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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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

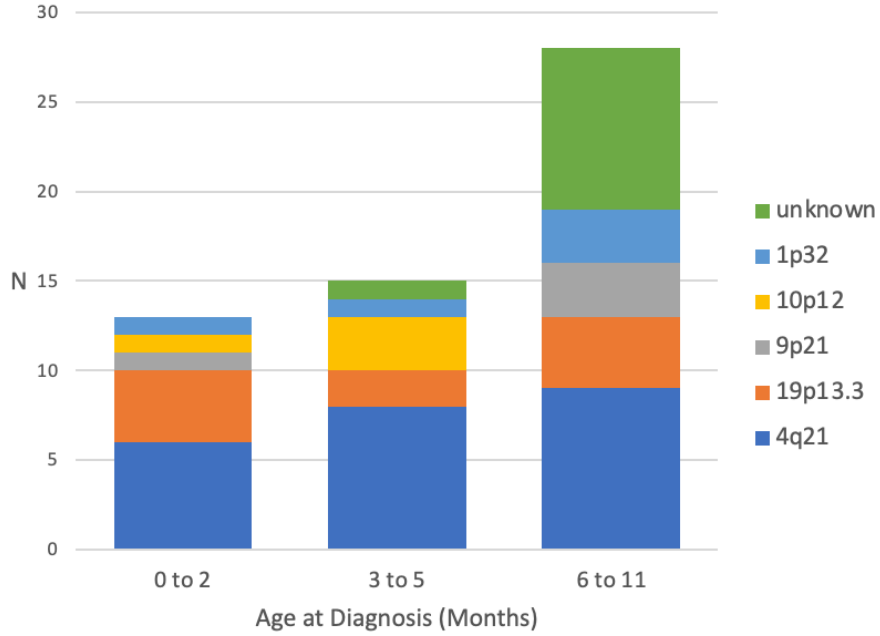
Abbreviations: *KMT2A*-r, *KMT2A*-rearrangement; IV, intravenous; mg, milligram; kg, kilogram

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

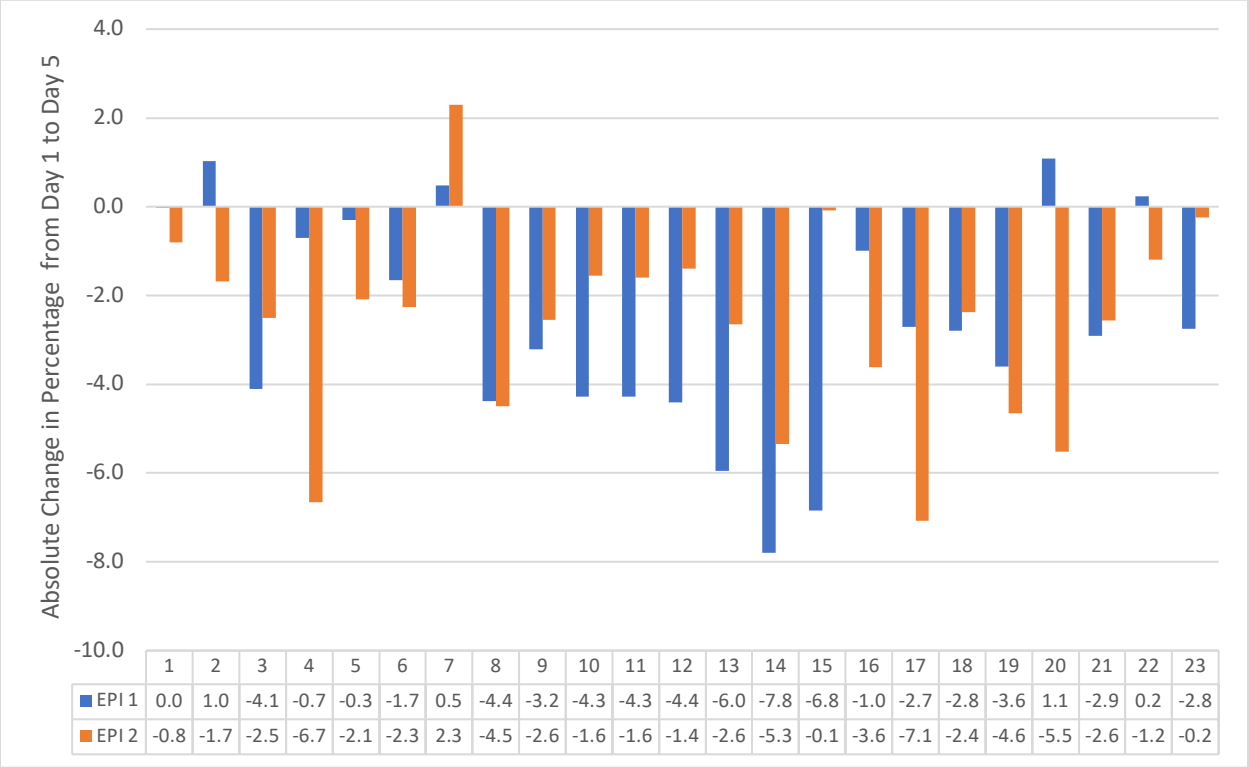
n	**b(n)**
≤6	3
7-10	4
11-16	5
17-21	6
22-28	7
29-30	8

