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ORIGINAL RESEARCH ARTICLE

Azacitidine (Vidaza®) in Pediatric Patients with Relapsed Advanced MDS and JMML: Results of a Phase I/II Study by the ITCC Consortium and the EWOG-MDS Group (Study ITCC-015)

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Abstract

Background Advanced myelodysplastic syndrome (MDS) and juvenile myelomonocytic leukemia (JMML) are rare hematological malignancies in children. A second allograft is recommended if a relapse occurs after hematopoietic stem cell transplantation, but the outcome is poor.

Objective We conducted a phase I/II multicenter study to evaluate the safety, pharmacokinetics, and activity of azacitidine in children with relapsed MDS/JMML prior to the second hematopoietic stem cell transplantation.

Methods Patients enrolled from June 2013 to March 2019 received azacitidine intravenously/subcutaneously once daily on days 1–7 of a 28-day cycle. The MDS and JMML cohorts followed a two-stage design separately, with a safety run-in for JMML. Response and safety data were used to evaluate efficacy and establish the recommended dose. Pharmacokinetics was also analyzed. The study closed prematurely because of low recruitment.

Results Six patients with MDS and four patients with JMML received a median of three and five cycles, respectively. Azacitidine 75 mg/m^2 was well tolerated and plasma concentration–time profiles were similar to observed in adults. The most prevalent grade 3–4 adverse event was myelotoxicity. No responses were seen in patients with MDS, but 83% achieved stable disease; four patients underwent an allotransplant. Overall response rate in the JMML cohort was 75% (two complete responses; one partial response) and all responders underwent hematopoietic stem cell transplantation. One-year overall survival was 67% (95% confidence interval 38–100) in MDS and 50% (95% confidence interval 19–100) in JMML.

Conclusions Azacitidine 75 mg/m^2 prior to a second hematopoietic stem cell transplantation is safe in children with relapsed MDS/JMML. Although the long-term advantage remains to be assessed, this study suggests that azacitidine is an efficacious option for relapsed JMML.

Clinical Trial Registration EudraCT 2010-022235-10.

1 Introduction

Myelodysplastic disorders in childhood comprise a group of hematological malignancies that include advanced myelodysplastic syndrome (MDS) and juvenile myelomonocytic leukemia (JMML) [1]. Juvenile myelomonocytic leukemia, which represents around 2–3% of all pediatric leukemias [2], is caused by the excessive proliferation of cells of monocytic and granulocytic lineages that lead to massive tumor cell infiltration of organs [3]. Myelodysplastic syndrome is a group of heterogeneous conditions associated with ineffective hematopoiesis, functional cellular defects, and persistent peripheral blood cytopenia that comprises about 3% of childhood cancers [4]. Pediatric MDS have different cytogenetic findings, somatic genetic landscapes, and therapeutic aims than found in adults [1, 5, 6].

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only potentially curative treatment option for children with advanced MDS and for most children with JMML, but there is currently no pre- or post-HSCT treatment established to avoid disease progression or prevent relapse [7, 8]. The role of irreversible DNA methyltransferase inhibitors such as azacitidine has been investigated in adults with MDS demonstrating significant

Key Points

This is the first prospective trial designed to assess the safety and efficacy of azacitidine in children with relapsed myelodysplastic syndrome and relapsed juvenile myelomonocytic leukemia.

Pharmacokinetic analyses show azacitidine exposure in subcutaneous and intravenous administration in children in line with results in adults.

Azacitidine 75 mg/m^2 prior to a second hematopoietic stem cell transplant is safe in children with relapsed myelodysplastic syndrome and relapsed juvenile myelomonocytic leukemia.

prolongation of survival [9]. Based on that, azacitidine has been approved by the US Food and Drug Administration (FDA) for the first-line treatment of adult patients with MDS [10], and by the European Medicines Agency for adult patients with MDS who are not eligible for HSCT [11]. Azacitidine in the first-line setting prior to HSCT showed promising results also in a pediatric MDS population, but only in retrospective real-world experience cohorts [12, 13]. In JMML, a recent trial demonstrated azacitidine is well tolerated and provides valuable clinical benefit prior to HSCT in the upfront line [14]. This study reported in May 2022 led to the approval of azacitidine for pediatric patients with newly diagnosed JMML by the FDA [15].

