementary Table S1*). Median time to achievement of CR was 113 days (range, 50-295 days), corresponding to a median of four treatment cycles (range, 1-8 cycles). Twenty (80%) of the 25 patients achieving CR received DLI with a median of three DLI (range, 1-9) per patient. Of these, ten patients (50%) were already in CR before the first DLI.

Of note, CR rates did not differ between patients treated at the stage of molecular relapse and those initiated at hematological relapse (56% *vs*. 44%; *P*=0.778), neither between those with early and late relapse nor between the two dosage levels (Table 5). Also the presence of a dele-

Table 1. Patient demographics (N=50).

Characteristic	N	2.5 mg (N=14)	5 mg (N=36)	*P*
Age in years, median (range)	63 (30–75)	64.5 (43-73)	62.5 (30-75)	
Sex (%)				
female	19 (38)	4 (29)	15 (42)	0.52
male	31 (62)	10 (71)	21 (58)	
ECOG at screening (%)				
0	14 (28)	4 (29)	10 (28)	0.31
1	32 (64)	8 (57)	24 (67)	
2	4 (8)	2 (14)	2 (6)	
HCT-CI (N=49) (%)				
low	18 (37)	6 (43)	12 (34)	0.50
intermediate	16 (33)	5 (36)	11 (31)	
high	15 (30)	3 (21)	12 (34)	
WHO 2016 diagnosis (%)				
AML	23 (46)	3 (21)	20 (56)	0.049
MDS	24 (48)	10 (71)	14 (39)	
CMML	3 (6)	1 (7)	2 (6)	
IPSS at diagnosis (N=19) (%)				
low	0 (0)	0 (0)	0 (0)	0.65
intermediate-1	8 (42)	3 (33)	5 (50)	
intermediate-2	6 (32)	3 (33)	3 (30)	
high	5 (26)	3 (33)	2 (20)	
IPSS-R at diagnosis (N=21) (%)				
intermediate	9 (43)	4 (57)	5 (36)	0.40
high	8 (38)	1 (14)	7 (50)	
very high	4 (19	2 (29)	2 (14)	
Karyotype (N=45) (%)				
normal	20 (44)	6 (46)	14 (44)	0.99
abnormal	25 (56)	7 (54)	18 (56)	
complex	12 (24)	3 (23)	9 (28)	0.99
non-complex	13 (32)	4 (31)	9 (28)	
Molecular/genetic risk* (N=45) (%)				
favorable	15 (33)	5 (38)	10 (31)	0.32
intermediate	14 (31)	5 (38)	9 (28)	
adverse	16 (36)	3 (24)	13 (41)	
Disease status at Tx (%)				
remission	14 (28)	4 (29)	10 (28)	>0.99
no remission	36 (72)	10 (71)	26 (72)	
primary refractory	9 (18)	0 (0)	9 (25)	
no response	8 (16)	4 (29)	4 (11)	
relapse	5 (10)	0 (0)	5 (14)	
untreated	14 (28)	6 (43)	8 (22)	0.17
Conditioning (%)				
standard-dose	34 (68)	9 (64)	25 (69)	0.75
reduced-intensity	16 (32)	5 (36)	11 (31)	
Donor/HLA-match (%)				
related	9 (18)	1 (7)	8 (22)	0.41
unrelated	41 (82)	13 (93)	28 (78)	
10/10	44 (88)	12 (86)	32 (89)	
9/10	6 (12)	2 (14)	4 (11)	
In vivo T-cell depletion (%)				
yes	41 (82)	13 (93)	28 (78)	0.41
no	9 (18)	1 (7)	8 (22)	
Graft source (%)				
PBSC	49 (98)	14 (100)	35 (97)	>0.99
BM	1 (2)	0 (0)	1 (3)	

*For acute myeloid leukemia (AML) patients we used the ELN AML 2017 Genetic Risk Stratification. For patients with myelodysplastic syndrome (MDS) we used the International Prognostic Scoring System - revised (IPSS-R) genetic risk categories summarizing very good and good as well as high and very high. For chronic myelomonocytic leukemia (CMML), we used the genetic risk categories of the CMML-specific prognostic scoring system (CPSS). Molecular/genetic risk confers to the results obtained at the time of primary diagnosis. Numbers in parentheses display patients with available information. Data are given for the entire cohort as well as for the 2 daily dosage levels (2.5 mg and 5 mg). BM: bone marrow; ECOG: Eastern Cooperative Oncology Group; ELN: European Leukemia Net; HCT-CI: hematopoietic cell transplantation - specific comorbidity index; HLA: human leukocyte antigen; PBSC: peripheral blood stem cells; Tx: transplantation.

tion 5q (n=7, including 6 with a complex karyotype) had no impact on the likelihood to achieve CR.

