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LEADING ARTICLE

# Azacitidine for treatment of imminent relapse in MDS or AML patients after allogeneic HSCT: results of the RELAZA trial

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**This study evaluated azacitidine as treatment of minimal residual disease (MRD) determined by a sensitive donor chimerism analysis of CD34$^+$ blood cells to pre-empt relapse in patients with CD34$^+$ myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML) after allogeneic hematopoietic stem cell transplantation (HSCT). At a median of 169 days after HSCT, 20/59 prospectively screened patients experienced a decrease of CD34$^+$ donor chimerism to <80% and received four azacitidine cycles (75 mg/m$^2$/day for 7 days) while in complete hematologic remission. A total of 16 patients (80%) responded with either increasing CD34$^+$ donor chimerism to ⩾80% ($n=10$; 50%) or stabilization ($n=6$; 30%) in the absence of relapse. Stabilized patients and those with a later drop of CD34$^+$ donor chimerism to <80% after initial response were eligible for subsequent azacitidine cycles. A total of 11 patients (55%) received a median of 4 (range, 1–11) additional cycles. Eventually, hematologic relapse occurred in 13 patients (65%), but was delayed until a median of 231 days (range, 56–558) after initial decrease of CD34$^+$ donor chimerism to <80%. In conclusion, pre-emptive azacitidine treatment has an acceptable safety profile and can substantially prevent or delay hematologic relapse in patients with MDS or AML and MRD after allogeneic HSCT.**
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## Introduction

Among standard therapeutic strategies in patients with advanced myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML), allogeneic hematopoietic stem cell transplantation (HSCT) remains the only potentially curative treatment option.[1–3] Nevertheless, in patients eligible to undergo allogeneic HSCT, disease relapse still remains a major cause of treatment failure.[4–6] Treatment algorithms for relapsed disease are currently limited to conventional salvage chemotherapy, second allogeneic HSCT, and donor lymphocyte infusion (DLI).[5,7–9] However, the majority of patients who relapse after allogeneic HSCT do not achieve long-term survival.[5,8,10] Furthermore, although DLI can induce sustained second remission in some patients, severe graft-versus-host disease (GvHD) is a major complication, often resulting in significant morbidity and mortality.[10,11]

In recent years, techniques such as flow cytometric assessment of residual disease, donor chimerism and molecular analyses of leukemia-specific fusion genes have been used to facilitate the detection and monitoring of minimal residual disease (MRD).[12–16] Although determination of MRD by these methods may precede morphologic disease detection by weeks, they are often only applicable in a distinct population of MDS or AML patients with specific molecular markers.[13–15,17,18] Donor chimerism analyses of whole blood or bone marrow cells harbor a low sensitivity.[13–15,17] In contrast, CD34$^+$ donor chimerism analyses are useful and very specific for monitoring MRD in patients with MDS or AML following prior allogeneic HSCT,[19,20] because CD34$^+$ blasts at diagnosis are observed in the majority of these patients.[13,21,22] This makes CD34$^+$ donor chimerism analysis an attractive tool in the management of these patients, irrespective of leukemia-specific markers. In fact, we have shown previously that a decrease of CD34$^+$ donor cells below 80% in the peripheral blood predicts almost unavoidable relapse in all patients after a median of 61 days.[23] Relapse occurs in the majority of the patients even in the presence of interventions such as immediate interruption of immunosuppression or administration of DLI.[10,11,23]

One major reason for treatment failure in patients with MRD is the fact that the kinetics of disease recurrence are usually very rapid,[11,18] which limits the time for graft-versus-leukemia effects, for example when using DLI. Furthermore, these interventions often result in clinically significant and life-threatening GvHD.[10,11] Therefore, to prevent subsequent hematologic relapse with high efficacy, it would be optimal to integrate novel treatment strategies that are not only effective at modifying the natural history of disease and thus preventing relapse, but that are also well tolerated after HSCT. Azacitidine, a nucleoside analog of cytidine, is the first drug therapy to show a significant survival advantage compared with conventional care regimens in patients with higher-risk MDS and World Health Organization (WHO)-defined AML with 20–30% bone marrow blast count.[24,25] Its favorable toxicity profile further supports its use for pre-emptive treatment in higher-risk MDS or AML patients with detectable MRD after allogeneic HSCT.[24–26] Recent results of low-dose azacitidine as maintenance or salvage therapy in higher-risk MDS and AML patients after allogeneic HSCT pointed to azacitidine being well tolerated and associated with prolonged progression-free and overall survival.[27,28]

Herein, we report the results of an open-label, single-center phase II clinical trial evaluating the efficacy of azacitidine in the setting of MRD-triggered (decrease of CD34$^+$ donor chimerism below 80%) pre-emptive therapy to prevent or delay

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hematologic relapse in patients with CD34$^+$ MDS or AML after allogeneic HSCT.

