Azacitidine as epigenetic priming for chemotherapy is safe and well-tolerated in infants with newly diagnosed *KMT2A*-rearranged acute lymphoblastic leukemia: Children's Oncology Group trial AALL15P1

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Abstract

Infants less than 1 year old diagnosed with *KMT2A*-rearranged (*KMT2A*-r) acute lymphoblastic leukemia (ALL) are at high risk of failure to achieve remission, relapse, and death due to leukemia, despite intensive therapies. Infant *KMT2A*-r ALL blasts are characterized by DNA hypermethylation. Epigenetic priming with DNA methyltransferase inhibitors increases the cytotoxicity of chemotherapy in preclinical studies. The Children's Oncology Group trial AALL15P1 tested the safety and tolerability of 5 days of azacitidine treatment immediately prior to the start of chemotherapy on day 6, in four post-induction chemotherapy courses for infants with newly diagnosed *KMT2A*-r ALL. The treatment was well-tolerated, with only two of 31 evaluable patients (6.5%) experiencing dose-limiting toxicity. Whole genome bisulfite sequencing of peripheral blood mononuclear cells demonstrated decreased DNA methylation in 87% of samples tested following 5 days of azacitidine treatment. Event-free survival was similar to that in prior studies of newly diagnosed infant ALL. Azacitidine is safe and results in decreased DNA methylation of peripheral blood mononuclear cells in infants with *KMT2A*-r ALL, but the incorporation of azacitidine to enhance cytotoxicity did not impact survival. Clinicaltrials.gov identifier: NCT02828358.

Introduction

Acute lymphoblastic leukemia (ALL) with *KMT2A* rearrangement (*KMT2A*-r) in infants younger than 1 year of age is a high-risk subtype, with an historically poor event-free survival (EFS) of approximately 35% when treated with intensive chemotherapy with or without hematopoietic stem cell transplant (HSCT) in multinational cooperative group

trials.[1-4] Two recent trials have shown improved survival in comparison to historical outcomes. The Japanese Pediatric Leukemia/Lymphoma Study Group MLL-10 trial provided intensified chemotherapy, allocated high-risk patients (75% of *KMT2A*-r infants in the trial) to HSCT, and resulted in a 3-year EFS of 66.2% for infants with *KMT2A*-r.[5] A pilot trial of blinatumomab immunotherapy in combination with chemotherapy and HSCT, conducted by the Interfant study group, demonstrated safety and a promising efficacy signal, with substantial improvement in early disease-free survival.[6] Additional targeted therapies are needed to improve cure rates further and to reduce both the short- and long-term toxicities of chemotherapy and HSCT in infants.

Infant *KMT2A*-r ALL is challenging to treat because the leukemic blasts can develop rapid resistance to chemotherapy and relapses frequently occur early, often while infants are still receiving intensive therapies. Infant ALL blasts with *KMT2A*-r are characterized by unique biological features, including very few additional somatic genomic alterations,[7] overexpression of the receptor tyrosine kinase *FLT3*,[8,9] and global DNA hypermethylation.[10,11] Targeting of *FLT3* signaling with lestaurtinib did not lead to improved survival in the Children's Oncology Group (COG) trial AALL0631, although improved EFS was observed in a subset of infants with evidence of adequate plasma inhibition of *FLT3* activity.[1]

Epigenetic modification of DNA methylation is a treatment strategy with strong biological rationale and preclinical evidence of efficacy for treatment of *KMT2A*-r ALL. Global DNA hypermethylation is hypothesized to contribute to chemoresistance in infant ALL blasts by altering transcriptional regulation of gene expression.[10-13] Epigenetic therapy with DNA methyltransferase inhibition induces broad cell reprogramming by reactivation of tumor suppressor genes, has established efficacy in other hematologic malignancies, and is previously untested in infants with *KMT2A*-r ALL.[14,15] Azacitidine, a pyrimidine nucleoside analog of cytidine, is a DNA methyltransferase (DNMT) inhibitor that is approved by the Food and Drug Administration (FDA) for the treatment of acute myeloid leukemia, myelodysplastic syndrome, and juvenile myelomonocytic leukemia.[16-18] At higher doses, it induces DNA damage and is cytotoxic.[19] When used as monotherapy demethylating agents, DNMT inhibitors have been observed to induce rapid demethylation of specific tumor suppressor gene promoters, in addition to genome-wide demethylation of DNA.[14,15] In preclinical studies of *KMT2A*-r leukemia cell lines, epigenetic priming with a DNMT inhibitor – azacitidine, decitabine, or zebularine – has been shown to reverse the methylation pattern of silenced genes and induce selective toxicity for *KMT2A*-r cells.[20-22] In a mouse patient-derived xenograft model of *KMT2A*-r infant ALL, single-agent treatment with azacitidine or decitabine significantly prolonged survival.[21] Azacitidine or decitabine has been safely used as epigenetic priming prior to treatment with cytarabine, daunorubicin, and etoposide,[23] fludarabine and cytarabine,[24] and cytarabine alone[25] in children with hematologic malignancies. In combination studies, azacitidine or decitabine has been well tolerated when given with fludarabine, cytarabine, and vorinostat,[26,27] venetoclax,[28] doxorubicin and cyclophosphamide,[29] and amsacrine and etoposide[30,31] in the treatment of children with relapsed/refractory hematologic or solid tumor malignancies.

