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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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ABSTRACT

Novel treatment strategies are needed to improve cure for all children with acute lymphoblastic leukemia (ALL). To this end, we investigated the therapeutic potential of clofarabine in primary ALL in trial CoALL 08-09 (clinicaltrials gov. identifier: NCT01228331). The primary study objective was the minimal residual disease (MRD)-based comparative assessment of cytotoxic efficacies of clofarabine 5x40 mg/m^2 *versus* high-dose cytarabine (HIDAC) 4x3g/m^2, both in combination with PEG-ASP 2,500 IU/m^2 as randomized intervention in early consolidation. The secondary objective was an outcome analysis focused on treatment arm dependence and MRD after randomized intervention. In B-cell precursor (BCP)-ALL, eradication of MRD was more profound after clofarabine compared to cytarabine, with 93 *versus* 79 of 143 randomized patients per arm reaching MRD-negativity (χ^2 test *P*=0.03, left-sided *P* [Fisher's exact test]=0.04). MRD status of BCP-ALL after randomized intervention maintained its prognostic relevance, with a significant impact on event-free survival (EFS) and relapse rate. However, no difference in outcome regarding EFS and overall survival (OS) between randomized courses was observed (5-year EFS: clofarabine 85.7, SE=4.1 *vs.* HIDAC 84.8, SE=4.7 [*P*=0.96]; OS: 95.7, SE=1.9 *vs.* 92.2, SE=3.2 [*P*=0.59]), independent of covariates or overall risk strata. Severe toxicities between randomized and subsequent treatment elements were also without significant difference. In conclusion, clofarabine/PEG-ASP is effective and safe, but greater cytotoxic efficacy of clofarabine compared to HIDAC did not translate into improved outcomes indicating a lack of surrogacy of post-intervention MRD at the trial level as opposed to the patient level, which hampers a broader implementation of this regimen in the frontline treatment of ALL.

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Introduction

The prevention of relapse without increasing toxicity is a challenging goal of frontline treatment in acute lymphoblastic leukemia (ALL), which is unlikely to be achieved by recombination or intensification of established chemotherapeutic agents. Besides immunotherapeutical approaches, novel compounds must be probed to prevent the development of resistant clones or to efficiently overcome those that already exist.

To this end, we evaluated clofarabine as one of the latest chemotherapeutic drugs to receive authoritative approval for the treatment of relapsed/refractory ALL in childhood. Clofarabine is a second-generation purine nucleoside analogue that combines the positive characteristics of first-generation purine nucleosides fludarabine and cladribine by retaining 2-halogenated adenines, resulting in improved resistance against deamination and phosphorolysis.[1-3] Several studies have been launched which scrutinized clofarabine in combination with other cytostatic drugs as second- or third-line therapy, or as a bridging regimen to hematopoietic stem cell transplantation.[4-6]

In the Children's Oncology Group (COG) trial AALL1131, clofarabine was administered in combination with etoposide and cyclophosphamide, which were associated with severe infections and persistent myelotoxicity leading to premature closure of the experimental clofarabine arm.[7]

In order to assess the value of the frontline usage of clofarabine, the Cooperative Acute Lymphoblastic Leukemia Study Group (CoALL) conducted a sequential phase II/III trial embedded into the CoALL 08-09 regimen for newly diagnosed ALL patients for whom end-of-induction (EOI) minimal residual disease (MRD) imposed a greater risk of relapse.

During the non-randomized phase II, all eligible patients with quantifiable EOI MRD received the combination of clofarabine 5x40 mg/m^2 and pegylated asparaginase (PEG-ASP) 2,500 IU/m^2 as early consolidation treatment. The results were compared to a high-dose cytarabine (HIDAC)/PEG-ASP control group in predecessor trial CoALL 03-07. Combined administration of clofarabine and PEG-ASP was feasible and exhibited acceptable toxicities without unexpected severe side effects.[8]

Herein, we describe the results of the subsequent phase III trial within CoALL 08-09, comparing the efficacy and tolerability of clofarabine/PEG-ASP *versus* HIDAC/PEG-ASP at early consolidation in a randomized fashion.

