Chemotherapy in 998 Unselected Childhood Acute Lymphoblastic Leukemia Patients. Results and Conclusions of the Multicenter Trial ALL-BFM 86

By Alfred Reiter, Martin Schrappe, Wolf-Dieter Ludwig, Wolfgang Hiddemann, Siegfried Sauter, Günter Henze, Martin Zimmermann, Fritz Lampert, Werner Havers, Dietrich Niethammer, Edelgard Odenwald, Jörg Ritter, Georg Mann, Karl Welte, Helmut Gadner, and Hansjörg Riehm

In trial ALL-BFM 86, the largest multicenter trial of the Berlin-Frankfurt-Münster (BFM) study group for childhood acute lymphoblastic leukemia (ALL), treatment response was used as an overriding stratification factor for the first time. In the previous trial ALL-BFM 83, the in vivo response to initial prednisone treatment was evaluated prospectively. A blast cell count of ≥1,000/μL peripheral blood after a 7-day exposure to prednisone and one intrathecal dose of methotrexate (MTX) identified 10% of the patients as having a significantly worse prognosis. In trial ALL-BFM 86 patients with ≥1,000/μL blood blasts on day 8 were included in an experimental branch EG. Patients with <1,000/μL blood blasts on day 8 were stratified by their leukemic cell burden into two branches, Standard Risk Group (SRG) and Risk Group (RG). SRG patients received an eight-drug induction followed by consolidation protocol M (6-mercaptopurine, high-dose [HD] MTX 4 × 5 g/m²) and maintenance. RG patients were treated with an additional eight-drug reinduction element. For EG patients protocol M was replaced by protocol E (prednisone, HD-MTX, HD-cytarabine, ifosfamide, mitoxantrone). All patients received intrathecal MTX therapy; only those of branches RG and EG received cranial irradiation. In branch RG, patients were randomized to receive or not to receive late intensification (prednisone, vindesine, teniposide, ifosfamide, HD-cytarabine) in the 13th month. During the trial reinduction therapy was introduced in branch SRG, because in the follow-up of trial ALL-BFM 83 the randomized low-risk patients receiving reinduction did significantly better. Nine hundred ninety-eight evaluable patients were enrolled, 28.6% in SRG, 61.1% in RG, 10.3% in EG. At a median follow-up of 5.0 (range 3.4 to 6.9) years, the estimated 6-year event-free survival was 72% ± 2% for the study population, 58% ± 5% in branch SRG for the first 110 patients without reinduction therapy, 87% ± 3% for the next 175 patients with reinduction therapy, 75% ± 2% in branch RG, and 48% ± 5% in branch EG. Late intensification did not significantly affect treatment outcome of RG patients; however, only 23% of the eligible patients were randomized. Prednisone poor response remained a negative prognostic parameter despite intensified therapy. The results confirmed the benefit of intensive reinduction therapy even for low-risk patients. The strategy of induction, consolidation, and intensive reinduction may offer roughly 75% of unselected childhood ALL patients the chance for an event-free survival.

THE TRIAL ALL-BFM 86 is the sixth multicenter trial in childhood acute lymphoblastic leukemia (ALL), conducted by the Berlin-Frankfurt-Münster (BFM) group. In the Berlin pilot study initiated in 1970[1] the basic rationale of the treatment strategy was to rapidly achieve a maximum cell kill to prohibit the acquisition of drug resistance by residual leukemic blasts that may give rise to subsequent treatment failure. Therefore, maximum tolerated doses of all active agents were delivered as early as possible in the course of therapy. Based on this concept, in trial ALL-BFM 76 an intensive reinduction therapy shortly after induction was introduced for patients with increased risk of failure. This turned out to be a very important step toward further improvement of results.[2,3]

The initial leukemic cell burden, retrospectively estimated as a continuous risk factor from the blast count in the blood and size of liver and spleen, was the most important prognostic factor in the unstratified pilot study ALL-BFM 70, and was used for stratification of therapy in trials ALL-BFM 81, 83, and 86.[2,4] The kinetics of the initial response to therapy as a predictor of treatment outcome was first described by Jacquillat et al in 1973.[5] In the trial ALL-BFM 83, the in vivo response to prednisone was prospectively evaluated for its prognostic significance. A blast cell count of ≥1,000/μL in the blood on day 8 after a 7-day exposure to prednisone and one initial intrathecal (i. th.) dose of methotrexate (MTX) identified 10% of the patients as having a significantly worse prognosis.[6]

Therefore, in trial ALL-BFM 86, high-risk ALL was defined by poor in vivo response to prednisone replacing the leukemic cell burden. Patients with ≥1,000/μL blasts in the blood on day 8 after a 7-day exposure to prednisone and one initial i. th. dose of MTX then received an experimental therapy protocol immediately after induction therapy. This experimental protocol was based on high-dose (HD) MTX, HD-cytarabine, ifosfamide, and mitoxantrone that had shown activity in refractory leukemia and lymphoma.[7-10] Because the majority of relapses still occurred in the large group of patients with good response to prednisone but with

From the Department of Pediatric Hematology and Oncology, Medizinische Hochschule Hannover; the Department of Medical Oncology and Applied Molecular Biology, Free University of Berlin; the Department of Hematology and Oncology, Georg-August-University, Göttingen; the Department of Pediatrics, Albert-Ludwigs-Universität, Freiburg; the Department of Pediatric Hematology and Oncology, Free University of Berlin; the Department of Pediatrics, Justus-Liebig-Universität, Giessen; the Department of Pediatric Hematology and Oncology, Gesamthochschule Essen; the Department of Pediatric Hematology and Oncology, Eberhard-Karls-Universität, Tübingen; the Department of Pediatric Hematology and Oncology, Westfälische Wilhelms-Universität, Münster, Germany; and St Anna Kinderspital, Wien, Austria.

Submitted February 7, 1994; accepted July 5, 1994.

Address reprint requests to Alfred Reiter, MD, Kinderklinik der Medizinischen Hochschule Hannover, Konstanty-Gutschow-Strasse 8, D-30625 Hannover, Germany.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

0006-4971/94/8409-0041$3.00/0

an increased leukemic cell mass we planned to test by randomization whether late intensification in the 13th month of therapy would improve the outcome for these patients. Furthermore, HD-MTX was introduced as consolidation therapy for all patients for better control of extramedullary leukemia and to allow further reduction of central nervous system (CNS) radiotherapy.

Initially, reinduction therapy was omitted for patients with low leukemic cell mass in trial ALL-BFM 86. However, it was re-introduced when, with longer follow-up of the preceding trial ALL-BFM 83, the event-free interval became significantly worse for low-risk patients without reinduction compared to those with reinduction therapy.[11] After this protocol amendment, the trial ALL-BFM 86 provided a basis to evaluate the effects of this intensive therapy regimen on a large group of unselected children with ALL.

MATERIALS AND METHODS

Patients. From October 1986 through March 1990, 1,114 patients up to 18 years of age were enrolled in trial ALL-BFM 86 from 61 pediatric hospitals in Germany and Austria. Informed consent was obtained for each patient. Forty-one patients (3.7%), diagnosed with acute B-cell leukemia (French-American-British [FAB] L3 cytomorphology) are reported elsewhere.[12] Of the remaining 1,073 patients, 75 (7.0%) were nonprotocol patients who were excluded from evaluation of treatment results for the following reasons: no therapy applied (2 patients, 1 death, 1 patient without follow-up data); in vivo response to prednisone not evaluable because of previous antileukemic treatment (19 patients, 3 adverse events) or exchange transfusion (3 patients, 2 early death on day 1 and 2, 1 relapse); major protocol violation that was not enforced by the course of disease or treatment, eg, complete therapy protocols were not applied or replaced for different treatment (35 patients, 20 adverse events); premature withdrawal from therapy (3 patients, no information on follow-up); lack of essential data (2 patients, in continuous complete remission [CCR]); patients treated according to the pilot protocol ALL-BFM 90 (5 patients, 3 adverse events); patients treated in nonmember hospitals (6 patients, 3 adverse events). Finally, 998 protocol patients were evaluable for results of treatment.

Diagnosis. ALL was diagnosed when 25% lymphoblasts or more were present in the bone marrow (BM). BM and blood smears as well as liquor cytospin preparations were stained using a modified Wright-staining technique and cytochemistry reactions (periodic acid Schiff reaction, acid phosphatase, α naphthyl acetate esterase, and myeloperoxidase reaction) and reviewed in the study center using FAB criteria.[13]

The definition of CNS involvement was based on $\geq 5/\mu L$ cells in the cerebrospinal fluid (CSF) and the presence of lymphoblasts or the detection of intracerebral infiltrates on cranial computed tomography.

