haematologica
Journal of the Ferrata Storti Foundation

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

by Erin M. Guest, John A. Kairalla, Meenakshi Devidas, Emily Hibbitts, Andrew J. Carroll, Nyla A. Heerema, Holly R. Kubaney, Margaret A. August, Sidharth Ramesh, Byunggil Yoo, Midhat S. Farooqi, Melinda G. Pauly, Daniel S. Wechsler, Rodney R. Miles, Joel M. Reid, Cynthia D. Kihei, Lia Gore, Elizabeth A. Raetz, Stephen P. Hunger, Mignon L. Loh, and Patrick A. Brown

Received: January 31, 2024.
Accepted: June 5, 2024.

Citation: Erin M. Guest, John A. Kairalla, Meenakshi Devidas, Emily Hibbitts, Andrew J. Carroll, Nyla A. Heerema, Holly R. Kubaney, Margaret A. August, Sidharth Ramesh, Byunggil Yoo, Midhat S. Farooqi, Melinda G. Pauly, Daniel S. Wechsler, Rodney R. Miles, Joel M. Reid, Cynthia D. Kihei, Lia Gore, Elizabeth A. Raetz, Stephen P. Hunger, Mignon L. Loh, and Patrick A. Brown. 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.
Haematologica. 2024 June 13. doi: 10.3324/haematol.2024.285158 [Epub ahead of print]

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

Erin M. Guest[1], John A. Kairalla[2], Meenakshi Devidas[3], Emily Hibbitts[2], Andrew J. Carroll[4], Nyla A. Heerema[5], Holly R. Kubaney[6], Margaret A. August[7], Sidharth Ramesh[8], Byunggil Yoo[9], Midhat S. Farooqi[10], Melinda G. Pauly[11], Daniel S. Wechsler[11], Rodney R. Miles[12], Joel M. Reid[13], Cynthia D. Kihei[14], Lia Gore[15], Elizabeth A. Raetz[16], Stephen P. Hunger[17], Mignon L. Loh[18], Patrick A. Brown[19]

[1]Division of Hematology, Oncology, Bone Marrow Transplant, Children's Mercy Kansas City, University of Missouri-Kansas City School of Medicine, Kansas City, MO, USA
[2]Department of Biostatistics, University of Florida, Gainesville, FL, USA
[3]St. Jude Children's Research Hospital, Memphis, TN, USA
[4]Department of Genetics, University of Alabama at Birmingham, Birmingham, AL, USA
[5]Department of Pathology, The Ohio State University, Columbus, OH, USA
[6]Dell Children's Medical Center of Central Texas, Austin, TX, USA
[7]Health Informatics and Technology, Children's Mercy Kansas City, Kansas City, MO, USA
[8]University of Pennsylvania, Philadelphia, PA, USA
[9]Research Informatics, Children's Mercy Kansas City, Kansas City, MO, USA
[10]Department of Pathology & Laboratory Medicine, Children's Mercy Kansas City, University of Missouri-Kansas City School of Medicine, Kansas City, MO, USA
[11]Aflac Cancer & Blood Disorders Center at Children's Healthcare of Atlanta and Emory University, Atlanta, GA, USA
[12] University of Utah, Department of Pathology, Salt Lake City, UT, USA
[13]Mayo Clinic, Rochester, MN, USA
[14]Saint Mary's Hospital, West Palm Beach, FL, USA
[15]Children's Hospital Colorado, Center for Cancer & Blood Disorders, Denver, CO, USA
[16]New York University Langone Health, New York, NY, USA
[17]Division of Oncology and Center for Childhood Cancer Research, Children's Hospital of Philadelphia, Philadelphia, PA, USA
[18]Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Institute and the Department of Pediatrics, Seattle Children's Hospital, University of Washington, Seattle, WA, 98105, USA
[19]Bristol Myers Squibb, Princeton, NJ, USA

Running head: Azacitidine in infant ALL

Corresponding author: Erin M. Guest, eguest@cmh.edu

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 data should be sent to: datarequest@childrensoncologygroup.org.

Word count: Abstract 173, Main text 3194, Tables 2, Figures 4, Supplementary files 1

Trial registration: ClinicalTrials.gov identifier: NCT02828358

Funding: This study was supported by National Institutes of Health (NIH) grants U10 CA180886 (NCTN Operations Center Grant), U10 CA98413 and U10 CA180899 (COG Statistics and Data Center Grants), St. Baldrick's Foundation, Noah's Bandage Project Foundation, and Alex's Lemonade Stand Foundation.

Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author 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 was 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.

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

Acknowledgements: We thank Dr. Mary Shago for providing expert input in the cytogenetic classification of cases.

ABSTRACT

Infants less than 1 year old diagnosed with *KMT2A*-rearranged (*KMT2A*-r) acute lymphoblastic leukemia (ALL) are at high risk of remission failure, 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 five days of azacitidine immediately prior to the start of chemotherapy on day six, 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 (PBMCs) demonstrated decreased DNA methylation in 87% of samples tested following five days of azacitidine. Event-free survival was similar to prior studies of newly diagnosed infant ALL. Azacitidine is safe and results in decreased DNA methylation of PBMCs 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 historically poor event-free survival (EFS) of approximately 35% when treated with intensive chemotherapy with or without hematopoietic stem cell transplant (HSCT) in multi-national 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 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 biologic 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, though 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 biologic 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 inhibitor (DNMTi) that is FDA approved for use 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 a monotherapy demethylating agent, DNMTi 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 DNMTi – 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 for 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] 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 biologic activity of azacitidine by pharmacodynamic assessment of global DNA methylation in peripheral blood mononuclear cells (PBMCs) 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 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 chemotherapy. Fluorescence in situ hybridization (FISH) testing of leukemia blasts for *KMT2A*-r was required in a COG-approved laboratory. 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 five consecutive days, preceding the start of chemotherapy on day six (Figure 1). Interfant-06 standard chemotherapy was selected as the post-induction backbone (Supplemental 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 was 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 the starting level exceeded the boundary of dose limiting toxicity (DLT) (Supplemental Tables S2 and S3). Adverse events were graded according to Common Terminology Criteria 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 five-day course, a four-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). Minimal residual disease (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 Supplemental 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 end of consolidation. EFS was defined as time from enrollment to first event (treatment failure, relapse, second malignant neoplasm, death) or censored at date of last contact. Overall survival (OS) was defined as time from enrollment to death or censored at last contact. Estimates of EFS and OS were calculated using the

Kaplan-Meier method with standard errors (SE) using Peto's formula. Two-sided log rank tests were used to compare survival between curves. Fisher's 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*-value 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 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 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.

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: four infants (7%) less than seven days of age, 13 (23%) less than 90 days of age, 18 (32%) with white blood cell count ≥300,000/μL, and 36 (64%) with CNS2 or CNS3 involvement. The median age was younger, median WBC 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 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 six months) and older infants (six to 11 months), while 10p12 (*MLLT10*), identified in four infants, was the only partner limited to infants younger than 6 months (Supplemental Figure S1).

Safety

Of 53 infants with *KMT2A*-r who continued on study after induction, 31 completed at least 3 courses of azacitidine and were evaluable for DLT, and 22 were inevaluable for DLT (Figure 2). Two infants (6.5%) experienced a DLT. The reported DLTs were both grade 4 neutropenia associated with a greater than four-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 DLTs, 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 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 in PBMCs following both EPI1 and EPI2 in 18 of the 23 infants. The mean percentage of CpG sites methylated pre-azacitidine was 77.9% in EPI1 (median 78.2%, range 73.3% to 80.2%) vs. 77.2% in EPI2 (median 77.3%, range 73.5% to 78.9%) ($p<0.001$), and the mean number methylated post-azacitidine was 75.3% in EPI1 (median 75.8%, range 66.4% to 79.1%) vs. 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 (Supplemental 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 assessment to relate the findings with 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 median (range) of time to relapse 0.7 (0.1-2.7) years. Of those, 16 were isolated bone marrow, 10 isolated extramedullary, and 4 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 per the treating physician's discretion and died of multi-organ failure approximately four months after initial diagnosis. Of the 21 deaths post-event, 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 and 4B).