Nevertheless, disease recurrence represents the main cause of treatment failure in children with advanced MDS and JMML. The 5-year probability of event-free survival for children after the first allograft is 59% [16] in MDS and 52% in JMML [17]. Furthermore, there are no standard treatments for advanced MDS and JMML at relapse, and treatment strategy is limited to achieve a second allogeneic HSCT. However, the majority of patients who relapse after a first allogeneic HSCT do not achieve long-term survival [18–20].

Given the relatively high number of relapses after HSCT [16, 17], and the lack of a consensus on the therapy recommended in relapses, additional therapeutic options for children with relapsed MDS and relapsed JMML are needed. Here, we report the results of a prospective, open-label, phase I/II study evaluating the safety, pharmacokinetics, and activity of azacitidine monotherapy in children with relapsed advanced MDS or JMML.

2 Methods

2.1 Study Design

This phase I/II study of azacitidine in pediatric patients with relapsed advanced MDS or JMML (ITCC-015/EWOG-MDS-Azacytidine-2010) was a collaborative study between the European Working Group of Myelodysplastic Syndromes in Childhood (EWOG-MDS) Working Group and the Innovative Therapies for Children with Cancer Consortium, with Erasmus MC as the international sponsor, and with the free drug provided by Celgene, who was also responsible for the bioanalysis of the pharmacokinetic samples. Financial support was provided by the Go4Children foundation (EUDRACT: 2010-022235-10). The trial was open at five centers in five European countries and enrolled from June 2013 to March 2019. The study was closed prematurely in February 2020 because of a low inclusion rate for different reasons.

This study was approved by the appropriate national research ethics committee of each participating country. It was conducted in accordance with the guidelines of Good Clinical Practice and the principles of the Declaration of Helsinki. The investigators obtained informed consent from each participant's legal guardian and each participant where applicable.

2.2 Patients

The study was open for two indications: relapsed advanced MDS and relapsed JMML. Diagnostic criteria for relapsed MDS were determined as the reappearance of blasts in the peripheral blood, or ≥ 5% blasts in the bone marrow (BM) not attributable to any other cause and confirmed with flow cytometry after a documented complete response (CR) or partial response (PR).

Diagnostic criteria for relapsed JMML were specified as the reappearance of organomegaly in combination with elevated white blood cells with peripheral blood monocytosis (greater than 1×10^9/L), and/or the reappearance of a cytogenetic or molecular lesion indicative of prior disease, and/or blast crises/transformation to acute myeloid leukemia (AML) after a documented CR or PR. In addition, clinical criteria may be used, which include objective parameters such as an increase in spleen size of > 50% from baseline, and/or the appearance of new skin lesions, and/or oxygen need.

Main inclusion criteria were: age 1 month to 18 years; Lansky play score or Karnofsky Performance Status > 60; normal renal function (National Cancer Institute Common

Terminology Criteria for Adverse Events version 4.0 grade 1, maximum 1.5× the upper limit of normal), and normal liver function (National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 grade 1, maximum 2.5× the upper limit of normal for transaminases and bilirubin); no prior treatment with a demethylating agent, no chemotherapy within 3 weeks of the start of the study medication, and recovery of all acute toxic effects of prior chemotherapy/HSCT. Exclusion criteria included germline molecular aberrations indicative of AML; patients with JMML in whom a diagnosis of Noonan syndrome was suspected; patients with secondary MDS with underlying BM failure syndromes or with familial MDS and isolated extramedullary disease. Detailed inclusion and exclusion criteria are provided in the Electronic Supplementary Material (ESM).

2.3 Treatment

Eligible patients were treated with intravenous (IV) or subcutaneous (SC) azacitidine on days 1–7 of a 28-day cycle, with preference for a minimum of three cycles, after which retransplantation was allowed. Dose level 1 (DL1) [starting dose] was 75 mg/m^2/day (2.5 mg/kg/day for patients < 10 kg body weight or < 1 year of age), dose level 2 (DL2) was 100 mg/m^2/day (3.3 mg/kg/day for patients < 10 kg body weight or < 1 year of age), and dose level-1 (DL-1) was 50 mg/m^2/day (1.7 mg/kg/day for patients < 10 kg body weight or < 1 year of age). Azacitidine administration was allowed to be IV or SC, and the decision on method of administration was made by the patient and the local investigator.

