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To the editor:

Protocol II vs protocol III given twice during reinduction therapy in children with medium-risk ALL

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Reinduction therapy (also known as delayed intensification, DI) is an essential part of childhood acute lymphoblastic leukemia (ALL) treatment.[1,2] In Berlin-Frankfurt-Münster (BFM) studies, it includes same/similar drugs as those employed in induction therapy. The Children Cancer Group (CCG)-1891 study, conducted in the early 1990s in ALL children with intermediate-risk (IR) clinical features, demonstrated that double DI, based on that used in the BFM 76/79 study,[3] improved event-free survival of patients younger than 10 years of age, although treatment-related toxicities were more frequent in patients given double DI than single DI.[4] The Associazione Italiana di Ematologia/Oncologia Pediatrica (AIEOP)-ALL95 study performed in high-risk (HR) children documented that reinduction therapy consisting of protocol II repeated twice was associated with improved outcome of patients with prednisone-poor response as only HR feature.[5]

In light of these findings, we designed a randomized clinical trial for children allocated to the medium-risk (MR) group of the study AIEOP-BFM ALL 2000 to evaluate if repeated reinduction therapy with protocol III, a slightly less intensive scheme than protocol II, resulted into better disease-free survival (DFS) than single exposure to protocol II.

Enrolled in the AIEOP-BFM ALL 2000 trial were 4741 patients, aged 1 to 18 years, with Philadelphia-chromosome–negative ALL diagnosed/treated in 1 of the participating centers (see supplemental Appendix 1, available on the *Blood* Web site, for details).[6]

The MR group included patients without HR features[6] (see supplemental data for details on risk-group assignment) with positive minimal residual disease (MRD) at 1 or both time points, but at a level $<5 \times 10^{-4}$ at day 78 (MRD-IR), and those for whom MRD evaluation was not available/noninformative. Because of the stringent criteria for MRD evaluation in this protocol (namely 2 markers required for evaluation),[6-8] 20% of patients could not be stratified by MRD, and because all these patients were allocated to the MR group unless they had other HR features, one-third of the MR patients did not have informative MRD data.

Overall, 2665 children (56.2% of the whole cohort) were allocated to the MR group and were eligible to the randomized study here reported if still in complete remission (CR) at time of randomization.

MR patients were randomly assigned to receive either a single protocol II (control group) or the shorter protocol III given, however, twice (experimental group; supplemental Figure 1). Details on protocol II and III are shown in supplemental Table 1. Compared with patients given protocol II, those randomized to protocol III repeated twice received the same cumulative doses of vincristine, doxorubicin, and cyclophosphamide, 4 weeks of dexamethasone vs 3 weeks, and 4 weeks of L-asparaginase, cytosine-arabinoside, 6-thioguanine, and intratechal methotrexate vs 2 weeks, given over 20 weeks with a 10-week interim maintenance phase (vs 7 weeks).

Randomization was performed in the 2 weeks preceding reinduction therapy (weeks 21-22) in a 1:1 ratio, stratified by country (and by center in Italy and Germany). Details on statistical analysis and definitions reported/used in this study are shown in the supplemental data.

Out of 2665 children with MR-ALL, 69 (2.6%) either relapsed (n = 5) or died (n = 64) before randomization and 555 (21.4%) patients were not randomized (supplemental Figure 2); thus, 2041 patients (77% of the MR population) were assigned to receive protocol III given twice or protocol II. Clinical characteristics of randomized or nonrandomized patients are shown in supplemental Table 2; their 5-year DFS was 79.3% vs 80.5%, respectively ($P = .23$).