Methods

Study design and patients

CoALL 08-09 was a multi-center, randomized trial for patients under the age of 18 years with a confirmed diagnosis of acute B- or T-cell precursor leukemia. Accrual was open from 1 October 2010 to 31 December 2019. The study was approved by the competent ethics boards (*Online Supplementary Table S1*) and conducted in accordance with the Declaration of Helsinki. The efficacy of clofarabine/PEG-ASP was compared with HIDAC/PEG-ASP in a randomized fashion as a primary study objective. An additional randomization of anthracyclines in delayed intensification was conducted from 2010 to 2016 with the primary objective of comparing toxicities.[9]

Stratification and treatment

All patients received the same three-drug induction with four weekly doses of daunorubicin (36 mg/m^2) and vincristine (1.5 mg/m^2) along with oral methylprednisolone (60 mg/m^2) over 28 days and a single dose of age-adapted intrathecal methotrexate. BCP-ALL with a discernible, but non-quantifiable, or quantifiable EOI MRD and T-ALL with ≥10-3 EOI MRD were eligible for randomization, receiving either clofarabine 5x40 mg/m^2 or HIDAC 4x3 g/m^2 in combination with PEG-ASP 2,500 IU/m^2 as the first or second course of consolidation in the treatment of BCP-ALL or T-ALL, respectively (Figure 1A).

Further treatment was administered according to respective strata (Figure 1B). By protocol, enrolled patients who achieved MRD-negativity at the end of induction or inversely showed an induction failure were not eligible for randomization (see the *Online Supplementary Appendix* for additional information).

Randomization

The randomization was performed by the coordinating trial center after stratification had been finalized according to EOI MRD status. Each stratum (high risk [HR] patients were subdivided according to immunophenotype) underwent independent randomization on the basis of randomly permuted blocks to avoid imbalances within risk strata.

Analysis of minimal residual disease

Real-time quantitative polymerase chain reaction (PCR) analyses were performed targeting immunoglobulin heavy chain (IGH) and T-cell receptor (TCR) gene rearrangements to assess MRD. Data were interpreted according to the guidelines developed by the European Study Group for MRD detection in ALL (EuroMRD ALL).[10]

Statistical analyses

The probability of event-free (pEFS) and overall survival (pOS) was estimated using the Kaplan-Meier method and compared between subgroups using the log-rank test.[11] Cumulative incidence functions of isolated CNS or any (isolated and combined) CNS relapse, as well as testicular relapse, treatment-related secondary malignancies and toxicity-related death were calculated using the Kalbfleisch and Prentice method and compared using Gray's test.[12] A χ^2 test, a Fisher's exact test, and Spearman's rank correlation analyses were applied to compare the distribution of parameters between subgroups and correlation between parameters.[13] A χ^2 test was applied to determine the difference in the rate of MRD-positive patients, as provided in the study protocol. This was complemented by a one-sided Fisher's exact test and a Cochran-Armitage trend test, the latter of which compared the trend in MRD values between randomized groups.[14]

The status of patients was monitored annually. The database was newly updated (1 December 2020) prior to usage for analysis. Analyses were carried out using SAS version 9.4. Further details of statistical analyses are provided in the *Online Supplementary Appendix*.

Results

Overall, 303 study patients were eligible and randomized, allocating 151 patients toward clofarabine/PEG-ASP and 152 patients toward HIDAC/PEG-ASP (Figure 2; Table 1; *Online Supplementary Appendix*). Of those patients, the main endpoint (i.e., MRD after randomized intervention) was reached by 296 patients, in close approximation to the planned sample size (n=295) (Table 2). There were no differences in patient characteristics regarding known risk factors other than a more frequent occurrence of ETV6-RUNX1 in the clofarabine-treated cohort (Table 1). The

incidence of hematopoietic stem cell transplantation (HSCT) in first complete remission due to persistent MRD was comparable between arms (n=11 *vs.* n=12 HSCT in clofarabine and HIDAC cohorts, respectively). T-ALL patients were similarly underrepresented in both randomized arms compared to the whole study cohort (5.3% [n=8] in the clofarabine and 5.9% [n=9] in the HIDAC cohort *vs.* 14.2% [n=67] in the total cohort), mainly due to a greater proportion of T-ALL in the induction failure cohort (n=24/31 patients [77%]) and in the HR-reduced cohort (n=15/51 patients [29%]), both of which were excluded from randomization according to the study protocol (Table 2; *Online Supplementary Appendix*).

Minimal residual disease response

In the randomized treatment arms, we observed a rate of 44% MRD-positivity after high-dose cytarabine *versus* 33% MRD-positivity after clofarabine in BCP-ALL (P_{chi}^2=0.03; left-sided Fisher test *P*=0.04). The overall reduction of MRD in BCP-ALL was significantly more profound after clofarabine compared to cytarabine, with 93 clofarabine-treated patients *versus* 79 HIDAC-treated patients reaching MRD