If, by cycle 3 day 28, a patient had a CR or PR, the patient could receive cycles up to six of azacitidine at the investigator's discretion. Patients were taken off the study when they did not show any response after the sixth course of treatment, or when there was clear progressive disease (PD). For advanced patients with MDS, PD was defined as evolution to MDS-related AML (> 30% BM blasts). In JMML, PD was defined as clinical progression including objective parameters such as an increase in spleen size of > 50% from baseline, and/or the appearance of new skin lesions, and/or oxygen need, and/or blast crises/transformation to AML.

Patients who showed a benefit and for whom no donor was available or who could not be transplanted for other reasons were offered to continue azacitidine as long as they received a benefit, in the absence of major safety concerns. The follow-up period was 1 year after HSCT or until 1 year after the last azacitidine administration when HSCT did not take place.

2.4 Objectives and Endpoints

The primary objective for this study was to establish the recommended dose and preliminary efficacy of azacitidine in children with relapsed MDS or JMML. Response data combined with safety data were used to evaluate efficacy and establish the recommended dose in both cohorts independently.

Dose-limiting toxicities (DLTs) were reported and considered in the dose escalation during the first course of azacitidine. A definition of DLTs for this study can be found in the ESM.

The primary efficacy endpoint was the overall response rate (ORR), defined as the number of patients with either a CR or PR, over the total number of patients evaluable for the analysis. The response definition was adapted from Cheson et al. [21] for patients with advanced MDS. For patients with JMML, the response definition was adapted from Chan et al. [22] Detailed response criteria are reported in the ESM. Secondary objectives included the safety and tolerability of azacitidine, additional efficacy measures, and the pharmacokinetic profile of azacitidine.

The safety analysis included the frequency, severity, and relatedness of all adverse events (AEs), frequency of dose interruptions, dose reductions and treatment discontinuation for toxicity, and use of concomitant medications. Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0 and recorded until 28 days after the last dose of azacitidine. As additional efficacy measures, HSCT rate, progression-free survival (PFS), and overall survival (OS) were analyzed. Pharmacokinetic parameters of azacitidine were calculated from the concentration–time profiles using non-compartmental analyses.

2.5 Statistical Analyses

The data cut-off for this analysis was 30 November, 2020. Each disease indication was considered an independent treatment arm that ran in parallel with each other. One dose escalation was allowed for each arm.

Dose escalation in both study arms followed a two-stage design, with the option of a safety run-in in the case of the JMML arm. Each stage (stage one and stage two) consisted of a minimum of three patients per disease indication.

Progression-free survival was defined as the time from the date of the first administration of azacitidine until progression, relapse, or death, whichever occurred first. Overall survival was defined as the time from the first dose of azacitidine until death due to any cause. Survival curves for PFS and OS were generated using the Kaplan–Meier method. For confidence intervals (CIs) of survival rates, the standard error was computed using Greenwood's formula. Median PFS and OS were obtained along with 95% CIs using the Brookmeyer and Crowley method.

3 Results

3.1 Patient Characteristics

From June 2013 to March 2019, ten pediatric patients were recruited: six with relapsed advanced MDS and four with relapsed JMML. All patients were included in all analyses as they all received at least one dose of the study medication and had at least one post-baseline assessment. Their demographics and baseline characteristics are shown in Table 1.

Three male individuals and three female individuals with MDS were recruited with a median age of 14.3 years (range 9.7–18.2 years). Median time from diagnosis to relapse was 458 days (range 214–755 days). All patients received prior HSCT.

Four male individuals with JMML were recruited with a median age of 4 years (range 3.1–7.3 years). Median time from diagnosis to relapse was 588 days (range 328–1645 days). Methylation class was high in three patients and low in one patient based on the JMML-specific DNA methylation score [23]. The spleen tip was palpable at a median of 4 cm (range 0–11 cm) below the costal margin. All patients received prior HSCT.

