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Response to treatment with azacitidine in children with advanced myelodysplastic syndrome prior to hematopoietic stem cell transplantation

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ABSTRACT

Advanced myelodysplastic syndrome harbors a high risk of progression to acute myeloid leukemia and poor prognosis. In children, there is no established treatment to prevent or delay progression to leukemia prior to hematopoietic stem cell transplantation. Azacitidine is a hypomethylating agent, which was shown to slow progression to leukemia in adults with myelodysplastic syndrome. There is little data on the efficacy of azacitidine in children. We reviewed 22 pediatric patients with advanced myelodysplastic syndrome from a single center, diagnosed between January 2000 and December 2015. Of those, eight patients received off-label azacitidine before hematopoietic stem cell transplantation. A total of 31 cycles were administered and modification or delay occurred in four of them due to cytopenias, infection, nausea/vomiting, and transient renal impairment. Bone marrow blast percentages in azacitidine-treated patients decreased significantly from a median of 15% (range 9-31%) at the start of treatment to 5.5% (0-12%, P=0.02) before hematopoietic stem cell transplantation. Following azacitidine treatment, four patients (50%) achieved marrow remission, and none progressed. In contrast, three untreated patients (21.4%) had progressive disease characterized by >50% increase in blast counts or progression to leukemia. Azacitidine-treated patients had significantly increased 4-year event-free survival (P=0.04); predicted 4-year overall survival was 100% *versus* 69.3% in untreated patients (P=0.1). In summary, azacitidine treatment prior to hematopoietic stem cell transplantation was well tolerated in pediatric patients with advanced myelodysplastic syndrome, led to partial or complete bone marrow response in seven of eight patients (87.5%), and correlated with superior event-free survival in this cohort.

Introduction

Myelodysplastic Syndrome (MDS) is a clonal disorder with cytopenias, cytogenetic aberrations, varying degrees of bone marrow dysplasia, and leukemic blasts. Pediatric MDS is rare and comprises about 3% of childhood cancers.[1] Advanced MDS in children with an increase in leukemic blasts in the bone marrow (5-29%)

and/or peripheral blood (2-29%), is termed either refractory cytopenia with excess blasts (RCEB),[2,3] or divided into refractory anemia with excess blasts (RAEB, bone marrow blasts 5-19% and/or peripheral blasts 2-19%) and refractory anemia with excess blasts in transformation (RAEB-T, bone marrow and/or peripheral blasts 20-29%).[4] RCEB harbors a high propensity of transformation to leukemia, mainly MDS-related acute myeloid leukemia (MDR-AML).[1,5]

The outcome of RCEB in childhood is poor, with a 5-year overall survival (OS) of about 35-63%,[6-9] which is inferior to survival in MDS without excess blasts[10,11] and *de novo* AML.[12] Allogeneic hematopoietic stem cell transplantation (HSCT) is currently the only curative treatment for RCEB.[8] Earlier HSCT correlates with better outcome,[13] but preparations for HSCT are often lengthy and appropriate stem cell donors may not be readily available. The main causes of poor outcome include progression to leukemia, high treatment-related toxicity and high relapse incidence of MDS or occurrence of MDR-AML after HSCT.[8] The administration of AML-type induction chemotherapy to children with RCEB prior to HSCT did not improve outcome.[13] However, the occurrence of progression to MDR-AML leads to reduction in survival by about 50%, and intensive pre-transplant chemotherapy is typically given, resulting in significant toxicity.[8] There is currently no pre-HSCT treatment that has been established in children with RCEB to prevent progression to MDR-AML, and patients are closely monitored and typically managed without any cytoreductive treatment prior to HSCT.

Aberrant methylation of critical genes was seen in adult and pediatric patients with advanced MDS, and is believed to be one of the driving alterations of the disease.[14,15] Implicated genes act as cell differentiation, cell cycle and cell growth regulators, or play roles in the stress response and apoptosis pathways. Hypermethylation leads to the silencing of regulatory genes and aberrant cell behavior. Furthermore, hypermethylation of the promoters of various genes, such as *p15*,[15,16] *DLX4*,[17] *p73*,[18] and *VTRNA1-3*,[19] has been associated with unfavorable prognosis in MDS.