Immunophenotyping. Immunophenotyping was performed as described elsewhere.[14] The criterion for marker positivity was expression by $\geq 20\%$ of the blasts (surface antigens) or intracytoplasmic (cy)/intranuclear detection (cyIgM, cyCD3, TdT) in $\geq 10\%$ of the leukemic cells. Immunophenotypic subgroups were defined as follows: Pre-pre-B ALL: TdT^+, $CD19^+$, $CD10^-$, $cyIgM^-$, surface Ig $(sIg)^-$; common (c) ALL: TdT^+, $CD19^+$, $CD10^+$, $cyIgM^-$, sIg^-; pre-B ALL: TdT^+, $CD19^+$, $CD10^{+/-}$, $cyIgM^+$, sIg^-; T-ALL: TdT^+, $cyCD3^+$, $CD7^+$. Coexpression of myeloid antigen(s) was defined as simultaneous expression of one or more of the myeloid-lineage associated molecules tested (CD13, CD33, CDw65) on at least 20% of the lymphoblasts. Mixed lymphoid/myeloid phenotype was defined as coexistence of two different blast populations, expressing either a lymphoid or a myeloid phenotype. An acute, undifferentiated leukemia (AUL) was diagnosed in cases with morphologically/cytochemically unclassifiable leukemia according to FAB criteria, CD45-positivity, but no evidence of B-, T- or myeloid lineage associated antigens, irrespective of HLA-DR-, TdT-, and CD34-reactivity.

Chromosome analysis. Cytogenetic studies were performed using standard techniques as described elsewhere.[15]

DNA index. Cellular DNA content was determined in one central laboratory using flow cytometry as previously described.[16] The DNA index of the leukemic blasts was defined as the ratio of DNA content in leukemic G0/G1 cells to that of normal diploid lymphocytes. A cut-off of 1.16 was used to distinguish prognostic categories.[16,17]

Estimation of the leukemic cell mass (risk factor [RF]). The leukemic cell mass estimate (RF) was calculated by the equation: RF = 0.2 × log (number of blood blasts/μL + 1) + 0.06 × liver (cm*) + 0.04 × spleen (cm*) (*below the costal margin).[4]

Definition of prednisone poor response. Therapy for all patients started with a 7-day monotherapy with prednisone and an i.th. MTX dose on day 1. Dosage of prednisone was adjusted according to leukemic cell mass, renal, and metabolic parameters to circumvent complications of acute cell lysis, then carefully increased to 60 mg/m^2 daily. The number of leukemic blasts in the blood on day 8 was calculated from the absolute leukocyte count and percentage of blasts in peripheral blood smears. All probes were reviewed in the study center. The presence of $\geq 1{,}000/\mu L$ blasts in the blood on day 8 was defined as prednisone poor response.[6]

Treatment and treatment stratification. Therapy was stratified into the three branches Standard Risk Group (SRG), Risk Group (RG), and Experimental Group (EG), mainly according to the leukemic cell mass estimate (RF) and the treatment response. SRG: $<1{,}000/\mu L$ blood blasts on day 8, risk factor <0.8, no CNS disease, no mediastinal mass; RG: $<1{,}000/\mu L$ blood blasts on day 8, risk factor ≥ 0.8, or risk factor <0.8 and CNS disease or/and presence of a mediastinal mass; EG: $\geq 1{,}000/\mu L$ blood blasts on day 8, or $>5\%$ marrow blasts on day 40, or acute undifferentiated leukemia.

The treatment strategy is shown in Fig 1. All patients received induction protocol I. In branch SRG, therapy was continued with the consolidation/extracompartment protocol M and maintenance therapy (oral 6-mercaptopurine, 50 mg/m^2 daily and MTX 20 mg/m^2 once a week). No CNS irradiation was performed. RG patients received protocol M, reinduction protocol II, CNS irradiation, and maintenance therapy. Patients of branch RG who were still in their first remission after 1 year were randomized to receive (stratum RG-2) or to not receive (stratum RG-1) late intensification protocol S, if parents gave informed consent. Patients of branch EG received protocol E after induction therapy, followed by reinduction protocol II, CNS irradiation, and maintenance therapy. Allogeneic BM transplantation (BMT) was optional in branch EG.

The protocol was amended in October 1988 because follow-up analysis of trial ALL-BFM 83 showed a significant difference between the randomized branches with and without reinduction therapy for patients with a risk factor RF < 0.8 in favor of the reinduction branch.[11] Therefore, reinduction protocol II was introduced in branch SRG. At the same time, results of a randomization comparing 18 versus 24 months total therapy duration in trials ALL-BFM 81 and 83 showed an advantage for a therapy duration of 24 months.[11] Thus, the total therapy duration was subsequently extended from 18 to 24 months in trial ALL-BFM 86.

The compositions of protocol I, M, E, II, and S are given in Table 1. In protocol M and in protocol E, 10% of the HD-MTX was given intravenously (IV) as a loading dose over 30 minutes. Ninety percent of the dose was administered as continuous IV infusion over 23.5 hours. I.th. MTX was administered at hour 2 in an age-adapted

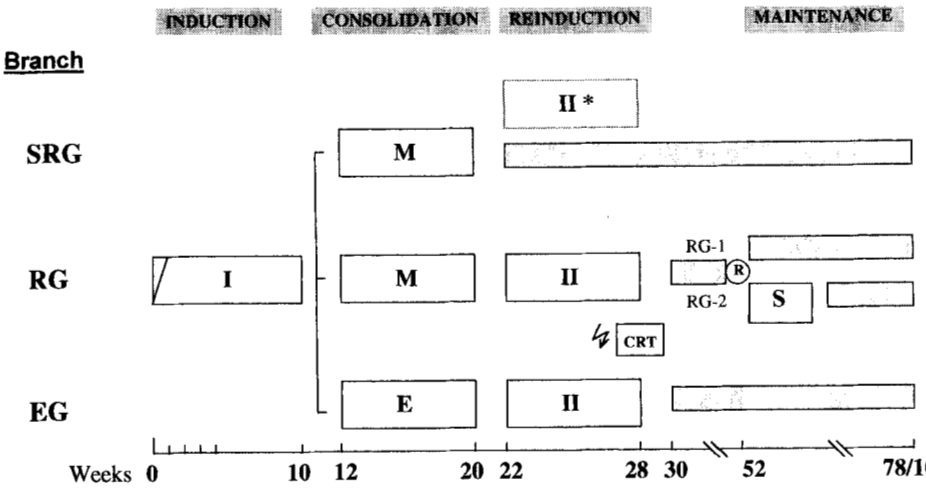

Fig 1. Treatment strategy of trial ALL-BFM 86. Therapy was stratified into the three therapy branches SRG, RG, and EG. The stratification criteria are given in the text. The therapy protocols are given in detail in Table 1. *The treatment plan was amended during study: reinduction protocol II was introduced in branch SRG and total therapy duration was extended from 18 to 24 months. CRT, cranial radiotherapy.

dosage. The citrovorum factor (CF) rescue was started at hour 36 with 75 mg/m^2 IV, followed by 5 doses of 15 mg/m^2 IV/po every 3 hours and additional 4 doses every 6 hours. In October 1988 the CF rescue was reduced to 6 doses of 15 mg/m^2 every 6 hours.

Cranial irradiation was given in branches RG and EG during the second phase of reinduction protocol II at age dosages adapted for age and leukemic cell mass (risk factor): age <year: no radiotherapy, even with overt CNS disease; age 1 − <2 years: 12 Gy (18 Gy if CNS positive); age ≥2 years: branch RG, risk factor 0.8 − <1.2, 12 Gy; risk factor ≥1.2, 18 Gy; branch EG, 18 Gy (24 Gy if CNS positive).

Local radiotherapy at a dose of 30 Gy was performed in two cases with a persistent mediastinal tumor after induction protocol I. In males with clinically overt testicular involvement, local irradiation (24 Gy) was performed.

Response criteria. Complete remission (CR) was defined as the absence of leukemic blasts in blood and CSF, fewer than 5% lymphoblasts in marrow aspiration smears, and no evidence of localized disease. Relapse was defined as recurrence of lymphoblasts or localized leukemic infiltrates at any site.

Statistical analysis. Patients of branch RG who were still in CCR after 1 year were randomized to receive (stratum RG-2) or not to receive (stratum RG-1) late intensification protocol S, if parents gave informed consent. The randomization was weighted for sex and participating hospitals. The following assumptions were made based on the results of the previous studies: Ten percent of the patients of branch RG will suffer from relapse during the first year, another 20% of patients will have relapse hazards subsequently until the end of the fifth year. Sample size considerations resulted in 200 patients needed for each of the randomization branches RG-1 and RG-2 to detect a decrease of the relapse incidence during the second and the subsequent years by late intensification in the 13th month from 20% to 10% with a test power of 0.80 (α error = 0.05).[18]

The Kaplan Meier method[19] was used to estimate survival rates with differences compared by the two-sided log-rank test.[20] Event-free survival (EFS) was calculated from the first day of treatment to the time of analysis or to the first event. Failure to achieve remission (early death, resistent leukemia), relapse, death during CCR, and second malignancy were evaluated as events. Patients lost to follow-up were censored at the time of their withdrawal. For the patients of the randomized strata RG-1 and RG-2 the event-free interval was calculated from the beginning of the 13th month to termination of first remission (relapse, death in CCR, second malignancy) or to the time of analysis.

