Clofarabine increases the eradication of minimal residual disease of primary B-precursor acute lymphoblastic leukemia compared to high-dose cytarabine without improvement of outcome. Results from the randomized clinical trial 08-09 of the Cooperative Acute Lymphoblastic Leukemia Study Group.

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Supplementary Information:

- **Supplemental Table 1:**

  Participating trial sites

- **Supplemental Table 2:**

  Comparison of toxicities after clofarabine vs high-dose cytarabine (HIDAC) according to CTC grades.

  A: Comparison of all CTC grades

  B: Comparison of CTC grades 0-2 vs 3 and 4.

  Data are presented as No. (%).

- **Supplemental Table 3:**

  MRD response in BCP-ALL patients according to their ETV6-RUNX1 status.

- **Supplemental Table 4:**

  Distribution of relapses in the randomized arms according to the MRD level

- **Supplemental Figure 1:**

  Comparison of MRD response in BCP-ALL

- **CoALL 08-09 stratification algorithm**

- **Definition of event-free and overall survival**

- **Statistical analyses**

- **Study recruitment**

Supplemental Table 1: Participating trial sites

Site	City	Country
University Medical Center Hamburg-Eppendorf, Clinic of Pediatric Hematology and Oncology	Hamburg	Germany
Protestant Hospital of Bethel Foundation, Department of Pediatric Hematology and Oncology	Bielefeld	Germany
University Hospital Bonn, Department of Pediatric Hematology/Oncology	Bonn	Germany
Hospital Bremen-Mitte, Department of Pediatric Hematology and Oncology	Bremen	Germany
Helios Hospital Krefeld, Department of Pediatric Hematology and Oncology	Krefeld	Germany
University Medical Center of the Johannes Gutenberg University Mainz, Department of Pediatric Hematology/Oncology	Mainz	Germany
University Hospital, Ludwig Maximilian Munich, Dr. von Hauner Children's Hospital	Munich	Germany
Medical Faculty, Heinrich Heine University Düsseldorf, Pediatric Oncology, Hematology and Clinical Immunology	Düsseldorf	Germany

Supplemental Table 2A,B ：Comparison of toxicities

Table 2A:

		high-dose Cytarabine, No. (%)	Clofarabine, No. (%)	*P* Value (Fisher)
General condition	**Grade 0**	13 (8.7)	15 (10.0)	.38493
	Grade 1	90 (60.0)	92 (61.3)	
	Grade 2	37 (24.7)	40 (26.7)	
	Grade 3	8 (5.3)	3 (2.0)	
	Grade 4	2 (1.3)	0	
	Total	150	150	
Hemoglobin	**Grade 0**	1 (0.7)	1 (0.7)	.03460
	Grade 1	6 (4.0)	4 (2.6)	
	Grade 2	33 (21.9)	56 (37.1)	
	Grade 3	87 (57.6)	75 (49.7)	
	Grade 4	24 (15.9)	15 (9.9)	
	Total	151	151	
WBC	**Grade 0**	0	0	< .0001
	Grade 1	0	0	
	Grade 2	5 (3.3)	1 (0.7)	
	Grade 3	60 (39.5)	8 (5.3)	
	Grade 4	87 (57.2)	142 (94.0)	
	Total	152	151	
Neutrophils	**Grade 0**	1 (0.8)	1 (0.8)	.37821
	Grade 1	0	1 (0.8)	
	Grade 2	1 (0.8)	0	
	Grade 3	8 (6.1)	3 (2.3)	
	Grade 4	122 (92.4)	123 (96.1)	
	Total	132	128	
Platelets	**Grade 0**	1 (0.7)	15 (9.9)	< .0001
	Grade 1	4 (2.6)	12 (7.9)	
	Grade 2	9 (5.9)	17 (11.3)	
	Grade 3	87 (57.2)	83 (55.0)	
	Grade 4	51 (33.6)	24 (15.9)	
	Total	152	151	
Number of platelet transfusions	**Grade 0**	46 (30.7)	106 (70.7)	< .0001
	Grade 1	84 (56.0)	34 (22.7)	
	Grade 2	16 (10.7)	8 (5.3)	
	Grade 3	4 (2.7)	2 (1.3)	
	Grade 4	0	0	
	Total	150	150	
Infections	**Grade 0**	44 (29.1)	65 (43.0)	.07835
	Grade 1	11 (7.3)	12 (7.9)	
	Grade 2	82 (54.3)	63 (41.7)	
	Grade 3	13 (8.6)	11 (7.3)	
	Grade 4	1 (0.7)	0	
	Total	151	151	
Fever	**Grade 0**	41 (27.2)	67 (44.4)	.01048
	Grade 1	65 (43.0)	55 (36.4)	
	Grade 2	41 (27.2)	28 (18.5)	
	Grade 3	3 (2.0)	1 (0.7)	