## Patients and methods

### *Study design*

The RELapse prevention with AZAcitidine (RELAZA) trial was a prospective, open-label, single-center phase II study performed at the University Hospital 'Carl Gustav Carus', Technical University of Dresden in Germany. The study was conducted in accordance with the Declaration of Helsinki and received Institutional Review Board approval by the Ethical Board of the University Hospital of Dresden. The trial was sponsored by the Technical University of Dresden and registered at http://clinicaltrials.gov (NCT00422890). Written informed consent was given by all patients. UP, CT, and FS analyzed the data, and all authors had access to primary clinical trial data.

### *Patients*

Patients aged >18 years with CD34$^+$ MDS or AML (according to WHO criteria)[29,30] after allogeneic HSCT, irrespective of other molecular markers, were eligible for screening at any time point after HSCT if they had shown a documented CD34 positivity of their leukemic blasts at diagnosis or subsequent relapse. Key exclusion criteria were as follows: presence of hematologic relapse; severe hepatic impairment, including cirrhosis and malignant tumor; renal impairment defined as serum creatinine more than two times the normal value or creatinine clearance <50 ml/min or participation in a drug study outside the indication of allogeneic HSCT up to 4 weeks before study initiation.

### *Screening*

Up to 100 patients were intended to be screened after allogeneic HSCT to enroll up to 20 patients to the treatment phase. During screening, CD34$^+$ donor chimerism in the peripheral blood was monitored at intervals of 3–4 weeks during the first 8 months after HSCT, and every 7–8 weeks during months 8–24. However, patients could also enter the screening phase at later time points after HSCT. On the basis of the data from a previous study,[23] patients who experienced a drop in CD34$^+$ donor chimerism to values below 80% without concurrent hematologic relapse (that is, patients with <5% bone marrow blasts as obtained at that time point) entered the treatment phase and were offered treatment with azacitidine.

### *Treatment*

During the 3 weeks before the start of azacitidine treatment, patients had an initial visit at which they underwent laboratory tests as well as a bone marrow examination to confirm that they were still in complete hematologic remission (CR) and therefore eligible for treatment.

Patients received four cycles of azacitidine (Vidaza; Celgene Corporation, Summit, NJ, USA) 75 mg/m$^2$/day subcutaneously on days 1–7, with repeated cycles beginning on day 29 ± 2. A limited number of azacitidine cycles were administered because of concerns of sustained myelosuppression as well as possible induction of GvHD by the study drug at the time of study design in 2006.

Patients with a major response, defined as an increase of CD34$^+$ donor chimerism in the peripheral blood above 80%, were continuously monitored for a consecutive decrease in CD34$^+$ donor chimerism, which, according to protocol, resulted in re-treatment with four cycles of azacitidine. Patients with a minor response, defined as an increase of CD34$^+$ donor chimerism but to <80%, or a stabilization or further decrease of CD34$^+$ donor chimerism in the absence of relapse after four cycles of treatment, were eligible for an additional four cycles of azacitidine. Additional treatment was permitted if feasible and required because of lack of a major response. CD34$^+$ donor chimerism was determined 4–6 weeks after completion of the initial four cycles; therefore, there was a gap in azacitidine administration of at least 1 month before continuation of treatment.

Before the start of any subsequent azacitidine cycle, patients were required to have a leukocyte count $>3 \times 10^9$/l and platelet count $>50 \times 10^9$/l (independent of transfusions). If necessary, the dose of azacitidine for subsequent cycles was adjusted based on the leukocyte and platelet nadirs of the preceding cycle, with 67% of the planned azacitidine dose administered for a leukocyte nadir of $1–3 \times 10^9$/l and a platelet nadir of $25–50 \times 10^9$/l. In all, 50% of the planned dose was administered for lower leukocyte or platelet nadirs. Study medication with azacitidine was discontinued in any patient with hematologic relapse, renal or hepatic toxicity of grade 3 or higher, or grade 4 hematologic toxicity (defined as leukocyte count $<1 \times 10^9$/l or platelet count $<25 \times 10^9$/l) for >4 weeks after the scheduled date for continuation of therapy.

In addition, immune suppression could be withdrawn to support relapse prevention. In the absence of GvHD symptoms, tacrolimus or cyclosporine could be cautiously reduced to a maximum of 10% of the total dose per week, starting at any time from the first day of azacitidine treatment. In the case of a new manifestation of GvHD, any clinically feasible immunosuppressive treatment could be administered.

During azacitidine therapy, antibiotic prophylaxis was permitted in line with local standards, for example, ciprofloxacin $2 \times 500$ mg/day orally in case of severe neutropenia ($<0.5 \times 10^9$/l neutrophil count).