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

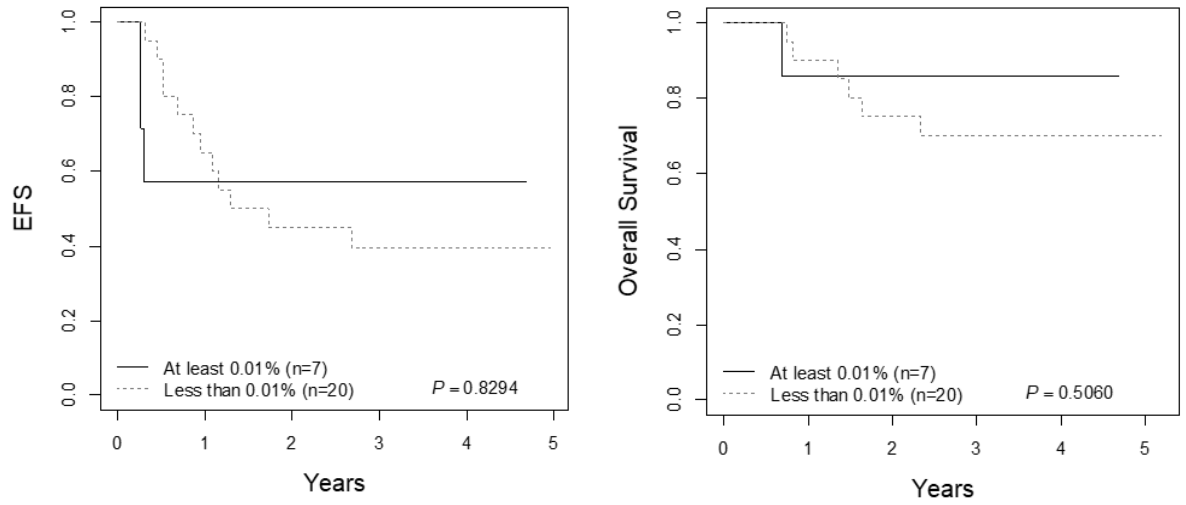


Supplemental Figure S2 Change in Percentage of in Total CpG Methylation Per Patient



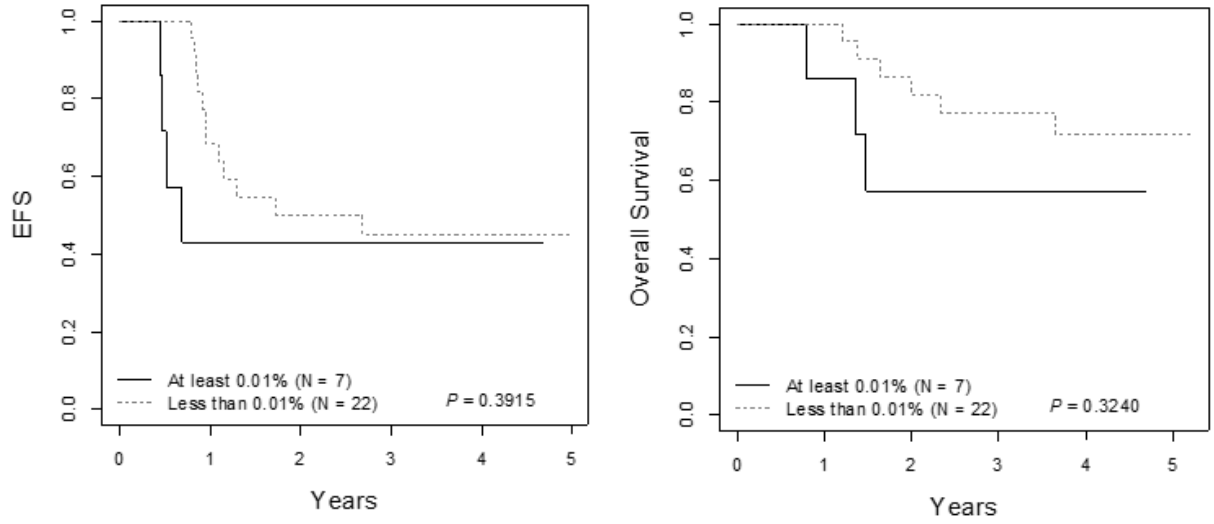
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
EPI 1	0.0	1.0	-4.1	-0.7	-0.3	-1.7	0.5	-4.4	-3.2	-4.3	-4.3	-4.4	-6.0	-7.8	-6.8	-1.0	-2.7	-2.8	-3.6	1.1	-2.9	0.2	-2.8
EPI 2	-0.8	-1.7	-2.5	-6.7	-2.1	-2.3	2.3	-4.5	-2.6	-1.6	-1.6	-1.4	-2.6	-5.3	-0.1	-3.6	-7.1	-2.4	-4.6	-5.5	-2.6	-1.2	-0.2

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