VOLUME 31 · NUMBER 5 · FEBRUARY 10 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Improved Outcome in Pediatric Relapsed Acute Myeloid Leukemia: Results of a Randomized Trial on Liposomal Daunorubicin by the International BFM Study Group

Gertjan J.L. Kaspers, Martin Zimmermann, Dirk Reinhardt, Brenda E.S. Gibson, Rienk Y.J. Tamminga, Olga Aleinikova, Hortensia Armendariz, Michael Dworzak, Shau-Yin Ha, Henrik Hasle, Liisa Hovi, Alexei Maschan, Yves Bertrand, Guy G. Leverger, Bassem I. Razzouk, Carmelo Rizzari, Petr Smisek, Owen Smith, Batia Stark, and Ursula Creutzig

Author affiliations appear at the end of this article.

Published online ahead of print at www.jco.org on January 14, 2013.

Presented in part at the American Society of Hematology 52nd Annual Meeting, December 4-7, 2010, Orlando, FL; at the International Society of Paediatric Oncology XXXXII Congress, October 21-24, 2010, Boston, MA; and at the 7 Bi-annual Childhood Leukemia Symposium, October 4-6, 2010, Antalya, Turkey.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: ISRCTN94206677.

Corresponding author: Gertjan J.L. Kaspers, MD, PhD, Pediatric Oncology/Hematology, VU University Medical Center, De Boelelaan 1117, NL-1081HV Amsterdam, the Netherlands; e-mail: gjl.kaspers@vumc.nl.

0732-183X/13/3105-599/$20.00

DOI: 10.1200/JCO.2012.43.7384

ABSTRACT

Purpose
In pediatric relapsed acute myeloid leukemia (AML), optimal reinduction therapy is unknown. Studies suggest that liposomal daunorubicin (DNX; DaunoXome; Galen, Craigavon, United Kingdom) is effective and less cardiotoxic, which is important in this setting. These considerations led to a randomized phase III study by the International Berlin-Frankfurt-Münster Study Group.

Patients and Methods
Patients with relapsed or primary refractory non–French-American-British type M3 AML who were younger than 21 years of age were eligible. Patients were randomly assigned to fludarabine, cytarabine, and granulocyte colony-stimulating factor (FLAG) or to FLAG plus DNX in the first reinduction course. The primary end point was status of the bone marrow (BM) sampled shortly before the second course of chemotherapy (the day 28 BM). Data are presented according to intention-to-treat for all 394 randomly assigned patients (median follow-up, 4.0 years).

Results
The complete remission (CR) rate was 64%, and the 4-year probability of survival (pOS) was 38% (SE, 3%). The day 28 BM status (available in 359 patients) was good (≤ 20% leukemic blasts) in 80% of patients randomly assigned to FLAG/DNX and 70% for patients randomly assigned to FLAG ($P = .04$). Concerning secondary end points, the CR rate was 69% with FLAG/DNX and 59% with FLAG ($P = .07$), but overall survival was similar. However, core-binding factor (CBF) AML treated with FLAG/DNX resulted in pOS of 82% versus 58% with FLAG ($P = .04$). Grade 3 to 4 toxicity was essentially similar in both groups.

Conclusion
DNX added to FLAG improves early treatment response in pediatric relapsed AML. Overall long-term survival was similar, but CBF-AML showed an improved survival with FLAG/DNX. International collaboration proved feasible and resulted in the best outcome for pediatric relapsed AML reported thus far.

J Clin Oncol 31:599-607. © 2013 by American Society of Clinical Oncology

INTRODUCTION

Survival is currently achieved in more than 60% of children with acute myeloid leukemia (AML).[1-3] The most frequent type of treatment failure is relapse, occurring in 30% to 40% of patients. Pediatric relapsed AML has a probability of overall survival (pOS) of 16% to 34%, with more recent studies showing relatively better outcome.[4-12] There have been no randomized studies in these patients, and much is unknown, including optimal reinduction therapy.

Cardiotoxicity owing to higher cumulative doses of anthracyclines is a concern, especially in children.[13-15] Moreover, Webb et al[11] reported that different reinduction regimens with and without an anthracycline had more or less similar efficacy in pediatric relapsed AML, although numbers were small. Fludarabine, cytarabine, and granulocyte colony-stimulating factor (FLAG) is a well-known reinduction regimen in AML.[16,17] Because there is no evidence that anthracyclines improve survival from relapsed AML, whereas there is evidence that higher cumulative doses of anthracyclines increase the risk of late cardiotoxicity, study Relapsed AML 2001/01 was designed to randomly assign for FLAG versus FLAG plus an anthracycline, instead of comparing different anthracyclines added to FLAG.

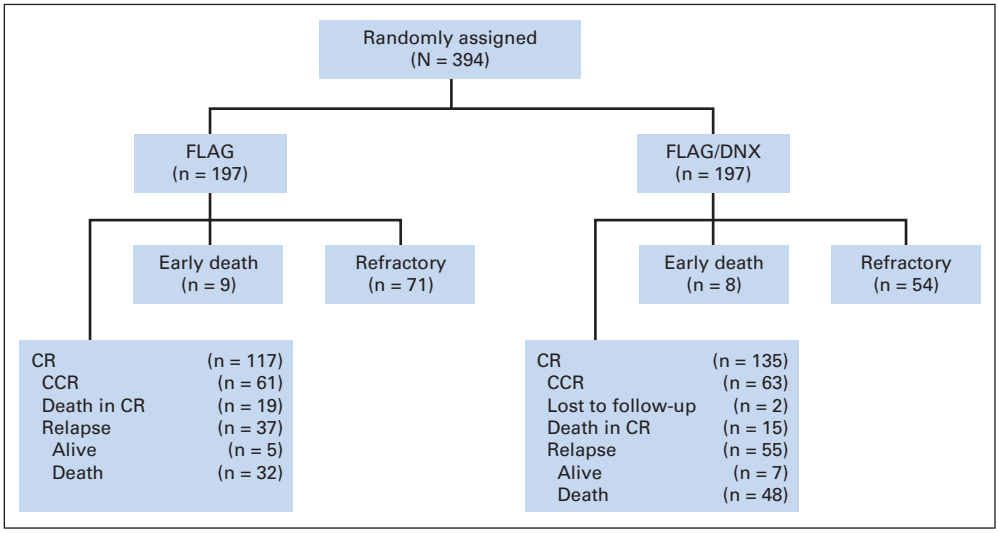

Fig 1. Flow diagram of randomly assigned patients enrolled in study Relapsed AML 2001/01. CR, complete remission; CCR, complete continuous remission; DNX, liposomal daunorubicin; FLAG, fludarabine, cytarabine, and granulocyte colony-stimulating factor.