Twenty of the 25 patients (80%) achieving CR in the 8-month treatment period remained in ongoing remission for a median of 15 months (range, 6-21 months) without any additional antileukemic treatment at last follow-up, while five patients (20%) relapsed again in median 12 months (range, 3-20 months) after achieving CR (Figure 1). Three of the former patients with ongoing remissions died due to non-relapse-related causes (ischemic stroke, organ failure and sepsis).

In addition to that, another eight patients (16%), who did

Table 2. Relapse characteristic (N=50).

Characteristic	N All (N=50)	2.5 mg (N=14)	5 mg (N=36)	*P*
Time to relapse in days, median (range)	233 (61-2,659)	193 (91-2,659)	243 (61-1,487)	0.83
Type of relapse (%) molecular hematological	 26 (52) 24 (48)	 9 (64) 5 (36)	 17 (47) 19 (53)	0.35
WBC x10^9/L, median (range)	3.57 (1.2-20.3)	3.6 (1.69-6.9)	3.57 (1.2-20.3)	0.74
PB blasts %, median (range)	0 (0-32)	0 (0-32)	0 (0-19)	0.08
BM blasts %, median (range)	4 (0-70)	4 (0-70)	4 (0-60)	0.77
Hb g/dL, median (range)	11.1 (8.1-16.5)	10.1 (8.1-13.2)	11.4 (8.1-16.5)	0.39
Platelets x10^9/L, median (range)	87 (8-824)	83 (8-468)	90 (9-824)	0.91
LDH U/L, median (range)	197 (128-501)	175 (153-363)	202 (128-501)	0.08
BM chimerism %, median (range)	76 (6-100)	77.5 (30-100)	71.5 (6-100)	0.98
PB chimerism %, median (range)	95.5 (23-100)	87 (35-100)	96 (23-100)	0.61
GvHD before relapse (%) acute grade 1 grade 2 unknown grade chronic mild	 9 (18) 4 (8) 4 (8) 1 (2) 4 (8) 4 (8)	 1 (7) 0 (0) 1 (7) 0 (0) 0 (0) 0 (0)	 8 (22) 4 (11) 3 (8) 1 (3) 4 (11) 4 (11)	0.08
Immunosuppression at study entry (%) yes no taper/stop*	 15 (30) 35 (70) 11 (73)	 5 (36%) 9 (64%) 5 (100%)	 10 (28) 26 (72) 6 (60)	0.99

*Percentage refers to the number of patients, who received immunosuppression at study entry. Numbers in parentheses display patients with available information. The cohort of patients with molecular relapse included 5 individuals with isolated loss of complete donor chimerism (DC). Data are given for the entire cohort as well as for the 2 daily dosage levels (2.5 mg and 5 mg). Hb: hemoglobin; GvHD: graft-*versus*-host disease; Hb: hemoglobin; LDH: lactate dehydrogenase; PB: peripheral blood; BM: bone marrow; WBC: white blood cells.

Table 3. Hematotoxicity and laboratory findings during the study.

Grade	Parameters									
	Absolute neutrophil count		Platelets		Anemia		Bilirubin		Creatinine	
	N (%)	Pat.	N (%)	Pat.	N (%)	Pat.	N (%)	Pat.	N (%)	Pat.
No toxicity	22 (8)	1	22 (8)	2	20 (7)	2	239 (87)	34	162 (59)	17
1	8 (3)	1	86 (31)	2	107 (39)	6	25 (9)	9	94 (34)	27
2	36 (13)	2	42 (15)	6	107 (39)	23	6 (2)	2	19 (7)	6
3	62 (23)	3	49 (18)	7	41 (15)	19	4 (1)	4	0 (0)	0
4	144 (53)	43	76 (28)	33	0 (0)	0	1 (0)	1	0 (0)	0