### *Cell sorting and CD34$^+$ donor chimerism analysis from peripheral blood*

Pre-enrichment of CD34$^+$ cells from peripheral blood using magnetic beads and subsequent fluorescence-activated cell sorting was performed as described previously.[23,31] All detectable CD34$^+$ cells were sorted, with a maximum of 10 000 CD34$^+$ cells used for polymerase chain reaction (PCR). In selected patients, additional CD34$^+$ cells were used for confirmatory analysis of cytogenetic markers. The median number of sorted cells in patients in the screening phase before treatment was 5000. The median purity, as measured by repeated fluorescence-activated cell sorting analysis and in patients with sufficient cell numbers, was >95% (range, 90–100%). CD34$^+$ donor chimerism analysis was performed as recently described in detail.[32] The HumanType Chimera kit (Biotype, Dresden, Germany) was used to amplify 12 short tandem repeat loci and the *amelogenin* gene by PCR from patients' CD34$^+$ cell DNA.

### *Study end points*

The primary end point was the proportion of patients with a major response after completing four cycles of azacitidine. Secondary end points were the proportion of patients with a minor response after completing four cycles of azacitidine, the proportion of patients with a major or a minor response at

6 months after the last azacitidine cycle, and the incidence of infectious complications or any of the following toxicities up to 3 months after the last azacitidine cycle: allergy, hepatic or renal toxicity, acute gastrointestinal toxicity and hematologic toxicity. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. The criteria for premature termination of the clinical trial were grade 5 infectious complications in over 40% of patients during azacitidine treatment.

### *Statistical analysis*

Patients were evaluated on an intent-to-treat basis, with all patients who received at least one dose of study drug included in the analyzed population. Major and minor response rates after completing four cycles of azacitidine are reported with exact Clopper–Pearson confidence intervals. SPSS and SAS were used as the statistical software. The cutoff date for data inclusion was 1 January 2011.

## Results

### *Patient demographics and disposition*

A total of 59 patients entered the screening phase between January 2007 and February 2010. The majority of patients (90%) were included immediately after allogeneic HSCT with screening for $CD34^+$ donor chimerism starting within 100 days after HSCT (median 85 days; range, 56–2931 days). A total of 20 patients experienced a drop of $CD34^+$ donor chimerism to below 80% without concurrent signs of hematologic relapse during screening, and were subsequently enrolled into the treatment phase. All 20 patients received study treatment and comprised the intent-to-treat population. Their demographics and baseline characteristics are shown in Table 1 and Supplementary Table S1. The median age of the treated population was 58 years (range, 20–74 years). Three patients had International Prognostic Scoring System-defined High-risk MDS and 17 patients had AML, including 3 patients with secondary AML after MDS. Two patients had already relapsed after a first allogeneic HSCT and had undergone a second transplantation. All but two patients had received reduced intensity conditioning before HSCT. Decrease of $CD34^+$ donor chimerism to below 80% occurred at a median of 169 days (range, 61–2919 days) after allogeneic HSCT. Patients had a median residual $CD34^+$ donor chimerism of 25% (range, 0–79%) in peripheral blood. To confirm the specificity of the $CD34^+$ donor chimerism analysis for detection of MRD, we identified leukemia-specific markers (del(5q) and −7) by fluorescence *in situ* hybridization in $CD34^+$ cells of two representative patients before azacitidine initiation (data not shown). Additionally, there were two patients in whom the decrease in $CD34^+$ donor chimerism was accompanied by a significant increase in MRD as determined by PCR of molecular markers (nucleophosmin $1^+$ mutation and t(8;21); data not shown). Pre-emptive treatment with azacitidine was started at a median of 13 days (range, 2–37 days) after the first detection of MRD.