Differences in the distribution of variables among patient subsets were analyzed using the χ^2 test for categorized variables and the Wilcoxon rank-sum test for continuous variables.

Differences between EFS distributions for patient subpopulations were evaluated using two-sided log-rank tests[20] for subsets characterized by categorical variables. The Cox regression analysis[21] was used to test for a trend of EFS distributions between patient subsets defined by scores of the classified variables age, white blood cell count (WBC), and leukemic cell mass estimate (RF). For the variable age, the squared deviation from the mean score was added to the model. The prognostic relevance of clinical and biologic variables in the whole group was examined by a stepwise Cox regression analysis.[21] For the continuous variables age, WBC, and RF multiple cut-off points were used, each of which divided all patients into two complementary subsets. Data on the DNA index of the blasts were available from 42% of patients. The variables with significant prognostic influence in the whole group ($P < .05$) were used as covariables in a Cox regression model to test the prognostic relevance of the DNA index in that subset of patients.

The first 110 patients of branch SRG enrolled from October 1986 to March 1988 did not receive reinduction therapy. The following 175 patients of branch SRG enrolled since April 1988 received reinduction protocol II because of the protocol amendment of October 1988. Treatment results for patients of branch SRG were analyzed separately for those who received reinduction therapy and those who did not. For the analysis of the prognostic relevance of clinical and biologic variables, the first 110 patients of branch SRG who did not receive reinduction therapy were excluded because their treatment is to be considered inadequate. One hundred forty-four patients (30 of branch SRG, 90 of branch RG, 24 of branch EG) enrolled from October through March 1987 received only 18 months of therapy. All patients enrolled since April 1987 received maintenance therapy up to 24 months. In the statistical analyses no adjustments were made for those patients receiving only 18 months' therapy duration. All statistical tests were explorative and descriptive with the exception of the planned test RG-1 versus RG-2. Computations were performed using the SAS program PHREG (SAS-PC, Version 6.04; SAS Institute Inc, Cary, NC). The cut-off for all analyses of treatment results was September 1, 1993.

RESULTS

Patient characteristics. Table 2 presents the clinical and biologic features of the 998 evaluable protocol patients and

Table 1. Treatment Protocols

Drug	Dose	Administered on Days*
Induction protocol I		
Prednisone (orally)	60 mg/m²	1-28
Vincristine (IV)	1.5 mg/m² (max 2 mg)	8, 15, 22, 29
Daunorubicin (IV)	40 mg/m²	8, 15, 22, 29
L-Asparaginase (IV)	10,000 IU/m²	19, 22, 25, 28, 31, 34, 37, 40
Cyclophosphamide (IV)	1,000 mg/m²	43, 71
Cytarabine (IV)	75 mg/m²	45-48, 52-55, 59-62, 66-69
6-Mercaptopurine (orally)	60 mg/m²	43-70
Methotrexate (IT)†	12 mg†	1, 45, 59
Consolidation protocol M		
6-Mercaptopurine (orally)	25 mg/m²	1-56
Methotrexate (24-h INF)‡	5 g/m²	8, 22, 36, 50
Methotrexate (IT)†	12 mg†	8, 22, 36, 50
Experimental protocol E		
Prednisone (orally)	100 mg/m²	1-7, 15-21, 29-35, 43-49
Cytarabin (3-h INF)	2,000 mg/m² every 12 h	1, 2, 29, 30
Ifosfamide (1-h INF)	1,000 mg/m² every 12 h	15, 16, 43, 44
Mitoxantrone (IV)	10 mg/m²	1, 15, 29, 43
Methotrexate (24-h INF)‡	5 g/m²	8, 22, 36, 50
Methotrexate (IT)†	12 mg†	8, 22, 36, 50
Reinduction protocol II		
Dexamethasone (orally)	10 mg/m²	1-21
Vincristine (IV)	1.5 mg/m² (max 2mg)	8, 15, 22, 29
Doxorubicin (IV)	30 mg/m²	8, 15, 22, 29
L-Asparaginase (IV)	10,000 IU/m²	8, 11, 15, 18
Cyclophosphamide (IV)	1,000 mg/m²	36
Cytarabine (IV)	75 mg/m²	38-41, 45-48
6-Thioguanin (orally)	60 mg/m²	36-49
Methotrexate (IT)†	12 mg†	38, 45
Late intensification protocol S		
Prednisone (orally)	100 mg/m²	1-7, 15-21
Vindesine (IV)	3 mg/m² (max 5 mg)	1, 8, 15, 22
Teniposide (IV)	150 mg/m²	1, 8, 15, 22
Ifosfamide (IV)	1,000 mg/m² every 12 h	1, 2
Cytarabine (3-h INF)	2,000 mg/m² every 12 h	15, 16

Abbreviations: IT, intrathecally; INF, intravenous infusion.

* Adjustments of time schedule were made for clinical condition and marrow recovery.

† Doses were adjusted for children <3 years of age, ‡ with citrovorum factor rescue.

the 75 nonprotocol patients excluded from analysis. The median age of the evaluable patients was 4.7 (range 0.0 to 18.0) years and the median WBC at diagnosis was 10,100/μL (range 350 to 1,055,000/μL). Six boys (1%) had testicular involvement at diagnosis. A mediastinal mass was present in 102 (10%) of the evaluable patients and in 7 (9%) of the patients excluded from analysis of treatment results. Five children were black, four of them among the evaluable patients.

Ninety-five protocol patients (9.5%) had 1,000/μL or more blasts in the blood on day 8 defined as prednisone poor

Table 2. Patient Characteristics

	No. (%) of Patients Evaluable N = 998	No. (%) of Patients Excluded N = 75
Sex		
Male	550 (55)	38 (51)
Female	448 (45)	37 (49)
Age (yrs)		
<1	34 (3)	5 (7)
1-9	774 (78)	45 (60)
10-14	122 (12)	12 (16)
15-18	68 (7)	13 (17)
Leukemic cell mass (RF)		
<0.8	303 (30)	23 (31)
0.8-<1.2	345 (35)	24 (32)
1.2-<1.7	283 (28)	18 (24)
≥1.7	67 (7)	10 (13)
WBC (10^3/μL)		
<10	490 (49)	36 (48)
10-<50	312 (31)	22 (29)
≥50	196 (20)	17 (23)
CNS disease	29 (3)	6 (8)
Immunophenotype		
Pre-pre-B-ALL	51 (5)	12 (16)
c-ALL	635 (64)	30 (40)
Pre-B-ALL	155 (15)	11 (15)
T-ALL	126 (13)	9 (12)
AUL	2	0
Mixed lineage	1	3 (4)
Not determined	28 (3)	10 (13)
Myeloid Antigen pos	51 (5)	10 (13)
Myeloid Antigen neg	924 (93)	63 (84)
Not examined	23 (3)	2 (3)
DNA index		
<1.16	318 (76)*	25 (83)*
≥1.16	99 (24)*	5 (17)*
Not examined	581 (58)	45 (60)
Genotype		
t(9;22)	7 (2)*	0
t(4;11)	11 (3)*	2 (9)*
t(1;19)	10 (3)*	0
Not examined	665 (67)	53 (71)
Down's Syndrome	15 (2)	2 (3)
Prednisone response (blood blasts on day 8)		
<1,000/μL	903 (90)	62 (83)
≥1,000/μL	95 (9)	13 (17)

* Percent of patients examined.

response. Prednisone poor response was associated with age less than 1 year, increased leukemic cell mass (risk factor ≥ 1.2), WBC $\geq 50{,}000/\mu L$, and the immunophenotypes pre-pre-B ALL and T-cell ALL ($P < .001$, χ^2 test). The initial prednisone dose was adjusted according to WBC counts at diagnosis to circumvent acute cell lysis complications. The cumulative prednisone dose during the 7-day prephase was available from 947 of the 998 protocol patients. The interrelationships between WBC at diagnosis, cumulative prednisone dose during the 7-day test, and the number of blasts per microliter of blood on day 8 were as follows: The cumulative prednisone dose was inversely correlated with the WBC counts at diagnosis (Spearman correlation coefficient $-.31672$, $P < .0001$); the number of blood blasts on day 8 was correlated with the WBC at diagnosis (Spearman correlation coefficient .47218, $P < .0001$); the number of blasts per microliter blood on day 8 was inversely correlated with the cumulative prednisone dose, but the correlation was low (Spearman correlation coefficient $-.13170$, $P < .0001$). The median cumulative dose of prednisone administered during days 1 through 7 was 318 mg/m^2 (25% quantile 268 mg/m^2, 75% quantile 392 mg/m^2) for patients with $\geq 1{,}000/\mu L$ blood blasts at day 8 compared with 363 mg/m^2 (25% quantile 300 mg/m^2, 75% quantile 412 mg/m^2) for patients with $<1{,}000/\mu L$ blood blasts on day 8 ($P < .0004$, Wilcoxon test). Among patients with WBC of $\geq 50{,}000/\mu L$ at diagnosis the cumulative dose of prednisone during the 7-day test was not significantly different between patients with a blast count in the blood of $\geq 1{,}000/\mu L$ on day 8 (median 315 mg/m^2, 25% quantile 259 mg/m^2, 75% quantile 390 mg/m^2) and those with a blast count of $<1{,}000/\mu L$ on day 8 (median 307 mg/m^2, 25% quantile 255 mg/m^2, 75% quantile 362 mg/m^2), $P = .7361$ (Wilcoxon test).