Minimal residual disease (MRD) levels of marrow blasts by flow cytometry in COG-approved laboratories were submitted for 49 *KMT2A*-r patients at the end of induction. Of those, 32 were MRD negative <0.01% (65%), eight MRD positive 0.01% to <1% (16%) and nine MRD positive ≥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) vs. 40.4% (+/-0.09) (p=0.0182) for those with MRD <0.01% (Figure 4C and 4D). At end of consolidation, 20 patients with data were MRD negative (<0.01%) and 7 were MRD positive (≥0.01%), and at end of interim maintenance, 22 patients were MRD negative (<0.01%) and 7 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 end of consolidation or end of interim maintenance (Supplemental Figures S3 and S4).

DISCUSSION

Epigenetic priming with azacitidine prior to standard chemotherapy was well tolerated in infants with *KMT2A*-r ALL. Treatment with five days of azacitidine resulted in reduced DNA methylation of PBMCs in the majority of infants with samples 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 PMBCs 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 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] Though 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 PBMCs because azacitidine was administered post-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 end of induction and 12 of 14 at end of consolidation). Thus, methylation changes in the leukemia cells were not able to 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 of 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 U.S. until FDA approvals (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 hematopoietic stem cell transplant.[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 biologic 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 2 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.

REFERENCES

1. Brown PA, Kairalla JA, Hilden JM, et al. FLT3 inhibitor lestaurtinib plus chemotherapy for newly diagnosed KMT2A-rearranged infant acute lymphoblastic leukemia: Children's Oncology Group trial AALL0631. Leukemia. 2021;35(5):1279-1290.
2. Pieters R, De Lorenzo P, Ancliffe P, et al. Outcome of Infants Younger Than 1 Year With Acute Lymphoblastic Leukemia Treated With the Interfant-06 Protocol: Results From an International Phase III Randomized Study. J Clin Oncol. 2019;37(25):2246-2256.
3. Pieters R, Schrappe M, De Lorenzo P, et al. A treatment protocol for infants younger than 1 year with acute lymphoblastic leukaemia (Interfant-99): an observational study and a multicentre randomised trial. Lancet. 2007;370(9583):240-250.
4. Dreyer ZE, Hilden JM, Jones TL, et al. Intensified chemotherapy without SCT in infant ALL: Results from COG P9407 (Cohort 3). Pediatr Blood Cancer. 2015;62(3):419-426.
5. Tomizawa D, Miyamura T, Imamura T, et al. A risk-stratified therapy for infants with acute lymphoblastic leukemia: a report from the JPLSG MLL-10 trial. Blood. 2020;136(16):1813-1823.
6. van der Sluis IM, de Lorenzo P, Kotecha RS, et al. Blinatumomab Added to Chemotherapy in Infant Lymphoblastic Leukemia. N Engl J Med. 2023;388(17):1572-1581.
7. Andersson AK, Ma J, Wang J, et al. The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. Nat Genet. 2015;47(4):330-337.
8. Armstrong SA, Kung AL, Mabon ME, et al. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. Cancer Cell. 2003;3(2):173-183.
9. Stam RW, den Boer ML, Schneider P, et al. Targeting FLT3 in primary MLL-gene-rearranged infant acute lymphoblastic leukemia. Blood. 2005;106(7):2484-2490.
10. Schafer E, Irizarry R, Negi S, et al. Promoter hypermethylation in MLL-r infant acute lymphoblastic leukemia: biology and therapeutic targeting. Blood. 2010;115(23):4798-4809.
11. Stumpel DJ, Schneider P, van Roon EH, et al. Absence of global hypomethylation in promoter hypermethylated Mixed Lineage Leukaemia-rearranged infant acute lymphoblastic leukaemia. Eur J Cancer. 2013;49(1):175-184.
12. Bergmann AK, Castellano G, Alten J, et al. DNA methylation profiling of pediatric B-cell lymphoblastic leukemia with KMT2A rearrangement identifies hypomethylation at enhancer sites. Pediatr Blood Cancer. 2017;64(3).
13. Stumpel DJ, Schneider P, van Roon EH, et al. Specific promoter methylation identifies different subgroups of MLL-rearranged infant acute lymphoblastic leukemia, influences clinical outcome, and provides therapeutic options. Blood. 2009;114(27):5490-5498.
14. Jones PA, Issa JP, Baylin S. Targeting the cancer epigenome for therapy. Nat Rev Genet. 2016;17(10):630-641.
15. Sigalotti L, Fratta E, Coral S, et al. Epigenetic drugs as pleiotropic agents in cancer treatment: biomolecular aspects and clinical applications. J Cell Physiol. 2007;212(2):330-344.

16. Niemeyer CM, Flotho C, Lipka DB, et al. Response to upfront azacitidine in juvenile myelomonocytic leukemia in the AZA-JMML-001 trial. Blood Adv. 2021;5(14):2901-2908.
17. Waespe N, Van Den Akker M, Klaassen RJ, et al. Response to treatment with azacitidine in children with advanced myelodysplastic syndrome prior to hematopoietic stem cell transplantation. Haematologica. 2016;101(12):1508-1515.
18. Cseh AM, Niemeyer CM, Yoshimi A, et al. Therapy with low-dose azacitidine for MDS in children and young adults: a retrospective analysis of the EWOG-MDS study group. Br J Haematol. 2016;172(6):930-936.
19. Pechalrieu D, Etievant C, Arimondo PB. DNA methyltransferase inhibitors in cancer: From pharmacology to translational studies. Biochem Pharmacol. 2017;129:1-13.
20. Stumpel DJ, Schotte D, Lange-Turenhout EA, et al. Hypermethylation of specific microRNA genes in MLL-rearranged infant acute lymphoblastic leukemia: major matters at a micro scale. Leukemia. 2011;25(3):429-439.
21. Cheung LC, Aya-Bonilla C, Cruickshank MN, et al. Preclinical efficacy of azacitidine and venetoclax for infant KMT2A-rearranged acute lymphoblastic leukemia reveals a new therapeutic strategy. Leukemia. 2023;37(1):61-71.
22. Nishi M, Eguchi-Ishimae M, Wu Z, et al. Suppression of the let-7b microRNA pathway by DNA hypermethylation in infant acute lymphoblastic leukemia with MLL gene rearrangements. Leukemia. 2013;27(2):389-397.
23. Gore L, Triche TJ Jr, Farrar JE, et al. A multicenter, randomized study of decitabine as epigenetic priming with induction chemotherapy in children with AML. Clin Epigenetics. 2017;9:108.
24. Sun W, Triche T, Jr., Malvar J, et al. A phase 1 study of azacitidine combined with chemotherapy in childhood leukemia: a report from the TACL consortium. Blood. 2018;131(10):1145-1148.
25. Kearns P, Zwaan CM, Reinhardt D, et al. Phase 1-2 safety, efficacy and pharmacokinetic study of decitabine in sequential administration with cytarabine in children with relapsed or refractory acute myeloid leukaemia. Br J Haematol. 2019;186(3):e7-e11.
26. Pommert L, Schafer ES, Malvar J, et al. Decitabine and vorinostat with FLAG chemotherapy in pediatric relapsed/refractory AML: Report from the therapeutic advances in childhood leukemia and lymphoma (TACL) consortium. Am J Hematol. 2022;97(5):613-622.
27. Schafer ES, Chao K, Stevens AM, et al. Real-world experience in treating pediatric relapsed/refractory or therapy-related myeloid malignancies with decitabine, vorinostat, and FLAG therapy based on a phase 1 study run by the TACL consortium. Pediatr Blood Cancer. 2022;69(10):e29812.
28. Winters AC, Maloney KW, Treece AL, et al. Single-center pediatric experience with venetoclax and azacitidine as treatment for myelodysplastic syndrome and acute myeloid leukemia. Pediatr Blood Cancer. 2020;67(10):e28398.
29. George RE, Lahti JM, Adamson PC, et al. Phase I study of decitabine with doxorubicin and cyclophosphamide in children with neuroblastoma and other solid tumors: a Children's Oncology Group study. Pediatr Blood Cancer. 2010;55(4):629-638.