Azacitidine (5-azacytidine, AZA) is a hypomethylating agent that has been approved for the treatment of MDS in adults. The mechanism of action of AZA is related to interference with DNA methyltransferases leading to DNA hypomethylation and subsequent cell cycle exit and differentiation of blast cells. High doses of AZA result in direct cytotoxicity.[20] Response to AZA was seen in adults with high-risk MDS after a median of 2-3 cycles with a maximum efficacy after 4-6 cycles.[21] AZA reduced the percentage of leukemic blasts in the bone marrow and the rate of transformation to MDR-AML, it also prolonged survival and improved quality of life.[22] In adult patients eligible for HSCT, treatment with AZA prior to HSCT improved survival[23-25] or resulted in similar outcomes,[26] compared to standard AML-type induction chemotherapy prior to HSCT. The short- and long-term side effect profile of AZA is better than that of AML-type chemotherapy.[25,27]

AZA prior to HSCT was shown to improve outcome in a smaller MDS cohort, including pediatric patients without separate evaluation of this subgroup.[25] In a single case report, AZA was shown to induce complete remission in a child with treatment-related MDS and signs of early relapse after HSCT, however, long-term follow-up was not reported.[28] The European Working Group of Myelodysplastic Syndromes in Childhood recently reported their experience with AZA in MDS and MDR-AML.[29] Their retrospective analysis comprised a heterogeneous group of children with low-grade MDS (refractory cytopenia of childhood), advanced MDS, secondary MDS, MDR-AML, and relapsed disease after HSCT. Among the seven patients with RAEB/-T in this report, only one was declared alive in remission at 24 months of follow-up; the remaining 6/7 patients died after 1-6 months. Notwithstanding this, AZA was given in 4/7 patients after relapse only, and half of those received AZA with palliative intent. Furthermore, there was no description of what the inclusion criteria were in order to offer AZA treatment. Finally, this study did not report details of the response criteria, such as changes in leukemic blast counts, and did not have a comparative analysis to a non-treated group.

Herein we report on the use of AZA in pediatric patients with RCEB prior to HSCT and compare their outcomes to patients who were not treated with AZA.

Methods

Patient population

All consecutive patients from 1 to 18 years of age who fulfilled the diagnostic criteria of pediatric refractory cytopenia with excess blasts (RCEB) according to the Category Cytology Cytogenetics (CCC) classification of childhood MDS,[3] and were treated, at least partially, at the Hospital for Sick Children, Toronto, Canada between January 1st 2000 and December 31st 2015, were identified. The definition of RCEB included the categories of "refractory anemia with excess blasts (RAEB)" and "refractory anemia with excess blasts in transformation (RAEB-T)" as defined by Hasle *et al.*[4] In summary, patients were included if they had bone marrow blast counts of 5-29% in addition to one or more of the following: (i) sustained unexplained cytopenia, (ii) prominent multilineage dysplasia, and (iii) acquired clonal cytogenetic abnormality in hematopoietic cells. Throughout the article the term RCEB is used for the patient population.

Patients with Down syndrome (DS)-related MDS were excluded since DS-related MDS is associated with different genetic alterations, and leads to more favorable outcomes after AML-type chemotherapy alone without proceeding to HSCT.[30] Patients deemed not eligible for HSCT at the time of evaluation for MDS treatment were also excluded.

The charts of all identified patients were retrospectively reviewed with a collection of demographic and clinical data, laboratory test results, and outcome. Bone marrow blast percentages as determined by morphological examination of bone marrow aspirate samples were included. Bone marrow morphology was assessed by expert hematopathologists at the Hospital for Sick Children, Toronto (n=18), or by expert hematopathologists of other Canadian centers for pediatric hematology and oncology (n=4), not blinded for diagnosis or treatment. Most patients also had flow cytometric evaluation. Response to treatment was assessed using the 2006 revision of the International Working Group (IWG) response criteria in myelodysplasia.[31] Bone marrow blast percentages at presentation, after AZA cycles, at progression if present, or before HSCT were used to assign remission status. Adverse events to administered treatment were graded using the common terminology criteria of adverse events score (CTCAE v4.0).[32] The study was approved by the institution's research ethics board and abided by the principles of the Declaration of Helsinki.

Statistical analysis

Results were presented using descriptive statistics, including calculation of the median and range for continuous variables, and the percentage for categorical variables. Overall survival (OS) was defined as the time from diagnosis of RCEB until death from any cause. Event-free survival (EFS) was defined as the time from diagnosis until evidence of progression to leukemia, relapse of MDS or leukemia after HSCT, or death from any cause. OS and EFS data were compared using the Kaplan-Meier estimate and Mantel-Cox log-rank test to assess statistical difference. Fisher's exact test was used to compare dichotomous variables, Mann-Whitney U test to compare continuous variables, and Wilcoxon signed-rank test to compare repeated measurements. A *P*-value of <0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism 6.0h.