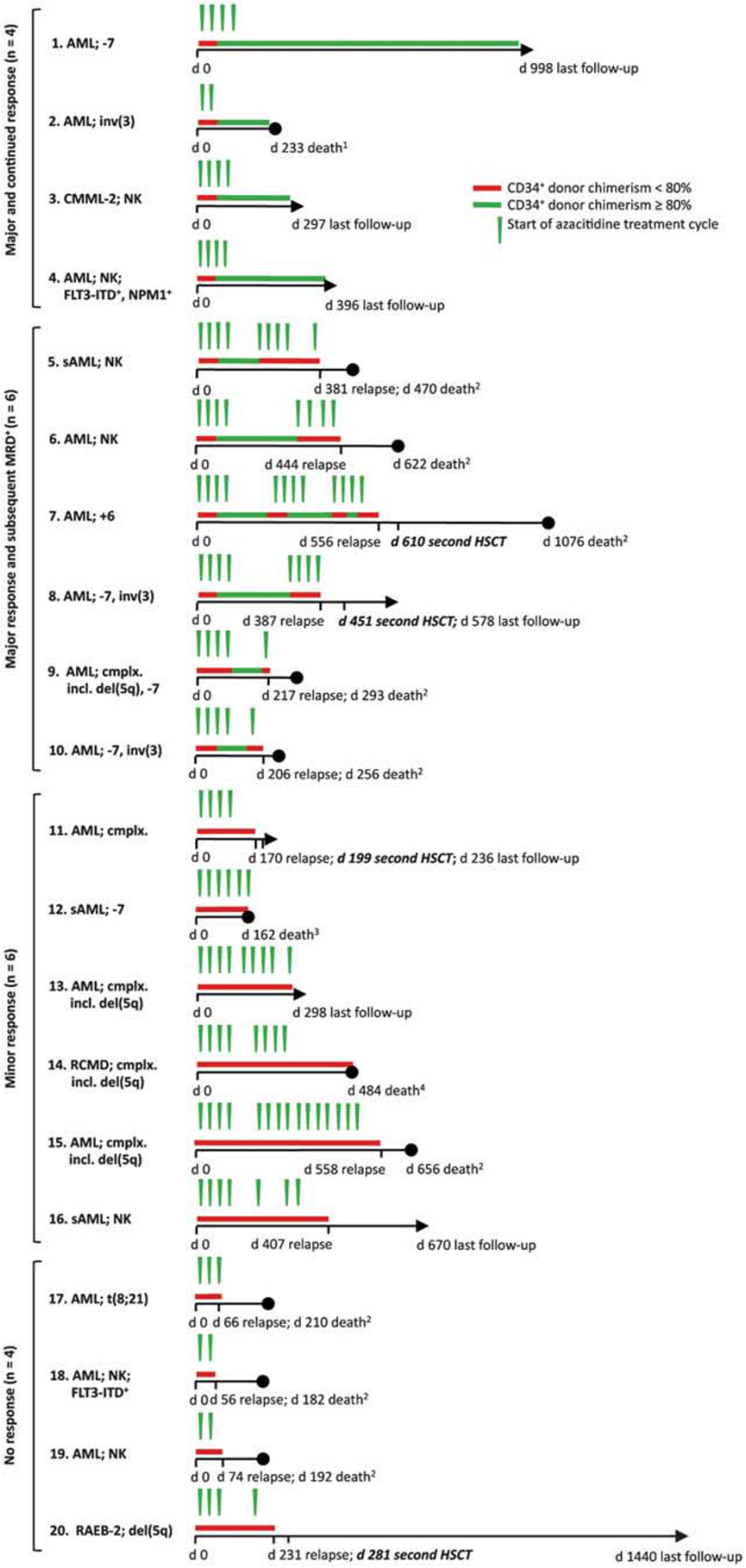
### *Clinical response after the first four azacitidine cycles*

Details of the individual patients' clinical responses after the first four azacitidine cycles are shown in Figure 1 and Supplementary Table S1, and are summarized as a flow chart in Figure 2. Ten of 20 patients (50%; 95% confidence interval 27–73) achieved an increase in $CD34^+$ donor chimerism to over 80% (that is, major response). Six patients (30%; 95% confidence interval 12–54) had no increase in $CD34^+$ donor chimerism to over 80% but they did not experience hematologic relapse while on azacitidine therapy (that is, minor response). Of these, one patient (5%) had an increase in $CD34^+$ donor chimerism, but not to a level above 80%, whereas the other five patients (25%) had stabilization or further decrease of $CD34^+$ donor chimerism. Four patients (20%) experienced hematologic relapse during or after the first four azacitidine cycles, with relapse occurring after the second cycle in two patients, although one of these two patients showed a temporary increase of $CD34^+$ donor chimerism to over 80%. The two other patients relapsed after the third and fourth azacitidine cycles, respectively. The latter patient had refractory anemia with excess blasts type 2 with a

**Table 1** Patient baseline demographics and disease characteristics

| *Characteristic* | n |
|---|---|
| *Total number of patients* | 20 |
| *Sex* | |
| Male | 11 |
| Female | 9 |
| *Median age, years (range)* | 58 (20–74) |
| *Disease* | |
| MDS | 3 |
| sAML | 3 |
| *De novo* AML | 14 |
| *Karyotype* | |
| Normal | 7 |
| −7 and/or inv(3) | 5 |
| Complex[a] | 5 |
| Other | 3 |
| *Molecular alterations* | |
| $FLT3\text{-}ITD^+$ | 2 |
| $NPM1^+$ | 1 |
| *Disease status prior to HSCT* | |
| First CR | 5 |
| Second CR | 6[b] |
| Third CR | 1 |
| First PR | 2 |
| Second PR | 2 |
| Primary refractory | 2 |
| Untreated | 2 |
| *Donor* | |
| Unrelated | 14 |
| Related | 6 |
| *HLA match* | |
| HLA-identical | 15 |
| One-allele mismatch | 3 |
| Haplo-identical | 2 |
| *Conditioning* | |
| Standard | 2 |
| Reduced | 18 |

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; $FLT3\text{-}ITD^+$, FMS-like tyrosine kinase 3 internal tandem duplication; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; inv, inversion; MDS, myelodysplastic syndromes; $NPM1^+$, nucleophosmin $1^+$; PR, partial remission; sAML, secondary AML; −7, monosomy 7.
[a]Defined as ⩾3 chromosomal abnormalities.
[b]Includes two patients who had already relapsed after first allogeneic HSCT.