COG AALL15P1 (NCT02828358) was a single-arm, open label, groupwide pilot trial that tested the hypothesis that azacitidine in addition to standard chemotherapy would be well tolerated in infants with newly diagnosed *KMT2A*-r ALL. The major secondary aim of the trial was to evaluate the biological activity of azacitidine by pharmacodynamic assessment of global DNA methylation in peripheral blood mononuclear cells of infants treated with azacitidine. Estimation of 5-year EFS and correlation of EFS with minimal residual disease (MRD) following induction were exploratory aims, given the small sample size.

Methods

Eligibility

Eligibility criteria included a diagnosis of B-ALL or acute leukemia of ambiguous lineage with at least 50% B-lymphoblasts, less than 1 year of age at diagnosis, and greater than 36 weeks gestational age at enrollment.

Central nervous system (CNS) status was determined prior to the administration of any systemic or intrathecal che-

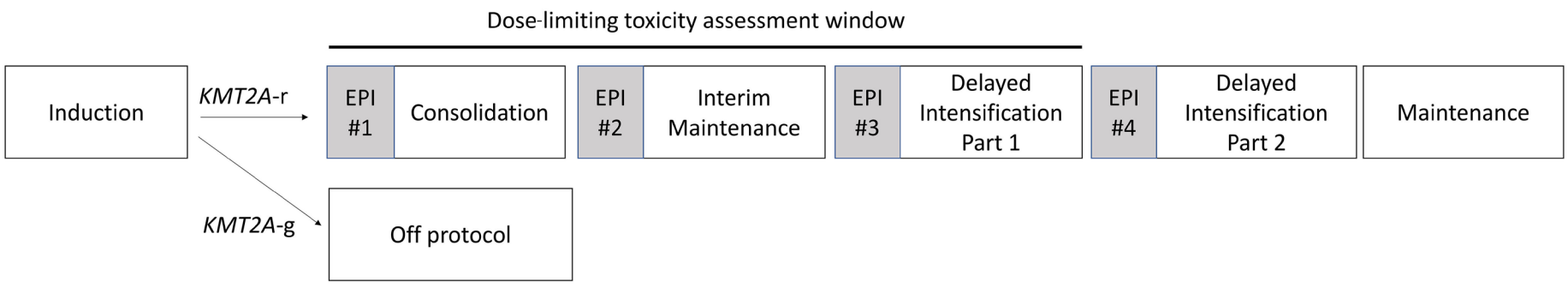

Figure 1. Treatment schema. Patients with *KMT2A*-r received four azacitidine (EPI) courses, each immediately prior to a chemotherapy course. The dose-limiting toxicity evaluation period extended from the start of EPI1 to the completion of Delayed Intensification Part 1 until the patient met parameters to begin EPI4.

Table 1. Patients' characteristics.

	KMT2A-r	*KMT2A*-g	P*
Total, N	56	22	
Age at diagnosis in days			
Median (range)	177 (1-342)	286 (64-357)	<0.001
Sex, N (%)			
Male	21 (37.5)	10 (45.5)	0.61
Female	35 (62.5)	12 (54.6)	
Race, N (%)			
White	40 (71.4)	15 (68.2)	0.46
Black or African American	3 (5.4)	2 (9.1)	
Asian	4 (7.1)	1 (4.5)	
American Indian	0 (0.0)	1 (4.5)	
Unknown	9 (16.1)	3 (13.6)	
Ethnicity, N (%)			
Hispanic or Latino	11 (19.6)	4 (18.2)	1.0
Not Hispanic or Latino	40 (71.4)	17 (77.3)	
Unknown	5 (8.9)	1 (4.6)	
WBC count at diagnosis, $x10^9$/L			
Median (range)	167.15 (3.2-1115.0)	22.65 (3.0-299.0)	<0.001
Diagnosis, N (%)			
B-lymphoblastic leukemia	51 (91.1)	22 (100.0)	0.31
Acute leukemia of ambiguous lineage	5 (8.9)	0 (0.0)	
CNS status, N (%)			
CNS1	19 (33.9)	13 (59.1)	0.09
CNS2	32 (57.1)	7 (31.8)	
CNS3	4 (7.1)	2 (9.1)	
Unknown	1 (1.8)	0 (0.0)	
KMT2A chromosomal partner, N (%)			
4q21	23 (41.1)	-	-
19p13.3	10 (17.9)	-	-
1p32	5 (8.9)	-	-
9p21	4 (7.1)	-	-
10p12	4 (7.1)	-	-
Unknown	10 (17.9)	-	-

*Wilcoxon rank sum test for continuous variables and Fisher exact tests for categorical frequency comparisons. *KMT2A*-r: *KMT2A*-rearrangement; *KMT2A*-g: *KMT2A*-germline; WBC: white blood cell; CNS: central nervous system.

motherapy. Fluorescence *in situ* hybridization testing of leukemia blasts, in a COG-approved laboratory, for *KMT2A*-r was required. Patients with known absence of *KMT2A*-r prior to enrollment, Down syndrome, treatment-related ALL, or prior cytotoxic therapy, with exceptions for corticosteroids and/or intrathecal chemotherapy, were excluded. The trial was approved by the Institutional Review Boards at participating COG member institutions and conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from parents or legal guardians according to federal and local regulations.