3.2 Therapy Delivered

A total of 36 cycles of azacitidine at 75 mg/m^2/day (DL1) were administered, 25 intravenously and 11 subcutaneously. No patients were dose escalated to DL2.

Patients with relapsed advanced MDS received a total of 17 cycles. The median duration of treatment was three cycles (range 2–4 cycles). Four patients (67%) completed three cycles of azacitidine but two patients (33%) discontinued therapy before completing three cycles because of disease progression and complications (Table 2). Patients with relapsed JMML received 19 cycles. The median duration of treatment was five cycles (range 3–6 cycles). All patients with JMML completed at least three cycles of azacitidine (Table 2).

3.3 Safety

All patients had more than one AE. The most common grade 3 or 4 treatment-related AEs were neutropenia, thrombocytopenia, anemia, and febrile neutropenia reported in seven, five, four, and four patients each, respectively. Other related grade 3 and 4 events consisted of elevation of liver functions, anorexia, and headache (Table 3).

Five patients (50%) experienced a serious AE, due to 12 grade 2/3 AEs that required hospitalization (Table 4). Five out of 12 serious AEs were considered as suspected unexpected serious adverse reactions, all clustered in the same

Table 1 Population baseline characteristics

Patient ID	Stratum	Sex	Time between diagnosis and relapse (months)	Age at inclusion (years)	Weight (kg)	Height (cm)	Spleen size (cm)	Methylation class	Somatic mutation	Karyotype	Platelet count ($\times 10^9$/L)	Prior Allo-HSCT
1	MDS	Female	18	14.2	48.1	159.0	0			47,XX, +mar[17]	26	Yes
2	JMML	Male	23	3.1	12.7	87.0	11	High	NRAS	46,XY	8	Yes
3	JMML	Male	11	4.8	16.8	108.0	0	High	PTPN11	46,XY	106	Yes
4	JMML	Male	16	3.2	15.9	96.0	4	High	NRAS	Not obtained	63	Yes
5	MDS	Male	13	14.4	34.0	147.0	0			46,XY	17	Yes
6	MDS	Female	7	9.7	28.8	133.5	0			− 7	23	Yes
7	MDS	Female	25	18.2	58.0	161.8	2			− 7	115	Yes
8	MDS	Male	9	15.2	64.0	181.0	0			46,XY,t(3;5)(p23;q1.4)	27	Yes
9	MDS	Male	19	11.5	77.7	165.6	0			46,XY	32	Yes
10	JMML	Male	54	7.3	28.8	129.0	0	Low		+ 8	27	Yes

Allo-HSCT allogeneic hematopoietic stem cell transplantation, *ID* identification number, *JMML* juvenile myelomonocytic leukemia, *MDS* myelodysplastic syndrome

Table 2 Dosing and response details per patient

Patient ID	Stratum	Method of administration	Number of cycles	Best response obtained	Time when best response was obtained	Status at second transplant if applicable	Status at last follow-up
1	MDS	IV	3	SD	C2D1	SD	Alive
2	JMML	SC	3	SD	C2D1	SD	Death
3	JMML	SC	5	PR	C2D1	–	Death
4	JMML	IV	6	CR	C2D1	CR	Alive
5	MDS	IV	2	PD	C2D1	–	Death
6	MDS	IV	2	SD	C2D1	–	Death
7	MDS	IV	3	SD	C2D1	SD	Alive
8	MDS	SC	3	SD	C2D1	SD	Death
9	MDS	IV	4	SD	C2D1	SD	Alive
10	JMML	IV	5	CR	C5D1	CR	Death

C cycle, *CR* complete response, *D* day, *ID* identification number, *IV* intravenous, *JMML* juvenile myelomonocytic leukemia, *MDS* myelodysplastic syndrome, *PD* progresive disease, *PR* partial response, *SC* subcutaneus, *SD* stable disease

