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ORIGINAL REPORT

Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group

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ABSTRACT

Purpose

Outcome of childhood acute lymphoblastic leukemia (ALL) improved greatly by intensifying chemotherapy for all patients. Minimal residual disease (MRD) levels during the first months predict outcome and may select patients for therapy reduction or intensification.

Methods

Patients 1 to 18 years old with ALL were stratified on the basis of MRD levels after the first and second course of chemotherapy. Thereafter, therapy was substantially reduced in patients with undetectable MRD (standard risk) and intensified in patients with intermediate (medium risk) and high (high risk) levels of MRD. Seven hundred seventy-eight consecutive patients were enrolled. The method of analysis was intention-to-treat. Outcome was compared with historical controls.

Results

In MRD-based standard-risk patients, the 5-year event-free survival (EFS) rate was 93% (SE 2%), the 5-year survival rate was 99% (SE 1%), and the 5-year cumulative incidence of relapse rate was 6% (SE 2%). The safety upper limit of number of observation years was reached and therapy reduction was declared safe.

MRD-based medium-risk patients had a significantly higher 5-year EFS rate (88%, SE 2%) with therapy intensification (including 30 weeks of asparaginase exposure and dexamethasone/vincristine pulses) compared with historical controls (76%, SE 6%). Intensive chemotherapy and stem cell transplantation in MRD-based high-risk patients resulted in a significantly better 5-year EFS rate (78%, SE 8% *v* 16%, SE 8% in controls). Overall outcome improved significantly (5-year EFS rate 87%, 5-year survival rate 92%, and 5-year cumulative incidence of relapse rate 8%) compared with preceding Dutch Childhood Oncology Group protocols.

Conclusion

Chemotherapy was substantially reduced safely in one-quarter of children with ALL who were selected on the basis of undetectable MRD levels, without jeopardizing the survival rate. Outcomes of patients with intermediate and high levels of MRD improved with therapy intensification.

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INTRODUCTION

In the last five decades, survival rates for children with acute lymphoblastic leukemia (ALL) have improved by intensifying chemotherapy for all patients.[1] By the 1970s, one-third of patients were being cured with moderately intensive therapies, implying that treatment could be reduced for some patients. Assessment of early therapy response by measuring minimal residual disease (MRD) is the strongest predictor of survival,[2-6] first shown in the late 1990s by different groups, including a study by Associazione Italiana Ematologia ed Oncologia Pediatrica (AIEOP)–Berlin-Frankfurt-Münster (BFM) and Dutch Childhood Oncology Group (DCOG).[4] Their BFM-based protocols, including the DCOG ALL8-study,[7] identified three risk groups by MRD levels after courses IA and IB: standard risk (SR), medium risk (MR), and high

risk (HR), with 5-year event-free survival (EFS) rates of 98% (SE 2%), 76% (SE 6%), and 16% (SE 8%), respectively.[2,8] The DCOG ALL10 protocol, which aimed to improve overall outcome, reduced therapy in patients with the lowest MRD levels without jeopardizing outcome, and improved outcome in patients with intermediate and high MRD levels by intensifying therapy, is presented here.

METHODS

Patients

Between November 2004 and April 2012, 778 consecutive children (1 to 18 years old) with newly diagnosed ALL were treated with the ALL10