Of the 998 protocol patients 285 (28.6%) were placed into branch SRG, 610 (61.1%) in branch RG, and 103 (10.3%) in branch EG. The clinical and biologic features of the patients of the three stratification branches are presented in Table 3. Within branch SRG the distributions of age, WBC at diagnosis, and immunophenotypes were comparable between the patients who did not receive reinduction therapy compared with patients who did.

Treatment results. At a median follow-up of 5.0 years (range 3.4 to 6.9 years) the estimate for a 6-year duration of EFS (pEFS) was 71% ± 1% for all 1,073 patients registered with non–B-cell ALL, 51% ± 8% for the 75 nonprotocol patients, and 72% ± 2% for the 998 evaluable protocol patients (Fig 2). The 75 nonprotocol patients were excluded from the analysis of treatment results. Table 4 presents treatment results of the 998 evaluable protocol patients. Of these, 985 patients (98.7%) achieved CR. Of the 13 patients who did not enter remission, 1 patient died of renal failure on day 3, 5 died an early toxic death, and 7 had resistant leukemia. Two hundred thirty-three patients (23.3%) suffered from relapse. Thirteen patients died while in CCR and 3 patients developed a second malignancy: acute myeloid leukemia in 2 patients (18 and 36 months after the diagnosis of ALL), and a brain tumor in 1 (4.2 years after the diagnosis of ALL). Seven hundred thirty-four patients (73.5%) were still in their first CCR. The 6-year EFS estimate according to treatment branch was 58% ± 5% for the first 110 patients of branch SRG who did not receive reinduction, 87% ± 3% for the next 175 patients of branch SRG who did receive reinduction therapy, 75% ± 2% for patients of branch RG, and 48% ± 5% for the patients of branch EG (Fig 3). When the first 110 patients of branch SRG who did not receive reinduction are excluded from analysis, the estimate for pEFS at 6 years for the remaining 888 patients of all branches together was 74% ± 2%.

Experimental branch EG. Two patients were placed into branch EG because of AUL and mixed-lineage leukemia, respectively (Table 3); both patients are in CCR. Ninety-five patients qualified for branch EG by prednisone poor response. Two of them died before day 40 of infection and progressive disease, respectively; 79 achieved CR on day 40; 41 of them (52%) were still in their first CCR. Fourteen of the prednisone poor responders did not achieve CR on day 40. Six patients who had less than $1{,}000/\mu L$ blood blasts on day 8 were included in branch EG because of the presence of greater than 5% blasts in the marrow on day 40. Twenty patients had more than 5% blasts in the marrow on day 40, 6 never achieved remission and died, 9 achieved remission after completion of induction protocol I, and 5 entered remission during protocol E. Of these 14 patients who remitted, 5 suffered from relapse, 2 died of BMT-related toxicity, and a third patient died from toxic death during maintenance. Allogeneic BMT in first remission was performed in another 4 patients of branch EG: 3 of them suffered from relapse and died; only 1 patient survived event-free. Almost all relapses in branch EG occurred within 2 years after diagnosis compared with a longer relapse cascade in branches SRG and RG (Fig 3). The estimated probability of a 6-year EFS for patients with prednisone poor response was 48% ± 5% ($n = 95$) in trial ALL-BFM 86 compared with 39 ± 7 ($n = 49$) in the preceding trial ALL-BFM 83 ($P > .1$, log-rank test; test power = 40%[22]).

The main toxicity of the investigational protocol E was severe myelosuppression leading to considerable delay in treatment realization. The median time for the completion of protocol E was 13 weeks (range 9 to 16 weeks). No patient died during protocol E because of therapy-related complications.

Randomized trial for late intensification in branch RG. By randomization, 62 patients entered stratum RG-1 without late intensification and 66 patients entered stratum RG-2 and received late intensification in the 13th month. There was no apparent difference in the distribution of immunophenotypes, age, and leukocyte counts among both randomization strata. Eleven of the 62 patients (18%) in branch RG-1 and 12 of the 66 patients (18%) in branch RG-2 suffered from relapse during the second and subsequent years. The median follow-up of patients is 6.1 (range 4.5 to 6.8) years in branch RG-1 and 5.6 (range 4.5 to 6.8) years in branch RG-2. In both strata no patient died a toxic death. The estimated probability for a 5-year duration of event-free interval beginning with the 13th month was 82% ± 5% for RG-1 and 80% ± 5% for RG-2 ($P = .8$).

Table 3. Patient Characteristics According to Stratification Branch

Branch	SRG No Reinduction N = 110 No. (%) of Patients	SRG Reinduction N = 175 No. (%) of Patients	RG N = 610 No. (%) of Patients	EG N = 103 No. (%) of Patients
Stratification parameters				
Blood blasts on day 8				
<1,000/μL	110	175	610	6*
≥1,000/μL	0	0	0	95
Day 40 marrow >5% blasts	0	0	0	20
AUL	0	0	1	1
Leukemic cell mass (RF)				
<0.8	108	172	20	3
≥0.8	2	3	590†	100
CNS positive	0	2	21	6
Mediastinal mass	0	0	73	29
Other features				
Age				
<1 yr	1 (1)	1 (1)	17 (3)	15 (15)
1-9 yrs	90 (82)	135 (77)	486 (80)	63 (63)
≥10 yrs	19 (17)	39 (22)	107 (18)	25 (25)
WBC				
<10,000/μL	105 (95)	162 (93)	216 (35)	7 (7)
10,000-<50,000/μL	5 (5)	13 (7)	259 (42)	35 (35)
≥50,000/μL	0	0	135 (22)	61 (61)
Immunophenotype				
Pre-pre-B-ALL	5 (5)	2 (1)	29 (5)	15 (15)
c-ALL	86 (78)	141 (81)	373 (61)	35 (35)
Pre-B-ALL	11 (10)	22 (13)	106 (17)	16 (16)
T-ALL	2 (2)	1 (1)	88 (14)	35 (35)
Mixed lineage	0	0	0	1 (1)
Not determined	6 (5)	9 (5)	13 (2)	0

* Patients had >5% blasts in the marrow on day 40.

† RF 0.8-<1.2, n = 315; RF 1.2-<1.7, n = 194; RF ≥1.7, n = 81.

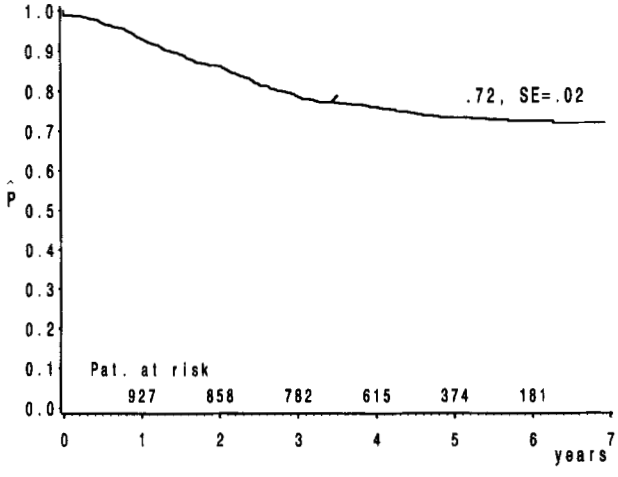

Fig 2. Kaplan Meier estimate of EFS for the study population (N = 998 protocol patients); / indicates last patient in CCR entering the trial. (—) Protocol patients (N = 998, 734 in CCR).

Relapses. BM was the most frequent site of relapse (Table 4). Only 1.8% of the patients suffered from isolated CNS relapse, and the overall rate of relapses with CNS involvement was 4.6%. CNS relapse rates in branch SRG (without cranial irradiation) and RG (with cranial irradiation) were comparable. However, in branch EG the proportion of patients who suffered from CNS relapses was higher (7% isolated CNS relapses). In branch RG, 323 of the 610 patients (53%) received cranial irradiation with the reduced dosage of 12 Gy because they had a risk factor of <1.2 and no CNS disease. One of them suffered from isolated CNS relapse and five suffered a combined CNS/BM relapse. The pattern of the site of failure was similar between immunophenotypes except for the pre-pre-B type in which CNS involvement was rare. However, the time periods at which relapses occurred after achieving remission were different for patients with different immunophenotypes: T-ALL, 2 to 29 (median 12) months; pre-pre-B ALL, 5 to 37 (median 15) months; common-ALL, 1 to 56 (median 25) months; pre-B ALL, 2 to 64 (median 21) months. The differences were significant between T-ALL and common-ALL ($P = .0002$), T-ALL and pre-B ALL ($P < .006$), and between pre-pre-B ALL and common-ALL ($P < .03$) using the Wilcoxon rank-sum test.