30. Look AT, Dahl GV, Kalwinsky D, et al. Effective remission induction of refractory childhood acute nonlymphocytic leukemia by VP-16-213 plus azacitidine. Cancer Treat Rep. 1981;65(11-12):995-999.
31. Steuber CP, Krischer J, Holbrook T, et al. Therapy of refractory or recurrent childhood acute myeloid leukemia using amsacrine and etoposide with or without azacitidine: a Pediatric Oncology Group randomized phase II study. J Clin Oncol. 1996;14(5):1521-1525.
32. Ivanova A, Qaqish BF, Schell MJ. Continuous toxicity monitoring in phase II trials in oncology. Biometrics. 2005;61(2):540-545.
33. Bhatla T, Wang J, Morrison DJ, et al. Epigenetic reprogramming reverses the relapse-specific gene expression signature and restores chemosensitivity in childhood B-lymphoblastic leukemia. Blood. 2012;119(22):5201-5210.
34. Kagan AB, Garrison DA, Anders NM, et al. DNA methyltransferase inhibitor exposure-response: Challenges and opportunities. Clin Transl Sci. 2023;16(8):1309-1322.
35. Fandy TE, Herman JG, Kerns P, et al. Early epigenetic changes and DNA damage do not predict clinical response in an overlapping schedule of 5-azacytidine and entinostat in patients with myeloid malignancies. Blood. 2009;114(13):2764-2773.
36. Voso MT, Santini V, Fabiani E, et al. Why methylation is not a marker predictive of response to hypomethylating agents. Haematologica. 2014;99(4):613-619.
37. Przepiorka D, Ko CW, Deisseroth A, et al. FDA Approval: Blinatumomab. Clin Cancer Res. 2015;21(18):4035-4039.
38. O'Leary MC, Lu X, Huang Y, et al. FDA Approval Summary: Tisagenlecleucel for Treatment of Patients with Relapsed or Refractory B-cell Precursor Acute Lymphoblastic Leukemia. Clin Cancer Res. 2019;25(4):1142-1146.
39. Wynne J, Wright D, Stock W. Inotuzumab: from preclinical development to success in B-cell acute lymphoblastic leukemia. Blood Adv. 2019;3(1):96-104.
40. Moskop A, Pommert L, Baggott C, et al. Real-world use of tisagenlecleucel in infant acute lymphoblastic leukemia. Blood Adv. 2022;6(14):4251-4255.
41. Clesham K, Rao VN, Bartram J, et al. Blinatumomab for Infant Acute Lymphoblastic Leukaemia. Blood. 2020;135(17):1501-1504.
42. Faulk KE, Kairalla JA, Dreyer ZE, et al. Minimal residual disease predicts outcomes in KMT2A-rearranged but not KMT2A-germline infant acute lymphoblastic leukemia: Report from Children's Oncology Group study AALL0631. Pediatr Blood Cancer. 2023:e30467.
43. Van der Velden VH, Corral L, Valsecchi MG, et al. Prognostic significance of minimal residual disease in infants with acute lymphoblastic leukemia treated within the Interfant-99 protocol. Leukemia. 2009;23(6):1073-1079.
44. Stutterheim J, van der Sluis IM, de Lorenzo P, et al. Clinical Implications of Minimal Residual Disease Detection in Infants With KMT2A-Rearranged Acute Lymphoblastic Leukemia Treated on the Interfant-06 Protocol. J Clin Oncol. 2021;39(6):652-662.
45. Robinson BW, Behling KC, Gupta M, et al. Abundant anti-apoptotic BCL-2 is a molecular target in leukaemias with t(4;11) translocation. Br J Haematol. 2008;141(6):827-839.

46. Benito JM, Godfrey L, Kojima K, et al. MLL-Rearranged Acute Lymphoblastic Leukemias Activate BCL-2 through H3K79 Methylation and Are Sensitive to the BCL-2-Specific Antagonist ABT-199. Cell Rep. 2015;13(12):2715-2727.
47. Khaw SL, Suryani S, Evans K, et al. Venetoclax responses of pediatric ALL xenografts reveal sensitivity of MLL-rearranged leukemia. Blood. 2016;128(10):1382-1395.
48. Krivtsov AV, Evans K, Gadrey JY, et al. A Menin-MLL Inhibitor Induces Specific Chromatin Changes and Eradicates Disease in Models of MLL-Rearranged Leukemia. Cancer Cell. 2019;36(6):660-673.
49. Issa GC, Aldoss I, DiPersio J, et al. The menin inhibitor revumenib in KMT2A-rearranged or NPM1-mutant leukaemia. Nature. 2023;615(7954):920-924.
50. Aldoss I, Ghayas CI, Thirman M, et al. Revumenib Monotherapy in Patients with Relapsed/Refractory KMT2Ar Acute Leukemia: Topline Efficacy and Safety Results from the Pivotal Augment-101 Phase 2 Study. Blood. 2023;142(Supplement 2):LBA-5.

Table 1. Patient Characteristics

	KMT2A-r	*KMT2A*-g	*P**
Total, N	56	22	
Age at diagnosis			
Median age (range)	177d (1 to 342d)	286d (64 to 357d)	<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 (cells x 10^9/L)			
Median (range)	167.15 (3.2 to 1115.0)	22.65 (3.0 to 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's Exact Tests for categorical frequency comparisons

Abbreviations: *KMT2A*-r, *KMT2A*-rearrangement; *KMT2A*-g, *KMT2A*-germline; d, days; WBC, white blood cell; CNS, central nervous system

Table 2. Reported toxicities, all grades

	Induction *KMT2A*-g (n=22) N (%)	Induction *KMT2A*-r (n=56) N (%)	EPI1 (n=53) N (%)	Consol (n=51) N (%)	EPI2 (n=38) N (%)	IM (n=37) N (%)	EPI3 (n=32) N (%)	DI1 (n=31) N (%)	EPI4 (n=30) N (%)	DI2 (n=30) N (%)	Maint (n=27) N (%)
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)

Abbreviations: *KMT2A*-g, *KMT2A*-germline; *KMT2A*-r, *KMT2A*-rearrangement; EPI, azacitidine course; Consol, Consolidation; IM, Interim Maintenance; DI, Delayed Intensification; Maint, Maintenance

FIGURE LEGENDS

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.

Figure 2. CONSORT diagram

Figure 3. Whole genome bisulfite sequencing data
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.

Figure 4. Outcomes for all patients and stratified by MRD status at the end of induction
Event free (4A) and overall (4B) survival for all eligible patients, stratified by *KMT2A*-r receiving azacitidine (Positive + Aza) and *KMT2A*-g (Negative). Event free (4C) but not overall (4D) survival was significantly better for *KMT2A*-r patients with negative Minimal Residual Disease (MRD) at the end of Induction.

Figure 1.