Major and continued response (n = 4)
1. AML; -7
d 0
d 998 last follow-up
2. AML; inv(3)
d 0
d 233 death[1]
3. CMML-2; NK
d 0
d 297 last follow-up
4. AML; NK; FLT3-ITD+, NPM1+
d 0
d 396 last follow-up
CD34+ donor chimerism < 80%
CD34+ donor chimerism ≥ 80%
Start of azacitidine treatment cycle
Major response and subsequent MRD+ (n = 6)
5. sAML; NK
d 0
d 381 relapse; d 470 death[2]
6. AML; NK
d 0
d 444 relapse
d 622 death[2]
7. AML; +6
d 0
d 556 relapse
d 610 second HSCT
d 1076 death[2]
8. AML; -7, inv(3)
d 0
d 387 relapse
d 451 second HSCT; d 578 last follow-up
9. AML; cmplx. incl. del(5q), -7
d 0
d 217 relapse; d 293 death[2]
10. AML; -7, inv(3)
d 0
d 206 relapse; d 256 death[2]
Minor response (n = 6)
11. AML; cmplx.
d 0
d 170 relapse; d 199 second HSCT; d 236 last follow-up
12. sAML; -7
d 0
d 162 death[3]
13. AML; cmplx. incl. del(5q)
d 0
d 298 last follow-up
14. RCMD; cmplx. incl. del(5q)
d 0
d 484 death[4]
15. AML; cmplx. incl. del(5q)
d 0
d 558 relapse
d 656 death[2]
16. sAML; NK
d 0
d 407 relapse
d 670 last follow-up
No response (n = 4)
17. AML; t(8;21)
d 0 d 66 relapse; d 210 death[2]
18. AML; NK; FLT3-ITD+
d 0 d 56 relapse; d 182 death[2]
19. AML; NK
d 0 d 74 relapse; d 192 death[2]
20. RAEB-2; del(5q)
d 0
d 231 relapse; d 281 second HSCT
d 1440 last follow-up

del(5q) abnormality, whereas the other three patients had AML with either normal karyotype (including one with FMS-like tyrosine kinase 3 internal tandem duplication) or t(8;21). The three AML patients ultimately died owing to disease progression; whereas the MDS patient successfully underwent a second allogeneic HSCT.