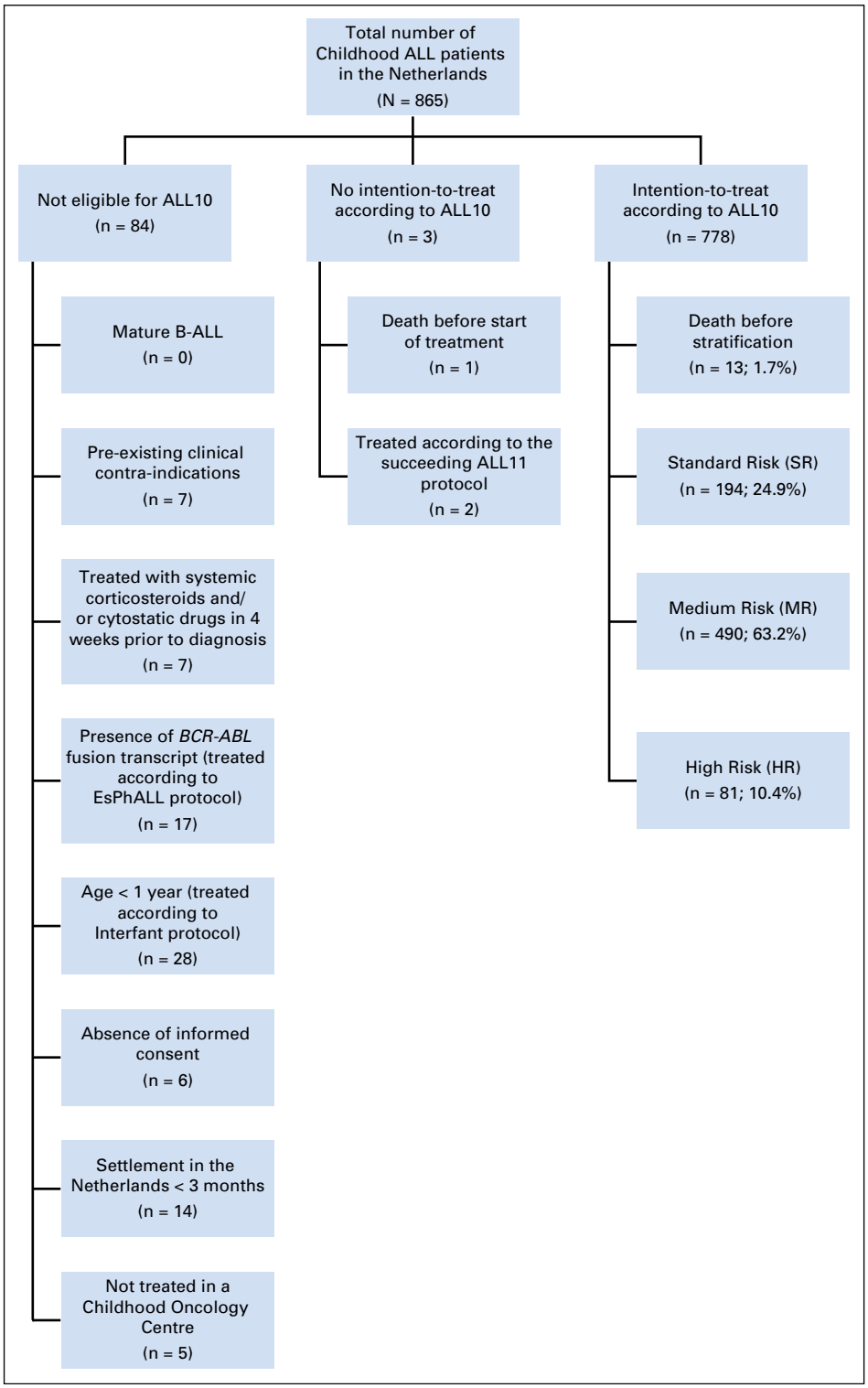

Fig 1. Overview of patients with acute lymphoblastic leukemia (ALL) in the Netherlands and treatment according to ALL10.

protocol (Fig 1). Infants younger than 1 year (Interfant protocol)[9] and *BCR-ABL*–positive ALL cases (EsPhALL protocol) were excluded.[10] The protocol was approved by institutional review boards. Informed consent was signed by parents and patients according to Dutch law.

Therapy

Initial therapy with courses IA, IB, and M, and MRD-based stratification, were identical to the historical DCOG ALL8 control group.[2,7,8] MRD levels were measured after IA (time point [TP] 1) and IB (TP2). Subsequent intensification therapy in ALL10 was substantially reduced in patients with SR, intensified in patients with MR, and strongly intensified in patients with HR (Appendix Fig 1, online only).