As shown in Table 1, the 2 randomization arms were well balanced by main prognostic features. Events and outcome curves by assigned treatment (intention-to-treat) are shown in supplemental Table 3 and Figure 1, respectively. No difference in 10-year DFS (79.8%, 95% confidence interval [CI] 77.1% to 82.2% vs 81.3%, 95% CI 78.7% to

Table 1. Patients characteristics in randomized patients (by intention to treat)

	2× P-III		P-II		Total	
	N	%	N	%	N	%
Total patients	1023		1018		2041	
Sex						
Male	601	58.7	561	55.1	1162	56.9
Female	422	41.3	457	44.9	879	43.1
Age, y						
1-9	795	77.7	795	78.1	1590	77.9
10-17	228	22.3	223	21.9	451	22.1
White blood cell count						
<20 000/μL	688	67.3	706	69.4	1394	68.3
20-100 000/μL	263	25.7	253	24.8	516	25.3
≥100 000/μL	72	7.0	59	5.8	131	6.4
CNS involvement						
No (CNS1-CNS2)	983	96.1	972	95.5	1955	95.8
Yes (CNS3)	21	2.1	28	2.8	49	2.4
Not known	19	1.8	18	1.7	37	1.8
Immunophenotype						
Bcp-ALL	896	87.6	887	87.1	1783	87.4
T-ALL	107	10.5	115	11.3	222	10.9
Not known	20	1.9	16	1.6	36	1.7
National Cancer Institute criteria						
Standard	686	67.1	676	66.4	1362	66.7
High	337	32.9	342	33.6	679	33.3
Random in induction						
Dexamethasone	397	38.8	404	39.7	801	39.2
Prednisone	430	42.0	419	41.1	849	41.6
Not performed	196	19.2	195	19.2	391	19.2
ETV6-RUNX1						
Positive	193	18.9	176	17.3	369	18.1
Negative	728	71.1	744	73.1	1472	72.1
Not known	102	10.0	98	9.6	200	9.8
MRD levels at TP1*						
$<5 \times 10^{-4}$	469	67.2	464	68.8	933	68.0
$\geq 5 \times 10^{-4}$	229	32.8	210	31.2	439	32.0
MRD levels at TP2*						
Negative	461	66.1	459	68.1	920	67.1
$<5 \times 10^{-4}$	237	33.9	215	31.9	452	32.9

Bcp, B-cell precursor; CNS, central nervous system; T-ALL, T-cell acute lymphoblastic leukemia.

*MRD was not known in 666 patients (32.6%) overall (322 in protocol [P]-III ×2 and 344 in protocol II).

83.7%, $P = .37$; Figure 1A) and survival (92.6%, 95% CI 90.6% to 94.2% vs 90.1%, 95% CI 87.7% to 92.0%, $P = .10$) was seen for the experimental arm compared with the control group. There was no difference in the proportion of toxic deaths (0.5% [n = 5] in the experimental arm vs 0.7% [n = 7] in the control arm) and of second malignancies (0.7% vs 1.0%; supplemental Table 3). In addition, no significant difference was seen in the cumulative incidence of relapse (Figure 1B; $P = .22$). There was a slightly lower incidence of very early relapse (<18 months from diagnosis) and a higher incidence of early or late relapses (≥18 months from diagnosis) in the protocol III × 2 arm, compared with the protocol II arm. This finding accounted for a better trend of overall survival in the former group, because it is well known that the longer the CR-1 duration, the better the chance of being rescued for relapsed patients.[9] The Children's Oncology Group AALL0232 trial showed a similar trend in patients with end-induction MRD ≥0.1% or with morphologic slow-early response who received second DI.[10] No difference by randomized arm was seen within Bcp-ALL or T-ALL (Figure 1C-D). Also, considering specific subsets of patients, we did not detect any advantage for repeating protocol III twice (see supplemental data and supplemental Figures 3 and 4).

Multivariable analysis performed on the larger cohort of Bcp-ALL patients classified at MR by the presence of informative MRD levels confirmed the lack of impact of the randomized reinduction therapy (hazard ratio = 1.03, 95% CI 0.80-1.31, $P = .83$) and the impact of known prognostic features, namely white blood cell count and MRD levels both at time point (TP)1 and TP2 (supplemental Table 4).

In T-ALL patients, MRD data were available in 152 cases. No difference by randomized arm was seen at TP1 and at TP2 for the same categories of MRD levels (see supplemental Data for details).