Results

A total of 29 patients with a diagnosis of RCEB were identified during the 16-year period. Six patients with Down syndrome-associated MDS were excluded. One

Table 1. Characteristics and outcome of the patients included in the present study.

Patient number	Predisposition or preceding disease	Age (y) /sex	Cytopenias	BM cytogenetics	Pre-HSCT treatment	BM blasts at presentation/ before treatment (if present)/ prior to HSCT (%)	BM treatment response	Last follow-up from presentation (months)/ status
1	Idiopathic	9.2/ M	A/T/N	t(3;5)(q25;q34)	AZA: 3 cycles	10/ 14/ 6	Cycle 1: SD; cycle 3: PR	15.5/ alive in remission
2	Idiopathic	10.3/ F	A/T/N	Normal	AZA: 3 cycles	13/ 9/ 12	Cycle 3: SD	54.5/ alive in remission
3	Idiopathic	11.7/ F	A/N	Normal	AZA: 3 cycles	13/ 10/ 0	Cycle 1 and 2: CR	10.5/ alive in remission
4	Idiopathic	17.2/ F	A/T	-7,+8 at diagnosis; then complex	AZA: 1 cycle	28/ 28/ 5	Cycle 1: CR	50/ alive in remission
5	Prior chemotherapy	6.6/ F	A/T	t(1;7)(p10;q10), t(11;17)(p15;q21); then additional clone: t(X;1)(q21;q12)	AZA: 13 cycles	15/ 17/ 0	Cycle 1: SD; cycle 3-13: CR	40/ alive in remission
6	Germline *RUNX1* mutation	11.1/ M	A/T/N	Complex	AZA: 2 cycles	13/ 14/ 3	Cycle 1: SD; cycle 2: CR	25/ alive in remission
7	Unclassified syndrome	12.3/ F	A/T	Normal	AZA: 2 cycles	15/ 16/ 8	Cycle 2: PR	50/ alive in remission
8	HLH	11.5/ M	T/N	Normal	AZA: 4 cycles	15/ 31/ 6	Before cycle 1: PD; cycles 1 and 3: PR	7/ alive in remission
9	Idiopathic	1.3/ M	A/T	-7/ i(11)(q10)/ del(5)(q15q31)	AML-I: 2 cycles	7/ 12/ 0	CR	66/ alive in remission
10	Idiopathic	2/ F	A/T	Complex	None	18/ -/ 18	NA	81/ alive in remission
11	Idiopathic	6.8/ F	A/T	-7	None	10/ -/ 6	NA	108/ relapse after first HSCT, salvaged
12	Idiopathic	7/ M	A/T	Normal	None	11/ -/ 8	NA	7.5/ died, DRM
13	Idiopathic	8.8/ M	A/N	-7	None	13/ -/ NA	NA	111/ relapse after first HSCT, salvaged
14	Idiopathic	9.8/ F	A/T	-7	None	5/ -/ 5	NA	38.5/ died, DRM
15	Idiopathic	10.6/ F	A/T/N	Normal	None	8/ -/ 18	NA	98/ alive in remission
16	Idiopathic	13.7/ M	T/N	t(3;12)(q26.2;p13)	ARA-C: 2 weeks	16/ 22/ 21	SD	58/ alive in remission
17	Idiopathic	15.1/ M	A/T/N	del9(q13q22)	AML-I: 2 cycles	14/ 93/ 4	CR	14/ died, DRM
18	Prior chemotherapy	16.6/ F	A/N	-7,r(6)(p22-23q22)	None	7/ -/ 10	NA	26.5/ alive in remission
19	Germline *RUNX1* mutation	16.5/ F	A/T/N	-7	None	9/ -/ 9	NA	13.5/ alive in remission
20	Constitutional trisomy 8	10.3/ F	A/N	+8c,+8	None	22/ -/ 14	NA	53.5/ died, DRM
21	Hepatitis-associated SAA	13/ F	A/T	-7 at diagnosis; then complex	None	9/ -/ 3	NA	7.5/ died, TRM post-HSCT
22	Behçet disease	13.3/ F	T	+8	None	5/ -/ 5	NA	53/ alive in remission