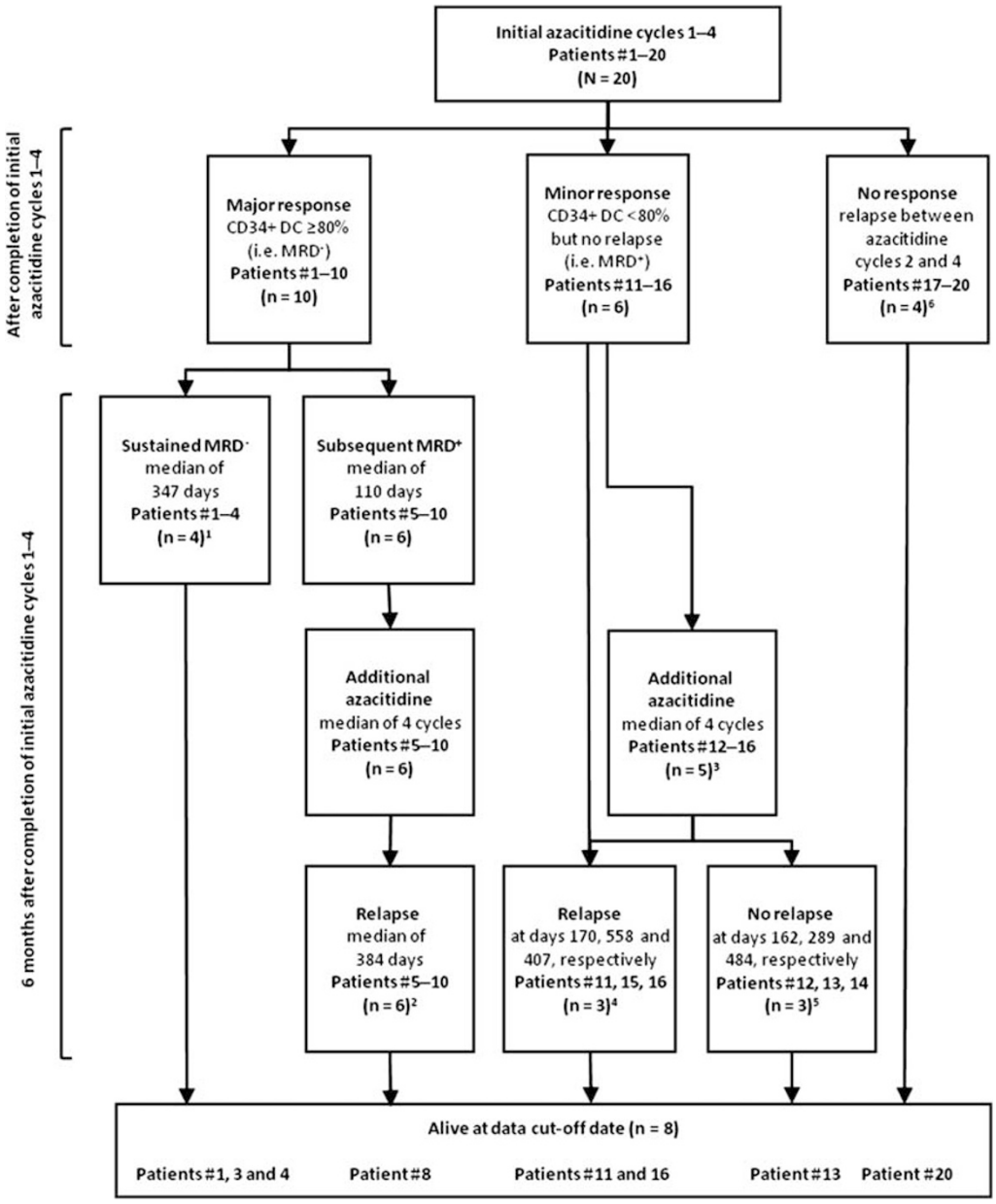



**Figure 2** Flow chart of the patients' clinical response and outcome during and after azacitidine treatment. DC, donor chimerism; MRD, minimal residual disease. [1]The patient no. 2 refused further azacitidine treatment and died due to non-azacitidine-related pneumonia, while sustaining $CD34^+$ donor chimerism above 80%. [2]The patients no. 7 and 8 underwent a second HSCT. Patients no. 5–7, 9 and 10 died due to progressive disease. [3]The patient no. 11 relapsed before additional azacitidine cycles could be initiated. [4]The patient no. 11 underwent a second HSCT. Patient no. 15 died due to progressive disease. [5]The patients no. 12 and 14 died due to non-azacitidine-related mesenterial infarction and progressive disease, respectively. [6]The patient no. 20 underwent a second HSCT. Patients no. 17–19 died due to progressive disease.

**Figure 1** Summary of clinical response during azacitidine treatment according to the four patterns observed: major and continued response, major response and subsequent $MRD^+$, minor response and no response. MRD was assessed every 4 weeks. AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; cmplx, complex (⩾3 karyotype abnormalities); d, day after first detection of $CD34^+$ donor chimerism below 80%; del, deletion; $FLT3\text{-}ITD^+$, FMS-like tyrosine kinase 3 internal tandem duplication; HSCT, hematopoietic stem cell transplantation; inv, inversion; MRD, minimal residual disease; NK, normal karyotype; $NPM1^+$, nucleophosmin 1 mutation; RAEB, refractory anemia with excess blasts; RCMD, refractory anemia with multilineage dysplasia; sAML, secondary AML; t, translocation; +6, trisomy 6; −7, monosomy 7. [1]The patient refused further azacitidine treatment and died due to non-azacitidine-related pneumonia, while sustaining $CD34^+$ donor chimerism >80%. [2]The patient died due to progressive disease. [3]The patient died due to non-azacitidine-related mesenterial infarction. [4]The patient stopped azacitidine treatment, was taken off protocol, received donor leukocyte infusions and died due to graft-versus-host disease.