Table 3 List of the grade 3–4 adverse events

	Number of patients		
	Grade 3	Grade 4	Total
Alanine aminotransferase increased	2	0	2
Anemia	4	0	4
Anorexia	1	0	1
Blood total bilirubin increased	1	0	1
Blood direct bilirubin increased	0	1	1
Bronchial infection	1	0	1
C-reactive protein increase	1	0	1
Erythema nodosum	1	0	1
Erythrocytosis	1	0	1
Febrile neutropenia	4	0	4
Flu-like symptoms	1	0	1
Headache	1	0	1
Lymphocyte count decreased	1	0	1
Lung infection	1	0	1
Multifocal leukoencephalopathy	1	0	1
Neutrophil count decreased	0	7	7
Parotitis	1	0	1
Platelet count decreased	0	5	5
Pulmonary hemosiderosis	1	0	1
Sepsis	1	0	1
Splenomegaly and extramedullary hematopoiesis	1	0	1

patient with MDS: grade 3 pulmonary hemosiderosis, grade 3 erythrocytosis, grade 3 splenomegaly, and grade 3 and grade 2 erythema nodosum. These events were considered related as there was no clear alternative explanation.

Only one patient with MDS experienced three DLTs: pulmonary hemosiderosis in cycle 1 and splenomegaly and erythrocytosis in cycle 2. The patient was discontinuing because of toxicity after cycle 2. No other DLTs were seen in any of the participants. No other AEs lead to a dose reduction or dose interruption in any other patient. No treatment-related deaths were recorded.

3.4 Efficacy

Details of the individual patients' clinical responses are shown in Table 2.

3.4.1 MDS Cohort

At C1D28, five out of six patients (83%) achieved stable disease and one patient (17%) presented with PD. No responses were achieved. Importantly, four of the five patients with stable disease (67%) underwent allogeneic HSCT. There were no graft failures. Grade I acute graft-versus-host disease was diagnosed in one patient and chronic graft-versus-host disease in another patient.

With a median follow-up of 8.7 months (interquartile range 4.09–9.57), three of six patients (50%) were alive at the end of the study. The patient who progressed during the treatment trial died before HSCT, one patient died before undergoing a transplantation following a splenectomy because of respiratory insufficiency and multi-organ failure, and one other patient died after a transplant because of disease progression despite HSCT. The 1-year OS from the start of treatment was 67% (95% CI 38–100) for the MDS cohort.

3.4.2 JMML Cohort

The ORR was 75%: two patients (50%) achieved CR at C1D28 and at C4D28, respectively, and one patient (25%)

Table 4 List and characteristics of the severe AEs

Patient ID	Stratum	AE term	SAE category	Timing	Outcome
1	MDS	Increased CRP level	Prolongation of hospitalization	Cycle 1	Resolved
1	MDS	Increased CRP level	Prolongation of hospitalization	Cycle 2	Resolved
2	JMML	Fever	Prolongation of hospitalization	Cycle 1	Resolved
2	JMML	Febrile neutropenia	Prolongation of hospitalization	Cycle 2	Resolved
3	JMML	Jaundice	Prolongation of hospitalization	Cycle 2	Resolved
6	MDS	Pulmonary hemosiderosis	Prolongation of hospitalization	Cycle 1	Resolved
6	MDS	Erythrocytosis	Prolongation of hospitalization	Cycle 2	Resolved
6	MDS	Febrile neutropenia	Prolongation of hospitalization	Cycle 2	Resolved
6	MDS	Erythema nodosum	Prolongation of hospitalization	Cycle 2	Resolved
6	MDS	Splenomegaly with extramedullary hematopoiesis	Prolongation of hospitalization	Cycle 2	Ongoing
6	MDS	Erythema nodosum	Prolongation of hospitalization	Cycle 1	Resolved
9	MDS	Febrile neutropenia	Prolongation of hospitalization	Follow-up	Resolved

AE adverse event, *CRP* C-reactive protein, *ID* identification number, *JMML* juvenile myelomonocytic leukemia, *MDS* myelodysplastic syndrome, *SAE* severe adverse event

achieved PR at C1D28. One (25%) patient achieved stable disease. There were no PDs during the study treatment. Three of the four patients (75%) underwent allogeneic HSCT. There was one graft failure and one patient presented with grade II acute graft-versus-host disease.

With a median follow-up of 14.7 months (interquartile range 6.59–14.42 months), only one of four patients was alive. After transplantation, one patient died following a graft failure and one because of veno-occlusive disease and macrophage activation syndrome. The other patient died after relapse before undergoing a transplant. The 1-year OS from the start of treatment was 50% (95% CI 19–100) for the JMML cohort (Fig. 1).