SR. Protocol IV was substantially reduced by deleting 14 days 6-thioguanine 60 mg/m^2/day, one dose cyclophosphamide 1,000 mg/m^2, 8 days cytarabine 75 mg/m^2/dose, two intrathecal doses methotrexate (MTX), and four doses doxorubicin (30 mg/m^2/dose). Vincristine (VCR) (1.5 mg/m^2/dose) was reduced from four to two doses and dexamethasone (10 mg/m^2/day) from 3 to 2 weeks. This resulted in a mild intensification course IV, with only 2 weeks dexamethasone, two doses VCR, and one dose PEG-asparaginase. This is a considerable decrease in intensity compared with, for instance, the contemporary intensification course of AIEOP-BFM 2000, which uses the same stratification[4] (Appendix Table A1, online only). Maintenance therapy consisted of only oral mercaptopurine/MTX.

MR. MR intensification and maintenance were based on the Dana-Farber Cancer Institute protocol,[11] including intensive PEG-asparaginase (15 doses) and dexamethasone/VCR pulses during maintenance.

HR. HR patients received six HR courses (HR1-HR6) according to the Australian and New Zealand Children's Haematology/Oncology Group protocol,[12] followed by course II and maintenance or three HR courses plus allogeneic stem cell transplantation (alloSCT; Appendix Fig A1, online only).

CNS-directed therapy. SR and MR patients received a total of nine and 14 intrathecal triple-therapy administrations, respectively. HR patients received nine to 12 and, if not transplanted, 12 to 15 intrathecal therapies. Patients with CNS2 (nontraumatic puncture, $\leq$ 5 WBC/μL CSF with identifiable leukemic cells), CNS3 (nontraumatic puncture, $>$ 5 WBC/μL CSF with identifiable leukemic cells), or TLP+ (traumatic lumbar puncture with leukemic cells) received two additional intrathecal therapies in protocol I, and HR patients received another two intrathecal therapies in protocol II. HR patients older than 3 years who were not receiving stem cell transplantation (SCT) received 12-Gy cranial irradiation.

MRD Stratification

Polymerase chain reaction (PCR)–detected MRD was evaluated according to EuroMRD guidelines.[13,14]

Patients were stratified to SR if they met all of the following criteria:

- cytomorphologic complete remission (CR) day 33
- MRD-negativity at TP1 and TP2 (at least one MRD-PCR target with a quantitative range of 10^{-4} and one MRD-PCR target with a quantitative range of 5×10^{-4} and sensitivity of 10^{-4})
- no *MLL-AF4* rearrangement
- no prednisone-poor response
- no CNS/testicular involvement (CNS status; Table 1)

Patients were stratified to HR if they met at least one of the following criteria:

- TP1 MRD level of $\geq 5 \times 10^{-4}$ or unknown and TP2 MRD level of $\geq 5 \times 10^{-4}$
- *MLL-AF4* rearrangements
- poor prednisone response
- no CR day 33

All others and patients with inconclusive/missing MRD data were stratified to MR. Children with Down syndrome (DS) were not eligible for HR therapy.

Statistical Analysis

Because therapy was reduced in SR, a noninferiority design was used to ensure no worse outcome compared with historical controls. In MR/HR, we intensified therapy and superiority questions were asked.

In SR, there was a 20% probability that ALL10 is incorrectly declared unsafe at a safe event rate of 2% per year and a 5% probability that ALL10 is incorrectly declared safe at an unsafe event rate of 3% per year. For every second event, the cumulative observation time (T) of all SR patients is compared with the predefined upper and lower safety limits, based on the sequential probability ratio test for exponential distribution (Appendix Table A2, online only).[15]

Events were induction failure (defined as event at day 0), relapse, death, or secondary malignancy. Analyses were by intention-to-treat. EFS was computed from diagnosis to first event or last follow-up; overall survival (OS) was computed from diagnosis to death or last follow-up. The Kaplan-Meier methodology was used to estimate EFS/OS, Cox models to assess effects of covariates on EFS/OS, and the local test to compare EFS between ALL10 and controls.