	Grade 4	1 (0.7)	0	
	Total	151	151	
Days in hospital	**Grade 0**	44 (29.1)	70 (46.4)	.03230
	Grade 1	34 (22.5)	23 (15.2)	
	Grade 2	61 (40.4)	48 (31.8)	
	Grade 3	8 (5.3)	8 (5.3)	
	Grade 4	4 (2.6)	2 (1.3)	
	Total	151	151	
Stomatitis	**Grade 0**	122 (80.3)	123 (88.7)	.09755
	Grade 1	15 (9.9)	12 (8.0)	
	Grade 2	12 (7.9)	3 (2.0)	
	Grade 3	2 (1.3)	1 (0.7)	
	Grade 4	1 (0.7)	1 (0.7)	
	Total	152	150	
Diarrhea	**Grade 0**	132 (87.4)	125 (83.9)	.54837
	Grade 1	15 (9.9)	16 (10.7)	
	Grade 2	4 (2.6)	6 (4.0)	
	Grade 3	0	2 (1.3)	
	Grade 4	0	0	
	Total	151	149	
Creatinine	**Grade 0**	142 (93.4)	148 (98.0)	.11123
	Grade 1	9 (5.9)	3 (2.0)	
	Grade 2	1 (0.7)	0	
	Grade 3	0	0	
	Grade 4	0	0	
	Total	152	151	
Bilirubine	**Grade 0**	103 (68.2)	95 (63.3)	.72543
	Grade 1	32 (21.2)	33 (22.0)	
	Grade 2	14 (9.3)	17 (11.3)	
	Grade 3	2 (1.3)	4 (2.7)	
	Grade 4	0	1 (0.7)	
	Total	151	150	
Transaminases	**Grade 0**	18 (12.1)	10 (6.7)	< .0001
	Grade 1	50 (33.6)	41 (27.3)	
	Grade 2	43 (28.9)	27 (18.0)	
	Grade 3	36 (24.2)	52 (34.7)	
	Grade 4	2 (1.3)	20 (13.3)	
	Total	149	150	
Peripheral neurotoxi-city	**Grade 0**	147 (96.7)	144 (96.6)	1.0000
	Grade 1	4 (2.6)	4 (2.7)	
	Grade 2	1 (0.7)	1 (0.7)	
	Grade 3	0	0	
	Grade 4	0	0	
	Total	152	149	
Central neurotoxicity	**Grade 0**	149 (98.0)	150 (99.3)	.24752
	Grade 1	3 (2.0)	0	
	Grade 2	0	0	
	Grade 3	0	0	
	Grade 4	0	1 (0.7)	
	Total	152	151	
Arrhythmia	**Grade 0**	135 (98.5)	133 (97.8)	.62177
	Grade 1	0	2 (1.5)	
	Grade 2	1 (0.7)	1 (0.7)	