A: anemia; AML-I: acute myeloid leukemia induction chemotherapy; ARA-C: low-dose cytarabine SC; AZA: azacitidine IV/ SC; BM: bone marrow; CR: complete marrow remission (blast decrease by ≥ 50% from baseline and reduction of blasts in the bone marrow to ≤5%); DRM: disease-related mortality; F: female; HLH: hemophagocytic lymphohistiocytosis; HSCT: hematopoietic stem cell transplantation; M: male; N: neutropenia; NA: not applicable; PD: progressive disease (blast increase by ≥ 50% from baseline or development of acute leukemia); PR: partial marrow response (blast decrease by ≥ 50% from baseline but >5% blasts); SD: stable disease; T: thrombocytopenia; TRM: treatment-related mortality; SAA: severe aplastic anemia.

additional patient was found to have multi-organ dysfunction following previous chemotherapies for a malignant solid tumor. The patient was deemed not to be in a condition to undergo HSCT at the time of initial evaluation and was excluded from our analysis. Ultimately, 22 patients were included in our analysis.

Azacitidine treatment

Since October 2010, off-label treatment with AZA was offered to all patients diagnosed with RCEB who did not have a matched related donor. Eight patients agreed to treatment with AZA and were compared to patients not having received this treatment (controls). Controls included children with RCEB before AZA was offered at the treating institutions (n=10), and after its introduction (n=4). The latter 4 patients were not treated with AZA due to family preference, drug coverage matters, or provision of pre-HSCT care at outside centers where AZA was not offered upfront. AZA was administered at a dose of 75mg/m^2 subcutaneously or intravenously for seven consecutive days every 28 days analogous to the dosing scheme in major studies for adult patients.[23,25,33] In 7/8 patients, pre-medication with ondansetron was given intravenously or orally prior to starting AZA, and concomitant IV hydration was administered. AZA treatment was started after a median of 41.5 days (range 19-99) from diagnosis and a median of 3 cycles (1-13) were administered.

Patient characteristics

Age at presentation, sex, and bone marrow blast percentages at presentation did not differ significantly between the two groups (Table 1). Time to HSCT was significantly longer in the group of patients who underwent treatment with AZA (*Online Supplementary Table S1*). One patient with treatment-related MDS was included in each group: one patient after autologous HSCT for a solid tumor and one after chemotherapy and radiotherapy for a lymphoma, respectively. One patient in the AZA treatment group, who had a previous short episode of hemophagocytic lymphohistiocytosis (HLH), had been treated successfully with corticosteroids alone and was in remission from HLH without ongoing treatment at the time of diagnosis of RCEB. A control group patient had Behçet disease and was treated with colchicine and corticosteroids at the time of diagnosis of RCEB.

Constitutional *RUNX1* mutations were identified in 1/5 tested AZA-treated and 1/5 tested control patients. One patient in the AZA treatment group was found to have a syndromic disease (not further classified) with short stature, developmental delay, and anhidrosis. Most patients were screened for Fanconi anemia by chromosome fragility testing in the blood or on skin fibroblasts. Of those tested, all were within the normal range (n=7 tested in the AZA treatment group and n=9 in the control group).

Hematological and bone marrow findings

Peripheral blood counts at diagnosis did not differ significantly between the two groups. Hypocellular bone marrow specimens (cellularity <50% of age-adjusted reference, n=1 in each group) and prominent dysplasia in at least two cell lineages were detected in a similar subgroup of patients (n=5 in the AZA-treated and n=8 in control patients). Two patients in the AZA treatment group had bone marrow findings consistent with acute erythroleukemia of mixed erythroid/myeloid subtype (AML M6a).[34] Both patients showed stable myeloid blast counts in repeated bone marrow aspirations within 21 days, and thus the decision was taken not to proceed to AML-type treatment but offer AZA treatment instead, as suggested previously by other authors.[35,36]

Four patients in the AZA treatment group and 12 patients in the untreated group had clonal marrow cytogenetic abnormalities. There were no significant group differences. Evolution of additional clones from the time of RCEB diagnosis to HSCT was seen in two patients in the AZA treatment group before the initiation of treatment and in one control after 16, 35, and 28 days, respectively.