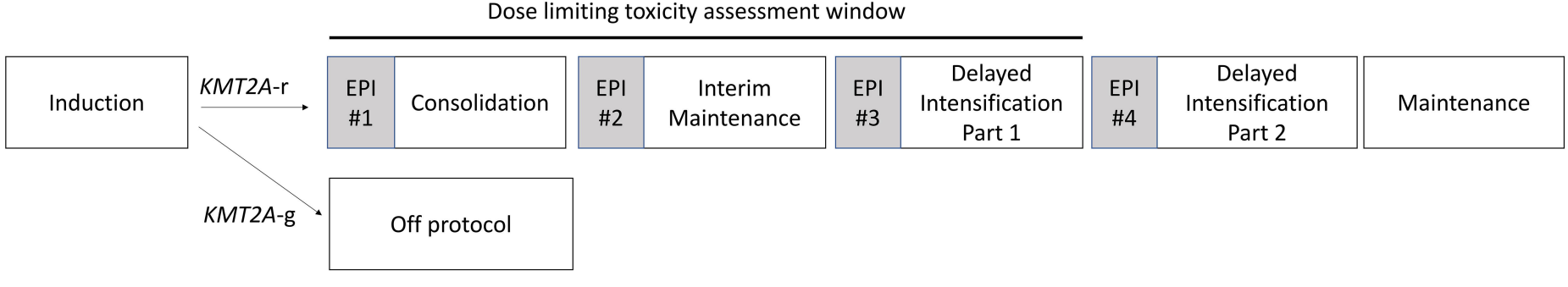

Dose limiting toxicity assessment window
Induction
KMT2A-r
KMT2A-g
EPI #1
Consolidation
EPI #2
Interim Maintenance
EPI #3
Delayed Intensification Part 1
EPI #4
Delayed Intensification Part 2
Maintenance
Off protocol

Figure 2.

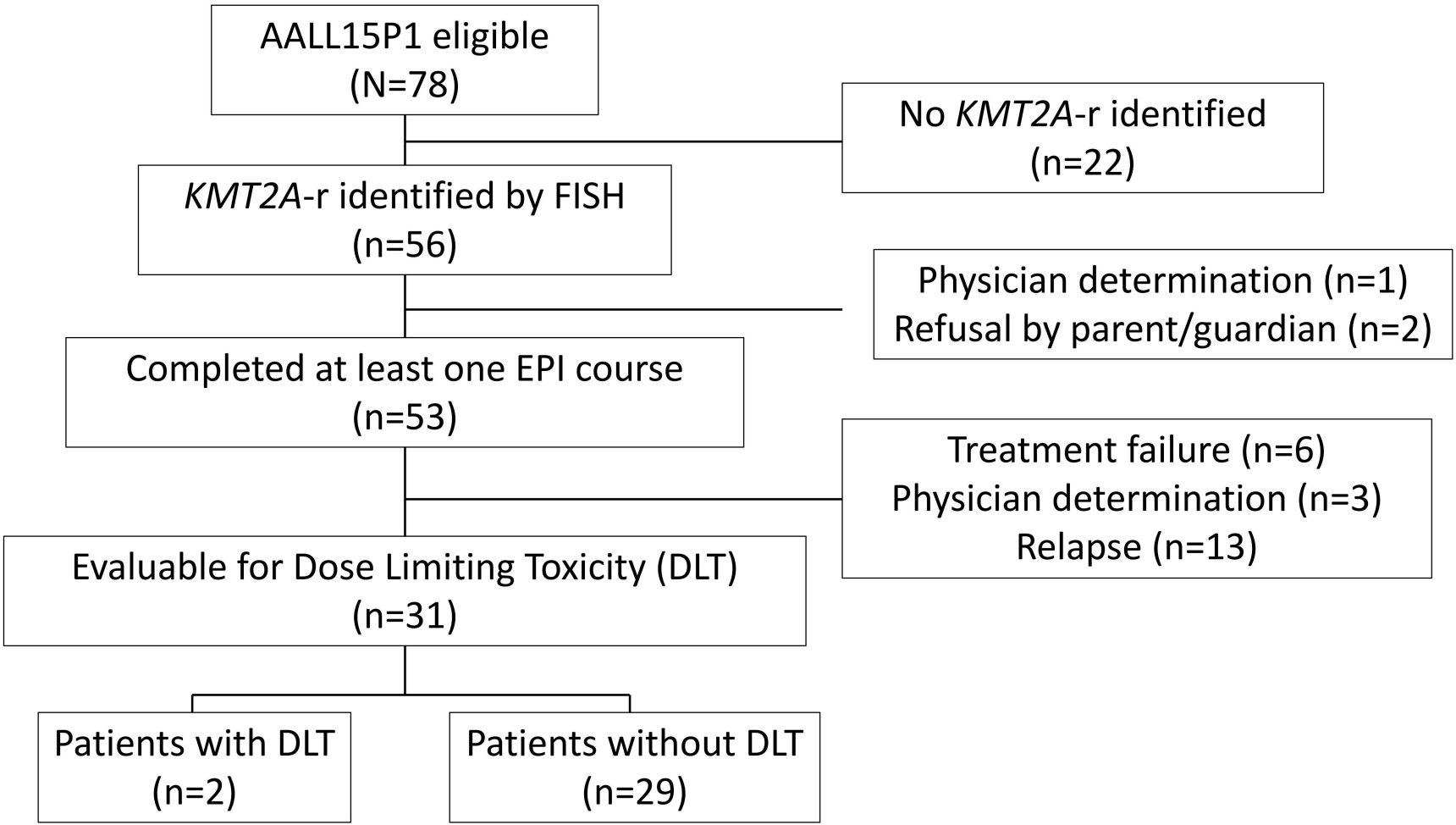
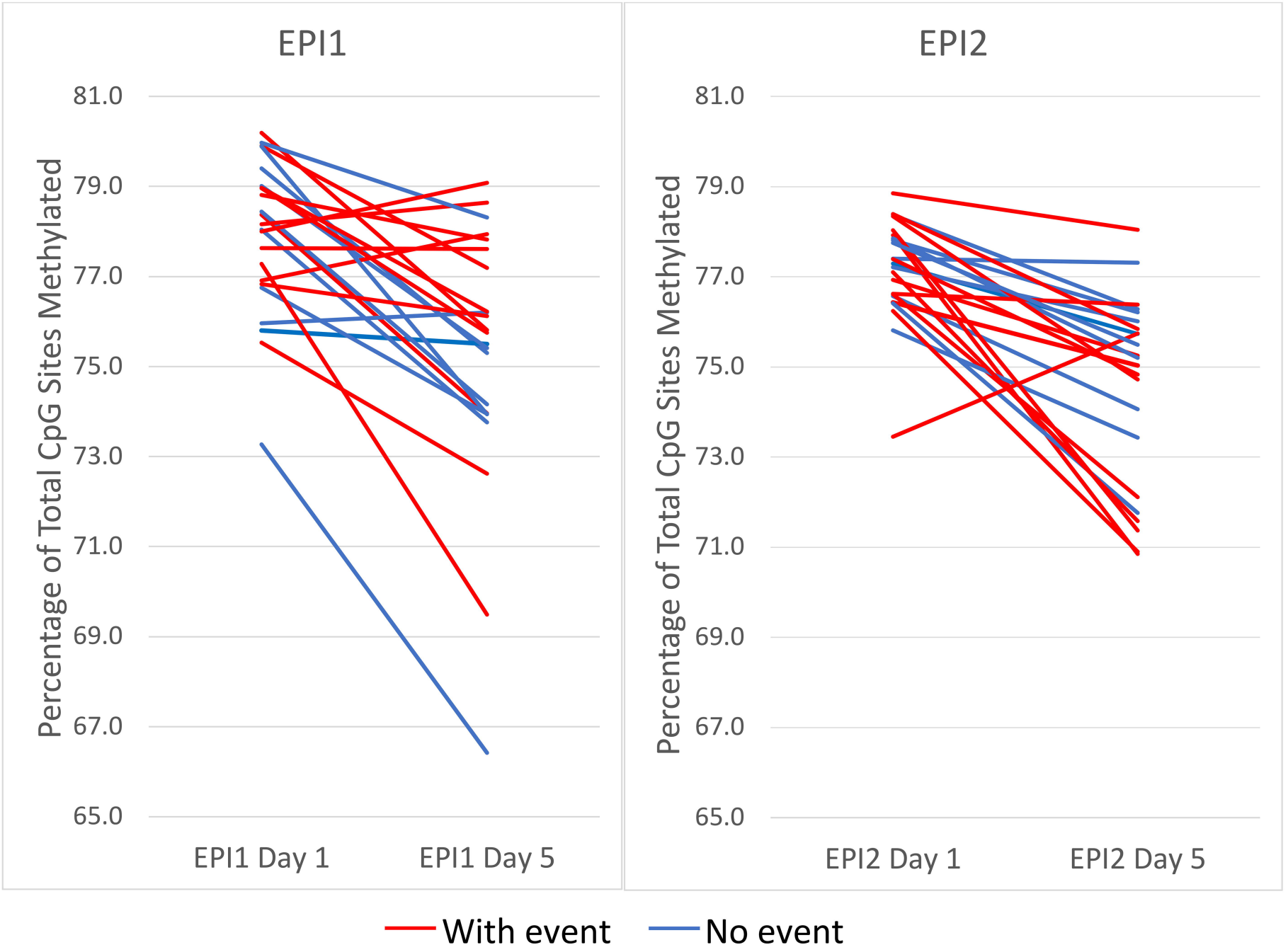
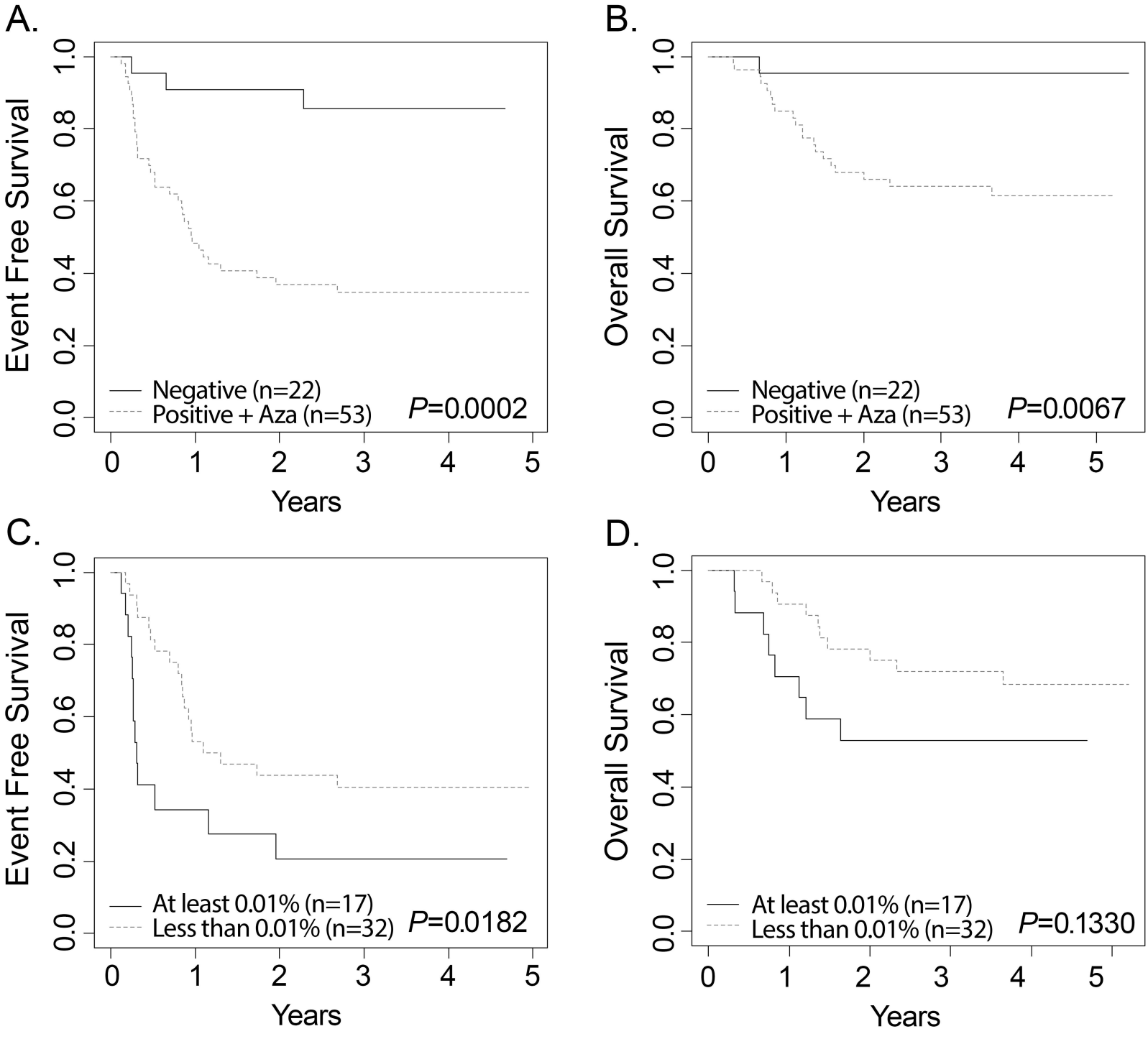