Liposomally entrapped daunorubicin (DNX; DaunoXome; Galen, Craigavon, United Kingdom) was selected, because preclinical and animal studies and limited clinical data suggest that DNX is less cardiotoxic.[18-22] Indeed, liposomal anthracyclines in general cause less cardiotoxicity than conventional anthracyclines.[23] This study was also based on the AML Berlin-Frankfurt-Münster (BFM) REZ97 study, which applied DNX plus cytarabine in reinduction, showing no clinically relevant cardiotoxicity.[24]

This worldwide study is the largest of its kind and is the first randomized study in pediatric relapsed AML.

PATIENTS AND METHODS

Patients

Patients were eligible if they were in first relapse or had primary refractory AML and were younger than 21 years. All randomly assigned patients with information on leukemic blast percentage in the bone marrow (BM) on day 28 were included, independent of that percentage pretreatment, the latter to allow evaluating any differential effect on progressive disease (Fig 1). Ineligible patients included those with acute promyelocytic leukemia, patients older than 18 years at initial diagnosis, and patients with symptomatic cardiac dysfunction, fractional shortening less than 29%, and/or performance status less than 40%. Patient enrollment (13 groups, 20 countries) lasted from November 2001 until April 1, 2009. Human investigations were performed after approval by a local human investigations committee and in accordance with an assurance filed with and approved by the Department of Health and Human Services. The study was conducted in accordance with the Declaration of Helsinki.

Treatment

Courses were not to commence less than 28 days from the start of the previous course and should start on blood cell count recovery (platelets $> 50 \times 10^9$/L, neutrophils $> 1.0 \times 10^9$/L; Fig 2A).

Reinduction. Patients were randomly assigned to FLAG or FLAG/DNX 1:1 in the first reinduction course (Fig 2B). The 5-day FLAG regimen without DNX was the second course. DNX was not provided free of charge for the international study, and there was no outside funding for the trial.

Consolidation. Consolidation was advised to bridge the time to transplant, if necessary. High-intensity consolidation consisted of cytarabine (500 mg/m^2/d via continuous infusion for 4 days) and etoposide (100 mg/m^2/dose twice daily for 4 days). Low-intensity consolidation, for patients with a poor condition and/or shorter time to transplant, consisted of thioguanine (100 mg/m^2/dose once daily, maximum of 4 weeks) and cytarabine (75 mg/m^2/dose once daily subcutaneously for 4 days, every other week).

CNS-directed treatment. Each course started with intrathecal cytarabine. In case of CNS involvement, triple (methotrexate, cytarabine, prednisolone) intrathecal medication at age-adjusted doses was given every 7 days until 1 week after complete clearance of leukemic blasts. Then two more doses were given. Cranial irradiation was not recommended.

Allogeneic stem-cell transplantation (alloSCT) was indicated in all patients achieving complete remission (CR), preferably using a matched sibling donor, alternatively with a matched unrelated donor. If not available, patients in early relapse and those with primary refractory disease were permitted to undergo haplo-identical donor SCT, whereas patients with late relapsed AML were to undergo autologous SCT. Guidelines for conditioning regimens consisted of either total-body irradiation with cyclophosphamide, or busulphan, cyclophosphamide, melphalan.

Treatment Response Monitoring

Early treatment response was documented by BM examination shortly before the start of the second reinduction course (the day 28 BM). If the day 28 BM showed more than 20% blasts, patients were removed from protocol and became eligible for more experimental therapy such as gemtuzumab ozogamicin[25] or methotrexate,[26] or received no further treatment. This was based on unpublished data from the BFM-AML group that such patients have a very poor outcome (Appendix Fig A1, online only), even with the limitation that blast percentage was determined by morphology. There were no guidelines for additional immunophenotyping of blasts, but control by flow cytometry was done in most patients. A second BM aspiration was performed 28 to 42 days after start of the second course of chemotherapy. Patients not achieving CR were taken off protocol but resumed in follow-up. BM and cytogenetic results were centrally reviewed at the national level.

Toxicity Monitoring

Toxicity monitoring was performed after each treatment course according to National Cancer Institute Common Toxicity Criteria version 2.0.

Definitions

Definitions were as reported previously (Appendix Table A1, online only).[9,27,28] CR was defined as $\leq$ 5% leukemic blasts in BM with signs of normal hematopoiesis and of regeneration of normal peripheral-blood cell production (platelets $> 50 \times 109$/L without transfusions, neutrophils $> 1.0 \times 109$/L) and no leukemic cells in the peripheral blood or anywhere else.