Treatment

Induction chemotherapy was based upon the induction of the COG predecessor trial, AALL0631, with a change from native L-asparaginase to pegaspargase. Following induction, infants with *KMT2A*-r received four courses of azacitidine (EPI), 2.5 mg/kg/dose intravenously over 10 to 40 minutes daily for 5 consecutive days, preceding the start of chemotherapy on day 6 (Figure 1). Interfant-06 standard chemotherapy was selected as the post-induction backbone (*Online Supplementary Table S1*), as it provided lower cumulative chemotherapy exposure than prior COG regimens and its outcomes were similar to those of COG P9407 and Interfant-99. Infants with *KMT2A* germline (*KMT2A*-g) ALL were not eligible to continue protocol therapy following induction and no data were collected regarding the treatment they received, but they were followed for events.

One dose level of azacitidine was tested. A step-down dose was planned in the event that the starting level exceeded the boundary of dose-limiting toxicity (DLT) (*Online Supplementary Tables S2* and *S3*). Adverse events were graded according to Common Terminology Criteria

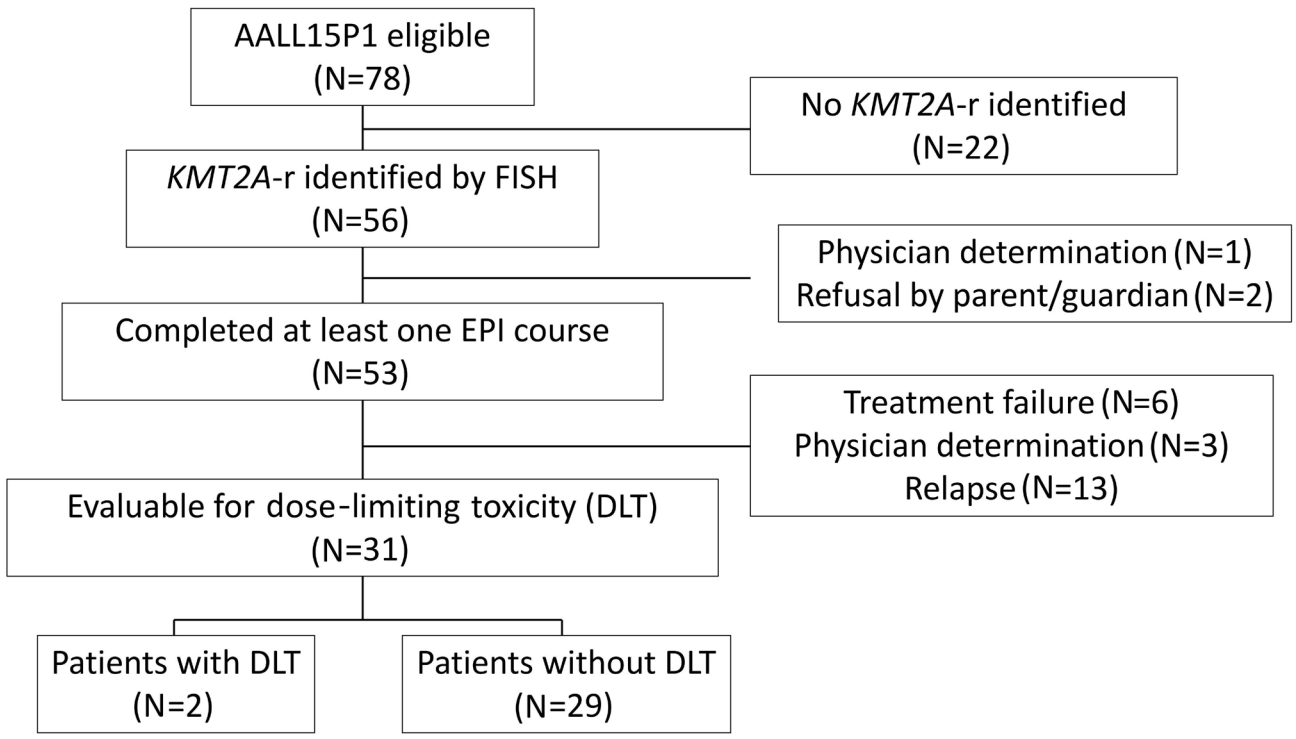

Figure 2. CONSORT (CONsolidated Standards of Reporting Trials) diagram. *KMT2A*-r: *KMT2A* rearranged; FISH: fluorescence *in situ* hybridization; EPI: azacitidine.

for Adverse Events (CTCAE) version 4.0. A DLT was defined as any grade 5 toxicity, or grade 3 or higher toxicity that led to omission of two or more doses of azacitidine in a 5-day course, a 4-week or greater delay in the start of the subsequent therapy course, or removal from protocol therapy. The DLT evaluation period consisted of the first three courses of azacitidine plus chemotherapy (Figure 1). MRD was measured by flow cytometry in COG-approved laboratories following induction, consolidation, and interim maintenance. Supportive care guidelines and pharmacodynamic assessment of DNA methylation are described in the *Online Supplementary Material*.