3.5 Pharmacokinetics

The pharmacokinetic data were analyzed by route of administration, seven patients received IV azacitidine and three patients received SC azacitidine. In one subject, the infusion duration was 48 min relative to 15 min per protocol and this patient had unusually high azacitidine concentrations and exposure parameters (maximum plasma concentration [C_{max}] more than ten-fold higher than highest C_{max} observed in the other subjects and an area under the concentration–time curve more than 35-fold higher than highest area under the concentration–time curve value observed in the other subjects), therefore summaries are provided excluding this subject (Fig. 2).

Following IV administration, azacitidine rapidly reached C_{max} with a mean C_{max} of 1920 ng/mL and then was rapidly eliminated with a mean terminal elimination half-life of 0.34 h. Following SC administration, azacitidine reached lower C_{max} with a mean C_{max} of 1263 ng/mL and showed slower elimination with a mean terminal elimination half-life of 0.54 h. High interpatient variability was observed for both routes of administration.

4 Discussion

This is the first prospective trial designed to assess the safety and efficacy of azacitidine in children with relapsed MDS and relapsed JMML. In this azacitidine monotherapy study, the dose of 75 mg/m^2/day on days 1–7 of a 28-day cycle was well tolerated. No patients were treated with 100 mg/m^2. Pharmacokinetic analyses showed drug exposure in line with results in adults, including SC administration that showed a half-life time approximately 1.5-fold greater than the IV half-life time. No responses were obtained in the MDS cohort, although five out of six patients achieved stabilization of the disease for a median duration of three cycles, enabling an HSCT in 80% of them. In the four patients with JMML, clear signs of activity were seen. The ORR was 75%, with a CR in two patients and a PR in one patient, which enabled all responders to undergo a second HSCT.

Azacitidine monotherapy was well tolerated in this patient population. Most prevalent grade 3–4 AEs were neutropenia, thrombocytopenia, anemia, and febrile neutropenia, which is consistent with the safety profile revealed in first-line pediatric patients [13, 14] and in adults [24, 25]. Intriguingly, a 9-year-old female child with relapsed MDS experienced three different DLTs with several as suspected unexpected serious adverse reactions, including pulmonary hemosiderosis and erythema nodosum. These findings have not been reported earlier with azacitidine. They are not without doubt attributable to azacitidine and may be related to an auto-immune phenomenon that can be observed in MDS;