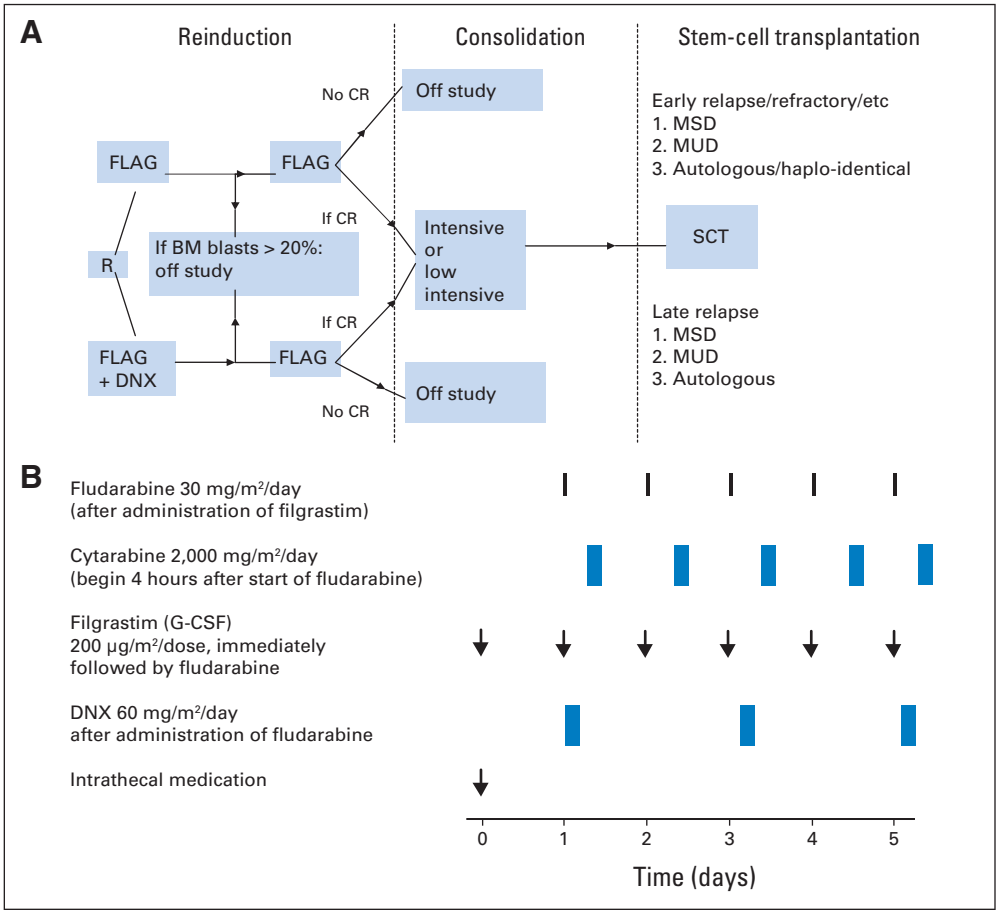

Fig 2. (A) Outline of pediatric study Relapsed Acute Myeloid Leukemia (AML) 2001/01; (B) fludarabine, cytarabine, and granulocyte colony-stimulating factor (FLAG) + liposomal daunorubicin (DNX) schedule. Consolidation chemotherapy guidelines were provided for patients in whom allogeneic stem-cell transplantation (alloSCT) was not available immediately after achieving complete remission (CR). Otherwise, alloSCT was advised immediately after the two reinduction courses. BM, bone marrow; G-CSF, granulocyte colony-stimulating factor; MSD, matched sibling donor; MUD, matched unrelated donor; R, random assignment.

Statistics

The primary end point of the study was the day 28 BM status. Secondary end points included CR after two courses, relapse, long-term survival, and toxicity. All living patients were censored at the time of last follow-up, but no later than January 1, 2011. The Kaplan-Meier method was used to estimate survival rates, and differences were compared with the two-sided log-rank test. The Cox proportional hazards model was used for univariate and multivariate analyses. Cumulative incidence functions for competing events were constructed by the method of Kalbfleisch and Prentice and compared with Gray's test. Results are presented as probability of death in CR, estimated cumulative incidence of relapse (pCIR), and estimated 4-year pOS with SE (± SE). Possible heterogeneity of the therapeutic effect of DNX in subgroups was analyzed post hoc by estimation of the odds ratios for early treatment response and overall survival (OS) for each of several subgroups, defined according to time to relapse (early and late relapse) and cytogenetics (favorable (t(8;21) and inv(16)) and nonfavorable; there were not enough patients to allow a subdivision of the nonfavorable subgroup). Differences in the distribution of individual parameters among patient subsets were analyzed using Fisher's exact test for categorized variables and the Mann-Whitney *U* test for continuous variables. Analyses were conducted using SAS (SAS-PC, version 9.1; SAS Institute, Cary, NC).

This open-label study needed to enroll 360 fully eligible and randomly assigned patients, with information on the primary end point, to demonstrate (with 80% power and a two-sided α level of .05) that adding DNX to FLAG resulted in at least a 15% higher good early treatment response rate. Block randomization with block size 4 was done with a central interactive computerized system, stratified by study group and time to relapse on a 1:1 basis. Ultimately, 394 randomly assigned patients resulted in 359 patients with information on the primary end point.

RESULTS

General Information

Data are presented according to intention to treat, with a median follow-up time of 4.0 years. Most data concern the group of 394 randomly assigned patients, including 35 patients in whom the primary end point was unknown (Fig 1). In total, 568 patients with refractory or relapsed AML were registered. Reasons not to randomize (n = 174) included lack of availability of DNX in two time periods and the decision to opt for palliative or experimental treatment. Registration of patients was done independent from the decision to participate in this trial. Treatment administered in the first course matched treatment assigned in 96% of patients. Adherence to FLAG as second course was lower, and 11% of patients received another type of second course of treatment.

Detailed Information on Treatment

The distribution among different types of consolidation and SCT was well-balanced (Appendix Table A2, online only). Type of alloSCT was also well-balanced among both treatment arms in the total group of patients as well as in the subgroup of patients with core-binding factor (CBF) AML. Time to transplant did not differ between patients who received FLAG (from CR, median 107 days) or FLAG/DNX (median, 105 days).

Characteristics of Randomly Assigned Patients and Overall Outcome Results

Randomly assigned patients seem to be an unselected cohort, except that fewer patients with primary refractory disease were randomly assigned. Patients with relapsed and refractory AML had to be registered, even if salvage treatment was not considered (Appendix Table A3, online only).

Table 1 shows characteristics of the randomly assigned patients, which were as expected, although CBF-AML was detected in 20% of all randomly assigned patients. Patients with less than 20% BM blasts before treatment were well-balanced between both arms (Table 1), as were patients with less than 5% BM blasts (eight with FLAG and 12 with FLAG/DNX). Table 2 shows outcome parameters in the total group of randomly assigned patients. Refractory disease and relapse were the most frequent events, occurring in 56% of patients. The overall 4-year pOS was 38% (SE, 3%). When analyzing the 568 patients together, to allow a comparison with outcome reported by other groups, overall 4-year pOS was 36% (SE, 2%; Fig 3A).