Statistics

The study required 30 infants with evaluable DLT assessment to meet its accrual goal to assess tolerability of azacitidine in combination with Interfant-06 standard chemotherapy. With continuous monitoring of DLT rates,[32] the study had 82.5%, 40.1%, and 4.3% probabilities of declaring the dose level as too toxic with true DLT rates of 30%, 20%, and 10%, respectively. Treatment failure was defined as failure to achieve M1 marrow status with resolution of extramedullary leukemia by the end of consolidation. EFS was defined as time from enrollment to first event (treatment failure, relapse, second malignant neoplasm, death) or censoring at the date of last contact. Overall survival (OS) was defined as time from enrollment to death or censoring at last contact. Estimates of EFS and OS were calculated using the Kaplan-Meier method with standard errors (SE) determined using the Peto formula. Two-sided log rank tests were used to compare survival between curves. Fisher exact tests were used to compare proportions and Wilcoxon rank-sum tests were used to compare distributions of continuous measures. One sample *t* tests were used to test for non-zero mean changes of CpG sites methylated. Statistical significance was defined as *P* values less than 0.05.

Results

Demographics

The study accrued patients from March 2017 to December 2019 and all protocol-directed treatment concluded in December 2021. Data frozen on December 31, 2022 are included in this report, with a median follow-up of 4.1 years. The trial was activated at 173 COG institutions and met expected accrual rates of 40 (actual rates of 41) infants per year. The initial accrual goal was 58 subjects on the starting dose level, to evaluate 30 infants for DLT. The study met the initial accrual goal in October 2018 and was temporarily closed. It re-opened in July 2019 to allow the enrollment of 20 additional subjects to replace subjects who went off protocol without completing the DLT window. No patients were ineligible.

The diagnostic clinical characteristics for all enrolled infants are shown in Table 1. Of the 78 infants enrolled, 56 (72%) had *KMT2A*-r lymphoblasts. Among those with *KMT2A*-r, additional baseline adverse prognostic features included: less than 7 days of age, four infants (7%); less than 90 days of age, 13 infants (23%); white blood cell counts ≥300,000/μL, 18 infants (32%); and CNS2 or CNS3 involvement, 36 infants (64%). The median age was younger, median white blood cell count higher, and CNS involvement more frequent in infants with *KMT2A*-r, in comparison to those with *KMT2A*-g. The most common *KMT2A* translocations identified were

Table 2. Reported toxicities, all grades.

Toxicities, N (%)	Induction *KMT2A*-g N=22	Induction *KMT2A*-r N=56	EPI1 N=53	Consol N=51	EPI2 N=38	IM N=37	EPI3 N=32	DI1 N=31	EPI4 N=30	DI2 N=30	Maint N=27
None	13 (59.1)	36 (64.3)	52 (98.1)	44 (86.3)	37 (97.4)	7 (18.9)	31 (96.9)	18 (58.1)	30 (100)	28 (93.3)	16 (59.3)
Blood and lymphatic system disorders	0 (0)	1 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cardiac disorders	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gastrointestinal disorders	1 (4.5)	3 (5.4)	0 (0)	0 (0)	0 (0)	13 (35.1)	0 (0)	3 (9.7)	0 (0)	0 (0)	1 (3.7)
Infections and infestations	6 (27.3)	13 (23.2)	0 (0)	5 (9.8)	1 (2.6)	15 (40.5)	1 (3.1)	9 (29.0)	0 (0)	2 (6.7)	6 (22.2)
Investigations	3 (13.6)	4 (7.1)	0 (0)	2 (3.9)	0 (0)	0 (0)	0 (0)	1 (3.2)	0 (0)	1 (3.3)	7 (25.9)
Metabolism and nutrition disorders	2 (9.1)	8 (14.3)	1 (1.9)	0 (0)	0 (0)	5 (13.5)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.7)
Musculoskeletal and connective tissue disorders	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.2)	0 (0)	0 (0)	0 (0)
Nervous system disorders	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Renal and urinary disorders	0 (0)	2 (3.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Respiratory, thoracic and mediastinal disorders	2 (9.1)	0 (0)	0 (0)	1 (2.0)	0 (0)	2 (5.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Skin and subcutaneous tissue disorders	0 (0)	1 (1.8)	0 (0)	0 (0)	0 (0)	1 (2.7)	0 (0)	1 (3.2)	0 (0)	0 (0)	0 (0)
Vascular disorders	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

KMT2A-g: KMT2A-germline; *KMT2A*-r: *KMT2A*-rearrangement; EPI: azacitidine course; Consol: consolidation; IM: interim maintenance; DI: delayed intensification; Maint: maintenance.

t(4;11)(q21;q23) and t(11;19)(p23;p13.3), representing 41% and 18% of *KMT2A*-r cases, respectively. Translocation partners 4q21 (*AFF1*) and 19p13.3 (*MLLT1*) were identified among both younger infants (less than 6 months old) and older infants (6 to 11 months old), while 10p12 (*MLLT10*), identified in four infants, was the only partner limited to infants younger than 6 months old (*Online Supplementary Figure S1*).

Safety

Of 53 infants with *KMT2A*-r who continued on study after induction, 31 completed at least three courses of azacitidine and were evaluable for DLT, and 22 were not evaluable for DLT (Figure 2).