Summary table indicates maximum common toxicity criteria (CTC) severity grades per patients (Pat.) and cycle. A total of 275 treatment cycles was administered. Number (N) depicts the affected treatment cycles, while patients indicates the number of affected patients according to the maximum adverse events CTC severity during treatment.

not respond and, therefore, prematurely terminated study treatment, achieved CR during follow-up. Median time to remission in these patients was 162 days (range, 73-554 days) calculated from treatment start. Two of these patients achieved CR after additional DLI, while the remaining six patients entered remission after salvage therapy with chemotherapy (n=3) or Decitabine/Venetoclax (n=2) followed by second transplantation (n=6). Of these eight patients, six were alive with ongoing remissions in four of them for a median of 8 months (range, 3-11 months).

At data lock 28 patients (56%) were alive including 17 patients, who were free of disease. Median OS of the entire cohort was 21 months and estimated 1-year OS rate was 65%. The median OS in patients who achieved CR was not reached compared to 9.7 months in non-responders (*P*=0.0004; Figure 1). Similar to response, the OS rate was not influenced by diagnosis, genetic risk, Lena dosage, time to as well as type of relapse (Table 5).

Twelve patients died during or after treatment due to disease progression, while ten patients, of which seven had active disease at the time of death, succumbed to due infections (n=6), cardiovascular complications (n=3) or liver failure (n=1).

Graft-*versus*-host disease

During the interval between transplantation and relapse a total of nine patients (18%) had suffered from acute GvHD (overall grade 1 n=4, grade 2 n=4, missing n=1) and four patients from mild chronic GvHD (8%). However, at relapse only one patient still suffered from grade 1 acute GvHD not requiring systemic immunosuppression. Fifteen patients were still on systemic immunosuppressive prophylaxis, which could be tapered or directly stopped in 11 of them.

Overall, 15 patients (30%) developed aGvHD (overall grade 1 n=3, grade 2 n=7, grade 3 n=2, grade 4 n=3) and 19 patients (38%) developed cGvHD (mild n=5, moderate n=10, severe n=4) in median 112 days (range, 5-810 days) after inclusion (*Online Supplementary Tables S3* and *S4*). In 11 patients *de novo* onset of cGVHD was observed, while cGvHD developed fom aGvHD in the remaining eight patients. Frequencies of GvHD in the 2.5 mg and 5 mg dosing cohort were 43% (n=6 patients) and 55% (n=20; *P*=0.533), respectively.

In 13 of the 26 patients that developed acute or chronic GvHD the first DLI was administered in median 203 days (range, 16-708 days) before GvHD onset, while seven patients received DLI after developing GvHD.

Table 4. Drug-related non-hematologic adverse events during the study.

Events	Grade 1 N (%)	Grade 2 N (%)	Grade 3 N (%)	Grade 4 N (%)	Patients
Blood and lymphatic system disorders	2 (15)	1 (8)	9 (69)	1 (8)	8
Ear and labyrinth disorders	1 (50)	1 (50)	0	0	2
Endocrine disorders	0	2 (100)	0	0	2
Eye disorders	1 (50)	0 (0)	1 (50)	0	2
Gastrointestinal disorders	40 (61)	23 (33)	4 (6)	0	28
General disorders and administration site conditions	26 (67)	7 (28)	2 (5)	0	26
Immune system disorders	8 (35)	12 (52)	3 (13)	0	13
Infections and infestations	4 (8)	39 (75)	9 (17)	2 (4)	24
Injury, poisoning and procedural complications	0 (0)	0	1 (100)	0	1
Investigations	25 (47)	15 (28)	12 (23)	1 (2)	22
Metabolism and nutrition disorders	6 (50)	5 (42)	1 (8)	0	7
Musculoskeletal and connective tissue disorders	3 (43)	3 (43)	1 (14)	0	6
Neoplasms, benign, malignant and unspecified	0	0	1 (100)	0	1
Nervous system disorders	6 (60)	1 (10)	3 (30)	0	6
Psychiatric disorders	1 (100)	0	0	0	1
Reproductive system and breast disorders	1 (100)	0	0	0	1
Respiratory, thoracic and mediastinal disorders	4 (57)	3 (43)	0	0	6
Skin and subcutaneous tissue disorders	17 (68)	8 (32)	0	0	11
Vascular disorders	3 (60)	1 (20)	1 (20)	0	4

Summary table indicates numbers and percentages of drug-related, non-hematologic adverse events (N=305) according to common toxicity criteria grades. Patients indicates the number of affected patients according to the respective adverse events term.