Table 1. Comparability of Both Treatment Arms for Patients Treated With FLAG or FLAG/DNX in the First Course of Reinduction Chemotherapy

Characteristic	FLAG (n = 197)		FLAG/DNX (n = 197)	
	No.	%	No.	%
Sex				
Female	79	40	84	43
Male	118	60	113	57
Age, years				
Median	10		9	
Range	0-19		0-19	
WBC, $\times 10^9$/L				
Median	4.2		3.7	
Range	0.4-267		0.4-950	
Pretreatment % blasts in bone marrow*				
Median	50		50	
Range	0-99		0-100	
Patients with ≤ 20% leukemic blasts pretreatment	39	20	31	16
Platelet count, $\times 10^9$/L				
Median	67		70	
Range	1-477		6-587	
FAB type (percentages calculated without "unknown")				
M0	13	8	14	8
M1	24	15	28	17
M2, all cases	52	32	36	22
With Auer rods	40		27	
M4, all cases	32	20	31	19
With eosinophilia	8		11	
M5	23	14	38	23
M6	6	4	8	5
M7	13	8	12	7
Unknown	34		30	
Cytogenetics† (percentages calculated without "unknown")				
Favorable‡	34	19	36	21
Nonfavorable	141	81	134	79
Unknown	22		27	
Site of relapse (percentages calculated without "unknown")				
Isolated BM	155	86	151	82
Combined including BM	19	11	26	14
Extramedullary	6	3	8	4
Unknown	17		12	
Disease status§				
Early relapse	90	46	95	48
Late relapse	95	48	87	44
Primary refractory disease	12	6	15	8

Abbreviations: BM, bone marrow; DNX, liposomal daunorubicin; FAB, French-American-British; FLAG, fludarabine, cytarabine, and granulocyte colony-stimulating factor.

*Unknown in 22 and 24 patients, respectively.

†In 43 patients, cytogenetics were known at initial diagnosis but not at relapse; in these patients, that information was used; in all other patients, cytogenetics at relapse was used.

‡Favorable: t(8;21), inv(16) or t(16;16); nonfavorable: all other patients with known cytogenetics. There were not enough patients with adverse cytogenetic features to allow a meaningful further subdivision of the nonfavorable subgroup into an adverse and intermediate one.

§Early relapse occurs within 1 year from initial diagnosis; late relapse occurs after 1 year.

Table 2. Clinical Outcome for the Total Group of Randomly Assigned Patients With Pediatric Relapsed AML and Per Treatment Arm in the First Course of Reinduction Chemotherapy

Parameter	Randomly Assigned Group			FLAG			FLAG/DNX			
	%	SE	95% CI	%	SE	95% CI	%	SE	95% CI	P*
No. of patients		394			197			197		
Early treatment response on day 28†										.04
Day 28 BM ≤ 20% leukemic blasts (good)										
No.		269			127			142		
%		75			70			80		
Day 28 BM > 20% leukemic blasts (poor)										
No.		90			54			36		
%		25			30			20		
Data on remission										
No CR										
No.		142			80			62		
%		36			41			32		
Early death rate										.80
No.		17			9			8		
%		4			5			4		
Refractory disease										.07
No.		125			71			54		
%		32			36			27		
CR after 2 courses										.07
No.		252			117			135		
%		64			59			69		
Survival data										
Probability of death in CR at 4 years	8	1		10	2		7	2		.48
pCIR at 4 years	24	2		19	3		29	3		.02
pOS at 4 years	38	3	33 to 43	36	4	29 to 43	40	4	33 to 48	.54
pOS at 4 years in subgroups										
Primary refractory	39	10	20 to 58	33	14	7 to 60	47	13	21 to 72	.69
Early relapse	28	3	21 to 35	29	5	19 to 39	28	5	18 to 37	.74
Late relapse	48	4	40 to 56	43	5	33 to 54	54	6	42 to 65	.41
Favorable cytogenetics	70	6	59 to 81	58	9	40 to 75	82	6	69 to 95	.04
Nonfavorable cytogenetics	32	3	26 to 38	34	4	26 to 42	30	4	21 to 38	.50
Unknown cytogenetics	34	4	26 to 42	17	8	2 to 33	38	9	20 to 56	.24

Abbreviations: BM, bone marrow; CR, complete remission; DNX, liposomal daunorubicin; FLAG, fludarabine, cytarabine, and granulocyte colony-stimulating factor; pCIR, estimated cumulative incidence of relapse; pOS, probability of overall survival.

*Based on comparisons between randomly assigned patients treated with FLAG or FLAG/DNX.

†Not available in 16 and 19 patients treated with FLAG and FLAG/DNX, respectively ($P = .60$), because of early death (nine and eight patients, respectively) and because of persisting or progressive disease in the peripheral blood or elsewhere leading to the decision not to sample BM (seven and 11 patients, respectively).

For comparison of both treatment arms in terms of characteristics and outcome, see Figure 1, Tables 1 and 2, and Appendix Table A4, online only.

Clinical and cell biologic features were not significantly different between both treatment arms for the total group (Table 1), nor for the subgroup of patients with favorable cytogenetics (Appendix Table A4).

Patients randomly assigned to FLAG/DNX had a 10% higher early good response rate than those randomly assigned to FLAG (80% *v* 70%; $P = .04$; difference 10%, 95% CI, 1% to 18%; odds ratio, 0.60; 95%, CI 0.37 to 0.97). Among patients with less than 20% BM blasts before randomization, 10 of 39 patients assigned to FLAG had more than 20% BM blasts on day 28, as compared with only three of 31 assigned to FLAG/DNX ($P = .088$). In patients with 10% to 20% BM blasts before treatment, four of seven patients assigned to FLAG had more than 20% BM blasts on day 28, as compared with one of 10 patients assigned to FLAG/DNX ($P = .62$). The median absolute percentages of leukemic blasts in the BM on day 28 was 4% (range, 0 to 100%) in the FLAG arm and 1% (range, 0 to 97%) in the FLAG/DNX arm ($P = .001$). In a logistic regression model with the variables treatment arm, time to relapse (early or late), and cytogenetics, the odds ratio for early treatment response was 0.48 (95% CI, 0.25 to 0.91, $P = .03$) in favor of FLAG/DNX.