Two infants (6.5%) experienced a DLT. The reported DLT were both grade 4 neutropenia associated with a greater than 4-week delay in therapy, one during consolidation and the other during delayed intensification. At no time did the DLT rate meet or exceed the pre-defined continuous stopping boundary.

Other than the two DLT, observed toxicities were within the expected range for infants receiving intensive ALL therapy. A review of the first five infants enrolled demonstrated delayed recovery periods following induction, without other excessive or unexpected adverse events. The trial was amended to extend the length of allowable induction recovery time from day 50 to day 64. The amendment also added blood cell count requirements (absolute neutrophil count ≥300/μL, platelets ≥30,000/μL) to begin cytarabine blocks in consolidation and delayed intensification.

Thirty-six (46.2%) infants experienced at least one grade 3+ infection, and infections occurred in nearly all blocks of chemotherapy (Table 2). Gastrointestinal disorders, including mucositis, and metabolism and nutrition disorders were common during interim maintenance. There were no grade 5 toxicities.

Pharmacodynamic assessment of DNA methylation

Protocol-required pre-azacitidine (day 1) and post-azacitidine (day 5) blood samples from both of the first two courses of azacitidine (EPI1 and EPI2) were submitted for 23 infants and were included in the pharmacodynamic assessment. Infants who went off protocol prior to completing at least two courses of azacitidine (N=18) and infants who completed EPI2 but did not submit all four blood samples (N=15) were excluded from the assessment. The reasons why some infants did not submit all required samples are unknown but are presumed to be related to site-specific sample collection and processing procedures. Whole genome bisulfite sequencing demonstrated demethylation

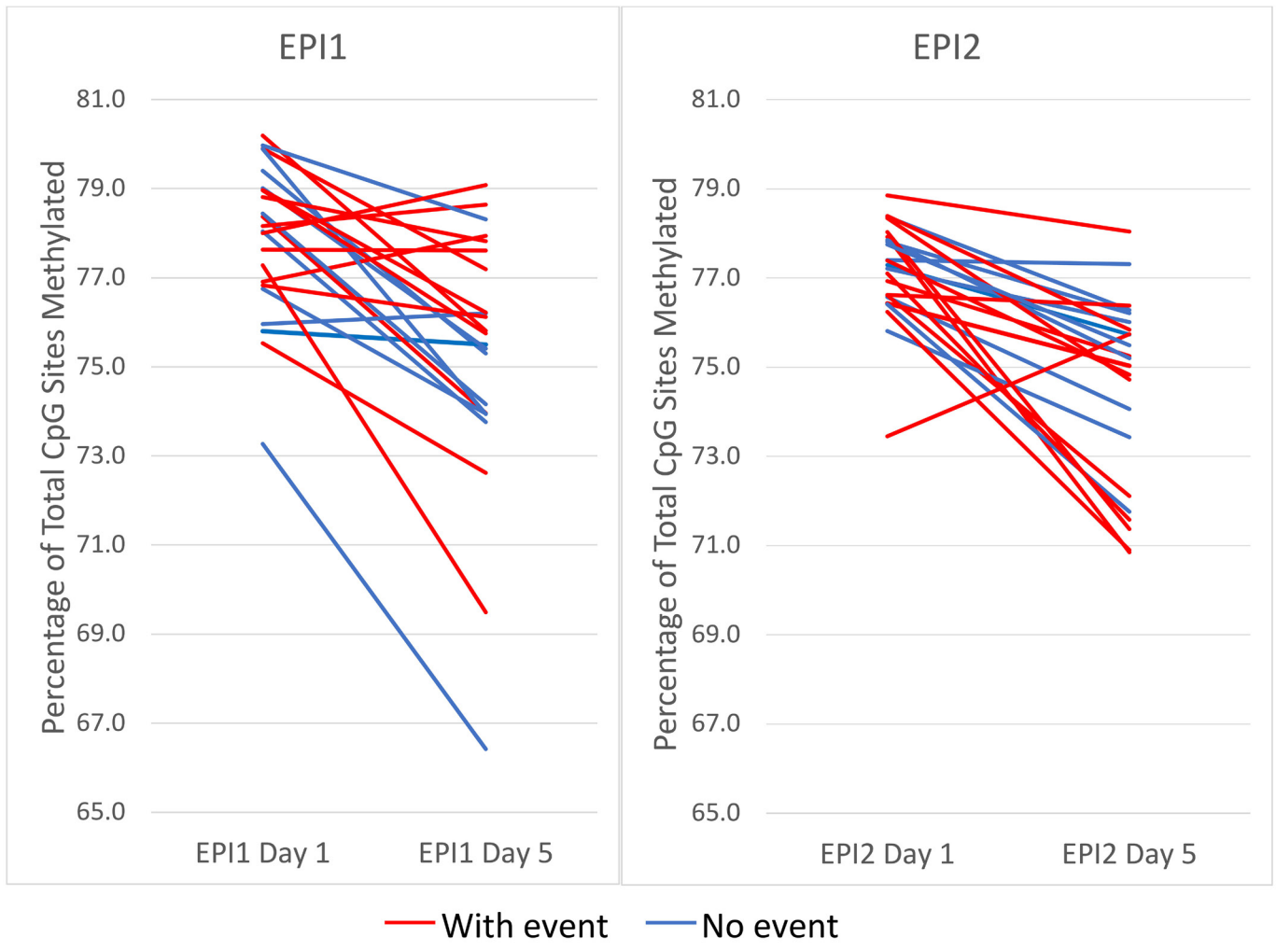

Figure 3. Whole genome bisulfite sequencing data. (A, B) Percentage of CpG sites methylated, measured by whole genome bisulfite sequencing, for EPI1 (A) and EPI2 (B). Twenty-three infants had samples submitted for both days 1 and 5 of both EPI1 and EPI2. Each line indicates an individual infant. Red indicates patients who experienced an event (treatment failure, relapse, second malignant neoplasm, or death) and blue indicates patients who did not experience an event. EPI1: first course of azacitidine treatment; EPI2: second course of azacitidine treatment.