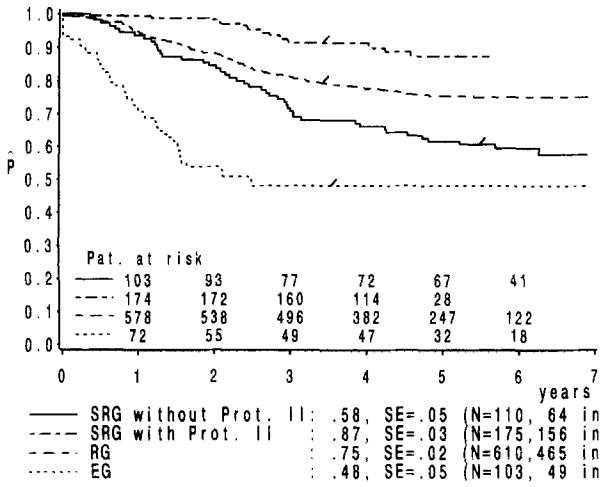

Fig 3. Kaplan Meier estimate of EFS according to stratification branch. / Indicates last patient in CCR entering the trial. Differences between all branches were significant ($P \leq .003$).

Toxic death. Five patients died an early toxic death during the first 40 days of induction therapy: three patients died of septicemia (one of them had Down's syndrome), a second patient with Down's syndrome died of varicella pneumonia, the fifth patient died because of cerebral hemorrhage. Thirteen patients died while in CCR. In branch EG, one patient died of fungal infection before protocol E was applied, two patients died of BMT-related toxicity, and three patients died after 6, 16, and 19 months, respectively, because of cardiomyopathy. Autopsy in one of them showed an aneurysm of the myocard. In branches SRG and RG three patients died of infections, and one patient each died due to Wernicke encephalopathy, hemophagocytic syndrome, cerebral bleeding because of arterio-venous angioma (after 39 months), and accident.

Prognostic parameters. Therapy results according to pretreatment and response parameters are given in Table 5. The first 110 patients of branch SRG who did not receive reinduction before the amendment of the protocol were excluded from that analysis because their treatment was considered inadequate. For the remaining group of 888 patients, all of whom received induction and reinduction therapy, the estimate of a 6-year EFS was 74% ± 2%. Variables that had an adverse association with EFS were age less than 1 year, increased leukemic cell mass (RF of 1.2 and more), WBC of 20,000/μL or more, the immunophenotype pre-pre-B, FAB L2 cytomorphology, and a prednisone poor response. Patients with more than 5% blasts in the marrow on day 40 of induction therapy had a worse outcome; only 6 of 20 of those patients remained in CCR. Features associated with favorable outcome were age 1 to 5 years old, low leukemic cell mass (RF <0.8), WBC below 20,000/μL, and a DNA index of the leukemic blasts ≥1.16.

In the Cox stepwise regression analysis a prednisone poor response, a day 40 marrow with more than 5% blasts, WBC ≥20,000/μL, ≥200,000/μL, age ≥6 years, male gender, a hemoglobin value ≥8 g/100 mL at diagnosis, Down's syndrome, and an increased leukemic cell mass (RF ≥0.8) re-

Table 4. Treatment Results and Site of Failure

		Treatment Branch			
		SRG Reinduction			
	All Patients	No	Yes	RG	EG
No. of Patients	998	110	175	610	103
Early death*	6	0	1	4	1
Remission failure	7	0	0	0	7
In first CCR	734 (73%)	64 (58%)	156 (89%)	465 (76%)	49 (47%)
Death in CCR	13†	1	1	5	6†
Second malignancy	3	0	1	1	1
No. of relapses	233 (23%)	44 (49%)	16 (9%)	135 (22%)	38 (37%)
Isolated relapses					
BM	150 (15.0%)	29	10	89	22
CNS	18 (1.8%)	2	2	7	7
Testes	17 (1.7%)	6	1	10	0
Other	3	0	0	1	2
Combined relapses					
BM/CNS	26 (2.6%)	4	1	16	5
BM/testes	16 (1.6%)	3	2	10	1
Other combinations	3‡	0	0	2	1

Median follow-up 5.0 years, range 3.4-6.9 years. (Median follow-up for patients of branch SRG without reinduction therapy: 6.2 years, range 5.5-6.9 years.) Two patients were lost to follow-up.

* Within the first 40 days.

† Two patients died due to BMT toxicity.

‡ CNS + testes; BM + CNS + testes, BM + other.

Table 5. Results by Pretreatment and Response Parameters. 110 Patients of Branch SRG Without Reinduction Therapy Are Excluded From Analysis

Variable	No. of Patients N = 888	% (SE) 6-yr EFS All Patients 74 (2)	P
Sex			
Male	493	71 (2)	.009
Female	395	79 (2)	
Age (yrs)			
<1	33	36 (8)	<.0001*
1-5	509	81 (2)	
6-9	175	71 (4)	
≥10	171	65 (4)	
Leukemic cell mass (RF)			
<0.8	195	88 (3)	<.0001*
0.8-<1.2	343	79 (2)	
1.2-<1.7	283	65 (3)	
≥1.7	67	52 (6)	
WBC ($10^3/\mu L$)			
<10	385	85 (2)	<.0001*
10-<20	134	82 (3)	
20-<50	173	65 (4)	
50-<200	137	64 (4)	
≥200	59	41 (6)	
Hemoglobin g/100 mL			
<8	486	78 (2)	.001
≥8	402	70 (2)	
CNS disease	29	71 (9)	.7
Mediastinal mass	102	76 (4)	.9
Cytology (FAB)			
L1	735	76 (2)	.013
L2	153	67 (4)	
Immunophenotype			
Pre-pre-B-ALL	46	54 (7)	<.0001†
c-ALL	549	77 (2)	
Pre-B-ALL	144	67 (5)	
T-ALL	124	73 (4)	
Myeloid antigen positive	49	66 (7)	.09
Myeloid antigen negative	821	75 (2)	
DNA index			
<1.16	289	71 (3)	.002
≥1.16	84	89 (3)	
Genotype			
t(9;22)	7	1‡	
t(4;11)	11	3‡	
t(1;19)	10	8‡	
Down's syndrome	15	6‡	
Prednisone response (blood blasts on day 8)			
<1,000/μL	793	78 (2)	<.0001
≥1,000/μL	95	48 (5)	
Day 40 marrow			
>5% blasts	20	6‡	

*Trend test by Cox regression analysis.[21]

† Pre-pre-B *v* c-ALL ($P < .0001$); pre-pre-B *v* pre-B ($P = .013$); pre-pre-B *v* T ($P = .029$); pre-B *v* c-ALL ($P = .038$); pre-B *v* T ($P = .9$); T *v* c-ALL ($P = .096$).

‡ No. of patients in CCR.

Table 6. Variables With Significant Influence ($P < .05$) on EFS by Cox Stepwise Regression Analysis in the Order of Risk Ratio

Variable*	Risk Ratio	P Value
Day 40 marrow >5% blasts	6.274	.0001
WBC ($10^3/\mu L$) ≥200	2.920	.0001
Down's Syndrome	2.462	.0156
WBC ($10^3/\mu L$) ≥20	2.060	.0001
Prednisone poor response (≥1,000/μL blood blasts on day 8)	1.864	.0016
Leukemic cell mass RF ≥0.8	1.766	.0358
Age ≥6 years	1.763	.0003
Male gender	1.709	.0003
Hemoglobin ≥8 g/100 mL	1.482	.0089
T-cell immunophenotype	0.350	.0001

Number of patients in the test: 848†, pEFS = 74% ± 2%. † Patients of branch SRG without reinduction therapy are excluded from analysis. From 40 of the 888 patients of Table 6, data on one or more of the variables to be included in the Cox stepwise regression analysis were lacking. WBC was analyzed at cut-off points ($\times 10^3/\mu L$): ≥20, ≥50, ≥100, ≥200; RF was analyzed at cut-off points ≥0.8, ≥1.2, ≥1.7.

* All variables of Table 6 were included in the model building process except cytogenetics, DNA index, and the presence or absence of a mediastinal mass.

mained significant predictors of an impaired prognosis in the whole group (Table 6), whereas age under 1 year lost predictive strength. The presence of a mediastinal mass was a predictor of better outcome in the multivariate analysis ($P = .0001$, risk ratio = .321). If the presence of a mediastinal mass was excluded as a covariable from the model the immunophenotype T cell gained predictive strength for a better outcome in the multivariate analysis (Table 6). Detailed analysis showed that in subsets of patients defined by the adverse prognostic factors male gender, higher WBC, and age ≥6 years old, patients with T-ALL had a better outcome than non–T-ALL patients. For instance, among patients with WBC ≥20,000/μL pEFS at 6 years was 69% ± 5% for T-ALL patients compared with 58% ± 3% for the complementary group of patients with an immunophenotype other than T cell. Other immunophenotypes had no predictive strength for treatment outcome in the multivariate analysis. The adverse association of the immunophenotype pre-pre-B with treatment results was abolished in the subset of patients 1 year of age and older (Fig 4). Although a prednisone poor response was associated with age less than 1 year, increased leukemic cell mass, increased WBC, and the immunophenotypes pre-pre-B ALL and T-cell ALL, the in vivo response to prednisone added prognostic strength in addition to these parameters. A blast cell count in the blood on day 8 of either <1,000/μL or ≥1,000/μL subdivided each patient group stratified by age, leukemic cell mass, WBC, and immunophenotype in two complementary subsets with a significant different EFS estimate (Table 7).