Data on severe adverse events showed that the rate of life-threatening events was slightly lower in patients given protocol III twice than in those receiving protocol II. Only 2 related fatal events occurred in patients given the experimental treatment vs 8 recorded in the control arm; 9 out of 10 fatalities were due to infections (supplemental Tables 3 and 5).

The 5-year rate of osteonecrosis during reinduction therapy, maintenance, or off-therapy was not influenced by the treatment received (3.2% and 2.7% in patients receiving protocol III twice [32 events over 946 patients] or protocol II [32 events over 1088 patients], $P = .36$). As expected,[11,12] the incidence of osteonecrosis was higher in children aged ≥10 years at diagnosis (11.9% [1.5]) than in younger children (0.4% [0.2], $P < .001$).

Children with MR ALL represent approximately half of patients enrolled in the MRD-based BFM protocols. Our results indicate that repeating reinduction therapy with protocol III did not give a significant

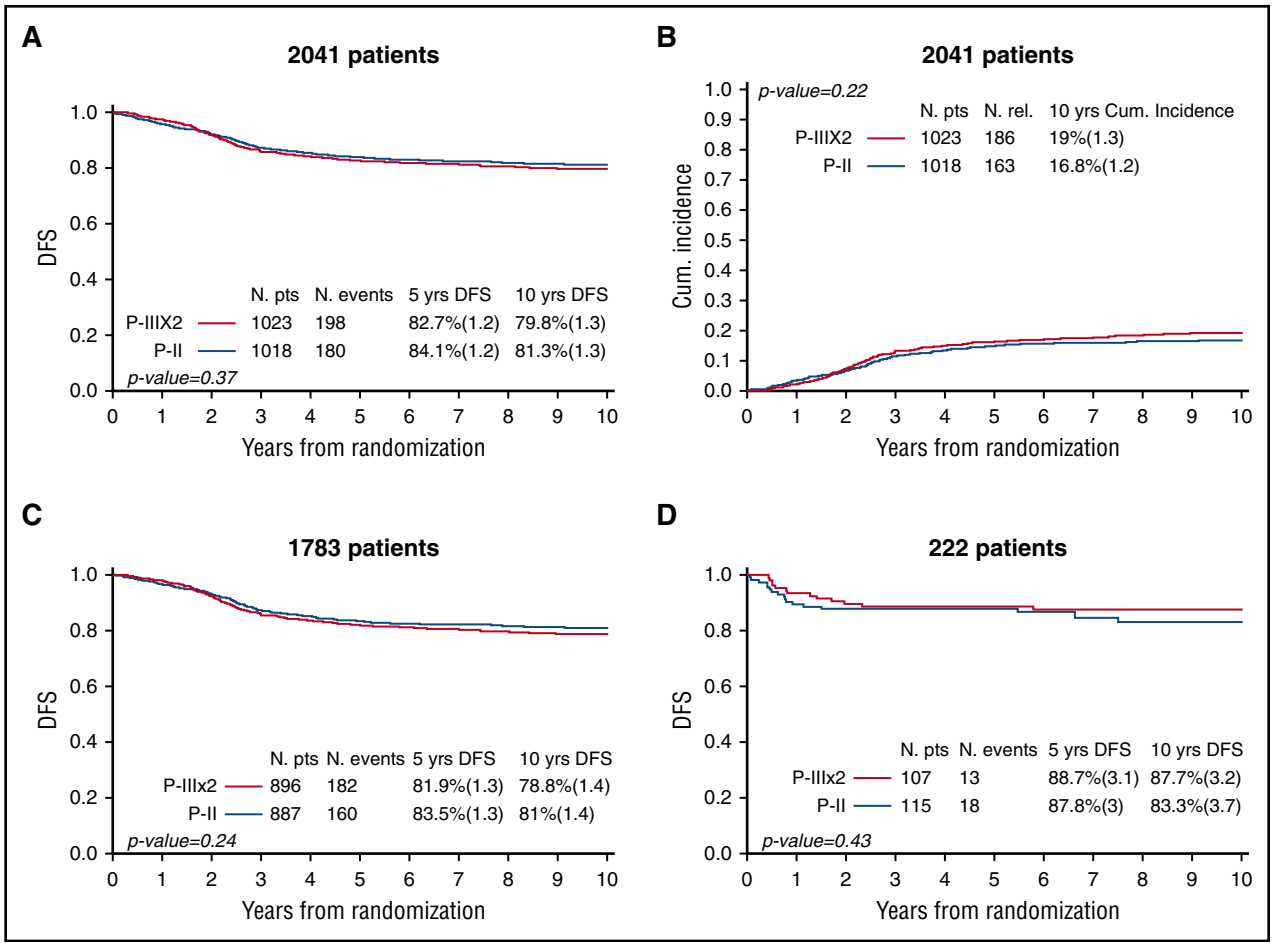

Figure 1. Outcome of patients enrolled in the study. (A) Probability of DFS for the whole cohort of patients. (B) Cumulative incidence of relapse for the whole cohort of patients; (C) probability of DFS for children with Bcp-ALL; (D) probability of DFS for children with T-ALL. Cum., cumulative; N., number; P, protocol; rel, relapse.

advantage over protocol II for children with MR-ALL, disease recurrence rate being similar between the 2 arms. This finding suggests that neither repeated exposure of leukemia cells to drugs employed during reinduction treatment nor an additional week of dexamethasone, or a twofold greater dosage of L-asparaginase, cytosine-arabinoside, and 6-thyoguanine, was able to eradicate blasts persisting after consolidation therapy. We cannot also exclude that protocol III is "less effective" than protocol II; the randomized trial comparing these DIs that we performed in standard-risk patients will answer this question.