in peripheral blood mononuclear cells following both EPI1 and EPI2 in 18 of the 23 infants. The mean percentage of CpG sites methylated before azacitidine was 77.9% in EPI1 (median 78.2%, range 73.3% to 80.2%) *versus* 77.2% in EPI2 (median 77.3%, range 73.5% to 78.9%) (P<0.001), and the mean number methylated after azacitidine was 75.3% in EPI1 (median 75.8%, range 66.4% to 79.1%) *versus* 74.5% in EPI2 (median 75.2%, range 70.9% to 78.0%) (P<0.001) (Figure 3). Decreased global methylation was detected in 40 of 46 (87%) EPI courses assessed (*Online Supplementary Figure S2*). Only one infant had no reduction in percentage of CpG sites methylated in either course. The mean absolute change in percentage of CpG sites methylated per infant was -2.6% in both EPI1 (median -2.8%, range: -7.8% to 1.1%) and EPI2 (median -2.4%, range: -7.1% to 2.3%). There were too few infants with pharmacodynamic assessments to relate the findings to disease outcomes.

Outcomes

There were 40 events among the 78 study participants, during therapy or in follow-up: six treatment failures, 31 relapses, one second malignant neoplasm, and two deaths as first events.

Thirty relapses were reported in infants with *KMT2A*-r with a median time to relapse of 0.7 years (range, 0.1-2.7 years). Of those, 16 were isolated bone marrow, ten isolated extramedullary, and four combined bone marrow and extramedullary. There were 23 deaths reported in total among study participants. The two deaths as first events were in infants who went off protocol therapy at the end of induction. One was an infant with *KMT2A*-g ALL who had an M2 marrow at the end of induction, received chemotherapy off protocol, and died following cord blood transplant. The other infant had *KMT2A*-r with severe multi-organ dysfunction related to treatment of sepsis, was removed from protocol at the treating physician's discretion and died of multi-organ failure approximately 4 months after initial diagnosis. Of the 21 post-event deaths, 19 were related to the leukemia and two were related to other causes: one due to progressive multi-system organ failure and one due to status epilepticus.

The 3-year EFS (±SE) and OS (±SE) rates for all eligible patients (*KMT2A*-r and *KMT2A*-g combined) were 47.9% (±0.06) and 71.6% (±0.06), respectively. For patients with *KMT2A*-r who received at least one dose of azacitidine (N=53), 3-year EFS and OS were 34.7% (±0.07) and 64.0% (±0.07), respectively. For patients with *KMT2A*-g (N=22), 3-year EFS and OS were 85.6% (±0.08) and 95.5% (±0.05), respectively (Figure 4A, B). MRD levels of marrow blasts by flow cytometry in COG-ap-