	Grade 3	1 (0.7)	0	
	Grade 4	0	0	
	Total	137	136	
Cardiac dysfunction	**Grade 0**	85 (98.8)	76 (98.7)	.72317
	Grade 1	0	0	
	Grade 2	0	1 (1.3)	
	Grade 3	1 (1.2)	0	
	Grade 4	0	0	
	Total	86	77	
Skin condition	**Grade 0**	141 (92.8)	96 (64.4)	< .0001
	Grade 1	6 (3.9)	33 (22.1)	
	Grade 2	5 (3.3)	20 (13.4)	
	Grade 3	0	0	
	Grade 4	0	0	
	Total	152	149	
Veno-occlusive dise-ase	**Grade 0**	..	..	
	Grade 1	1 (100)	..	
	Grade 2	..	..	
	Grade 3	..	..	
	Grade 4	..	..	
	Total	1	0	
Thrombosis	**Grade 0**	151 (100)	149 (99.3)	.49834
	Grade 1	0	1 (0.7)	
	Grade 2	0	0	
	Grade 3	0	0	
	Grade 4	0	0	
	Total	151	150	

Supplemental Table 2B:

		High-dose Cytarabine, No. (%)	Clofarabine, No. (%)	*P* Value (Fisher)
General condition	**Grade 0-2**	140 (93.3)	147 (98.0)	.08529
	Grade 3/4	10 (6.7)	3 (2.0)	
	Total	150	150	
Hemoglobin	**Grade 0-2**	40 (26.5)	61 (40.4)	.01451
	Grade 3/4	111 (73.5)	90 (59.6)	
	Total	151	151	
WBC	**Grade 0-2**	5 (3.3)	1 (0.7)	.21409
	Grade 3/4	147 (96.7)	150 (99.3)	
	Total	152	151	
Neutrophils	**Grade 0-2**	2 (1.5)	2 (1.6)	1.0000
	Grade 3/4	130 (98.5)	126 (98.4)	
	Total	132	128	
Platelets	**Grade 0-2**	14 (9.2)	44 (29.1)	< .0001
	Grade 3/4	138 (90.8)	107 (70.9)	
	Total	152	151	
Number of platelet transfusions	**Grade 0-2**	146 (97.3)	148 (98.7)	.68433
	Grade 3/4	4 (2.7)	2 (1.3)	
	Total	150	150	
Infections	**Grade 0-2**	137 (90.7)	140 (92.7)	.67696
	Grade 3/4	14 (9.3)	11 (7.3)	
	Total	151	151	
Fever	**Grade 0-2**	147 (97.4)	150 (99.3)	.37083
	Grade 3/4	4 (2.6)	1 (0.7)	
	Total	151	151	
Days in hospital	**Grade 0-2**	139 (92.1)	141 (93.4)	.82534
	Grade 3/4	12 (7.9)	10 (6.6)	
	Total	151	151	
Stomatitis	**Grade 0-2**	149 (98.0)	148 (98.7)	1.0000
	Grade 3/4	3 (2.0)	2 (1.3)	
	Total	152	150	
Diarrhea	**Grade 0-2**	151 (100)	147 (98.7)	.24584
	Grade 3/4	0	2 (1.3)	
	Total	151	149	
Bilirubine	**Grade 0-2**	149 (98.7)	145 (96.7)	.28247
	Grade 3/4	2 (1.3)	5 (3.3)	
	Total	151	150	
Transaminases	**Grade 0-2**	111 (74.5)	78 (52.0)	< .0001
	Grade 3/4	38 (25.5)	72 (48.0)	
	Total	149	150	
Central neurotoxicity	**Grade 0-2**	152 (100)	150 (99.3)	.49835
	Grade 3/4	0	1 (0.7)	
	Total	152	151	
Arrhythmia	**Grade 0-2**	136 (99.3)	136 (100)	1.0000
	Grade 3/4	1 (0.7)	0	
	Total	137	136	
Cardiac dysfunction	**Grade 0-2**	85 (98.8)	77 (100)	1.0000
	Grade 3/4	1 (1.2)	0	
	Total	86	77	

Supplemental Table 3: MRD response in BCP-ALL patients according to their ETV6-RUNX1 status.