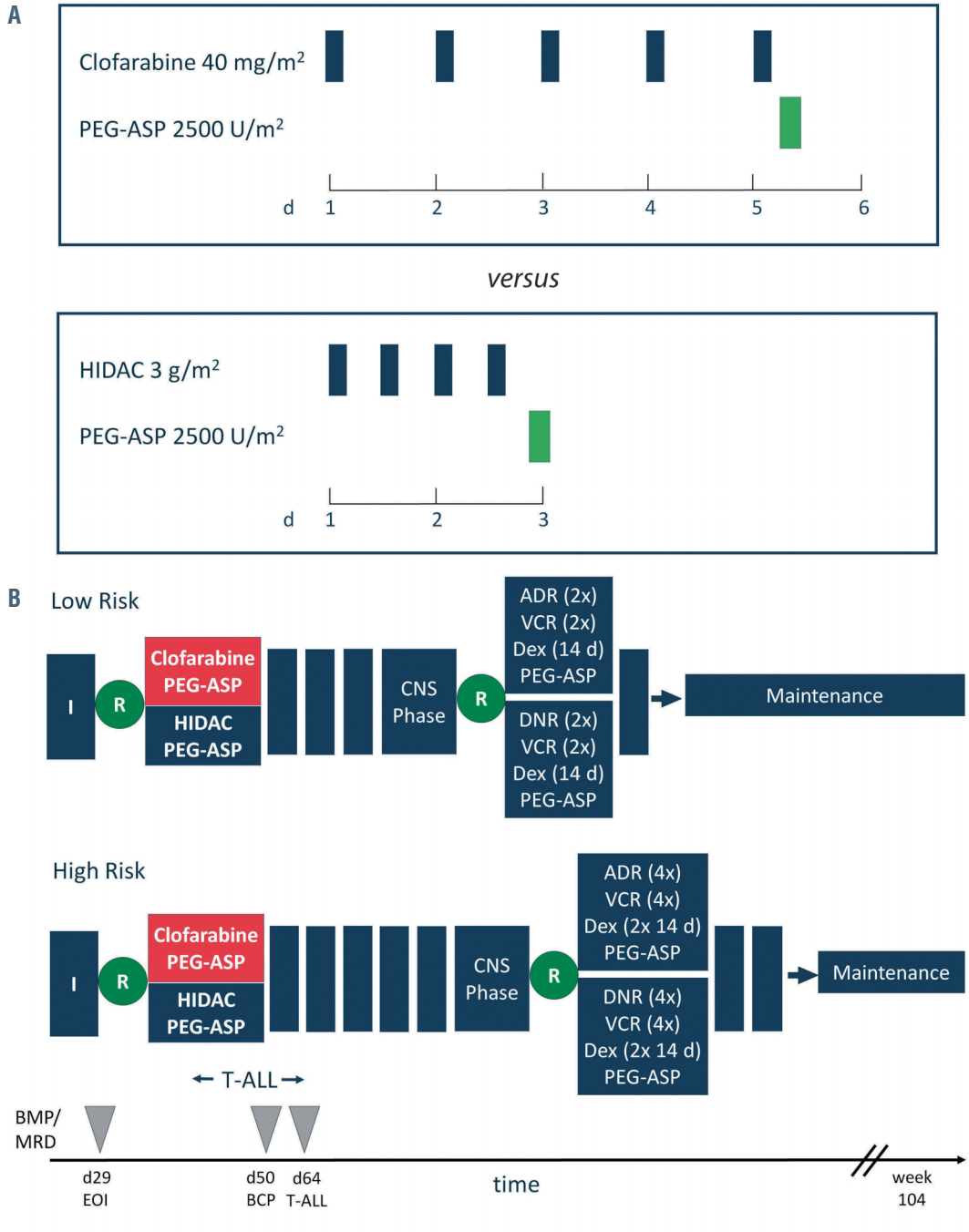

Figure 1. Treatment overview. (A) Randomized treatment block clofarabine *vs.* high-dose cytarabine, each combined with pegylated asparaginase (PEG-ASP). (B) Schematic overview of the CoALL 08-09 protocol. ADR: doxorubicin; BCP: B-cell precursor; BMP: bone marrow puncture; CNS: central nervous system; d: day; Dex: dexamethasone; DNR: daunorubicin; EOI: end of induction; HIDAC: high-dose cytarabine; I: induction; MRD: minimal residual disease; R: randomization; VCR: vincristine; CoALL: Cooperative Acute Lymphoblastic Leukemia study group.