AALL15P1 eligible
(N=78)
No *KMT2A*-r identified
(n=22)
KMT2A-r identified by FISH
(n=56)
Physician determination (n=1)
Refusal by parent/guardian (n=2)
Completed at least one EPI course
(n=53)
Treatment failure (n=6)
Physician determination (n=3)
Relapse (n=13)
Evaluable for Dose Limiting Toxicity (DLT)
(n=31)
Patients with DLT
(n=2)
Patients without DLT
(n=29)

Figure 3.


EPI1
EPI2
81.0
79.0
77.0
75.0
73.0
71.0
69.0
67.0
65.0
Percentage of Total CpG Sites Methylated
EPI1 Day 1
EPI1 Day 5
EPI2 Day 1
EPI2 Day 5
With event
No event


A.
Event Free Survival
0.0
0.2
0.4
0.6
0.8
1.0
Negative (n=22)
Positive + Aza (n=53)
P=0.0002
0
1
2
3
4
5
Years
B.
Overall Survival
0.0
0.2
0.4
0.6
0.8
1.0
Negative (n=22)
Positive + Aza (n=53)
P=0.0067
0
1
2
3
4
5
Years
C.
Event Free Survival
0.0
0.2
0.4
0.6
0.8
1.0
At least 0.01% (n=17)
Less than 0.01% (n=32)
P=0.0182
0
1
2
3
4
5
Years
D.
Overall Survival
0.0
0.2
0.4
0.6
0.8
1.0
At least 0.01% (n=17)
Less than 0.01% (n=32)
P=0.1330
0
1
2
3
4
5
Years

SUPPLEMENTAL MATERIAL

Central Nervous System (CNS) status

CNS 1: In cerebrospinal fluid (CSF), absence of blasts on cytospin preparation, regardless of the number of white blood cells (WBCs)

CNS 2: In CSF, presence < 5/μL WBCs and cytospin positive for blasts, or traumatic LP, > 5/μL WBCs, cytospin positive for blasts, but negative by Steinherz/Bleyer algorithm
CNS 2a: < 10/μL RBCs; < 5/μL WBCs and cytospin positive for blasts
CNS 2b: ≥ 10/μL RBCs; < 5/μL WBCs and cytospin positive for blasts
CNS 2c: ≥ 10/μL RBCs; ≥ 5/μL WBCs and cytospin positive for blasts but negative by Steinherz/Bleyer algorithm

CNS3: In CSF, after traumatic LP presence of ≥ 5/μL WBCs and cytospin positive for blasts and/or clinical signs of CNS leukemia
CNS 3a: < 10/μL RBCs; ≥ 5/μL WBCs and cytospin positive for blasts
CNS 3b: ≥ 10/μL RBCs, ≥ 5/μL WBCs and positive by Steinherz/Bleyer algorithm
CNS 3c: Clinical signs of CNS leukemia (such as facial nerve palsy, brain/eye involvement or hypothalamic syndrome)

Central review of cytogenetics/Fluorescence in situ hybridization (FISH)

Local bone marrow and/or peripheral blood evaluation to confirm *KMT2A*-r was required to remain on AALL15P1 post-induction. Both standard cytogenetic studies and FISH analysis were performed at a COG-approved cytogenetics lab, and results submitted for central review. The local institutions obtained the COG cytogenetics report forms and original karyotypes from two different cells from each abnormal clone from the approved laboratory and sent them by email to Dr Andrew Carroll (University of Alabama at Birmingham) or Dr Nyla Heerema (The Ohio State University).