Immune evaluation

As exposure to Aza and Lena both can modulate T-cell activity and functionality, we monitored T-cell numbers and functionality during study treatment in a subgroup of 11 patients. Consistent with the hypothesis of T-cell exhaustion, we observed a significant higher frequency of $CD3^+/CD8^+$ T cells expressing PD1, CTLA4 and TIM3 in patients at relapse compared with healthy controls ($P<0.05$; *Online Supplementary Figure S3*), which was not modulated during therapy. Furthermore, during study treatment the frequency of $CD3^+/CD4^+/CD25^+/FoxP3^+$ regulatory T cells significantly increased in comparison to the baseline level (*Online Supplementary Figure S3*).

Discussion

We here demonstrate that Lena can safely be added to the backbone of Aza and DLI as salvage therapy for relapse of myeloid malignancies after allo-SCT without excess of GvHD or other toxicities. The combination of immunodulatory drugs and cellular therapy induced a remarkable response rate of 56%, durable remissions in 20 patients, who have remained in remission for a median of 15 months, and a 1-year OS rate of 65%.

Generally, the outcome of relapse of AML or MDS after allo-SCT is dismal reflected by 2-year OS rates of 13.9%

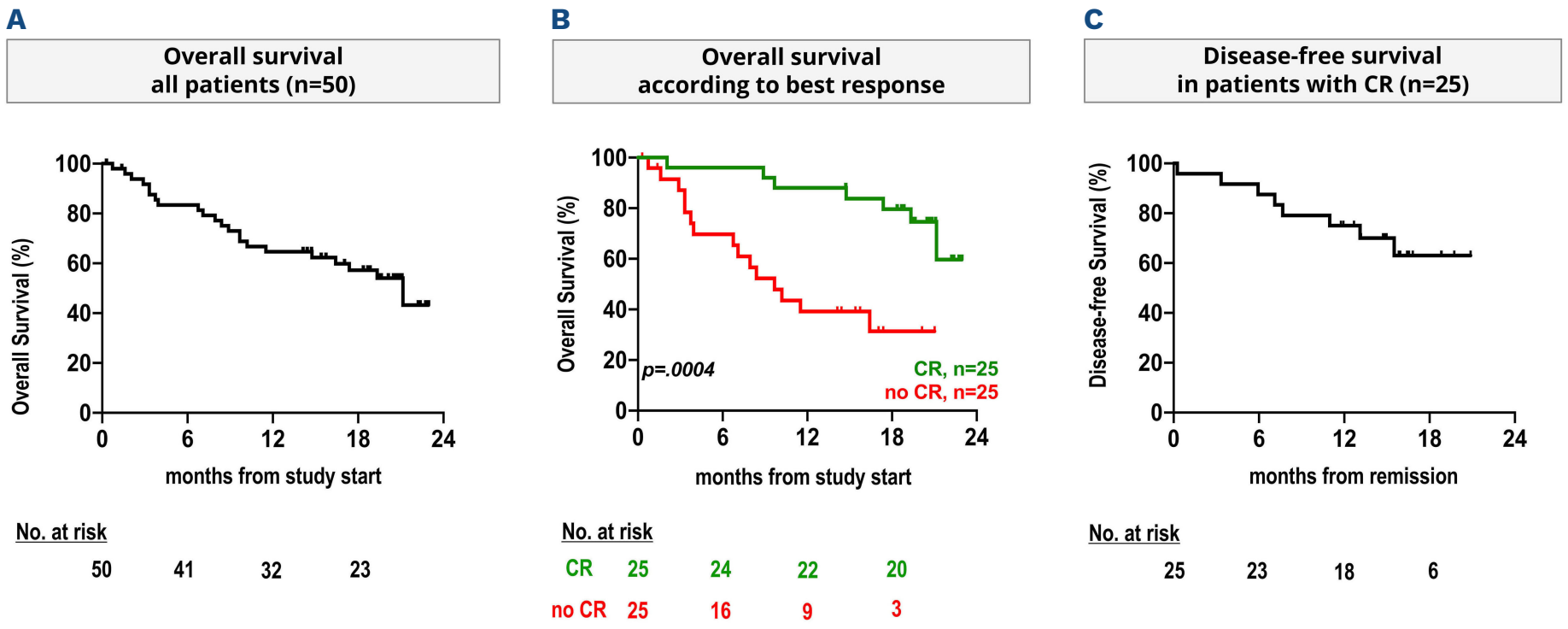

Figure 1. Overall survival and disease-free survival. Overall survival is displayed for all patients (A) and for patients separated according to response (B). Complete remission (CR) (n=25, green curve), no CR (n=25, red curve). (C) Disease-free survival (DFS) is displayed for the 25 CR patients. DFS was calculated as time from CR to relapse, death or last follow-up in those alive and still in remission.

Table 5. Predictors of response and survival – univariate analyses.

Variable	CR rate (%)	*P*	1-year OS (%)	*P*
Lena dosage 2.5 mg 5 mg	 43 53	0.75	 75 61	0.22
Diagnosis AML MDS/CMML	 48 52	>0.9999	 55 72	0.09
Type of relapse molecular hematologic	 56 44	0.78	 69 59	0.55
Time to relapse in days <233 ≥233	 48 52	>0.9999	 54 75	0.19
Genetic risk favorable/intermediate adverse	 56 44	>0.55	 84 50	0.07

AML: acute myeloid leukemia; CMML: chronic myelomonocytic leukemia; CR: complete remission; Lena: lenalidomide; MDS: myelodysplastic syndrome; OS: overall survival.