Adverse events on azacitidine treatment

In total, 31 cycles of AZA were administered. Treatment was delayed or dose reduced in four cycles (Table 2). Severe bilineage cytopenia (CTCAE grade 4) led to a prolonged delay of treatment in one patient, and after one cycle the patient proceeded directly to HSCT with improved blood counts and markedly reduced blast percentage in the bone marrow (reduction from 28% to 5%). Nausea and vomiting (CTCAE grade 3) led to treatment delay after three cycles in one patient with subsequent HSCT. One patient with severe infection (appendicitis with abdominal abscess, CTCAE grade 4) already had evidence of severe neutropenia prior to the initiation of AZA treatment, and another patient had a transient increase in creatinine (CTCAE grade 2). Treatment with the full dose of AZA was resumed in both, the patient with severe infection and the patient with transient rise in creatinine, subsequently. Both patients did not show further major toxicity. All other adverse events were managed with standard supportive care and without modifications or a significant delay in AZA treatment.

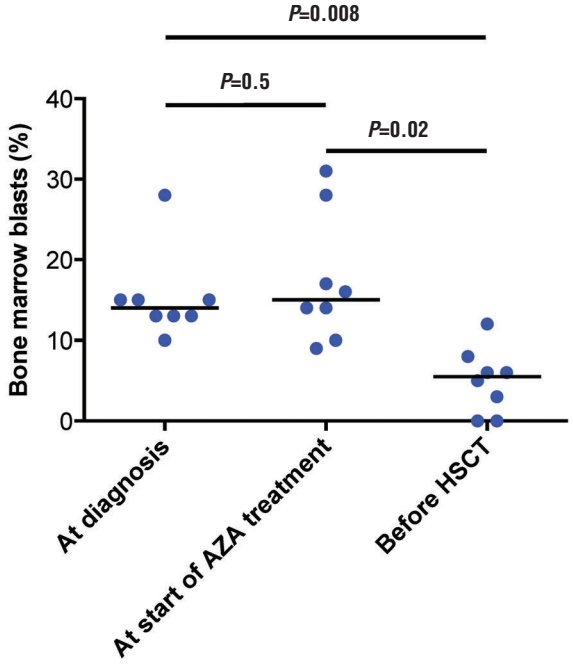


Figure 1. Bone marrow blast percentages at diagnosis, before start of azacitidine (AZA) treatment, and before hematopoietic stem cell transplantation (HSCT) in azacitidine-treated patients (n=8).

Table 2. Adverse events identified in patients treated with azacitidine (n=8).

	Highest CTCAE grade toxicity in a single patient			
	Grade 4	Grade 3	Grade 2	Grade 1
Hematological toxicity	2[†]		1	1
Fever and infection	1[†]			1
Nausea and vomiting		1[†]	1	
Urticaria and rash			2	
Acute kidney injury			1[†]	
Diarrhea			1	
Fatigue			1	
Insomnia			1	
Fluid overload with arterial hypertension			1	

[†]with treatment modification/delay (n=4, one patient with hematological toxicity and infection); CTCAE: common terminology criteria for adverse events.

Morphological response in the bone marrow

Of the AZA-treated patients, four (50%) achieved bone marrow remission (reduction in bone marrow blast counts by ≥50% from baseline and absolute bone marrow blasts ≤5% of nucleated cells) after a median of 2.5 cycles (range 1-6). Three patients (37.5%) had partial bone marrow response (reduction in bone marrow blast counts by ≥50% from baseline, but bone marrow blasts >5% of nucleated cells). One patient (12.5%) had stable disease, and none had progressive disease.

Among the controls, one patient did not have repeat bone marrow assessment of sufficient quality. Of the remaining 13, one patient (7.7%) with a history of hepatitis-associated severe aplastic anemia, immunosuppressive treatment, and intermittent G-CSF administration had a decline in bone marrow blasts from 9% to 3% after discontinuation of G-CSF without cytotoxic treatment; interestingly, this patient continued to have circulating blasts in the peripheral blood and the cytogenetic clones persisted in the bone marrow. Nine patients (69.2%) had stable disease, and three (23.1%) had progressive disease with an increase in marrow blast percentages (increase by ≥50% in bone marrow blast counts from baseline; n=2) or progression to MDR-AML (blast percentage in the bone marrow of >30%; n=1). One patient developed marked marrow erythroid hyperplasia with >20% myeloblasts in the non-erythroid precursors, and was diagnosed with AML M6a two weeks after presentation. This patient and the patient with progression to MDR-AML were subsequently treated with two cycles of AML-type induction chemotherapy, including intravenous cytarabine, daunorubicin, etoposide, and intrathecal cytarabine. Both patients achieved bone marrow morphological remission prior to HSCT. One control patient was treated with low-dose cytarabine (30mg/m^2/d SC) for five out of seven days per week. On repeat bone marrow testing before HSCT, blast counts were unchanged by morphological assessment but increased by flow cytometry.