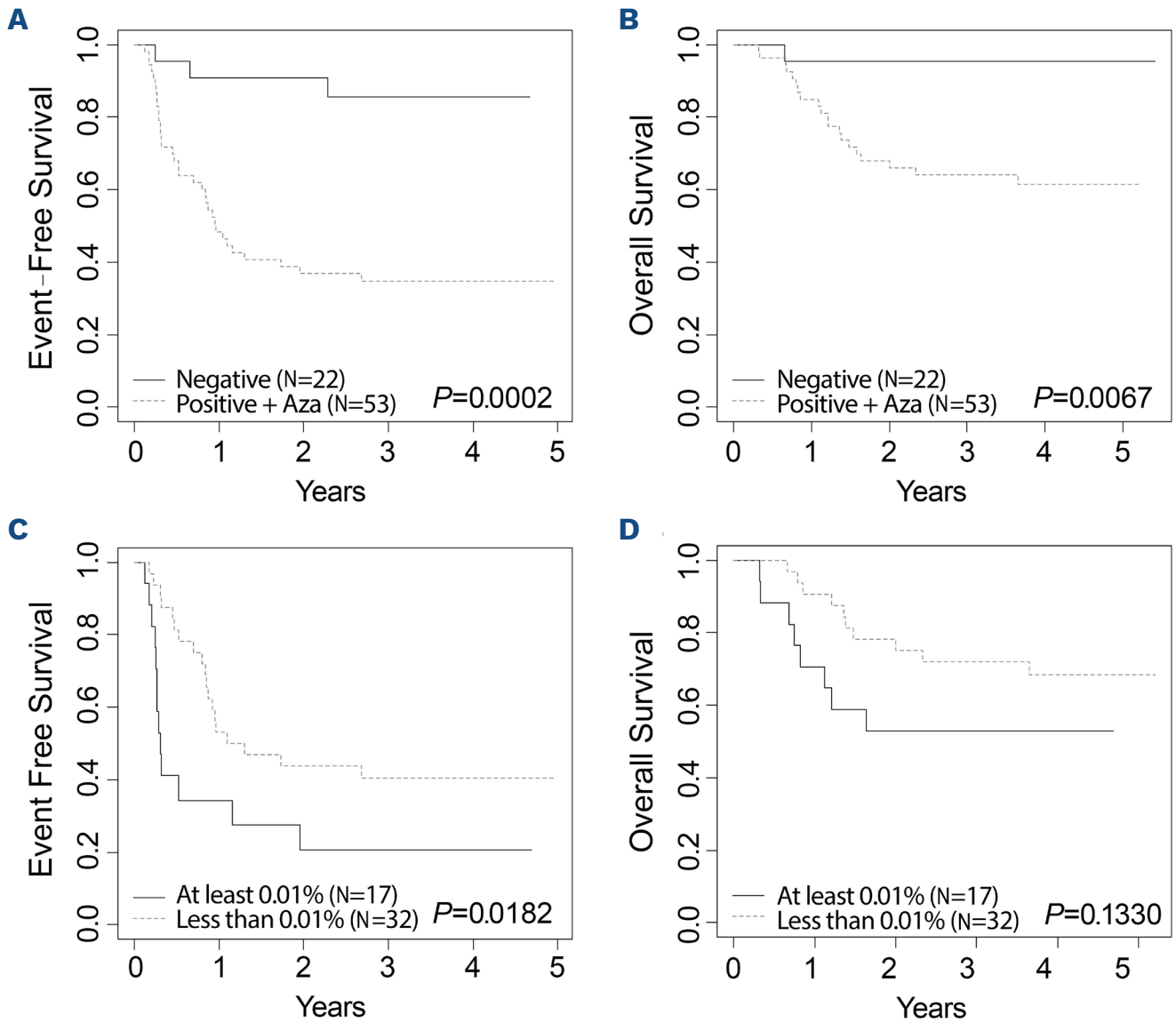

Figure 4. Outcomes for all patients and patients stratified by minimal residual disease status at the end of induction. (A, B) Event-free survival (A) and overall survival (B) for all eligible patients, stratified by *KMT2A*-rearrangement receiving azacitidine (Positive + Aza) and *KMT2A*-germline (Negative) status. (C, D) Event-free survival (C) but not overall survival (D) was significantly better for *KMT2A*-rearranged patients with negative minimal residual disease at the end of induction. Aza: azacitidine.

proved laboratories were determined for 49 *KMT2A*-r patients at the end of induction. Of those, 32 were MRD-negative at <0.01% (65%), eight were MRD-positive at 0.01% to <1% (16%) and nine were MRD-positive at ≥1% (18%). EFS was significantly associated with MRD; the 3-year EFS of patients with end-of-induction MRD ≥0.01% was 20.6% (±0.13) *versus* 40.4% (±0.09) (*P*=0.0182) for those with MRD <0.01% (Figure 4C, D). At the end of consolidation, 20 patients with data were MRD-negative (<0.01%) and seven were MRD-positive (≥0.01%), and at the end of interim maintenance, 22 patients were MRD-negative (<0.01%) and seven were MRD-positive (≥0.01%). There were no differences in EFS or OS for patients who were MRD-positive compared with patients who were MRD-negative at either the end of consolidation or the end of interim maintenance (*Online Supplementary Figures S3* and *S4*).

Discussion

Epigenetic priming with azacitidine prior to standard chemotherapy was well tolerated in infants with *KMT2A*-r ALL. Treatment with 5 days of azacitidine resulted in reduced DNA methylation of peripheral blood mononuclear cells in the majority of infants from whom samples were available. Despite evidence of pharmacodynamic response, the 3-year EFS results were consistent with the poor survival of historical outcomes with chemotherapy with or without HSCT, acknowledging that the study was not designed with sufficient power to detect a statistical improvement in survival. We conclude that azacitidine, despite demonstrating a global reduction in CpG site methylation in infants treated with the 2.5 mg/kg dose, would be unlikely to lead to improved survival in a larger study of the same treatment. Based upon other preclinical studies, it is plausible that azacitidine may have greater impact on *KMT2A*-r infant ALL outcomes if combined with synergistic agents, such as a BCL-2 inhibitor or histone deacetylase inhibitor.[21,33]

The pharmacodynamic change measured in peripheral blood mononuclear cells in response to azacitidine provides evidence of drug activity in infants at the administered dose. The trial did not pre-determine a benchmark for percentage of CpG sites with demethylation, as the degree of change in DNA methylation needed to target epigenetically regulated

genes in cancer cells is unknown.[14,34-36] In a preclinical study of hypomethylating agents in *KMT2A*-r infant ALL cell lines, treatment with either azacitidine or decitabine resulted in differential genome-wide methylation and alteration of global gene expression.[21] Although the degree of hypomethylation necessary for clinical response is undefined, the same study observed that *in vivo* efficacy of azacitidine in infant ALL mouse xenografts is dose-dependent, with a higher dose resulting in significantly longer survival.[21] In AALL15P1, the pharmacodynamic assessment was limited to the study of peripheral blood mononuclear cells because azacitidine was administered after induction. At the time of assessments, the majority of infants who submitted blood samples for pharmacodynamic testing and reported flow MRD results were MRD-negative (19 of 22 at the end of induction and 12 of 14 at the end of consolidation). Thus, methylation changes in the leukemia cells could not be measured directly. To better define the impact of azacitidine on cancer cell chemosensitivity in infants with ALL, both global and gene-specific methylation changes would ideally be measured in lymphoblasts, which would only be feasible in a clinical trial of hypomethylating agents in induction or in relapsed or refractory infant ALL.