negativity, and a lower rate of patients with quantifiable MRD levels (6 patients after clofarabine *vs.* 18 patients after HIDAC) (Cochran-Armitage trend test P=0.01; Table 2; *Online Supplementary Figure S1*). This observation holds true in a sub-analysis of the patients with a higher burden of EOI MRD (≥10^{-3}) who were stratified to the low risk (LR)- or HR-intensified arms. Among those 73 patients, 27 patients were MRD-negative after clofarabine compared to 16 patients randomized to the HIDAC arm (Cochran-Armitage trend test P=0.02). In *ETV6-RUNX1*-rearranged ALL, which occurred more frequently in clofarabine-treated patients by chance, we observed an equivalent efficacy of the randomized nucleosides, reflecting a generally high sensitivity toward asparaginase in this prognostically favorable genetic subgroup of ALL (Table 1; *Online Supplementary Table S3*). In order to address a potential skewing effect of misbalanced *ETV6-RUNX1* on the MRD outcome of randomized groups, *ETV6-RUNX1*-negative ALL was analyzed separately, which confirmed greater activity of clofarabine compared to HIDAC (P_{chi}^2=0.04210) (*Online Supplementary Table S3*).

Importantly, after the randomized course in early consolidation (day 50 in B-cell precursor (BCP)-ALL and day 64 in T-ALL patients), MRD maintained its prognostic relevance, with a significant impact on EFS and relapse rate in comparison to day 29 EOI MRD (Figure 3A and B).[15] T-ALL patients of both randomized arms achieved comparable MRD reductions by day 64, although the number of T-ALL patients was very small (Tables 1 and 2). Nevertheless, the test for trends in the overall cohort comprising both BCP-ALL and T-ALL confirmed that clofarabine was significantly more effective in MRD reduction compared to HIDAC (Cochran-Armitage trend test P=0.01) (Table 2).

Table 1. Demographics and clinical characteristics of randomized patients.

	High-dose cytarabine (n=152) No. (%)	Clofarabine (n=151) No. (%)	*P*
Immunophenotype			
B-precursor ALL	143 (94.1)	143 (94.7)	0.82
T-ALL	9 (5.9)	8 (5.3)	
Sex			
male	79 (52)	85 (56.3)	0.45
female	73 (48)	66 (43.7)	
Age at diagnosis			
< 10 years	123 (80.9)	119 (78.8)	0.65
≥ 10 years	29 (19.1)	32 (21.2)	
WBC			
< 25/nL	101 (66.4)	110 (72.8)	0.73
≥ 25/nL	51 (33.6)	41 (27.2)	
ETV6-RUNX1 rearrangement			
positive	30 (19.7)	47 (31.1)	0.02
negative	117 (77)	104 (68.9)	
unknown	5 (3.3)	0 (0)	
KMT2A rearrangement			
positive	2 (1.3)	2 (1.3)	1.0
negative	150 (98.7)	149 (98.7)	
Karyotype			
< 44 chromosomes	2 (1.3)	2 (1.3)	0.31
44-50 chromosomes	90 (59.2)	106 (70.2)	
> 50 chromosomes	48 (31.6)	38 (25.2)	
unknown	12 (7.9)	5 (3.3)	
Treatment response BM day 15			
M1	98 (64.5)	104 (68.9)	0.68
M2	28 (18.4)	23 (15.2)	
M3	4 (2.6)	5 (3.3)	
not available	22 (14.5)	19 (12.6)	
Risk Stratification			
Low-risk standard	57 (37.5)	62 (41.1)	
Low-risk intensified	20 (13.2)	19 (12.6)	
High-risk standard	47 (30.9)	43 (28.5)	
High-risk intensified	28 (18.4)	27 (17.9)	

ALL: acute lymphoblastic leukemia; WBC: white blood cell; BM: bone marrow.

Outcome of randomized groups

No significant differences in outcome regarding EFS and OS were observed between the randomized arms (Figure 3C and D), with a median observation time of 3.7 years. There were also no significant differences in Cox regression analyses regarding the covariates sex, age (<10 years *vs.* ≥10 years), WBC (< 25/nL *vs.* ≥25/nL), *ETV6-RUNX1*, and HSCT in first continuous remission as time-dependent variables. An additional stratified analysis confirmed that there were no significant differences in EFS or relapse rate between randomized courses according to the categories negative, positive non-quantifiable (n.q.), and quantifiable MRD on day 50. Besides events that were anticipated upon quantifiable MRD after randomized intervention, several relapses occurred in MRD-negative and MRD-positive n.q. patients in both randomized treatment arms, accounting for the observed lack of surrogacy of MRD in the outcome analysis (*Online Supplementary Table S4*). There was no evidence of a mutual impact between the randomizations at early consolidation and delayed intensification in this study, as shown by very similar pEFS in the latter randomized arms (log-rank test P=0.88 for patients receiving doxorubicin and log-rank test P=0.50 for patients receiving daunorubicin during delayed intensification).