and 29.7%, respectively.[2,3] In detail, CR rates following intensive chemotherapy or DLI alone range between 15% to 30% and translate into 2-year survival rates between 8% and 21%.[2,3,27,28] Similarly, a 2-year OS rate of 25% following second transplantation in selected patients has been reported, while targeted approaches such as Sorafenib or Enasidenib exert clinical activity, but are restricted to specific genotype-defined subtypes.[4,29,30] Following single-agent Aza with or without DLI, CR rates of 15% to 41% and 2-year survival rates ranging from 12% to 38% have been reported from one prospective and several retrospective studies.[6,7,10–14] Compared to conventional approaches, which can mostly be administered in an in-patient setting only, Aza and DLI is an outpatient approach resulting in similar or even better response and survival rates and has, therefore, become standard of care.

Although a direct comparison is not possible in the absence of a randomized trial and while the follow-up of this trial is still limited, the response and survival following Aza, Lena and DLI appears very promising. This is in accordance with the results of a recently published clinical trial investigating therapy of post-transplant relapse with a combination of Aza and Lena[22] indicating synergistic antileukemic and immunologic effects of the two compounds. Furthermore, the high response rate in our trial might also be related to the fact that, in contrast to the reports on single agent Aza,[6,7,10–14] a higher fraction of patients (52%) was treated at the stage of molecular relapse mirroring the currrent practice of preemptive, measurable-disease guided interventions. In addition, inclusion of patients merely based on decreasing chimerism as well as patients with MDS may also have contributed to the results observed in our trial. Disease burden and early relapse after transplant inversely correlate with response and survival following Aza monotherapy as reflected by a CR and 2-year OS rate of 29% and 27% in those with early hematological relapse.[14] In contrast, type of and time to relapse were no longer associated with response and survival in our actual trial as also demonstrated by five of 12 patients (42%) with early (<6 months) hematologic relapse achieving CR. Despite the limited patient number and the lack of a multivariate analysis, our data including a notable CR rate of 40% in patients with frank hematologic relapse suggest that patients with early hematologic relapse, who otherwise have a limited chance to respond to Aza monotherapy,[14] may benefit from the addition of Lena.