Changes in bone marrow blast counts among patients treated with AZA were assessed. The comparison of blast percentages before starting AZA treatment and before HSCT showed a statistically significant reduction in blast counts for the AZA treatment group from a median of 15% (range 9-31%) to 5.5% (range 0-12%; Figure 1). Patients without AZA treatment had similar medians of bone marrow blast percentages at presentation (9%; range 5-22%) and before HSCT or at progression (10%; range 3-93%; *Online Supplementary Figure S1*).

Peripheral blood counts and bone marrow cytogenetic response

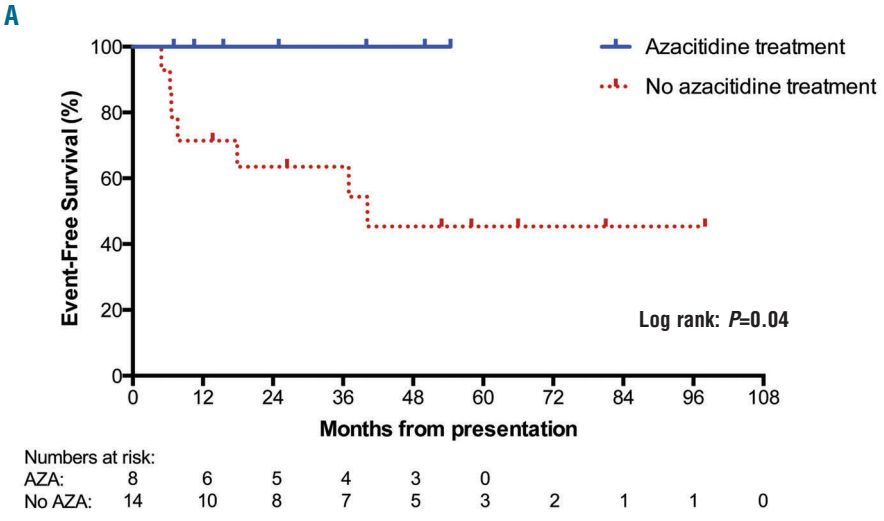
One patient was treated with AZA for 13 cycles in total. The patient became transfusion independent after six treatment cycles, and had morphological and complete cytogenetic remission (no clonal abnormalities detected with metaphase cytogenetics). In this case, the cytogenetic anomalies reappeared after cycle eleven; no increase in bone marrow blasts was seen. Hematological response was observed in two additional patients undergoing AZA treatment after two and three cycles respectively; one had an improvement in hemoglobin and platelet counts, and one showed normalization of absolute neutrophil counts from severe neutropenia at diagnosis. Despite a decrease in bone marrow blast percentages in four of the remaining five patients, no improvement in peripheral blood counts or transfusion requirements was seen after a median of three cycles of AZA (range 1-4). Clonal marrow cytogenetic abnormalities either persisted in these patients or information about repeat testing was not available.

Hematopoietic Stem Cell Transplantation

All patients included in this study proceeded to allogeneic HSCT. There was no statistical difference in the degree of HLA-matching and hematopoietic stem cell graft source between the groups (*Online Supplementary Table S1*). A majority of patients in both groups received busulfan-based myeloablative conditioning regimens with or without anti-thymocyte globulin, depending on the stem cell graft source. One patient in the AZA treatment group had primary engraftment failure after 5/8 mismatched unrelated cord blood transplantation. This patient proceeded to a second HSCT from a haploidentical parent, engrafted, and is alive and disease-free 15.5 months post diagnosis. No primary graft failures occurred in the control group. There were no significant differences in time to engraftment between the AZA treatment and control groups. One patient in the AZA treatment group was undergoing HSCT at the time of data analysis and did not have evaluable engraftment data available.