Supportive care

Supportive care guidelines were provided that recommended the following:

All infants should be placed on allopurinol (150-300 mg/m2/day or 10 mg/kg/day in 2-3 divided doses) when the diagnosis of leukemia is made or strongly suspected. Rasburicase may be indicated in some situations, per institutional guidelines.

Aggressive nutritional support should be provided, to maintain appropriate weight/height ratio. Caution is advised with early feeding in patients with difficult early courses or extensive mucositis or diaper area skin ulceration, as necrotizing enterocolitis and intestinal perforation are known risks in such infants. Total parenteral nutrition should be strongly considered in such infants, until it is certain there is no risk to the gut.

Hospitalization until evidence of marrow recovery is strongly recommended during induction, consolidation, interim maintenance, and delayed intensification. Antibiotic prophylaxis against gram-positive and gram-negative organisms, and antifungal prophylaxis, should be considered. Pneumocystis prophylaxis with trimethoprim-sulfamethoxazole or second-line agent should be started as soon as possible after the diagnosis of ALL is confirmed and continued until six months after all therapy is completed.

All respiratory syncytial virus (RSV) infections (upper and lower respiratory) should be treated per institutional guidelines. Additionally, palivizumab (15 mg/kg) intramuscular every month should be initiated at the start of RSV season and terminated at the end of RSV season. Intravenous immunoglobulin (IVIG) at a dose of 400 mg/kg if serum IgG level is below 500 mg/dL. Doses should be repeated every four weeks as needed to keep IgG level at 500 mg/dL or greater. Infants greater than or equal to six months of age should receive two doses of the influenza immunization per Center for Disease Control guidelines. Household contacts and out-of-home caregivers should also receive the influenza immunization.

For patients with moderate to severe mucositis, antifungal and antiviral therapy should be considered, based on the culture results and clinical evaluation. Daily oral antifungal prophylaxis with fluconazole should be strongly considered in patients not receiving vincristine. To prevent moderate to severe perineal irritation, placement of a Foley catheter is recommended for 48 to 72 hours during administration and urinary excretion of daunorubicin and high-dose methotrexate. Use of a strong barrier technique is also recommended.

Episodes of fever (>100.5^{o}F or 30.0^{o}C) should be aggressively managed, particularly during pre-maintenance phases of chemotherapy, or when the patient was neutropenic with ANC ≤1000. It is strongly advised that patients with fever and neutropenia (ANC <1000) not be managed with an outpatient antibiotic regimen. It is mandatory that patients with an ANC <500 and fever be hospitalized with immediate institution of broad-spectrum antibiotics adjusted appropriately for the causative organism.

Filgrastim or biosimilar may be used for severe infections with neutropenia, but routine use is discouraged. Filgrastim should not be given concurrently with azacitidine or chemotherapy and must be discontinued for at least 48 hours prior to the start of an azacitidine or chemotherapy course

Anti-emetics are strongly advised during days of azacitidine therapy and as needed during all phases of chemotherapy. The routine use of corticosteroids as antiemetics is discouraged.

Pharmacodynamic assessment of DNA methylation

Peripheral blood samples (3-4 mL in a green sodium heparin tube) were collected from infants on day one, prior to the first dose of azacitidine, and on day five, of the first two courses of azacitidine. Samples were shipped at room temperature to the Brown laboratory at Johns Hopkins University. Red blood cells were lysed, and peripheral blood mononuclear cells (PBMCs) were isolated and then frozen.

DNA was isolated from PBMCs and then treated with sodium bisulfite, which converts unmethylated cytosines into uracil while methylated cytosines remain unaltered. A library was then prepared using the treated DNA and sequenced with the Illumina NovaSeq 6000. The sequencing data was trimmed using the fastp tool and aligned to the GRCh37 reference genome using Illumina DRAGEN Bio-IT platform. After removing duplicates, the methylation level was computed as a fraction of methylated reads at each CpG site. The percent of methylated cytosines (mC) was compared between samples pre- and post- azacitidine.

Supplemental Table S1 Chemotherapy

	Route	Dose	Day(s) of phase
Induction (5 weeks)			
Methotrexate	IT	Age ≥1 year, 8 mg Age <1 year, 6 mg	1, 29
Predniso(lo)ne (or methylprednisolone IV at 80% of the predniso(lo)ne dose)	PO or NG	Age ≥6 months, 15 mg/m^2/dose TID Age ≥7 days to <6 months, 13 mg/m^2/dose TID Age <7 days, 10 mg/m^2/dose TID	1-7
Daunorubicin	IV over 1-15 min	Age ≥6 months, 23 mg/m^2 Age ≥7 days to <6 months, 20 mg/m^2 Age <7 days, 15 mg/m^2	8, 9
Cytarabine	IV over 30 min	Age ≥6 months, 60 mg/m^2 Age ≥7 days to <6 months, 50 mg/m^2 Age <7 days, 35 mg/m^2	8-21
Dexamethasone	PO or NG or IV	Age ≥6 months, 1.5 mg/m^2/dose TID Age ≥7 days to <6 months, 1.3 mg/m^2/dose TID Age <7 days, 1 mg/m^2/dose TID	8-28
Vincristine	IV over 1 min	Age ≥6 months, 1.2 mg/m^2 Age ≥7 days to <6 months, 1 mg/m^2 Age <7 days, 0.8 mg/m^2	8, 15, 22, 29
Pegaspargase	IV over 1-2 hours or IM	Age ≥6 months, 2000 IU/m^2 Age ≥7 days to <6 months, 1750 IU/m^2 Age <7 days, 1250 IU/m^2	12
Cytarabine	IT	Age ≥1 year, 20 mg Age <1 year, 15 mg	15
Hydrocortisone	IT	Age ≥1 year, 16 mg Age <1 year, 12 mg	15, 29
Consolidation (6 weeks)			
Cyclophosphamide	IV over 30-60 min	Age ≥12 months, 1000 mg/m^2 Age ≥6 months to <12 months, 750 mg/m^2 Age <6 months, 670 mg/m^2	1, 29

Mesna	IV over 15 min at hours 0, 4, and 8 from start of CPM infusion	Age ≥12 months, 200 mg/m^2/dose Age ≥6 months to <12 months, 150 mg/m^2/dose Age <6 months, 134 mg/m^2/dose	1, 29
Mercaptopurine	PO or NG	Age ≥12 months, 60 mg/m^2 Age ≥6 months to <12 months, 45 mg/m^2 Age <6 months, 40 mg/m^2	1-28
Cytarabine	IV push or SubQ	Age ≥12 months, 75 mg/m^2 Age ≥6 months to <12 months, 56 mg/m^2 Age <6 months, 50 mg/m^2	3-6, 10-13, 17-20, 24-27
Cytarabine	IT	Age ≥1 year, 20 mg Age <1 year, 15 mg	10
Hydrocortisone	IT	Age ≥1 year, 16 mg Age <1 year, 12 mg	10, 24
Methotrexate	IT	Age ≥1 year, 8 mg Age <1 year, 6 mg	24
Interim Maintenance (6 weeks)			
Mercaptopurine	PO or NG	Age ≥12 months, 25 mg/m^2 Age ≥6 months to <12 months, 19 mg/m^2 Age <6 months, 17 mg/m^2	1-14
High Dose Methotrexate	IV over 24 hours	Age ≥12 months, 5000 mg/m^2 Age ≥6 months to <12 months, 3750 mg/m^2 Age <6 months, 3300 mg/m^2	1, 8
Leucovorin	PO or IV at hours 42, 48 and 54 after the start of HD MTX and continued every 6 hours until serum MTX <0.1μM	15 mg/m^2/dose	3-4, 10-11
Methotrexate	IT	Age ≥1 year, 8 mg Age <1 year, 6 mg	1, 8
Hydrocortisone	IT	Age ≥1 year, 16 mg Age <1 year, 12 mg	1, 8