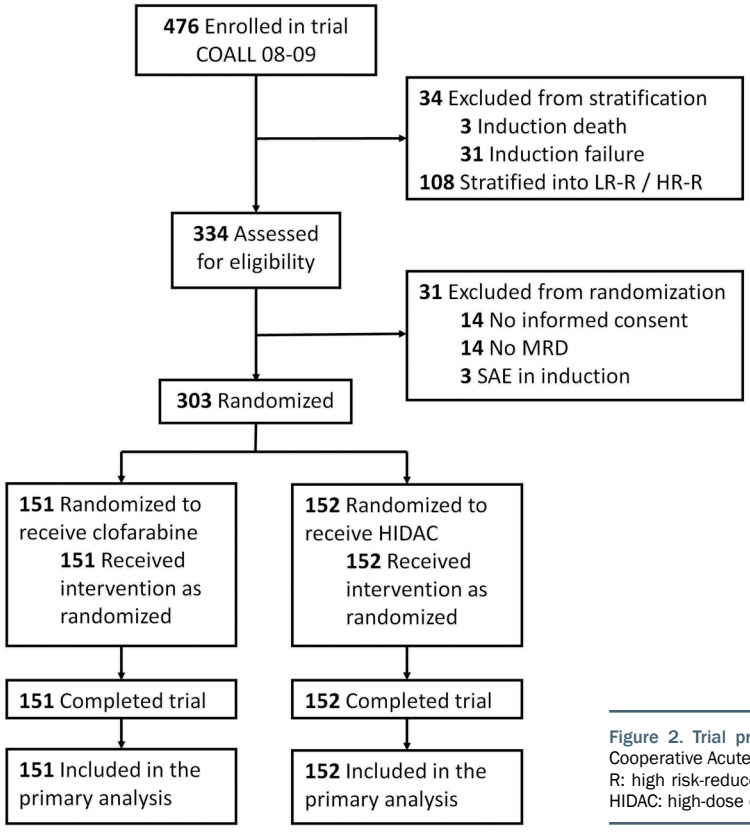

Figure 2. Trial profile. Flow diagram according to CONSORT guidelines. CoALL: Cooperative Acute Lymphoblastic Leukemia Study Group; LR-R: low risk-reduced; HR-R: high risk-reduced; MRD: minimal residual disease; SAE: serious adverse events; HIDAC: high-dose cytarabine.

Table 2. Minimal residual disease response toward clofarabine/PEG-ASP *versus* high-dose cytarabine/PEG-ASP.

		High-dose Cytarabine No. (%)	Clofarabine No. (%)	All	*P*
B-precursor ALL	MRD d50 pos.	61 (44)	45 (33)	106	0.03 χ^2 0.04 Fisher
B-precursor ALL	MRD d50 neg.	79 (56.4)	93 (67.4)	172	0.01
	MRD d50 pos. nq	43 (30.7)	39 (28.3)	82	Cochran-Armitage Trend Test
	MRD d50 ≥10^{-4}	18 (12.9)	6 (4.3)	24	
B-precursor ALL EOI MRD ≥10^{-3}	MRD neg.	16 (21.9)	27 (37)	43	0.02 Cochran-Armitage Trend Test
T-ALL	MRD d64 neg.	4 (44.4)	3 (37.5)	7	0.94
	MRD d64 pos. nq	3 (33.3)	4 (50.0)	7	Cochran-Armitage Trend Test
	MRD d64 ≥10^{-4}	2 (22.2)	1 (12.5)	3	

ALL: acute lymphoblastic leukemia; EOI: end-of-induction; MRD: minimal residual disease, d50: day 50; d64: day 64; neg: negative; pos: positive; nq: non-quantifiable. PEG-ASP: pegylated asparaginase.

Toxicity

No statistically significant differences in the incidence of severe or persistent toxicities between randomized treatment elements or in the subsequent treatment realization were documented (Figure 4; *Online Supplementary Table S2A* and *B*). In particular, severe grade 3 or 4 skin toxicities were not observed in either treatment arm, but clofarabine was more frequently associated with grade 2 skin toxicities. With regard to hepatotoxicity, an elevation of transaminases (aspartate and alanine transaminases [AST and ALT], respectively) was significantly more often reported after clofarabine than after HIDAC, and then spontaneously resolved without exception after each randomized treatment element before the start of subsequent chemotherapy. Accordingly, time intervals between the randomized courses and the subsequent treatment elements were similar, with a median of 22 days (range, 20–38 days) after clofarabine/PEG-ASP and 19 days (range, 18–38 days) after HIDAC/PEG-ASP. Incidence and degree of myelotoxicity differed slightly between clofarabine and HIDAC (Figure 4; *Online Supplementary Table S2A* and *B*). Remarkably, when comparing CTC grades 0 to 2 against grades 3 and 4 for hemoglobin and platelets, clofarabine was associated with significantly less severe toxicities (*Online Supplementary Table S2B*). Clofarabine caused a more frequent grade 4 depletion of white blood cells suggesting a greater lymphotoxicity given that grade 4 reduction in neutrophil counts was comparable between randomized arms (Figure 4; *Online Supplementary Table S2A*). Nevertheless, the incidence of severe infections after randomized treatment was comparable (Figure 4; *Online Supplementary Table S2A* and *B*). Finally, the incidence of serious adverse events (SAE) during the remaining treatment courses was very similar (18 and 19 SAE in the clofarabine *vs.* HIDAC arm, respectively).