The observed clinical synergism may be related to the additive antileukemic activity of the two drugs or alternatively to the pharmacologic manipulation of the graft-*versus*-leukemia (GvL) effect. While Lena directly enhances T-cell activity, Aza upregulates tumor antigen expression on leukemic cells and also induces $CD8^+$ T-cell response.[21] In order to further address this on the translational level, we investigated T cells with regard to subset composition and exhaustion/activation markers. In line with previous findings[22,31] we observed a higher frequency of $CD8^+$ T cells with exhausted phenotype ($PD\text{-}1^+$, $CTLA\text{-}4^+$, $TIM3^+$) at relapse, which was not reverted by the combined therapy with Aza and Lena (*Online Supplementary Figure S3*). Thus, the mechanism, how Lena synergizes with Aza remains elusive here and requires further investigations. Still, but alternatives to reverse T-cell exhaustion may be an additional option to prevent or treat relapse.

Aiming to decipher the role of DLI we looked at the 25 patients achieving CR, of whom 20 received DLI. Ten of these were already in remission prior the first DLI underlining the synergistic, antileukemic activity of Aza and Lena. This is in line with the results of Craddock *et al.*, who observed remissions following this pharmacological combination without the regular use of DLI.[22] The ten remaining patients achieved CR after first DLI suggesting an immunologic effect. Here, Aza and Lena had offered disease control and probably enhanced DLI-driven immune effect. Only five CR patients had lost response at last follow-up including three with previous DLI. Overall, we believe from our previous data[14] and reports from others,[2] that Aza and Lena can induce remissions, but additional donor-cell based consolidation is definitively required to achieve CR persistence. Nevertheless, the exact contribution of the two pharmacological compounds and the cellular therapy for remission induction and long-term disease control can only be dissected within a randomized trial.

Similar to the results reported by Craddock *et al.*,[22] response was not counterbalanced by severe toxicity including acute and chronic GvHD enabling outpatient treatment in most patients and an acceptable hospitalization rate. Drug-related, non-hematologic adverse event were mainly grade 1 and 2. Neutropenia and thrombocytopenia grade 3/4 occurred in a relevant proportion of patients, but were manageable and did not lead to omission of Lena in the majority of treatment cycles. This may be one advantage of the relatively low Lena dosages we used to take advantage of its immunomodulatory properties compared to other similar trials, where higher dosages were envisaged to excert its full antileukemic activity.[22]

Based on results from previous studies investigating Lena as maintenance therapy following transplant,[18,19] GvHD induction was a major concern when planning this trial and was addressed by two safety interim analyses, a dose escalating scheme and rather low dosages of Lena. We observed rates of aGvHD (30%) and cGvHD (38%), which are comparable to those after hypomethylating agent/DLI therapy and even slightly lower than aGvHD and cGvHD rates of 43% and 46% observed after DLI alone.[28] Thus, in combination with Aza, dosages of 2.5 and 5 mg Lena do not lead to an excess of GvHD. This might be related to the previously observed Aza-mediated expansion of regulatory T cells,[20,21] which we also found here in the trial population

treated with Aza combined with Lena (*Online Supplementary Figure S3*). Since an acceptable GvHD rate was reported even after 25 mg Lena,[22] one might speculate that an increase of the daily Lena dosage may further enhance the efficacy of this combined approach of Aza, Lena and DLI. However, hematologic toxicity may then become a concern. Hematologic toxicity and associated infections are the most serious side effects of combination therapy with Aza or Decitabine and Venetoclax, which has also become a treatment option for the relapse of myeloid malignancies after allo-SCT.[32] In addition Venetoclax, while enhancing the cytotoxicity of Aza on the one hand, may hinder the development of GvL effects and thereby long-term remissions due to its lymphotoxic properties on the other.[33]

Taken together, our data demonstrate that 5 mg Lena can be safely added to the combination of Aza plus DLI and exerts significant antileukemic and immune-modulatory activity in patients with relapse after allo-SCT, including those with early hematologic relapse. Our results establish the combination of Aza, Lena and DLI as valuable treatment alternative among other current treatment modalities for patients relapsing after allo-SCT.

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Contributions

GK and TS developed the concept and design of the study and wrote the manuscript. TS, CR, PSJ, ND and GK collected and assembled data. TS, CR, PSJ, ND and GK analyzed and interpreted data. All authors provided patient data and approved the final version of the manuscript.

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Data-sharing statement

Details on the data can be provided on personal request.

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