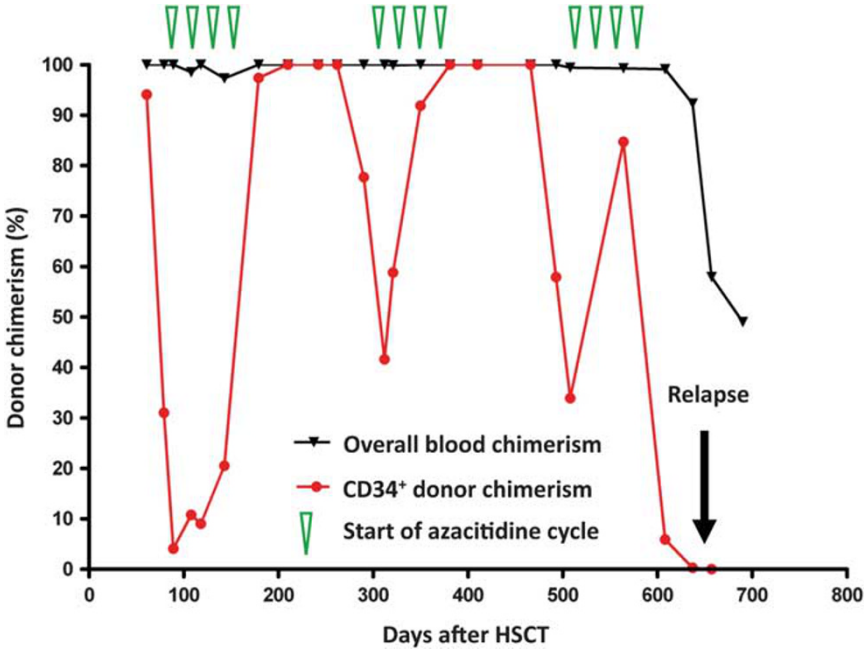



**Figure 3** Disease course of a patient with repeated major responses following azacitidine treatment. During the first four azacitidine cycles the patient's immunosuppressive prophylaxis (cyclosporine) was successfully tapered. Approximately 90 days after completing the first four cycles of azacitidine treatment, the patient experienced a second decrease of $CD34^+$ donor chimerism to <80%. The patient achieved a second major response with an additional four cycles of azacitidine and treatment was then stopped. Two months later, the patient's $CD34^+$ donor chimerism decreased a third time to below 80% and azacitidine was restarted, resulting in a third major response after two cycles. The patient subsequently experienced hematologic relapse ~3 weeks after cycle four (12 cycles of azacitidine received in total). HSCT, hematopoietic stem cell transplantation.

### *Clinical response after 6 months: follow-up of responding patients*

*Patients with major response* (n = *10*). Clinical response at 6 months after the first four azacitidine cycles is depicted in detail in Figure 1 and Supplementary Table S1, and is summarized in Figure 2. Of the 10 patients with major response after the first four cycles of azacitidine, three patients were still alive and maintained $CD34^+$ donor chimerism above 80% with no relapse at 297, 396 and 998 days after first MRD detection, respectively. An additional AML patient achieved major response after two initial cycles, but then refused any further treatment because of personal reasons not related to the study. This patient died due to pneumonia 8 months after first detection of MRD, still maintaining major response and in CR of the AML.

Six patients experienced a second decrease of $CD34^+$ donor chimerism to below 80% at a median of 110 days (range, 48–181 days) after the last cycle of azacitidine. These patients received a median of four additional cycles (range, 1–8 cycles) of azacitidine. One of these patients achieved a second major response after four further cycles of azacitidine (Figure 3). Two months later, this patient's $CD34^+$ donor chimerism dropped below 80% again and azacitidine was restarted. The patient achieved a third major response after two additional cycles, but experienced hematologic relapse ~3 weeks after the fourth cycle. This patient had received a total of 12 cycles of azacitidine during this study. The other five patients experienced a continuous decline in $CD34^+$ donor chimerism irrespective of azacitidine re-initiation and subsequently experienced hematologic relapse at 206, 217, 381, 387 and 444 days after first MRD detection, respectively.

*Patients with minor response* (n = *6*). Of the six patients with minor response after the first four cycles of azacitidine, one patient experienced hematologic relapse before a fifth cycle of azacitidine could be initiated (170 days after first MRD detection). The other five patients re-initiated therapy 4–8 weeks after completing the first four cycles. Patients received a median of four additional azacitidine cycles (range, 2–11 cycles), with one patient showing ongoing minor response and still receiving treatment at the data cutoff date (currently in cycle 9). Two patients relapsed after 3 and 11 additional cycles, respectively. Of the remaining two patients, one died after two additional cycles due to mesenteric infarction considered unrelated to azacitidine, while still in CR. The other patient stopped treatment with azacitidine after four additional cycles, while still in CR, but with no increase of $CD34^+$ donor chimerism. This patient was taken off the protocol, received DLI and subsequently died due to GvHD of the gut.

At the time of data cutoff, 13 patients (65%) in the intent-to-treat population had relapsed within a median of 231 days (range, 56–558 days) after first MRD detection. Four of these patients successfully underwent a second allogeneic HSCT at a median of 336 days (range, 199–610 days) after start of azacitidine. At data cutoff, eight patients (40%), including three of those who underwent a second allogeneic HSCT, were alive, with a median follow-up of 487 days (range, 236–1440 days) after the first detection of $CD34^+$ donor chimerism-defined MRD.

### *Safety and tolerability*

Reversible grade 3 or 4 neutropenia occurred in 16 of 20 patients (80%) and reversible grade 3 or 4 thrombocytopenia was observed in 13 of 20 patients (65%) during the first four cycles of azacitidine. Other adverse events included neutropenic fever ($n = 4$), pneumonia ($n = 3$) and cytomegalovirus reactivation ($n = 1$), which occurred in a total of six patients

(30%). Dose reductions because of myelosuppression were required in 9 of 20 patients (45%) by the end of the first four cycles of azacitidine, with at least one cycle of treatment delayed in 6 of 20 patients (30%). During azacitidine treatment, there was no manifestation of GvHD reported in patients without a prior history of GvHD. Complete cessation of immunosuppressive treatment was possible in four of six patients with no obvious exacerbation of GvHD. Interestingly, three of these four patients had a history of GvHD before treatment with azacitidine.