Table 3A: MRD response to clofarabine vs high-dose cytarabine in ETV6/RUNX1-negative BCP-ALL patients

p(chi)=.04210	high-dose Cytarabine		Clofarabine		All
	N	%	N	%	N
MRD Day 50					
MRD Day 50 neg.	54	50.0	58	62.4	112
MRD Day 50 pos. nq	37	34.3	30	32.3	67
MRD Day 50 pos.	17	15.7	5	5.4	22

Table 3B: MRD response to clofarabine vs high-dose cytarabine in ETV6/RUNX1-positive BCP-ALL patients

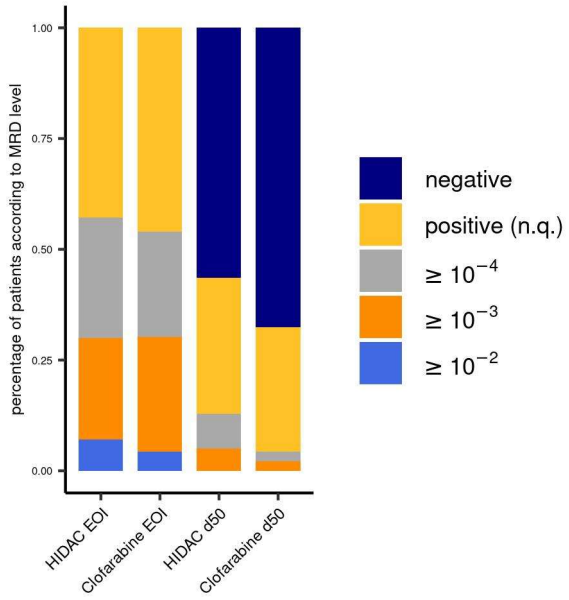
p(chi)=.94619	high-dose Cytarabine		Clofarabine		All
	N	%	N	%	N
MRD Day 50					
MRD Day 50 neg.	22	75.9	35	77.8	57
MRD Day 50 pos. nq	6	20.7	9	20.0	15
MRD Day 50 pos.	1	3.4	1	2.2	2

Supplemental Table 4**:** Distribution of relapses in absolute numbers in the randomized arms according to MRD level

	Clofarabine (n)	**high-dose Cytarabine (n)**
MRD negative after clofarabine/ high-dose cytarabine	**5**	**3**
BCP-ALL	4	2
T-ALL	1	1
MRD positive n.q. after clofarabine/ high-dose cytarabine	**8**	**5**
BCP-ALL	7	5
T-ALL	1	0
MRD ≥10^{-4} after clofarabine/ high-dose cytarabine	**2**	**6**
BCP-ALL	2	5
T-ALL	0	1
Total	**15**	**14**

Supplemental Figure 1: Comparison of MRD response in BCP-ALL

Comparison of MRD response after clofarabine vs high-dose cytarabine, each combined with PEG-ASP in BCP-ALL. MRD distribution at end of induction on day 29: clofarabine ≥ 1 x 10^{-2} 4.6%, ≥ 1 x 10^{-3} 26.5%, ≥ 1 x 10^{-4} 24.5%; ≥ 1 x 10^{-6} 43.7%; HIDAC ≥ 1 x 10^{-2} 7.2%, ≥ 1 x 10^{-3} 24.3%, ≥ 1 x 10^{-4} 27% ≥ 1 x 10^{-6} 41.4%; MRD distribution on day 50 after clofarabine: ≥ 1 x 10^{-2} 0%, ≥ 1 x 10^{-3} 2%, ≥ 1 x 10^{-4} 2.6%, ≥ 1 x 10^{-6} 28.5%, negative 64%; after HIDAC: ≥ 1 x 10^{-2} 0%, ≥ 1 x 10^{-3} 5.9% ≥ 1 x 10^{-4} 7.2%, ≥ 1 x 10^{-6} 30.3%, negative 54%.