The 3-year OS for *KMT2A*-r infants in AALL15P1 was superior to that in AALL0631, (AALL15P1 62.4% [SE 7.1] *vs*. AALL0631 42.8% [SE 4.1], *P*=0.034), despite similar 3-year EFS rates (AALL15P1 32.8% [SE 6.9] *vs*. AALL0631 35.6% [SE 4.0], *P*=0.194).[1] This trial did not prospectively collect treatment data for infants following disease-related events, so it remains unknown what salvage therapies were effective. Newer B-cell-directed immunotherapies, such as blinatumomab, inotuzumab ozogamicin, and chimeric antigen receptor T-cell therapies did not become widely available in the USA until FDA approval (blinatumomab in late 2014, tisagenlecleucel and inotuzumab ozogamicin [in adults only] in 2017).[37-39] AALL0631 completed accrual in 2014 and most infants who experienced relapse in that trial would not have had access to these potentially effective second-line therapies. Recent retrospective case series reports of blinatumomab or tisagenlecleucel in infants with relapsed or refractory ALL describe success with inducing remission and bridging to HSCT.[40,41]

Positive flow MRD at the end of induction predicted a higher risk of treatment failure in comparison to negative MRD. This finding is consistent with other trials of *KMT2A*-r infant ALL.[5,42-44] Considering that the survival of patients with negative MRD following induction, consolidation, and interim maintenance was still unacceptably poor, future trials should evaluate other MRD methodologies, such as high-throughput sequencing, to better classify response among infants. It is imperative to improve upon the sensitivity and predictive value of residual disease detection in infants, to facilitate allocation of infants at highest risk of treatment failure or relapse to novel therapies.

There remains an urgent need for improved therapies for infants with *KMT2A*-r ALL. In two consecutive trials, the COG tested new therapies with strong biological rationale: *FLT3* inhibition with lestaurtinib in combination with chemotherapy (AALL0631) and epigenetic priming with azacitidine (AALL15P1). Both treatment strategies were feasible and safe, but neither improved survival. In contrast, the Japanese MLL-10 study achieved remarkable success by intensifying chemotherapy, utilizing more stringent criteria for age-based dose reductions, and allocating more infants to HSCT, but this approach carries considerable risks of acute and long-term toxicities.[5] Anti-CD19 targeted immunotherapies, including blinatumomab and tisagenlecleucel, have been successfully used to induce remission in relapsed or refractory cases, and may represent a strategy to improve survival and reduce the use of cytotoxic agents.[40,41] As mentioned earlier, the Interfant study group published results from a pilot study of blinatumomab in combination with chemotherapy for newly diagnosed infants, demonstrating a dramatic improvement in 2-year disease-free survival.[6] Other investigational agents of interest include BCL-2 inhibitors and inhibitors of the menin-MLL protein-protein interaction.[21,45-50] The COG is developing phase II trials of venetoclax and blinatumomab on an Interfant-based chemotherapy backbone for newly diagnosed infant ALL, and revumenib in combination with chemotherapy for infants who fail to achieve remission or are in first relapse. The AALL15P1 trial concept of piloting a novel therapy on an Interfant-based chemotherapy backbone provides a model to design these and future clinical trials for infants with ALL.

Disclosures

The authors declare no competing financial interests in relation to the work described. EMG has served on advisory boards for Syndax and Jazz Pharmaceuticals. LG has served on unpaid advisory boards for Amgen, Janssen, Kura, Roche, Genentech, and Syndax. EAR has received institutional research funding from Pfizer and serves on a Data Safety and Monitoring Board for Bristol Myers Squibb. SPH has provided consulting for Novartis, received honoraria from Amgen, Jazz, and Servier, and owns common stock in Amgen. MLL has provided consulting for AbbVie. PAB is employed by and owns stock in Bristol Myers Squibb.

Contributions

The study was designed by EMG, JAK, LG, EAR, SPH, MLL, and PAB. The statistical design and analyses were performed by JAK, MD, and EH. The cytogenetics data were provided by AJC and NAH. The whole genome bisulfite sequencing design and analyses were performed by EMG, SR, BY, MSF, and PAB. EMG wrote the manuscript, with contributions from all authors. All authors gave final approval of the manuscript.

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Data-sharing statement

The Children's Oncology Group data-sharing policy describes the release and use of COG individual subject data for use in research projects in accordance with National Clinical Trials Network (NCTN) Program and National Cancer Institute (NCI) Community Oncology Research Program (NCORP) guidelines. Only data expressly released from the oversight of the relevant COG Data and Safety Monitoring Committee (DSMC) are available to be shared. Requests for access to COG protocol research data should be sent to: datarequest@childrensoncologygroup.org.

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