Cytogenetics and DNA index were excluded from the multivariate analysis of prognostic factors in the whole group because these parameters were available only from part of the patients. The DNA index of blasts was available from 42% of the patients. The distribution of clinical and biologic

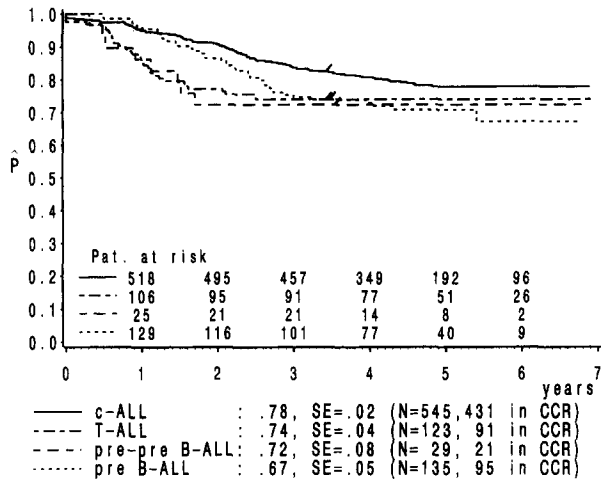

Fig 4. Kaplan Meier estimate of EFS according to immunophenotype for patients 1 year of age and older (*P* in all comparisons >.08). Patients of branch SRG without reinduction therapy were excluded from this analysis.

features was comparable among patients with and without available data on DNA index as was the estimate of EFS (75% ± 2% *v* 74% ± 2%, $P = .9$). By multivariate analysis, a DNA index ≥1.16 did not add significantly to the prediction of treatment outcome ($P = .0772$, risk ratio = .512) in this subset of patients if the variables with significant prognostic influence in the whole group (sex, age, WBC, leukemic cell mass, prednisone poor response) were used as covariates in the Cox regression model. However, a DNA index ≥1.16 gained predictive significance ($P < .05$), if WBC was excluded from the model.

DISCUSSION

Nine hundred ninety-eight evaluable patients were enrolled in study ALL-BFM 86. The median follow-up was 5 years at the time of analysis, so some conclusions can be drawn as to treatment outcome. The estimated 6-year EFS was 72% ± 2% for the whole study population including all subsets of childhood ALL with the exception of the small group of B-cell ALL. When the first 110 patients of branch SRG who did not receive reinduction were excluded from the analysis, the estimated 6-year EFS for the remaining 888 patients of all branches was 74% ± 2%. Except for the 10% of patients with a poor initial response to therapy and patients with a Philadelphia chromosome-positive ALL, the treatment strategy of trial ALL-BFM 86 provided all subsets of patients with an acceptable chance of surviving event-free. Even infants and patients with a very high leukemic cell mass had an estimated probability of EFS ≥ 50% if they had an adequate in vivo response during the first 7 days of treatment. Patients with T-cell ALL had a favorable EFS with this intensive treatment regimen, similar as observed in a Dana-Farber Institute study with a comparable intensive therapy.[23] Except for the experimental protocol E, treatment could be performed on an outpatient basis during most phases of therapy. The incidence rate of death because of therapy-related complications was less than 2% (including BMT-related mortality).

With intensive treatment for all patients resulting in an improved overall EFS, well-known parameters such as WBC, the leukemic cell mass (RF), age, and sex were still strong predictors of treatment outcome. However, the early response to treatment itself added prognostic strength to those parameters. Persistence of more than 5% blasts in the marrow on day 40 of induction therapy was the strongest predictor of a worse prognosis by Cox regression analysis. Most of these patients were among the prednisone poor responders. A prednisone poor response, defined as ≥1,000/μL blood blasts on day 8 after a 7-day exposure to prednisone and one i.th. MTX dose on day 1, seems to reflect highly resistant disease. It was the only variable that defined a patient subset of at least 10% of the whole group with an EFS of less than 50%.

Although a prednisone poor response was associated with other adverse prognostic parameters such as age less than 1 year and increased leukocyte count, it retained prognostic strength if those parameters were included as covariables in the Cox regression analysis. This powerful prognostic variable is able to be obtained easily and early in almost every patient, an important attribute for its use as stratification parameter in a large multicenter trial. Compared with the evaluation of the reduction of blasts in the marrow as a parameter of treatment response[24] the measurement of blasts in the blood is rarely altered by technical problems, eg, dilution of probes. The shortcoming of the so-defined prednisone poor response is certainly that "poor response" in patients with low blast count at diagnosis is missed. In vitro

Table 7. Treatment Outcome According to In Vivo Prednisone Response Stratified by Age, Leukemic Cell Mass, WBC, and Immunophenotype (N = 888)

	Blood Blasts on Day 8				
	<1,000/μL		≥1,000/μL		
Variable	n	% pEFS ± SE at 6 yrs	n	% pEFS ± SE at 6 yrs	*P*
Age					
<1	19	53 ± 11	14	14 ± 9	.002
1-9	622	80 ± 2	59	60 ± 6	<.0001
≥10	150	68 ± 4	22	36 ± 10	<.0001
Leukemic cell mass					
RF 1.2-<1.7	234	68 ± 3	50	50 ± 7	.001
RF ≥1.7	45	62 ± 7	22	32 ± 10	.005
WBC/μL					
<50,000	656	81 ± 2	36	57 ± 8	.0001
≥50,000	137	63 ± 4	59	42 ± 6	.004
Immunophenotype					
Pre-pre-B	33	67 ± 8	13	23 ± 12	.0022
c-ALL	516	79 ± 2	33	58 ± 9	.0002
Pre-B	128	69 ± 5	16	53 ± 13	.03
T	91	84 ± 4	33	45 ± 9	.0001

Patients of branch SRG without reinduction therapy were excluded from analysis.

assays such as the determination of the glucocorticoid receptor content of the leukemic blasts or in vitro steroid sensitivity tests may not have this limitation.[25,26] However, the relation of these in vitro methods to the in vivo prednisone sensitivity test remains to be determined in larger studies. Only the immunophenotype pre-pre-B was associated with an impaired treatment result in the whole group of patients, but the adverse impact on outcome was abolished when the infants were excluded from the analysis. Although EFS of patients with different immunophenotypes was similar, the time periods in which relapses occurred were different, an aspect of interest in terms of therapy stratification, eg, the duration of maintenance therapy. With this treatment regimen T-ALL was even a favorable prognostic variable in the multivariate analysis of the entire group of ALL patients. However, the prednisone response subdivided patients with T-ALL in a subset of 73% of patients ($<1,000/\mu L$ blood blasts on day 8) with an excellent 6-year EFS of 84% ± 4% and a subset of 27% of patients ($\geq 1,000/\mu L$ blood blasts on day 8) with poor outcome (Table 7). A DNA index ≥1.16 of the leukemic blasts was associated with a favorable treatment outcome as previously reported by others.[17,27] Unfortunately, data on the DNA index were available from only 42% of the patients. In this patient subset a DNA index ≥1.16 had a risk ratio below 1 in a multivariate analysis. However, statistical significance in predicting treatment outcome was lost after adjustments had been made for the in vivo response to prednisone, WBC, leukemic cell mass, age, and sex. The level of significance might have been different with a higher proportion of evaluable patients.

Prednisone poor responders had an unsatisfying treatment outcome although the experimental therapy component for this target group was mainly composed of drugs that have shown activity in refractory leukemia and lymphoma.[7-10,28] The EFS estimate of these patients was higher compared with that of prednisone poor responders in the preceding trial ALL-BFM 83, but the difference was not significant statistically. However, the test power was low because of the limited numbers of prednisone poor responders in both studies. Therefore, an improvement cannot be excluded. The experimental protocol E might have been placed too late in the course of treatment. Furthermore, the incorporation of mitoxantrone was rather disadvantageous because of its long-lasting myelosuppression prohibiting continuation of any treatment. Mitoxantrone might have added critical cardiotoxicity[29] because three patients of branch EG died of cardiomyopathy. This has never been experienced in patients of branches SRG and RG.