CoALL 08-09 stratification algorithm

At diagnosis, patients were stratified according to conventional risk criteria, allocating patients aged ≥10 years, with a T- or pro-B cell immunophenotype or with a white blood cell count (WBC) ≥25/nl to the high-risk (HR) arm, and all others to the low-risk (LR) arm. A second, more refined stratification was applied at EOI based on cytomorphological remission, molecular cytogenetics and *in vivo* MRD testing[11]. Patients not reaching remission at the end of induction, carrying a *KMT2A*-rearrangement or exhibiting a hypodiploid karyotype were also allocated to the HR arm. Ultimately, based on EOI MRD, three stratification arms were defined per risk group. B-precursor (BCP)-ALL patients with a negative MRD result and T-ALL patients with MRD EOI $<10^{-3}$ were stratified to receive reduced treatment (LR- or HR-reduced), and were not eligible for randomization at consolidation and reinduction. BCP-ALL patients with EOI MRD $\geq 10^{-3}$ and T-ALL patients with MRD $\geq 10^{-3}$ after the first course of consolidation were stratified to receive intensified treatment (LR- or HR-intensified). The remaining patients were assigned to standard treatment (LR- or HR-standard).

From 1 November 2013 to 31 December 2019, 476 protocol patients were enrolled in phase III of CoALL 08-09. All patients who were EOI MRD-negative (n=108) and patients with induction failure (n=31) were excluded from randomization, as were three patients who died during induction. 31 patients could not be randomized because of parental or patients' refusal (n=14), technical non-feasibility (n=14), or severe adverse events (SAE) during induction (n=3).

Definition of event-free and overall survival

EFS was the time from diagnosis to the first event, defined as failure of protocol treatment (non-remission: persistence of leukemic blasts ≥5% in the bone marrow (BM) until day 56 of treatment), induction death, relapse (re-emergence of blasts ≥25% or increasing blast counts in two consecutive BM biopsies after complete continuous remission and/or manifestation of ALL in CNS and/or any extramedullary site by cytomorphology), death by any cause while in remission, secondary malignancy or censoring at last follow-up. OS was defined as the time

from diagnosis to death by any cause or censoring at last follow-up. Cox regression was used for multivariate analysis of the randomization groups taking into account known risk factors as covariates.

Statistical analyses

Sample size for randomization was calculated according to estimations of the primary end-point, i.e. MRD of BCP-ALL after the first course of consolidation, based on the preceding trial, COALL 07-03. We estimated that 60% of patients in the control group who were MRD-positive prior to intensification would exhibit a detectable MRD level after the administration of HIDAC/PEG-ASP. We required 136 patients randomized to each group in order to demonstrate a 25% reduction yielding 45% MRD-positive patients with alpha=5% (one-sided) and beta=20%. Two interim analyses were planned in the study protocol, yielding a significance level of 0.042 in the final analysis.

For the randomized treatment element, local trial centers documented toxicities using a specific toxicity form based on NCI Common Toxicity Criteria, version 2.0. An additional field was implemented to capture the incidence and length of hospitalization. A lack of treatment-related hospitalization was defined as grade 0, <5 days:1, 5–10 days: 2, 10–15 days: 3, and >15 days of hospitalization corresponded to grade 4.

Study recruitment

From 1 November 2013 to 31 December 2019, 476 protocol patients were enrolled in phase III of CoALL 08-09. Among those, 108 patients achieved EOI MRD-negativity (n=108), 31 patients underwent an induction failure, and three patients died during induction who were not eligible for randomization. In addition, 31 patients could not be randomized because of parental or patients' refusal (n=14), technical non-feasibility (n=14), or severe adverse events (SAE) during induction (n=3).