In patients with early relapsed AML (47% of all patients), the difference in obtaining a good early treatment response was 16% in favor of FLAG/DNX (70% *v* 54%; $P = .04$; odds ratio, 0.49; 95% CI, 0.26 to 0.94), whereas in patients with late relapsed AML, it was 6% in favor of FLAG/DNX (90% *v* 84%; $P = .36$; odds ratio, 0.60; 95% CI, 0.25 to 1.52). In patients with CBF-AML, there were only two poor

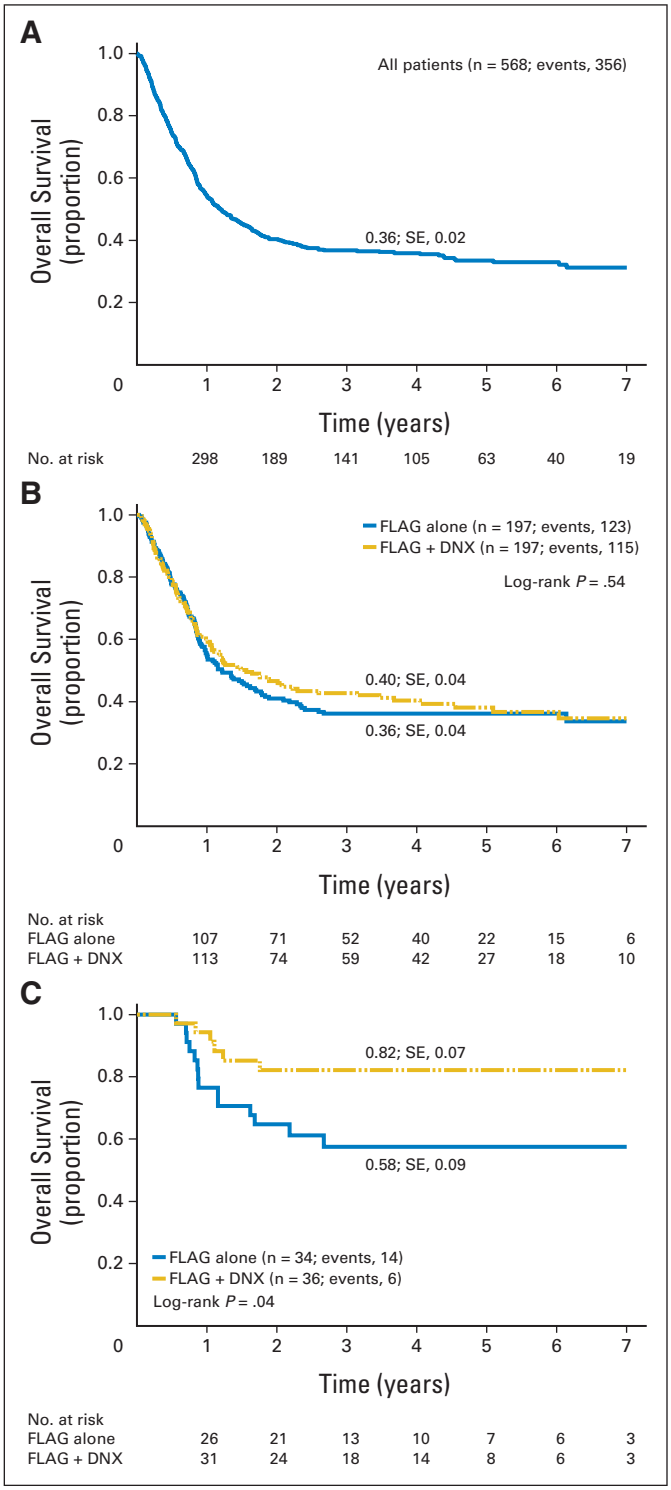

Fig 3. (A) Overall survival in pediatric study Relapsed Acute Myeloid Leukemia (AML) 2001/01 for 568 randomly assigned and nonrandomly assigned patients together. Median follow-up for patients at risk 4 years; (B) for fludarabine, cytarabine, and granulocyte colony-stimulating factor (FLAG) ± liposomal daunorubicin (DNX) in the total group of randomly assigned patients; (C) for FLAG ± DNX in the cytogenetically favorable subgroup with core-binding factor AML (ie, patients with t(8;21) and inv(16)). Unadjusted hazard ratios (HRs) regarding probability of overall survival indicate that treatment arm had no significant impact for the total group of patients (HR, 0.9; 95% CI, 0.7 to 1.2; $P = .56$), nor for the subgroup of patients with "other cytogenetics" (HR, 1.3; 95% CI, 0.9 to 1.8, $P = .20$), whereas patients with favorable cytogenetics seemed to have benefited from FLAG/DNX (HR, 0.37; 95% CI, 0.1 to 0.98; $P = .04$).

early treatment responders, both treated with FLAG only, whereas 31 of 33 and all 36 patients treated with FLAG and FLAG/DNX, respectively, achieved a good early treatment response. Within the relatively large subgroup of patients without favorable cytogenetics, the early good response rate was 10% higher with FLAG/DNX than with FLAG (76% *v* 64%; $P = .048$; odds ratio, 0.57; 95% CI, 0.33 to 0.99).