Survival

The estimated 4-year EFS was significantly higher in the AZA treatment group, with 100% of patients surviving in hematological remission at a median follow-up time of 32.5 months from diagnosis (range 7-54.4). EFS was 45.4%



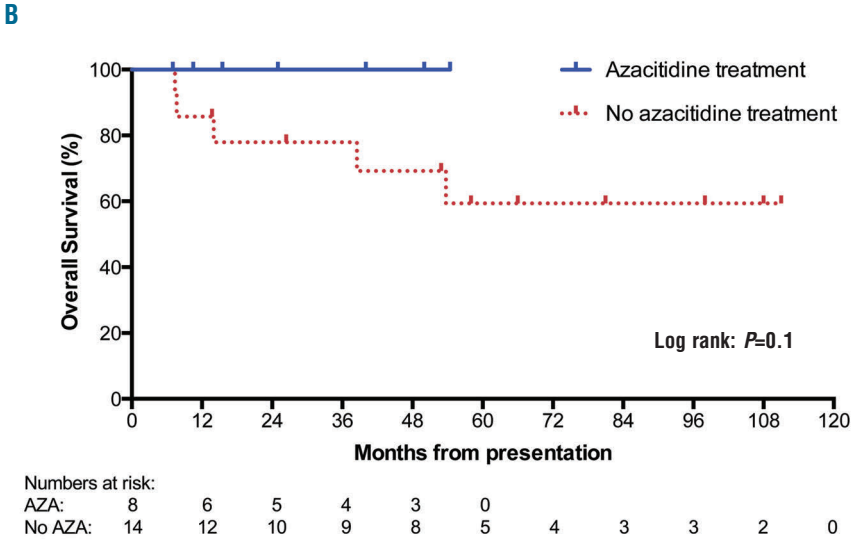


Figure 2. (A) Event-free survival of patients with azacitidine treatment compared to those without azacitidine treatment. (B) Overall survival of patients with azacitidine treatment compared to those without azacitidine treatment.

in control patients (P=0.04) and median time to event or last follow-up was 31.7 months (range 4.9-98; Figure 2A). Events in controls included progression to MDR-AML prior to HSCT (n=1), disease relapse, or progression to MDR-AML post HSCT (n=5), and treatment-related mortality (n=1). Two controls, who relapsed post-HSCT, were salvaged with AML-type induction chemotherapy followed by a second HSCT and are disease-free 108 and 111 months after diagnosis, respectively.

The estimated 4-year OS was 100% in the treatment group compared to 69.3% in the control group; however, the difference did not reach statistical significance (P=0.1, Figure 2B). We performed a subgroup analysis excluding all patients with matched related donors, which were only present in controls (n=9), and assessed OS and EFS compared to AZA-treated patients. The results were similar with significantly better EFS in AZA-treated patients (100% with AZA treatment *vs.* 40% without AZA treatment, P=0.03) with a trend towards better OS in patients with AZA treatment (100% with AZA treatment *vs.* 64.8% without AZA treatment, P=0.09).

Discussion

This study is the first to suggest that treatment with AZA prior to HSCT in pediatric patients with advanced MDS could decrease the percentage of bone marrow blasts in an important subset of patients. In our patient group this was associated with significantly improved 4-year EFS. The improved EFS may suggest that pre-HSCT treatment with AZA can reduce the incidence of relapse after HSCT without adding major toxicity.

AZA treatment was administered primarily in an outpatient setting and was well tolerated. Four patients needed treatment modifications and two of them resumed initial treatment dosage in subsequent courses without further delays or modifications. Thus, only two patients (25%) showed adverse events leading to prolonged treatment delay. All other adverse events were managed with standard measures (Table 2). This favorable side effect profile is in contrast to adverse events in AML induction chemotherapy, with typically high toxicity leading to grade 3 and 4 toxicities in about 80% of patients and treatment-related

mortality of around 10-20%.[37] Our findings are consistent with a recent report of good tolerability of AZA in 24 children and young adults with different types of MDS and MDR-AML.[29]

There was a trend towards higher bone marrow blast percentages at presentation in patients treated with AZA. A bias towards treating physicians favoring therapy with AZA in patients with higher blast counts at presentation cannot be completely excluded. The median blast percentage of patients included during the time period where AZA was available was not statistically different between the AZA-treated and untreated patients.