Discussion

As demonstrated in trial CoALL 08-09, clofarabine combined with PEG-asparaginase is effective in the eradication

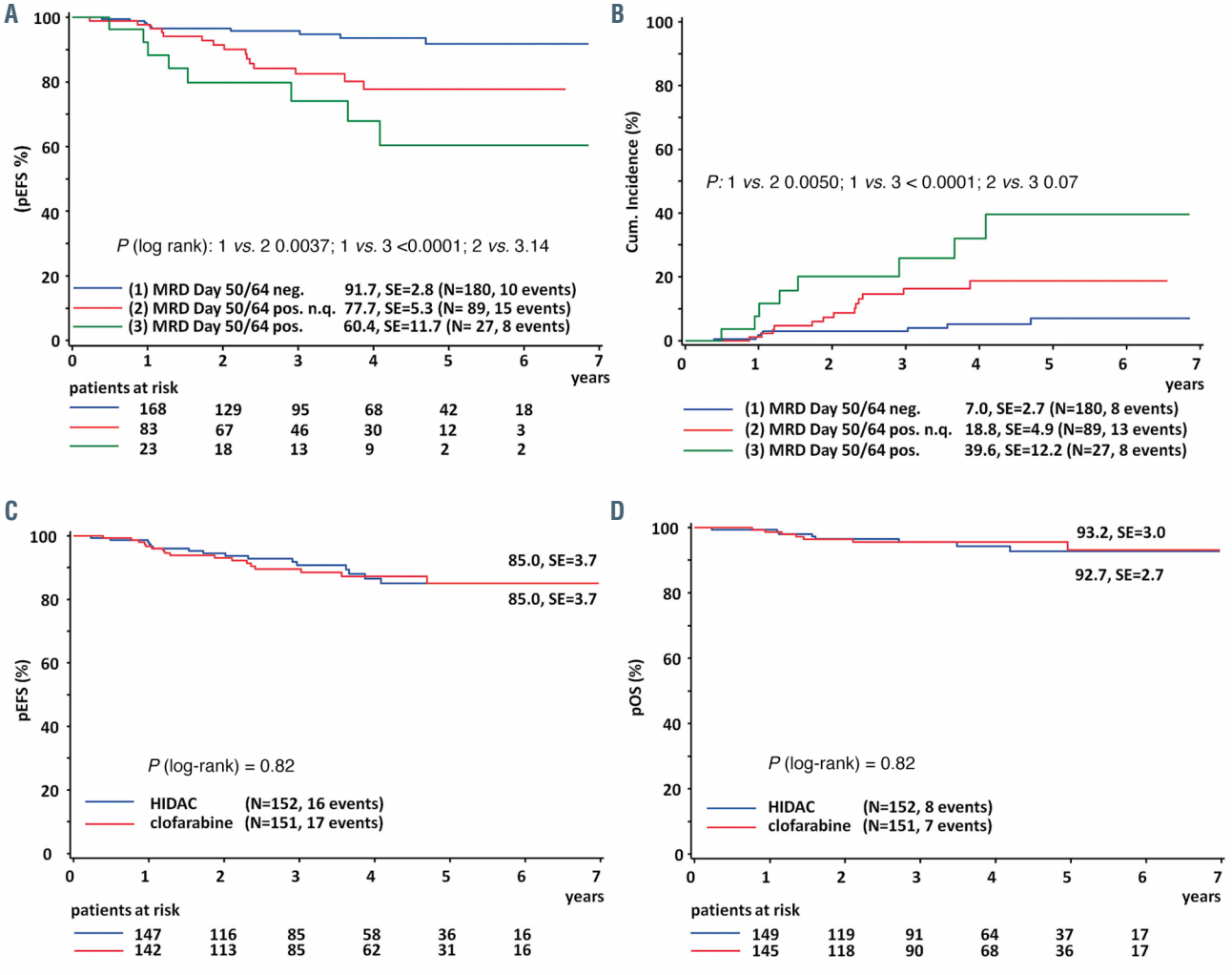

Figure 3. Outcome analyses in randomized patients. (A) Probability of event-free survival (pEFS) (5 years of follow-up) in randomized patients according to MRD on day 50/64 after completion of randomized treatment courses. For comparative outcome probability analyses according to MRD levels, MRD negativity is denoted as 1, non-quantifiable (n.q.) MRD positivity is denoted as 2, and MRD ≥ $1x10^{-4}$ is denoted as 3. (B) Cumulative relapse rate (5 years of follow-up) in randomized B-precursor and T-acute lymphoblastic leukemia (T-ALL) patients according to MRD on day 50/64. (C) and (D) legends are swoped. (C) Comparative probability of event-free (pEFS) (5 years of follow-up) analysis in clofarabine/PEG-ASP-treated