High Dose Cytarabine	IV over 3 hours	Age ≥12 months, 3000 mg/m^2/dose every 12 hours Age ≥6 months to <12 months, 2250 mg/m^2/dose every 12 hours Age <6 months, 2000 mg/m^2/dose every 12 hours	15-16, 22-23, total of 8 doses
Pegaspargase	IV over 1-2 hours or IM	Age ≥12 months, 2500 IU/m^2 Age ≥6 months to <12 months, 1875 IU/m^2 Age <6 months, 1650 IU/m^2	23
Delayed Intensification Part 1 (5 weeks)			
Pegaspargase	IV over 1-2 hours or IM	Age ≥12 months, 2500 IU/m^2 Age ≥6 months to <12 months, 1875 IU/m^2 Age <6 months, 1650 IU/m^2	1
Dexamethasone	PO or NG or IV	Age ≥12 months, 2 mg/m^2/dose TID Age ≥6 months to <12 months, 1.5 mg/m^2/dose TID Age <6 months, 1.3 mg/m^2/dose TID	1-14, then taper to 0 mg over days 15-21
6-Thioguanine	PO or NG	Age ≥12 months, 60 mg/m^2 Age ≥6 months to <12 months, 45 mg/m^2 Age <6 months, 40 mg/m^2	1-28
Vincristine	IV over 1 min	Age ≥12 months, 1.5 mg/m^2 Age ≥6 months to <12 months, 1.1 mg/m^2 Age <6 months, 1 mg/m^2	1, 8, 15, 22
Daunorubicin	IV over 1-15 min	Age ≥12 months, 30 mg/m^2 Age ≥6 months to <12 months, 23 mg/m^2 Age <6 months, 20 mg/m^2	1, 8, 15, 22
Cytarabine	IV push or SubQ	Age ≥12 months, 75 mg/m^2 Age ≥6 months to <12 months, 56 mg/m^2 Age <6 months, 50 mg/m^2	2-5, 9-12, 16-19, 23-26
Hydrocortisone	IT	Age ≥1 year, 16 mg Age <1 year, 12 mg	1, 15
Cytarabine	IT	Age ≥1 year, 20 mg Age <1 year, 15 mg	1, 15
Delayed Intensification Part 2 (3 weeks)			
6-Thioguanine	PO or NG	Age ≥12 months, 60 mg/m^2 Age ≥6 months to <12 months, 45 mg/m^2	1-14

		Age <6 months, 40 mg/m^2	
Cyclophosphamide	IV over 30-60 min	Age ≥12 months, 500 mg/m^2 Age ≥6 months to <12 months, 375 mg/m^2 Age <6 months, 330 mg/m^2	1, 15
Cytarabine	IV push or SubQ	Age ≥12 months, 75 mg/m^2 Age ≥6 months to <12 months, 56 mg/m^2 Age <6 months, 50 mg/m^2	2-5, 9-12
Maintenance Cycle 1 (12 weeks)			
Mercaptopurine	PO or NG	Age ≥12 months, 50 mg/m^2 Age ≥6 months to <12 months, 38 mg/m^2	1-84
Methotrexate	PO	Age ≥12 months, 20 mg/m^2 Age ≥6 months to <12 months, 15 mg/m^2	Once weekly
Methotrexate	IT	Age ≥1 year, 8 mg Age <1 year, 6 mg	1
Hydrocortisone	IT	Age ≥1 year, 16 mg Age <1 year, 12 mg	1, 57
Cytarabine	IT	Age ≥1 year, 20 mg Age <1 year, 15 mg	57
Maintenance Cycle 2 (12 weeks)			
Mercaptopurine	PO or NG	Age ≥12 months, 75 mg/m^2 Age ≥6 months to <12 months, 56 mg/m^2	1-84
Methotrexate	PO	Age ≥12 months, 20 mg/m^2 Age ≥6 months to <12 months, 15 mg/m^2	Once weekly
Methotrexate	IT	Age ≥1 year, 8 mg Age <1 year, 6 mg	15
Hydrocortisone	IT	Age ≥1 year, 16 mg Age <1 year, 12 mg	15
Maintenance Cycles 3+ (continue until 2 years from the start of Induction therapy)			
Mercaptopurine	PO or NG	Age ≥12 months, 75 mg/m^2	1-84
Methotrexate	PO	Age ≥12 months, 20 mg/m^2	Once weekly

Abbreviations: mg, milligram; IT, intrathecal; IV, intravenous; PO, oral; NG, nasogastric; m^2 square meters; TID, three times daily; min, minutes; IM, intramuscular; IU, international units; CPM, cyclophosphamide; SubQ, subcutaneous; HD MTX, high dose methotrexate

Supplemental Table S2 Experimental doses for infants with *KMT2A*-r

Dose level	**Azacitidine IV daily on days 1-5**
1 (starting dose, determined to be safe)	2.5 mg/kg/dose
0	1.8 mg/kg/dose

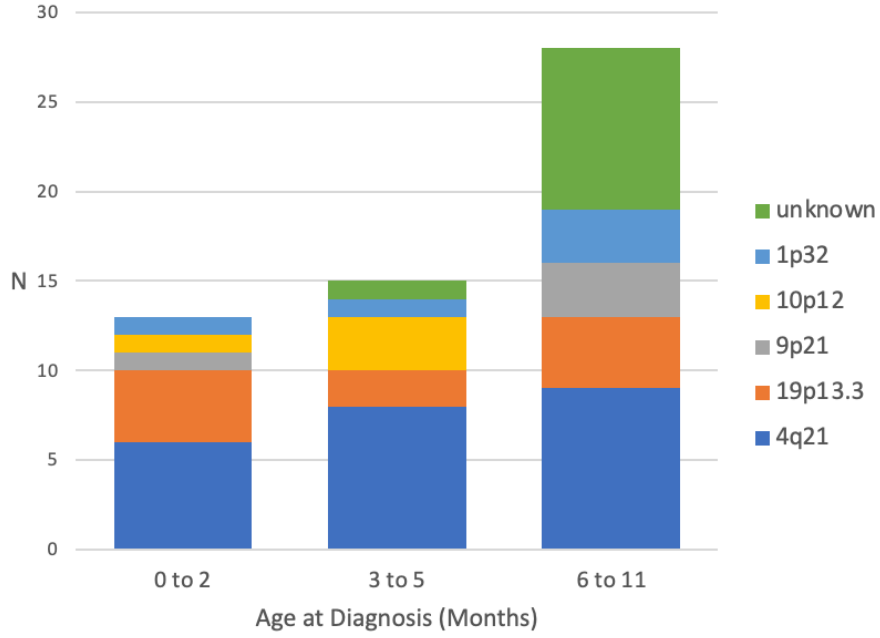
Abbreviations: *KMT2A*-r, *KMT2A*-rearrangement; IV, intravenous; mg, milligram; kg, kilogram

Supplemental Table S3 Continuous monitoring table for dose limiting toxicity (DLT)