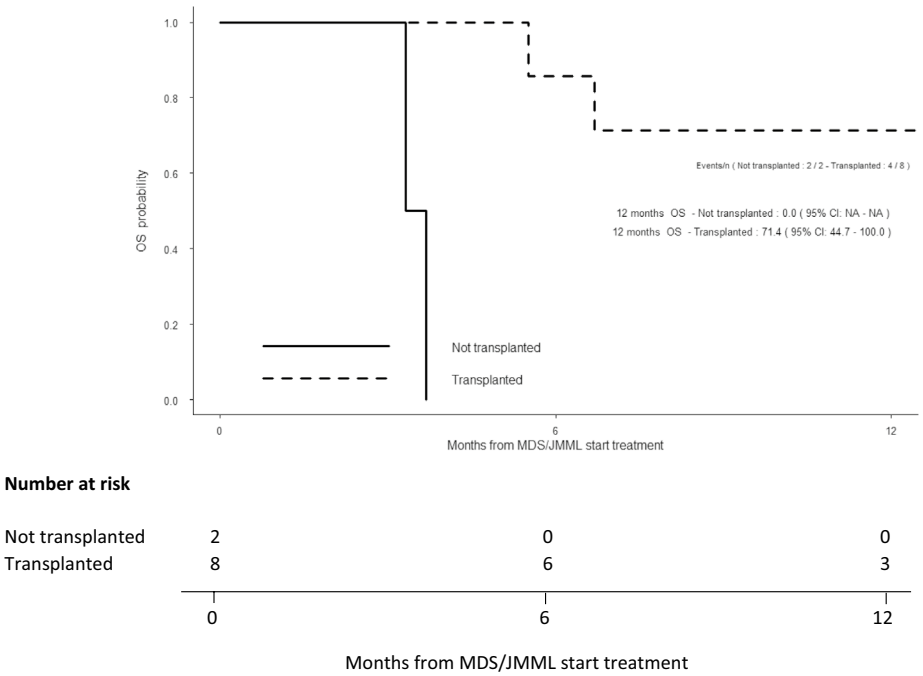

Fig. 1 Overall survival (OS) from the start of treatment for patients with juvenile myelomonocytic leukemia (JMML) and myelodysplastic syndrome (MDS) differentiated to transplantation status. The risk table shows the number of patients at a certain timepoint. *CI* confidence interval, *NA* MDS: Myelodysplastic syndrome, JMML: Juvenile myelomonocytic leukemia, OS: Overall survival

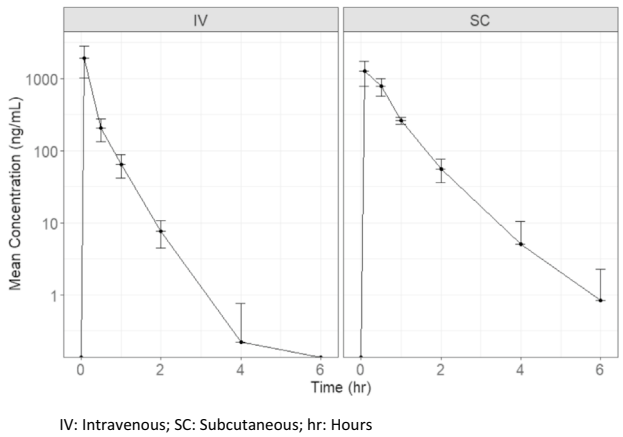

Fig. 2 Intravenous (IV) and subcutaneous (SC) log mean (± standard deviation) concentration versus time profile (excluding Subject 9). *hr* hours

however, the events occurred after drug exposure in both course 1 and course 2.

Unfortunately, we did not find responses for patients with MDS, but stabilization of the disease was achieved in five out of six patients. This is in accordance with the previous retrospective experience of the EWOG-MDS [13]. Cseh et al. reported a retrospective analysis of 24 children and young adults with MDS who received azacitidine at the time of the first diagnosis or relapse after an allotransplant. Ten children received azacitidine for relapsed MDS after an HSCT over 5–7 days at a single dose of 50–100 mg/m^2 per IV or SC route. Of these, seven experienced stable disease for a median of three cycles. Additionally, in adults, response to azacitidine occurs in less than 50% of treated patients, and duration of response is transient [26].

New drugs and innovative combinations with hypomethylating agents in MDS have emerged in recent years. One drug that has received a breakthrough therapy designation from the FDA for MDS in adults is magrolimab [27]. Magrolimab is a monoclonal antibody that blocks the macrophage inhibitory immune checkpoint CD47, a "do not eat me" signal overexpressed on tumor cells that allows for evasion of immune destruction [28]. In a phase Ib study of adults with newly diagnosed high-risk MDS, the combination of magrolimab and azacitidine was found to be tolerable and demonstrated encouraging activity with a 74.7% ORR [29]. Another drug that is currently under investigation is venetoclax. Venetoclax is a BCL2 inhibitor that can induce apoptosis in tumor cells that are dependent on BCL2 for survival [30]. The association of venetoclax and azacitidine has shown efficacy in adults patients with newly MDS who were not candidates for HSCT [31] and, in addition, the triplet is under development for high-risk de novo and secondary

AML (NCT05079230) [32]. Although these therapies seem promising also in children [33, 34], pediatric data are scarce and preliminary so far. Multicenter clinical trials should be developed to explore these novel regimens in pediatric subjects with newly and relapsed MDS.