The contribution of HD-MTX to the overall treatment results of our trial is difficult to judge. A major contribution of HD-MTX 5 g/m^2 as a 24-hour infusion may be protection of the CNS because cytotoxic steady-state concentrations are achieved in the cerebrospinal fluid[30,31] and are boosted by an intrathecal MTX application. Only 1.8% of our patients suffered from isolated CNS relapses. Patients of branch SRG were not irradiated. In trial ALL-BFM 81 it was proven by randomization that for patients with a low leukemic cell mass (risk factor < 0.8) cranial irradiation can be safely omitted.[11] About 70% of our patients (patients of branches RG and EG) received cranial irradiation which has considerable late risks.[32-34] However, roughly one half of the patients of branch RG (those with a risk factor of 0.8-<1.2 and no overt CNS disease) received a reduced dosage of 12 Gy, which had been shown in our previous trial ALL-BFM 83 to be as effective as 18 Gy to attain control of CNS leukemia in this patient subset when MTX was administered at an intermediate dose of 0.5 g/m^2.[11] The low incidence rate of CNS relapses in trial ALL-BFM 86 suggests that cranial radiotherapy could be omitted for most of the CNS^- patients, when HD-MTX as a 24-hour infusion and intrathecal MTX therapy is used. However, for patients characterized by poor initial response to treatment omission of cranial irradiation could be risky because the patients of branch EG had an increased incidence of CNS relapses in our trial (Table 4).

The importance of an intensive reinduction therapy even for patients with a low leukemic cell mass (RF <0.8), proven in a randomized trial in our previous study ALL-BFM 83,[11] was sustained. The estimated 6-year EFS for patients of branch SRG who did not receive reinduction therapy was almost 30% lower than in those with reinduction therapy. Unfortunately, the randomized trial to test whether an additional late intensification in the 13th month would reduce the relapse incidence for patients with increased leukemic cell burden was compromised by an inadequate acceptance. Although the follow-up of the patients is still too short for final analysis, no valid information can be expected because of the small number of patients in both randomization branches.

Comparison of our treatment results with those of concurrent therapy studies in childhood ALL is difficult because most reports are restricted to selected subsets of patients. Only a few reports of contemporary therapeutic trials in childhood ALL are available with comparable overall results for the whole unselected group of ALL patients.[23,35-37] The value of an intensification therapy after induction was meanwhile confirmed in trials of other groups at least for patients of intermediate- or increased-risk features.[36-38] In patients with favorable prognostic features, reductions of treatment intensity seem possible, maintaining the strategy of induction and reinduction therapy. In our regimen the cumulative dosage of anthracyclines in the induction and reinduction therapy was 280 mg/m^2, which potentially carries a late risk of cardiac function abnormalities in a proportion of the patients.[39] Therefore, its dosage was reduced in our current BFM-ALL protocol. Treatment regimens based on antimetabolite components, glucocorticoids, vincristine, and L-asparaginase resulted in an intriguing 4-year EFS rate in selected subgroups of childhood ALL such as precursor B-cell ALL excluding infants,[40,41] and in patients with WBC of less than $50,000/\mu L$ without mediastinal mass and without CNS disease.[42] Antimetabolite based regimens such as the ALinc 14 protocol of the Pediatric Oncology Group seem to be of particular efficacy for treatment of hyperdiploid ALL with a DNA index of >1.16[27,43] or, more specifically, for patients with a trisomy of chromosomes 4 and 10 of the leukemic blasts.[44] Parameters reflecting the in vivo sensitivity of leukemic cells to a treatment such as genetic mark-

ers[27,40,44,45] or direct sensitivity tests, eg, the prednisone response, will allow a more accurate tailoring of treatment modalities in the future.

In the current and the forthcoming BFM-ALL programs efforts are being undertaken to improve the treatment outcome of patients with initial poor response to treatment as well as to reduce the risk of long-term morbidity especially for favorable risk patients. However, the large group of children with ALL lacking known features of an extremely good or an extremely poor prognosis is of particular interest because, in absolute numbers, the majority of victims of ALL are found in this patient subset.

ACKNOWLEDGMENT

We thank Jennifer M. Meyers for proofreading the English text, and Andrea Brandt, Angelika Nehmer, and Christoph Hüstebeck for preparing the data of the ALL-BFM studies.

Principal Investigators of the Participating Hospitals: R. Mertens (Aachen); B. Höhmann (Aalen); A. Gnekow (Augsburg); R. Dickerhoff (St Augustin); G.F. Wündisch (Bayreuth); G. Henze (Berlin); U. Bode (Bonn); G. Mau (Braunschweig); H. Jacobi (Celle); J.-D. Thaben (Coburg); W. Andler (Datteln); T. Wagner (Delmenhorst); H. Breu (Dortmund); J.D. Beck (Erlangen); W. Havers (Essen); G. Müller (Feldkirch); B. Kornhuber (Frankfurt); A.H. Sutor (Freiburg); F. Lampert (Giessen); M. Lakomek (Göttingen); C. Urban (Graz); L. Reinken (Hamm); H. Riehm (Hannover); R. Ludwig (Heidelberg); N. Graf (Homburg); B. Ausserer (Innsbruck); G. Nessler (Karlsruhe); H. Wehinger (Kassel); M. Rister (Kiel); H. Messner (Klagenfurt); F. Berthold (Köln); W. Sternschulte (Köln); I. Mutz (Leoben); W. Tulzer (Linz); O. Stöllinger (Linz); C. Dominick (Ludwigshafen); J. Otte (Lübeck); C. Eschenbach (Marburg); W. Tillmann (Minden); S. Müller-Weihrich (München); C. Bender-Götze (München); G. Schellong (Münster); A. Feldmann (Neunkirchen); U. Schwarzer (Nürnberg); A. Jobke (Nürnberg); R. Geib-König (Saarbrücken); H. Grienberger (Salzburg); H. Haas (Schwarzach); F.J. Göbel (Siegen); R. Ploier (Steyr); J. Treuner (Stuttgart); H. Rauh (Trier); D. Niethammer (Tübingen); G. Gaedicke (Ulm); D. Franke (Vechta); H. Gadner (Wien); L. Thun Hohenstein (Wien); J. Kühl (Würzburg).

Immunophenotyping: W.-D. Ludwig, (Berlin); W. Knapp (Wien).

Chromosome Analyses: J. Harbott, F. Lampert (Giessen); O. Haas (Wien).

Statistics: A. Brandt, M. Zimmermann (Hannover).

REFERENCES

1. Riehm H, Gadner H, Henze G, Langermann HJ, Odenwald E: The Berlin childhood acute lymphoblastic leukemia therapy study, 1970-1976. Am J Pediatr Hematol Oncol 2:299, 1980

2. Riehm H, Feickert HJ, Schrappe M, Henze G, Schellong G: Therapy results in five ALL-BFM studies since 1970. Implications of risk factors for prognosis. Haematol Blood Transfus 30:139, 1987

3. Henze G, Langermann HJ, Ritter J, Schellong G, Riehm H: Treatment strategy for different risk groups in childhood acute lymphoblastic leukemia: A report from the BFM study group. Haematol Blood Transfus 26:87, 1981

4. Langermann HJ, Henze G, Wulf M, Riehm H: Abschätzung der Tumorzellmasse bei der akuten lymphoblastischen Leukämie im Kindesalter: Prognostische Bedeutung und praktische Anwendung. Klin Pädiat 194:209, 1982

5. Jacquillat C, Weil M, Gemon MF: Combination therapy in 130 patients with acute lymphoblastic leukemia (Protocol 06 LA 66-Paris). Cancer Res 33:3278, 1973

6. Riehm H, Reiter A, Schrappe M, Berthold F, Dopfer R, Gerein V, Ludwig R, Ritter J, Stollmann B, Henze G: Die Corticosteroid-abhängige Dezimierung der Leukämiezellzahl im Blut als Prognosefaktor bei der akuten lymphoblastischen Leukämie im Kindesalter (Therapiestudie ALL-BFM 83). Klin Pädiat 199:151, 1987

7. Herzig R, Wolff S, Lazarus H, Phillips G, Karanes C, Herzig G: High-dose cytosine arabinoside therapy for refractory leukemia. Blood 62:361, 1983

8. Rodriquez V, McCredie KB, Keating MJ, Valdivieso M, Bodey GP, Freireich EJ: Ifosfamide therapy for hematologic malignancies in patients refractory to prior treatment. Cancer Treat Rep 62:493, 1978

9. Patte C, Bernard A, Hartmann O, Kalifa C, Flamant F, Lemerle J: High-dose Methotrexate and Continuous Infusion Ara-C in Children's Non-Hodgkin's Lymphoma: Phase II Studies and Their Use in Further Protocols. Pediatr Hematol Oncol 3:11, 1986

10. Paciucci PA, Cuttner J, Holland JF: Mitoxantrone as a single agent and in combination chemotherapy in patients with refractory acute leukemia. Semin Oncol 11:36, 1984 (suppl 3)

11. Riehm H, Gadner H, Henze G, Kornhuber B, Lampert F, Niethammer D, Reiter A, Schellong G: Results and Significance of Six Randomized Trials in Four Consecutive ALL-BFM Studies. Haematol Blood Transfus 33:439, 1990

12. Reiter A, Schrappe M, Ludwig WD, Lampert F, Harbott J, Henze G, Niemeyer CM, Gadner H, Müller-Weihrich S, Ritter J, Odenwald E, Riehm H: Favorable Outcome of B-Cell Acute Lymphoblastic Leukemia in Childhood: A Report of Three Consecutive Studies of the BFM Group. Blood 80:2471, 1992