There was a trend towards more patients with chromosome 7 aberrations in the bone marrow in controls and a trend towards more normal cytogenetics in AZA-treated patients. Chromosome 7 or 7q abnormalities were not associated with poorer survival in pediatric MDS compared to other karyotypes in previous studies, which is in contrast to adult MDS.[38,39] Indeed, when we analyzed the differences in OS and EFS in controls with or without 7 or 7q abnormalities, we did not find significant differences. Normal cytogenetics were associated with better survival in one report from Japan[7] but not in a report from a European group.[6] In our series, the outcome of controls without cytogenetic changes (1/2 died of relapse) was not different from controls with cytogenetic abnormalities in the bone marrow. Complex karyotype is the main cytogenetic aberration associated with poor prognosis in children. Distribution in this regard was not different in the AZA treatment group compared to controls.

A significant reduction in median bone marrow blast percentages from diagnosis to HSCT was seen after AZA treatment. Half of the included patients achieved a morphological bone marrow remission prior to HSCT and another 37.5% of the children showed a partial response to AZA treatment, adding up to 87.5% responders. These are surprising results given the fact that a median of only three cycles were administered, and maximum response is expected in adults after four to six cycles.[21] Furthermore, two patients achieved complete marrow remission after only one cycle and another one after two cycles. Unless these differences are due to chance in a small cohort, these observations suggest that pediatric MDS may be more responsive to first-line azacitidine than adult MDS; possibly due to an absence of mutations in epigenetic genes. It is still debated if response to azacitidine treatment stems only from epigenetic changes or an additional cytotoxic effect. Recent data on chronic myelomonocytic leukemia in adult patients pointed towards epigenetic changes.[40] The only patient on long-term treatment with >4 cycles of azacitidine administered showed complete marrow and cytogenetic remission and became transiently transfusion-independent, but reappearance of the clone was detected after cycle eleven. In adults, approximately 60-70% of patients experienced partial response or complete remission after a median of four cycles of AZA.[21,26] These results suggest that AZA can induce an excellent bone marrow response in children, comparable to, or possibly better than in adults with advanced MDS.

EFS was significantly higher in the AZA treatment group. This was seen despite a trend towards a higher median percentage of blast cells in the bone marrow at presentation in the AZA treatment group and the longer time interval from diagnosis to HSCT, the latter having been previously associated with poorer survival.[13] OS showed similar trends towards better survival after AZA treatment, although not reaching statistical significance. In adults, survival was improved with AZA treatment compared to conventional care,[23] low-dose cytarabine prior to HSCT,[23] and was similar or possibly better than induction chemotherapy.[25,26] The increased EFS in our series in AZA-treated patients compared to no AZA treatment before HSCT was unexpected, since induction chemotherapy was not previously shown to positively influence the outcome in children unless they had progressed to MDR-AML.[8] The survival benefit might stem from the marked reduction in bone marrow blast loads without causing excess toxicity. It is also possible that AZA inhibits subclones that otherwise may contribute to relapse.

This study has several limitations. First, the study is retrospective and non-randomized. Therefore, we cannot exclude the non-random selection of patients. Nevertheless, there seems to be no major selection bias that would favor patients in the AZA treatment group. Particularly, patient characteristics were either similar (age at presentation, sex, and baseline hematological findings) or more favorable in control patients (shorter time to HSCT, trend towards lower blast counts at presentation, and less mismatched stem cell grafts). Second, all patients in the AZA group were treated after 2010, and only four patients that were not treated with AZA were cared for in the same time period. Thus, the possibility of improved outcome due to advances in HSCT donor selection and supportive care cannot be excluded. In this regard, it should be emphasized that HSCT protocols did not differ significantly between the AZA and the control group. Third, the differences in bone marrow cytogenetics might play a role in patient outcome, even though the differences were not statistically different. Subgroup analyses of patients affected with chromosome 7 or 7q abnormalities and normal cytogenetics did not show differences compared to other controls. Lastly, definite conclusions about drug efficacy should be drawn from prospective, randomized, multicenter trials with large numbers of patients; however, due to the rarity of pediatric advanced MDS, it is unlikely that an efficacy study of AZA would be feasible in a large number of children with RCEB.

In summary, this study suggests that introducing AZA as a bridging treatment while awaiting HSCT in advanced pediatric MDS is associated with a favorable side effect profile and reduction or stabilization of bone marrow blasts with remission induction in an important subset of patients. Our report shows for the first time that treatment with AZA could improve EFS in children with advanced MDS, and thus provides a basis to implement the use of AZA in pediatric patients with advanced MDS who are at risk of progression to MDR-AML and do not have a readily available bone marrow stem cell donor.

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