Although the number of patients with JMML is limited in our study, the findings support the hypothesis of efficacy in relapsed JMML. The EWOG-MDS study group presented a retrospective compilation of 12 children with JMML who received individual off-label treatment with azacitidine before HSCT ($N = 9$) or for relapsed disease ($N = 3$). Of three children with JMML who received azacitidine for disease recurrence after the second HSCT, all achieved clinical PR or could be maintained in a stable disease for four cycles before progressing [35]. Other investigators focused on patients with newly diagnosed JMML recently reported the results of a phase II trial with azacitidine prior to HSCT. Eighteen patients were treated with azacitidine (75 mg/m^2) administered intravenously once daily on days 1–7 of a 28-day cycle. After three cycles, 11 patients (61%) reached partial remission (PR) and in seven patients (39%), the disease had progressed [14]. In our study, fetal hemoglobin levels were not collected in patients with JMML. As high hemoglobin levels indicate aggressive disease [36], additional studies should be carried out to assess the role of hemoglobin levels in this setting.

Given the most favorable response in JMML than in MDS, one could speculate that distinct variations in the epigenetic make-up between these disorders might underlie a potential discrepancy in susceptibility to azacitidine. Additionally, our study reveals that, following IV and SC administration of azacitidine, plasma concentration–time profiles in pediatric patients were similar to profiles observed in adults. Adult studies assessed that the bio-availability of azacitidine was lower after SC administration when compared with 10 minutes IV administration [37]. Later studies showed that SC administration was as effective in adults [10, 38]. However, pharmacokinetic parameters in pediatric patients cannot simply be extrapolated from adult data because of differences in growth and development and changes in kidney and liver functions. Hence, in this study, pharmacokinetics was assessed within nine patients for IV and SC administration. Mean SC half-life time was 32 min (0.54 ± 0.20 h), compared with approximately 41 minutes (0.69 ± 0.14 h) in the adult population. Twenty minutes for mean half-life time after IV administration (0.34 ± 0.09 h) in children was comparable to 22 min (0.36 ± 0.02 h) in adult studies administration of 75 mg/m^2 azacitidine [36].

Limitations of the study are the small sample size and short follow-up time. The low recruitment rate, especially for patients with JMML, let to stop the study prematurely. Possible explanations for the lack of inclusion of patients with JMML are that the outcomes after transplantation for patients with primary JMML have improved [17, 39]. As a result, fewer patients were available than anticipated when setting up the study. In addition, azacitidine is also commercially available for pediatric patients, albeit not on-label, but this probably resulted in a lack of referrals to a study center to participate in the study. Furthermore, strict inclusion and exclusion criteria, such as the need of recovery of all acute toxic effects of prior treatments, could have made the inclusion of patients even more difficult. Another limitation is the short follow-up time. Whether a response to azacitidine prior to a second HSCT will translate into improved long-term OS remains to be determined.

5 Conclusions

Although the long-term advantage of azacitidine therapy remains to be fully assessed, this prospective clinical trial suggests that azacitidine therapy prior to a second HSCT is safe in children and an efficacious option for patients with JMML. In children with relapsed advanced MDS, new strategies have to be evaluated.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40272-023-00588-5.

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Declarations

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Conflicts of interest/competing interests Charlotte M. Niemeyer had a consultant role for BMS, Novartis, and Apriligen. Eric J. Laille is an employee of Celletics and a former employee of BMS and Celgene. Alba Rubio-San-Simón, Natasha K.A. van Eijkelenburg, Raoull Hoogendijk, Henrik Hasle, Michael N. Dworzak, Marco Zecca, Marta Lopez-Yurda, Julie M. Janssen, Iwin D.R. Huitema, Marry M. van den Heuvel-Eibrink, Harm van Tinteren, and Christian M. Zwaan have no conflicts of interest that are directly relevant to the content of this article.

Ethics approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Ethik-Kommission der Albert-Ludwigs-Universität Freiburg in Germany, Ethik-Komission der Medizinischen Universitat Wien und des Allgemeinen Krankenhauses der Stadt Wien AKH in Austria, METC Erasmus MC Rotterdam in the Netherlands, Comitato Bioetica Fondazione Policlinico San Matteo Pavia in Italy, Eticka Komise pro Multicentricke Klinicke Hodnoceni Fakultni Nemocnice v Motole in Czech Republic, and Videnskabsetisk komite Region Midt in Denmark.

Consent to participate Informed consent was obtained from all participants' legal guardians included in the study.

Consent for publication Not applicable.

Availability of data and material The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Authors' contributions All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by ARSS, NKAvE, RH, and CMZ. The first draft of the manuscript was written by ARSS, NKAvE, and CMZ and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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