## Discussion

Hematologic relapse is a major challenge during the care of patients with MDS or AML undergoing allogeneic HSCT and treatment options are currently limited, particularly in patients with early disease recurrence.[7–9] Previous clinical studies in patients with MDS and AML after allogeneic HSCT have shown that low doses of azacitidine (8–40 mg/m$^2$/day for 5 days every 4 weeks) as salvage or maintenance therapy could be a potential treatment option to prevent or delay hematologic relapse.[27,28] In both trials, the progression-free and overall survival seemed to be improved compared with historical approaches without maintenance, which supports the concept of our study. To our knowledge, this is the first prospective trial investigating a pharmacological intervention in MDS and AML patients with MRD and imminent relapse as assessed by sensitive CD34$^+$ donor chimerism analysis in peripheral blood. Tracking MRD after allogeneic HSCT via peripheral blood CD34$^+$ donor chimerism monitoring, as performed in this clinical study, provides the advantage of tailoring azacitidine therapy in a pre-emptive setting only when MRD is detected, thus avoiding unnecessary toxicity in patients with ongoing CR. After only four cycles of azacitidine, MRD was diminished or stabilized in 80% of patients in the absence of hematologic relapse. Furthermore, responses were continuous in four of these patients without any further treatment. This is a remarkable observation, given that the majority of patients had advanced disease characteristics, including unfavorable cytogenetic aberrations. It also points to the unique mode of action of this DNA-methyltransferase inhibitor.

Nevertheless, for the majority of patients, hematologic relapse after an initial response could not be finally prevented, even with azacitidine re-treatment; 13 patients relapsed at a median of 231 days after first MRD detection (65%). This relapse rate seems to be comparable to our previous study, which used only immunotherapeutic interventions to prevent relapse in MDS or AML patients who had a drop of CD34$^+$ donor chimerism to below 80% after HSCT ($n=28$).[23] However, when comparing with the overall historic cohort, the present patient population is older, and comprises more patients with poor risk karyotype and advanced disease stages before HSCT. Additionally, in our previous study relapse was delayed by a median of 61 days from first MRD detection,[23] which is in accordance with other reports that MRD-guided immunomodulatory measures are often without long-term efficacy in the majority of high-risk diseases.[33] In this trial the time to relapse was considerably prolonged, by $\sim$6 months (median: 231 days), which further supports the potential of azacitidine to prevent or at least delay relapse in advanced MDS or AML patients.

Our results also show that a delay of relapse may be of substantial benefit to patients by allowing for a successful second HSCT following recovery from early toxicities of the first transplantation. As previous retrospective analyses suggest that the time interval between first transplantation and relapse is of major relevance for the outcome of second transplantations or DLI,[5,10] the current strategy could open a window for patients in whom relapse can be prolonged for another 6–12 months until DLI or a second allogeneic HSCT can be scheduled.

Because of safety reasons, including possible exacerbation of GvHD, treatment with azacitidine was limited to four consecutive cycles for this first phase II study, with the option of re-treatment if CD34$^+$ donor chimerism decreased again to below 80% without hematologic relapse. Severe GvHD was, however, uncommon and azacitidine treatment was generally well tolerated in our trial. This represents a substantial improvement in quality-of-life for these patients when compared with our historical cohort,[23] where relapse could be delayed with immunotherapeutic intervention only at the expense of an extensive chronic GvHD. Furthermore, some patients could potentially benefit from continued azacitidine treatment, as seen in other reports.[24,25,34]

Hematologic toxicity and infectious complications during azacitidine treatment were acceptable, with myelosuppression being both reversible and manageable with dose reduction or interruption. The use of azacitidine was not associated with exacerbation of GvHD even in the minority of patients in whom systemic immunosuppression could be completely tapered during azacitidine treatment. Recent results in mice suggest that azacitidine induces FOXP3 expression in naïve T-cells, which in turn induces a regulatory T-cell population that mitigates GvHD while preserving a graft-versus-leukemia effect.[35,36] Additionally, azacitidine may also augment the graft-versus-leukemia effect by induction of cytotoxic T-cell responses or enhance presentation of tumor antigens.[37] These data provide additional rationale for the combination of DLI with azacitidine in patients not responding after 4–6 cycles.

In conclusion, this phase II study demonstrated that MRD-triggered treatment with azacitidine appears to be an effective strategy for preventing or substantially delaying hematologic relapse with an acceptable safety profile in patients with MDS or AML after HSCT. These results support further investigations of this strategy in larger patient populations using more and continuous azacitidine cycles, which could potentially further increase the number of long-term responders.

## Conflict of interest

Dr Platzbecker has received honoraria and research funding from Celgene Corporation, and is a member of board of directors or advisory committees for Celgene Corporation. Dr Thiede co-owns a company commercially performing chimerism analyses (AgenDix GmbH). Dr Bornhäuser has received honoraria from Celgene Corporation. The remaining authors declare no conflict of interest.

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## Author Contributions

UP, XG and CT designed this study; UP wrote and conducted the clinical protocol, collected, analyzed and interpreted the data and wrote the paper. CT performed the MRD detection (chimerism analyses and NPM1 real-time PCR), collected and analyzed data, and edited the paper. GE and MB provided patient support, collected data and edited the paper. AK, BM, CR, HK, I-MK, JR, JS, MW and UO provided patient support and collected data. FS collected and analyzed data. All authors were involved in critical review of the paper.

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Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)