vs. HIDAC/PEG-ASP-treated ALL patients. (D) Comparative analysis of overall survival (pOS) (5 years of follow-up) in clofarabine/PEG-ASP-treated *vs.* HIDAC/PEG-ASP-treated ALL patients. PEG-ASP: pegylated asparaginase; HIDAC: high-dose cytarabine.

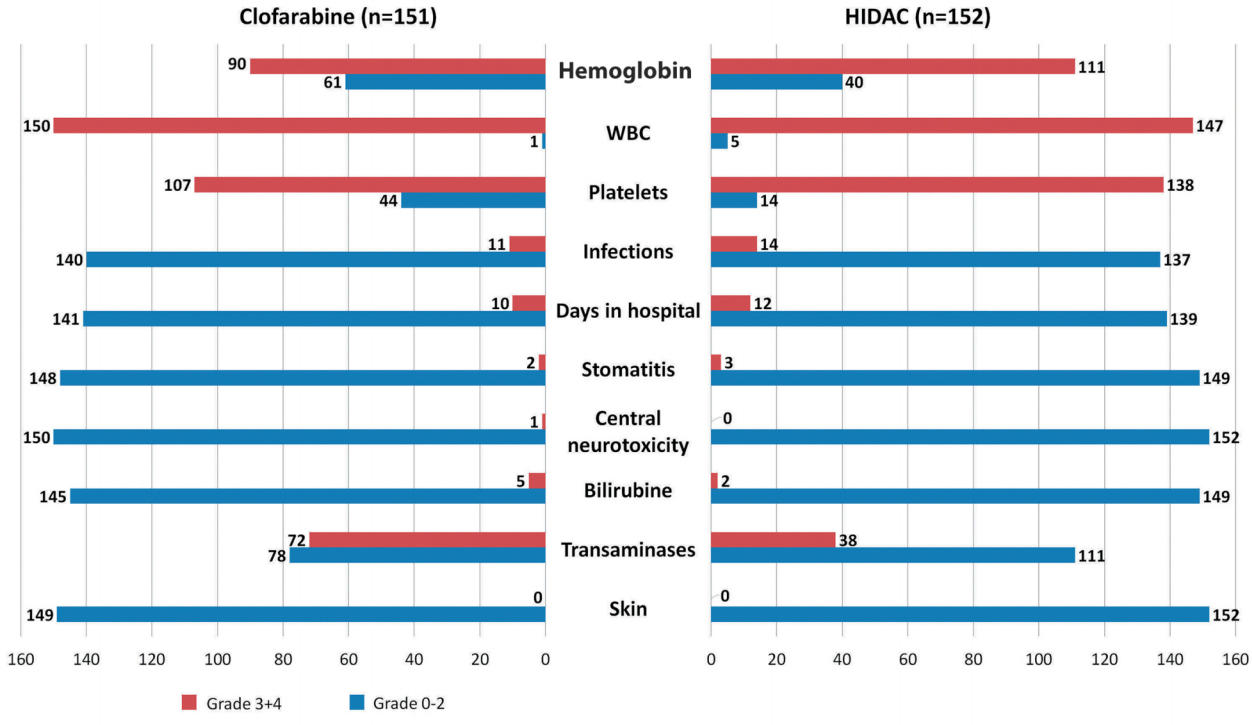

Figure 4. Treatment-related toxicities in randomized patients according to treatment arm and common terminology criteria. HIDAC: high-dose cytarabine; WBC: white blood cell.

of MRD and well tolerated in the frontline treatment of ALL. In comparison to high-dose cytarabine/PEG-ASP, clofarabine/PEG-ASP was superior in the overall reduction of an MRD burden. The frequency of MRD-positive BCP-ALL patients in the standard arm was lower than the predicted rate of 60%, likely due to a smaller sample size and the different distribution of risk strata in the preceding trial, CoALL 03-07.