Our results mimic those published by Seibel and colleagues, who showed that stronger intensity, but not prolonged duration of post-induction intensification (ie, double DI), improved outcome for ALL children with either M1 or M2 on day +7 after diagnosis.[13] No advantage for a second DI was also found in the Children Cancer Group 1991 trial[14] and in the low-risk patients enrolled in the UKALL 2003 trial.[15] It is reasonable to speculate that, with contemporary intensive backbone therapies, residual leukemic clones after 1 reinduction therapy/DI probably represent intrinsic drug-resistant disease; in these circumstances, further exposure to the same agents is not beneficial.

In conclusion, our data show that, for children with MR ALL defined considering also MRD levels, repeating protocol III with the schedule adopted in this study does not improve long-term outcome, when compared with conventional protocol II. Future studies will evaluate whether the addition of monoclonal antibodies targeting Bcp-ALL blasts[16] to conventional reinduction therapy can reduce relapse rate and improve DFS probability in MR patients.

The online version of this article contains a data supplement.

Acknowledgments: This work was supported by the Associazione Italiana per la Ricerca sul Cancro (special grant "5xmille"-9962 and IG-17200; F.L.), (IG 14634; M.G.V.), Comitato M.L. Verga and Fondazione Tettamanti (Monza, Italy), and Fondazione Città della Speranza (Padova, Italy).

Contribution: M.S., V.C., and F.L. planned and coordinated the study; F.L., A.M., B.G., A.B., A.E.K., N.B., M.C.P., S. Burdach, C.M., A.T.-S., J.R., A.P., S. Bielack, T.K., L.V., C.R., A.A., N.S., R.P., G.M., L.K., V.C., and M.S. contributed to protocol development and application; M.G.V. was the study statistician for AIEOP; D.S. contributed to study conduction and data analysis; M.Z. was the study statistician for BFM; A.M. contributed to data collection and verification; O.A.H., G.B., A.B., and G.C. were responsible for biological investigations; F.L., V.C., and M.G.V. wrote the paper; and all other authors reviewed the paper, approving the final version, and have also participated in the study conduction and supervision as well as in data interpretation stages of this study.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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DOI 10.1182/blood-2017-05-782086