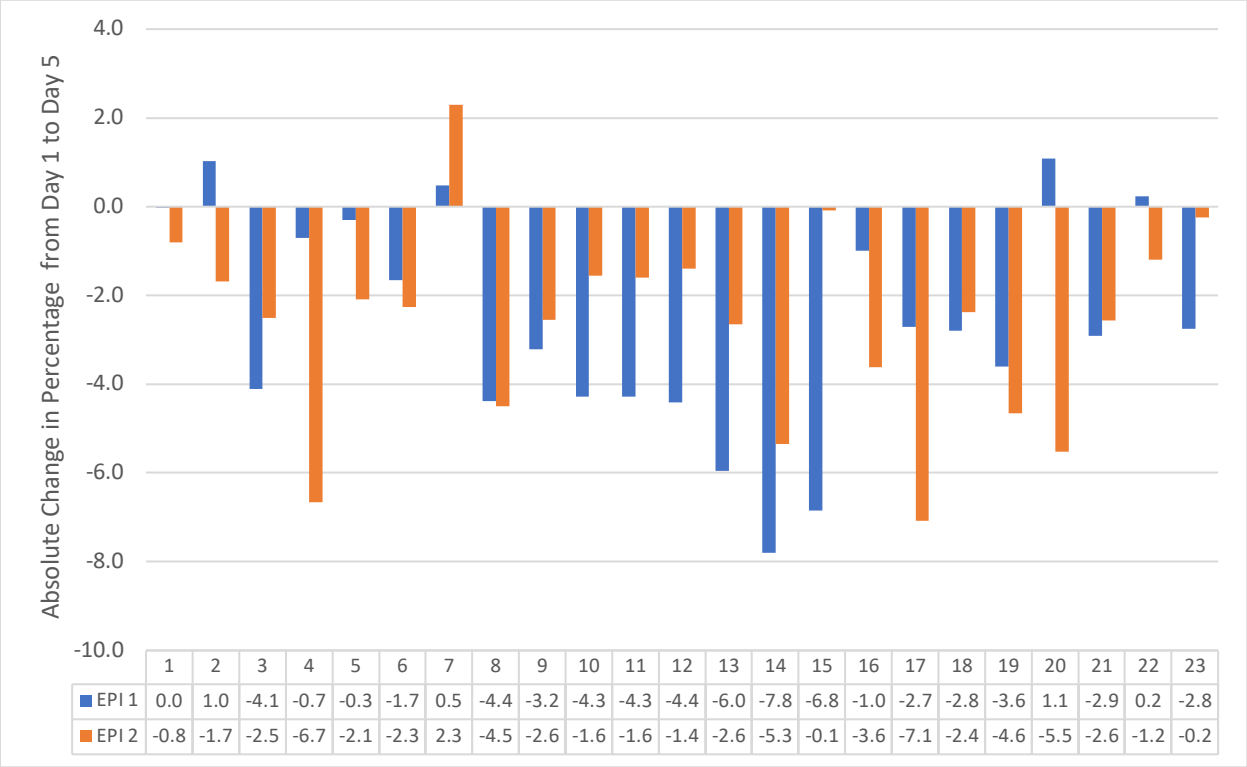
n	**b(n)**
≤6	3
7-10	4
11-16	5
17-21	6
22-28	7
29-30	8

n = number of evaluable patients treated on any single dose level
b(n)=toxicity boundary (if the number of patients with at least one DLT is ≥b(n) on any single dose level, then that dose is deemed excessively toxic)

Supplemental Figure S1 Frequency of *KMT2A* Chromosomal Partners by Age Groups at Diagnosis

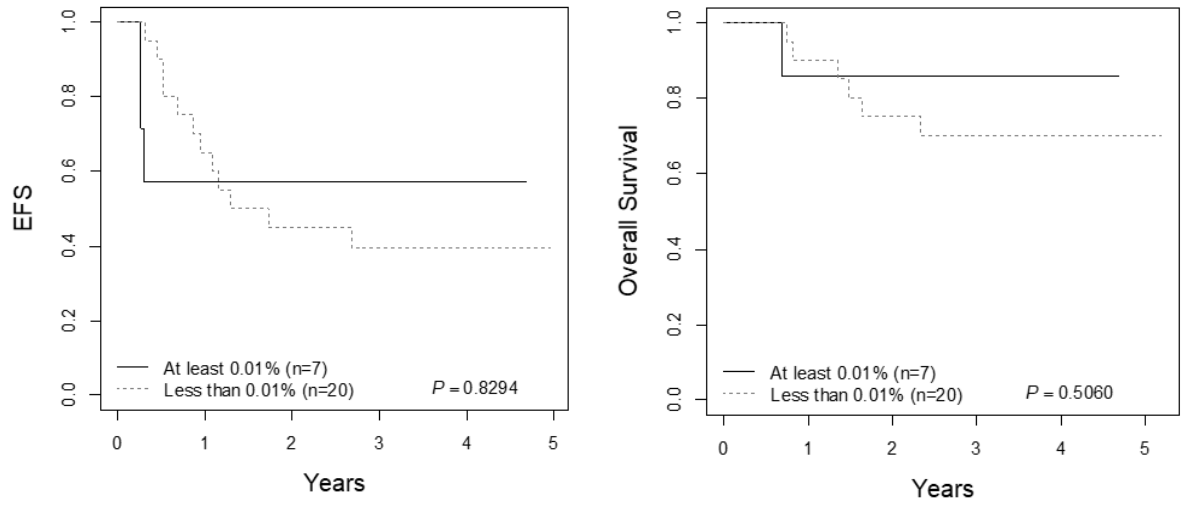


Supplemental Figure S2 Change in Percentage of in Total CpG Methylation Per Patient



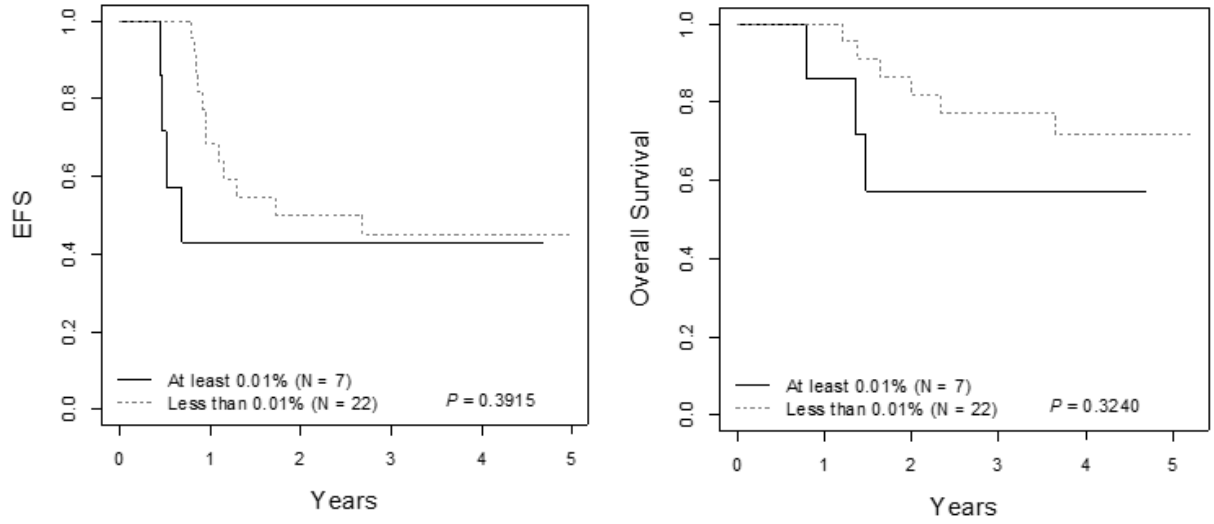
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
EPI 1	0.0	1.0	-4.1	-0.7	-0.3	-1.7	0.5	-4.4	-3.2	-4.3	-4.3	-4.4	-6.0	-7.8	-6.8	-1.0	-2.7	-2.8	-3.6	1.1	-2.9	0.2	-2.8
EPI 2	-0.8	-1.7	-2.5	-6.7	-2.1	-2.3	2.3	-4.5	-2.6	-1.6	-1.6	-1.4	-2.6	-5.3	-0.1	-3.6	-7.1	-2.4	-4.6	-5.5	-2.6	-1.2	-0.2

Supplemental Figure S3 Survival based on End of Consolidation MRD



MRD, minimal residual disease; EFS, event-free survival

Supplemental Figure S4 Survival based on End of Interim Maintenance MRD



MRD, minimal residual disease; EFS, event-free survival