13. Bennett JM, Catovski D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan C: Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 33:451, 1976

14. Ludwig WD, Bartram CR, Ritter J, Raghavachar A, Hiddemann W, Heil G, Harbott J, Seibt-Jung H, Teichmann JV, Riehm H: Ambiguous phenotypes and genotypes in 16 children as characterized by multiparameter analysis. Blood 71:1518, 1988

15. Harbott J, Ritterbach J, Ludwig W-D, Bartram CR, Reiter A, Lampert F: Clinical Significance of Cytogenetic Studies in Childhood Acute Lymphoblastic Leukemia: Experience of the BFM Trials. Recent Results in Cancer Res 131:123, 1993

16. Hiddemann W, Wörmann B, Ritter J, Thiel E, Göhde W, Lahme B, Henze G, Schellong G, Riehm H, Büchner Th: Frequency and Clinical Significance of DNA Aneuploidy in Acute Leukemia. Ann N J Acad Sci 468:227, 1986

17. Look AT, Roberson PK, Williams DL, Rivera G, Bowman WP, Pui CH, Ochs J, Abromowitch J, Kalwinsky D, Dahl GV, George S, Murphy SB: Prognostic importance of blast cell DNA content in childhood acute lymphoblastic leukemia. Blood 65:1079, 1985

18. Casagrande JT, Pike MC, Smith PG: An improved approximate formula for calculating sample sizes for comparing two binominal distributions. Biometrics 34:483, 1978

19. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457, 1958

20. Mantel N: Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50:163, 1966

21. Cox DR: Regression models and life tables. J R Stat Soc 34:187, 1972

22. Bernstein D, Lagakos W: Sample Size and power determination for stratified clinical trials. J Statist Comput Sinn 8:65, 1978

23. Schorin MA, Blattner S, Gelber RD, Tarbell NJ, Donnelly

M, Dalton V, Cohen HJ, Sallan SE: Treatment of childhood acute lymphoblastic leukemia: Results of Dana-Farber Cancer Institute/ Children's Hospital Acute Lymphoblastic Leukemia Consortium Protocol 85-01. J Clin Oncol 12:740, 1994

24. Gaynon PS, Bleyer WA, Steinherz PG, Finklestein JZ, Littman P, Miller DR, Reaman G, Sather H, Hammond GD: Day 7 marrow response and outcome for children with acute lymphoblastic leukemia and unfavorable presenting features. Med Pediatr Oncol 18:273, 1990

25. Pieters R, Huismans DR, Loonen AH, Hahlen K, van der Does-van den Berg A, van Wering ER, Veerman AJR: Relation of cellular drug resistance to long-term clinical outcome in childhood acute lymphoblastic leukaemia. Lancet 338:399, 1991

26. Kato GJ, Quddus FF, Shuster JJ, Boyet J, Pullen JD, Borowitz MJ, Whitehead VM, Crist WM, Leventhal BG: High glucocorticoid receptor content of leukemic blasts is a favorable prognostic factor in childhood acute lymphoblastic leukemia. Blood 82:2304, 1993

27. Trueworthy R, Shuster J, Look T, Crist W, Borowitz M, Carroll A, Frankel L, Harris M, Wagner H, Haggard M, Mosijczuk A, Pullen J, Steuber P, Land V: Ploidy of Lymphoblasts Is the Strongest Predictor of Treatment Outcome in B-Progenitor Cell Acute Lymphoblastic Leukemia of Childhood: A Pediatric Oncology Group Study. J Clin Oncol 10:606, 1992

28. Moe PJ, Seip M, Finne PH, Kolmannskog S: Methotrexate infusions in poor prognosis acute lymphoblastic leukemia in children: I. The Norwegian methotrexate study in acute lymphoblastic leukemia in childhood, August 1975-December 1980. Med Pediatr Oncol 14:187, 1986

29. Cassidy J, Merrick MV, Smyth JF, Leonard RCF: Cardiotoxicity of mitoxantrone assessed by stress and resting nuclear ventriculography. Eur J Cancer Clin Oncol 24:935, 1988

30. Milano G, Thyss A, Serre Debeauvais F, Laureys G, Benoit Y, Deville A, Dutour C, Robert A, Otten J, Behar C, Frappaz D: CSF drug levels for children with acute lymphoblastic leukemia treated by 5 g/m^2 methotrexate. Eur J Cancer 26:492, 1990

31. Hryniuk WM, Bertino JR: Treatment of leukemia with large doses of methotrexate and folinic acid: Clinical-biochemical correlates. J Clin Invest 48:2140, 1969

32. Ochs JJ: Neurotoxicity Due to Central Nervous System Therapy for Childhood Leukemia. Am J Ped Oncol 11:93, 1989 (suppl 1)

33. Siimes MA, Lie SO, Andersen O, Marky I, Rautonen J, Hertz H: Prophylactic Cranial Irradiation Increases the Risk of Testicular Damage in Adult Males Surviving ALL in Childhood. Med Ped Oncol 21:117, 1993

34. Nygaard R, Garwicz S, Haldorsen T, Hertz H, Jonmundsson GK, Lanning M, Moe PJ: Second malignant neoplasms in patients treated for childhood leukemia. A population-based cohort study from the Nordic countries. The Nordic Society of Pediatric Oncology and Hematology (NOPHO). Acta Paediatr Scand 80:1220, 1991

35. Niemeyer CM, Gelber RD, Tarbell NJ, Donnelly M, Clavell LA, Blattner SR, Donahue K, Cohen HJ, Sallan SE: Low-Dose Versus High-Dose Methotrexate During Remission Induction in Childhood Acute Lymphoblastic Leukemia (Protocol 81-01 Update). Blood 78:2514, 1991

36. Rivera GK, Raimondi SC, Hancock ML, Behm FG, Pui CH, Abromowitch M, Mirro J Jr, Ochs JS, Look AT, Williams DL, Murphy SB, Dahl GV, Kalwinsky DK, Evans WE, Kun LE, Simone JV, Crist WM: Improved outcome in childhood acute lymphoblastic leukaemia with reinforced early treatment and rotational combination chemotherapy. Lancet 337:61, 1991

37. Chessells JM, Bailey CC, Richards S: MRC UKALL X. The UK protocol for childhood ALL: 1985-1990. Leukemia 6:157, 1992 (suppl 2)

38. Tubergen DG, Gilchrist GS, O'Brien RTO, Coccia PF, Sather HN, Waskerwitz MJ, Hammond GD: Improved outcome with delayed intensification for children with acute lymphoblastic leukemia and intermediate presenting features: A Childrens Cancer Group phase III trial. J Clin Oncol 11:527, 1993

39. Lipshultz SE, Colan SD, Gelber RD, Perez-Atayde AR, Sallan SE, Sanders SP: Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukemia in childhood. N Engl J Med 324:808, 1991

40. Crist W, Carroll A, Shuster J, Jackson J, Head D, Borowitz M, Behm FG, Link M, Steuber P, Ragab A, Hirt A, Brock B, Land V, Pullen J: Philadelphia Chromosome Positive Childhood Acute Lymphoblastic Leukemia: Clinical and Cytogenetic Characteristics and Treatment Outcome. A Pediatric Oncology Group Study. Blood 76:489, 1990

41. Crist W, Shuster J, Look T, Borowitz M, Behm F, Bowman P, Frankel L, Pullen J, Krance R, Steuber P, Camitta B, Amylon M, Link M, Land V: Current results of studies of immunophenotype-, age- and leukocyte-based therapy for children with acute lymphoblastic leukemia. Leukemia 6:162, 1992 (suppl 2)

42. Veerman AJP, Hählen K, Kamps WA, Vanleeuwen EF, de Vaan GAM, Vanwering ER, van der Does-van den Berg A, Solbu G, Suciu S: Dutch Childhood Leukemia Study Group: Early Results of Study ALL VI (1984-1988). Haematol Blood Transfus 33:473, 1990

43. Whitehead VM, Vuchich MJ, Lauer SJ, Mahoney D, Carroll AJ, Shuster JJ, Esseltine DW, Payment C, Look AT, Akabutu J, Bowen T, Taylor LD, Camitta B, Pullen DJ: Accumulation of high levels of methotrexate polyglutamates in lymphoblasts from children with hyperdiploid (greater than 50 chromosomes) B lineage acute lymphoblastic leukemia: A Pediatric Oncology Group Study. Blood 80:1316, 1992

44. Harris MB, Shuster JJ, Carroll A, Look AT, Borowitz MJ, Crist WM, Nitschke R, Pullen J, Steuber CP, Land VJ: Trisomy of Leukemic Cell Chromosomes 4 and 10 Identifies Children With B-Progenitor Cell Acute Lymphoblastic Leukemia With a Very Low Risk of Treatment Failure: A Pediatric Oncology Group Study. Blood 79:3316, 1992

45. Arthur DC, Bloomfield CD, Lindquist LL, Nesbit ME: Translocation 4;11 in acute lymphoblastic leukaemia: Clinical characteristics and prognostic significance. Blood 59:96, 1982