Data on secondary end points are shown in Table 2. The CR rate was 10% higher with FLAG/DNX (69% *v* 59%; $P = .07$). The same trend was seen within the early and late relapsed AML subgroups, with respectively a 14% and 10% higher CR rate with FLAG/DNX. Within cytogenetic subgroups, comparing outcome in the FLAG and FLAG/DNX arms, the CR rate was 91% and 97%, respectively, for CBF-AML; 55% and 62%, respectively, for the subgroup with other cytogenetics; and 39% and 61%, respectively, for the subgroup with unknown cytogenetics. The better initial treatment response with FLAG/DNX was counterbalanced by a higher CIR, with a pCIR at 4 years of 29% (SE, 3%) versus 19% (SE, 3%) with FLAG only ($P = .02$). Probability of death in CR at 4 years was similar in both treatment arms (7% [SE, 2%] with FLAG/DNX and 10% [SE, 2%] with FLAG only; $P = .46$). Thus 4-year pOS was not significantly better with FLAG/DNX than with FLAG (40% [SE, 4%] and 36% [SE, 4%], respectively; $P = .54$; Fig 3B). Within the subgroup of patients with CBF-AML, the 4-year pOS with FLAG/DNX was 82% (SE, 6%) versus 58% (SE, 9%) with FLAG ($P = .04$; Fig 3C). pCIR at 4 years was 17% (SE, 7%) versus 22% (SE, 8%), respectively ($P = .59$). Treatment after course 1 did not differ between both arms in this subgroup either (data not shown).

At univariate and multivariate analysis, treatment with FLAG or FLAG/DNX was not a prognostic factor regarding OS. At Cox regression analysis of all randomly assigned patients regarding pOS, including treatment arm, time to relapse, and cytogenetics, the *P* value for the interaction of favorable risk by cytogenetics and the effect of DNX/FLAG is 0.034 (hazard rate, 0.3; 95% CI, 0.1 to 0.9), indicating a favorable effect of liposomal daunorubicin in the subgroup of patients with favorable cytogenetics.

Toxicity

Toxicity was essentially similar in both groups, except for skin toxicity, which occurred in 4% of patients treated with FLAG/DNX and in 1% in patients treated with FLAG ($P = .04$; Appendix Table A5, online only). Grade 3 and 4 acute cardiotoxicity was seen in five patients (2.7%) treated with FLAG/DNX (grade 4, n = 1; grade 3, n = 4) and in one patient (0.6%) treated with FLAG (grade 3), which was not statistically significantly different. Moreover, cardiotoxicity always coincided with fever and infections, and no episode was fatal. Long-term cardiotoxicity data are not yet available.

DISCUSSION

We report the first (to our knowledge) randomized study in pediatric relapsed AML. In this multinational setting, we achieved the best outcome for these children reported thus far, with a 4-year pOS of 38% in a group of 394 children.[4-12]

DNX added to FLAG in the first reinduction course significantly improved the early response rate as primary end point from 70% to 80%. The CR rate also improved from 59% to 69%. OS was similar with FLAG and FLAG/DNX. However, patients with CBF-AML had a 24% higher 4-year pOS if treated with FLAG/DNX ($P = .04$; Fig 3C). Subgroup analyses were not predefined in the original protocol, and this finding must be confirmed. However, it is not unexpected that a subgroup with a generally more favorable prognosis benefits from additional therapy. A recent study in adult AML showed a clear benefit of gemtuzumab ozogamicin added to chemotherapy in prognostically more favorable subgroups, such as patients with CBF-AML.[29] Webb et al[11] have also reported that within pediatric relapsed AML, patients with CBF-AML have a better outcome. However, early treatment response was better with FLAG/DNX in relapsed patients with and without CBF-AML in this study. The fact that a better early treatment response did not translate into a significantly better OS may partly be explained by the fact that several patients were treated with FLAG plus an anthracycline or gemtuzumab ozogamicin, introducing an unintended cross-over effect. In addition, the quality of remissions achieved with FLAG/DNX may have been relatively poor in many patients, and subsequent therapy could not prevent second relapse. The latter is likely to be true in view of the high incidence of second relapse, being even higher in patients who achieved CR after FLAG/DNX. This hypothesis is also supported by literature demonstrating the clinical relevance of minimal residual disease (MRD) in childhood AML and showing increased relapse rates in patients with poor-quality remissions with higher MRD levels.[3,30,31] Obviously, maintaining the better early treatment response achieved with FLAG/DNX requires additional therapeutic improvements.

The increased antileukemic activity with FLAG/DNX was not associated with increased toxicity during chemotherapy, except for a modest increase in skin toxicity. Concerns for late cardiotoxicity associated with higher cumulative doses of anthracyclines and related drugs are valid.[13-15] DNX is a liposomal anthracycline that might be less cardiotoxic because of its pharmacology, as discussed elsewhere.[13] The present study has detailed guidelines for long-term follow-up with special attention to cardiac function. Cumulative doses of anthracyclines, including information on exposure to anthracyclines in the upfront therapy, will then be taken into account.

This investigator-initiated, multicenter study has several limitations. It was not possible to have close monitoring of all details. This limited the possibility to study the significance of factors such as type of consolidation and quality of response by sophisticated MRD monitoring. We also could not determine the CR rate after one course of therapy because of insufficient data on regeneration of normal hematopoiesis. However, the main study end point is robust and can be used in future studies in relapsed AML, even though more detailed immunophenotyping of blasts to discriminate them from normal regenerating normal blasts and information on MRD will be useful in addition. One may argue that the clinical benefit of DNX is unknown at best, because OS did not improve significantly. However, it should be taken into account that therapy after induction was heterogeneous and may have been intensified in patients with a poor early treatment response. Moreover, a better early treatment response and an improved CR rate are clinically positive findings, as is the significantly higher pOS with FLAG/DNX for CBF-AML. Apparently, treatment after the first course of reinduction therapy should improve further for patients with nonfavorable cytogenetics. Another limitation of this study is the heterogenous first-line treatment used in the study patients. On the other hand, all groups applied cytarabine-based treatment combined with an anthracycline, and alloSCT had been used in a limited number of patients.

Despite an encouraging improved outcome in pediatric relapsed AML in this and several other recent studies, prognosis is unsatisfactory. Targeting leukemia-specific abnormalities such as mutated tyrosine kinases is an important area of clinical research, although instability of the targets must be taken into account.[32] In our next international randomized pediatric Relapsed AML 2010/01 study, the benefit of adding gemtuzumab ozogamicin to a backbone of fludarabine, cytarabine, and liposomal daunorubicin will be studied, and patients with *FLT3*-mutated AML cells will be treated with sorafenib.