Although the prognostic impact of MRD in BCP-ALL is still clearly discernible in early consolidation after the randomized courses of clofarabine *versus* HIDAC, the greater cytotoxic efficacy of clofarabine did not translate into an obvious improvement of outcome at the trial level after a median follow-up period of 3.7 years. This lack of surrogacy of MRD at early consolidation in a survival endpoint analysis could be explained by a small effect size, taking into account that only a single course of clofarabine was compared with high-dose cytarabine as a part of a complex multiagent chemotherapy backbone, the entirety of which determines treatment efficacy. Our trial design allowed for the detection of a ~10% difference in outcome between randomized treatment arms at a power of 80%. Hence, the small sample size has to be considered with regard to the number of randomized patients required in order to perform a meaningful comparative analysis of survival in CoALL 08-09, which was a priori defined as a secondary objective in the study protocol.

Overall, clofarabine increased the rate of MRD negativity by 25% compared to HIDAC, which is an incremental improvement with borderline significance in contrast to a statistically more robust overall reduction of MRD after clofarabine (Table 2; *Online Supplementary Figure S1*). The occurrence of relapsing disease in MRD-negative patients after clofarabine (and HIDAC) observed in this trial points at MRD as a time-dependent variable. In this regard, early achievement of MRD negativity at the end of induction is more predictive of outcome than achievement of MRD negativity later in treatment, most likely due to the emergence of resistant clones, i.e., MRD negativity does not necessarily imply true eradication of the disease, but simply reflects a decrease to a level below the detection limit of the PCR-based MRD assay. Inversely, MRD positivity more reliably reflects outcome when measured later in treatment.[15,16]

In addition, the rarity of events after treatment of ALL in childhood might generally compromise surrogacy of MRD as a prognostic marker of outcome at the trial level. A previous multi-trial approach including 4,830 patients with ALL demonstrated that EOI MRD failed as a surrogate for treatment effects on EFS at the trial level, when dexamethasone and prednisone were compared in induction treatment of AIEOP-BFM ALL and COG trials.[17-19] This meta-analysis raised caution with regard to MRD as a surrogate marker for treatment decisions in randomized trials. In contrast to these trials, in which the stratifying decision was made after randomization, we can exclude that the evaluation of MRD after randomized intervention impacted a decision on the subsequent treatment in CoALL 08-09, since the ultimate stratification had been done before randomization on d29 in BCP-ALL and on d43 in T-ALL.

In this trial, we applied clofarabine at a dose of 40 mg/m^2 daily x 5 corresponding to the previously established single agent maximum-tolerated dose (MTD) in adult acute leukemia which is lower than the MTD of 52 mg/m^2 x 5 determined in pediatric patients with acute leukemia.[2,20] The administration of high-dose clofarabine in conjunction with PEG-asparaginase in early consolidation of CoALL 08-09 was feasible largely due to almost non-overlapping

toxicities. By contrast, clofarabine given at a reduced dose level of 30 mg/m^2 x 5 or 20 mg/m^2 x 5, respectively, was associated with unacceptably severe infections and myelotoxicities in heavily pretreated pediatric patients with relapsed/refractory leukemia when combined with cyclophosphamide, etoposide, vincristine, and PEG-ASP in the COG trial AALL1131.[7]

Since MRD fell short as a surrogate marker in a true endpoint analysis of survival of randomized patient cohorts in CoALL 08-09, standard cytarabine treatment has not been replaced by clofarabine, despite its superior cytotoxic efficacy. Notwithstanding, given its favorable risk/benefit ratio, a further evaluation of clofarabine in combination with PEG-ASP might be warranted as a second-line replacement or add-on strategy in specific patients, to reduce treatment-related morbidities or to augment the depth of molecular remission after antibody-based immunotherapy.[21,22] In particular, clofarabine/PEG-ASP could be tested in high-risk patients and compared with other established anti-leukemic agents that are burdened with severe acute and long-term toxicities, such as anthracyclines or the anti-metabolite methotrexate.[23,24]

Disclosures

No conflicts of interest to disclose.

Contributions

MZ, MAH and GE designed the study with input from FS and UzS; DD, JF, TF, TI, NJ, AP, IS and FS recruited patients; MAH, GE and FS collected, analysed, and interpreted data.

Acknowledgments

Genzyme/Sanofi provided the investigational drug clofarabine. We thank Kseniya Bakharevich for her assistance in collecting and interpreting the data. We gratefully acknowledge all patients, their families and care providers who participated in this study. Finally, we thank all the clinicians, as well as diagnostics and research personnel who were actively involved in this clinical trial.

Data sharing

Individual patient data from the trial will not be shared publicly, since a data-sharing plan had not been included when ethical approval was requested. All original data can be obtained by the corresponding authors, please contact Dr. Gabriele Escherich: escherich@uke.de

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