Meanwhile, pediatric relapsed AML is curable in a significant proportion of patients. International collaboration is feasible in the treatment of pediatric relapsed AML and should be pursued, even with the current challenges of the European Union directive in the setting of investigator-initiated studies.[33]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
Employment or Leadership Position: None **Consultant or Advisory Role:** Gertjan J.L. Kaspers, Galen (C); Dirk Reinhardt, Galen (C) **Stock Ownership:** None **Honoraria:** None **Research Funding:** None **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Gertjan J.L. Kaspers, Martin Zimmermann, Dirk Reinhardt, Brenda E.S. Gibson, Rienk Y.J. Tamminga, Michael Dworzak, Henrik Hasle, Guy G. Leverger, Carmelo Rizzari, Owen Smith, Ursula Creutzig
Administrative support: Ursula Creutzig
Provision of study materials or patients: Gertjan J.L. Kaspers, Dirk Reinhardt, Olga Aleinikova, Hortensia Armendariz, Michael Dworzak, Henrik Hasle, Liisa Hovi, Alexei Maschan, Yves Bertrand, Guy G. Leverger, Bassem I. Razzouk, Carmelo Rizzari, Batia Stark
Collection and assembly of data: Gertjan J.L. Kaspers, Martin Zimmermann, Dirk Reinhardt, Rienk Y.J. Tamminga, Olga Aleinikova, Hortensia Armendariz, Michael Dworzak, Shau-Yin Ha, Henrik Hasle, Alexei Maschan, Yves Bertrand, Guy G. Leverger, Bassem I. Razzouk, Carmelo Rizzari, Petr Smisek, Batia Stark, Ursula Creutzig
Data analysis and interpretation: Gertjan J.L. Kaspers, Martin Zimmermann, Dirk Reinhardt, Rienk Y.J. Tamminga, Olga Aleinikova, Hortensia Armendariz, Michael Dworzak, Henrik Hasle, Liisa Hovi, Alexei Maschan, Yves Bertrand, Owen Smith, Ursula Creutzig
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Kaspers GJ, Creutzig U: Pediatric acute myeloid leukemia: International progress and future directions. Leukemia 19:2025-2029, 2005

2. Kaspers GJ, Zwaan CM: Pediatric acute myeloid leukemia: Towards high-quality cure of all patients. Haematologica 92:1519-1532, 2007

3. Rubnitz JE, Inaba H, Dahl G, et al: Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: Results of the AML02 multicentre trial. Lancet Oncol 11:543-552, 2010

4. Abrahamsson J, Clausen N, Gustafsson G, et al: Improved outcome after relapse in children with acute myeloid leukaemia. Br J Haematol 136:229-236, 2007

5. Aladjidi N, Auvrignon A, Leblanc T, et al: Outcome in children with relapsed acute myeloid leukemia after initial treatment with the French Leucemie Aique Myeloide Enfant (LAME) 89/91 protocol of the French Society of Pediatric Hematology and Immunology. J Clin Oncol 21:4377-4385, 2003

6. Goemans BF, Tamminga RY, Corbijn CM, et al: Outcome for children with relapsed acute myeloid leukemia in the Netherlands following initial treatment between 1980 and 1998: Survival after chemotherapy only? Haematologica 93:1418-1420, 2008

7. Gorman MF, Ji L, Ko RH, et al: Outcome for children treated for relapsed or refractory acute myelogenous leukemia (rAML): A Therapeutic Advances in Childhood Leukemia (TACL) Consortium study. Pediatr Blood Cancer 55:421-429, 2010

8. Rubnitz JE, Razzouk BI, Lensing S, et al: Prognostic factors and outcome of recurrence in childhood acute myeloid leukemia. Cancer 109:157-163, 2007

9. Sander A, Zimmermann M, Dworzak M, et al: Consequent and intensified relapse therapy improved survival in pediatric AML: Results of relapse treatment in 379 patients of three consecutive AML-BFM trials. Leukemia 24:1422-1428, 2010

10. Stahnke K, Boos J, Bender-Götze C, et al: Duration of first remission predicts remission rates and long-term survival in children with relapsed acute myelogenous leukemia. Leukemia 12:1534-1538, 1998

11. Webb DK, Wheatley K, Harrison G, et al: Outcome for children with relapsed acute myeloid leukaemia following initial therapy in the Medical Research Council (MRC) AML 10 trial. MRC Childhood Leukaemia Working Party. Leukemia 13:25-31, 1999

12. Wells RJ, Adams MT, Alonzo TA, et al: Mitoxantrone and cytarabine induction, high-dose cytarabine, and etoposide intensification for pediatric patients with relapsed or refractory acute myeloid leukemia: Children's Cancer Group Study 2951. J Clin Oncol 21:2940-2947, 2003

13. Creutzig U, Diekamp S, Zimmermann M, et al: Longitudinal evaluation of early and late anthracycline cardiotoxicity in children with AML. Pediatr Blood Cancer 48:651-662, 2007

14. Kremer LC, van Dalen EC, Offringa M, et al: Anthracycline-induced clinical heart failure in a cohort of 607 children: Long-term follow-up study. J Clin Oncol 19:191-196, 2001

15. Temming P, Qureshi A, Hardt J, et al: Prevalence and predictors of anthracycline cardiotoxicity in children treated for acute myeloid leukaemia: Retrospective cohort study in a single centre in the United Kingdom. Pediatr Blood Cancer 56:625-630, 2011

16. Gandhi V, Estey E, Keating MJ, et al: Fludarabine potentiates metabolism of cytarabine in patients with acute myelogenous leukemia during therapy. J Clin Oncol 11:116-124, 1993

17. Fleischhack G, Hasan C, Graf N, et al: IDA-FLAG (idarubicin, fludarabine, cytarabine, G-CSF), an effective remission-induction therapy for poor-prognosis AML of childhood prior to allogeneic or autologous bone marrow transplantation: Experiences of a phase II trial. Br J Haematol 102:647-655, 1998

18. Corbett TH, Griswold DP Jr, Roberts BJ, et al: Biology and therapeutic response of a mouse mammary adenocarcinoma (16/C) and its potential as a model for surgical adjuvant chemotherapy. Cancer Treat Rep 62:1471-1488, 1978

19. Forssen EA, Tökès ZA: Use of anionic liposomes for the reduction of chronic doxorubicin-induced cardiotoxicity. Proc Natl Acad Sci U S A 78:1873-1877, 1981

20. Forssen EA, Coulter DM, Proffitt RT: Selective in vivo localization of daunorubicin small unilamellar vesicles in solid tumors. Cancer Res 52:3255-3261, 1992

21. Gill PS, Wernz J, Scadden DT, et al: Randomized phase III trial of liposomal daunorubicin versus doxorubicin, bleomycin, and vincristine in AIDS-related Kaposi's sarcoma. J Clin Oncol 14:2353-2364, 1996

22. Pouna P, Bonoron-Adèle S, Gouverneur G, et al: Development of the model of rat isolated perfused heart for the evaluation of anthracycline cardiotoxicity and its circumvention. Br J Pharmacol 117:1593-1599, 1996

23. Levitt G: Cardioprotection. Br J Haematol 106:860-869, 1999

24. Reinhardt D, Hempel G, Fleischhack G, et al: [Liposomal daunorubicine combined with cytarabine in the treatment of relapsed/refractory acute myeloid leukemia in children]. Klin Padiatr 214:188-194, 2002

25. Zwaan CM, Reinhardt D, Zimmerman M, et al: Salvage treatment for children with refractory first or second relapse of acute myeloid leukaemia with gemtuzumab ozogamicin: Results of a phase II study. Br J Haematol 148:768-776, 2010

26. Kaspers GJ, Reinhardt D, Fleischhack G, et al: Low efficacy of methotrexate in childhood acute myeloid leukemia (AML): Single-agent therapeutic window study in relapsed AML. Pediatr Blood Cancer 47:539-542, 2006

27. Creutzig U, Kaspers GJ: Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol 22:3432-3433, 2004

28. Kardos G, Zwaan CM, Kaspers GJ, et al: Treatment strategy and results in children treated on three Dutch Childhood Oncology Group acute myeloid leukemia trials. Leukemia 19:2063-2071, 2005

29. Burnett AK, Hills RK, Milligan D, et al: Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: Results of the MRC AML15 trial. J Clin Oncol 29:369-377, 2011

30. MRD-AML-BFM Study Group, Langebrake C, Creutzig U, et al: Residual disease monitoring in childhood acute myeloid leukemia by multiparameter flow cytometry: The MRD-AML-BFM Study Group. J Clin Oncol 24:3686-3692, 2006

31. van der Velden VH, van der Sluijs-Geling A, Gibson BE, et al: Clinical significance of flowcytometric minimal residual disease detection in pediatric acute myeloid leukemia patients treated according to the DCOG ANLL97/MRC AML12 protocol. Leukemia 24:1599-1606, 2010

32. Cloos J, Goemans BF, Hess CJ, et al: Stability and prognostic influence of FLT3 mutations in paired initial and relapsed AML samples. Leukemia 20:1217-1220, 2006

33. Pritchard-Jones K, SIOP Europe: Clinical trials for children with cancer in Europe: Still a long way from harmonization—A report from SIOP Europe. Eur J Cancer 44:2106-2111, 2008

Affiliations

Gertjan J.L. Kaspers, Pediatric Oncology/Hematology, VU University Medical Center, Amsterdam; Gertjan J.L. Kaspers and Rienk Tamminga, Dutch Childhood Oncology Group, the Hague; Rienk Tamminga, Pediatric Oncology/Hematology, Beatrix Children's Hospital/University Medical Centre Groningen, Groningen, the Netherlands; Martin Zimmermann, Dirk Reinhardt, and Ursula Creutzig, Berlin-Frankfurt-Munster Acute Myeloid Leukemia (BFM-AML) Group, Medizinische Hochschule Hannover, Hannover, Germany; Brenda Gibson, United Kingdom Children's Cancer and Leukaemia Group (UK CCLG), Royal Hospital for Sick Children, Glasgow, Scotland, United Kingdom; Olga Aleinikova, Belarus Research Center of Pediatric Oncology and Hematology, Minsk Region, Belarus; Hortensia Armendariz, Grupo Argentino de Tratamiento de la Leucemia Aguda, Hospital Sor M. Ludovica, La Plata, Argentina; Michael Dworzak, BFM-AML Group, St Anna Children's Hospital and Children's Cancer Research Institute, Medical University of Vienna, Vienna, Austria; Shau-yin Ha, Hong Kong Paediatric Haematology & Oncology Study Group, Queen Mary Hospital, Pokfulam, Hong Kong, China; Henrik Hasle, Nordic Society for Pediatric Hematology and Oncology (NOPHO), Aarhus University Hospital Skejby, Denmark; Liisa Hovi, NOPHO, Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland; Alexei Maschan, Russian Institute for Pediatric Hematology, Moscow, Russia; Yves Bertrand, Children's Leukemia Cooperative Group/European Organisation for Research and Treatment of Cancer, Institut d'Hématologie et d'Oncologie Pédiatrique, Lyon; Guy Leverger, French Acute Lymphoblastic

Leukemia Study Group, Unité d'Onco-Hématologie Pédiatrique, Hôpital d'Enfants Armand Trousseau, Paris, France; Bassem Razzouk, St Jude's Children Research Hospital, Memphis, TN; Bassem Razzouk, Children's Center for Cancer and Blood Diseases, Peyton Manning Children's Hospital at St Vincent, Indianapolis, IN; Carmelo Rizzari, Associazione Italiana Ematologia Oncologia Pediatrica, S. Gerardo Hospital, Monza, Italy; Petr Smisek, Czech Republic Pediatric Hematology Working Group, University Hospital Motol, Charles University Prague, Prague, Czech Republic; Owen Smith, UK CCLG, Our Lady's Children's Hospital, Crumlin, Dublin, Ireland; and Batia Stark, Center of Pediatric Hematology/Oncology, Schneider Children's Medical Center of Israel, Petah Tiqwa, Israel.