The cumulative incidence of relapse (CIR) from diagnosis to relapse was estimated by a competing-risk model.[16] CIR curves were compared by Gray's log-rank test.[17] Regression on CIR function was performed by Fine and Gray's model.[18] Landmark analysis was performed at day 79 (MRD TP2), including only patients who survived up to day 79. The median follow-up time was assessed using the reverse Kaplan-Meier method.[19] Statistical analyses were performed in SPSS-Rel. 20.0.2012 (SPSS, Chicago, IL) and competing-risks models by the mstate library in R-15.[20-22]

RESULTS

Patient Characteristics

Table 1 presents an overview of patient characteristics, outcome data, and the univariate survival analysis. ALL10 enrolled more adolescents 15 to 18 years of age (7.3%) than previous protocols (1.4%, 4.1%, and 3.6% on ALL7, ALL8, and ALL9, respectively) as a result of better outcomes of adolescents on pediatric versus adult ALL protocols.[23] For unknown reasons, the number of patients with DS (5.1%) was higher when compared with 1.8%, 1.9%, and 2.8% in ALL7, ALL8, and ALL9, respectively.

Stratification

In 92 of 778 cases (11.8%), MRD classification was not feasible because of a lack of material and early events (6.9%) or an absence of (sensitive) MRD targets (4.9%). Among 686 MRD-stratified cases, 198 were SR, 460 MR, and 28 HR (Fig 2). Including all 778 patients and all stratification criteria, the final stratification was 194 SR (24.9%), 490 MR (63.0%), 81 HR (10.4%), and 13 deaths (1.7%) that occurred before stratification.

Outcomes

Table 2 presents an overview of events and 5-year outcome data in ALL10. The median follow-up was 80 months (range 36 to 125 months). Thirteen patients (1.7%, including seven with DS) died during induction, two (0.3%) did not achieve CR after HR1, and 763 (98.0%) achieved CR. Induction deaths were as a result of infection in eight patients, intracerebral bleeding/infarction in two, multiorgan failure in one, and unknown causes in two. Twenty patients (2.6%) died during CR: 13 as a result of infection (including four patients with DS), one pancreatitis, two sudden death,

Table 1. Overview of Patient Characteristics, Outcome Data, and Univariate Survival Analysis

Variable	No. of Patients	EFS 5-Year, % (SE)	EFS Estimated Hazard Ratio (Univariate Cox CI)	EFS *P*	OS 5-year, % (SE)	OS Estimated Hazard Ratio (Univariate Cox CI)	OS *P*	CIR 5-Year, % (SE)	CIR Estimated Hazard Ratio (Univariate Fine and Gray CI)	CIR *P*
Sex										
Male	420	85.3 (1.7)	1.00 (Ref)		91.0 (1.4)	1.00 (Ref)		9.6 (1.5)	1.00 (Ref)	
Female	358	89.2 (1.7)	0.76 (0.52 to 1.12)	.16	92.8 (1.4)	0.84 (0.52 to 1.34)	.46	6.7 (1.4)	0.74 (0.46 to 1.19)	.22
Age, years										
1-4	361	91.5 (1.5)	1.00 (Ref)		94.7 (1.2)	1.00 (Ref)		4.2 (1.1)	1.00 (Ref)	
5-9	230	86.1 (2.3)	1.77 (1.10 to 2.85)	.018	91.8 (1.9)	1.54 (0.84 to 2.83)	.16	10.9 (2.1)	2.90 (1.58 to 5.31)	< .001
10-14	130	80.1 (3.6)	2.73 (1.65 to 4.51)	< .001	88.4 (2.8)	2.49 (1.32 to 4.66)	.005	13.4 (3.1)	3.44 (1.76 to 6.73)	< .001
15-18	57	78.9 (5.4)	2.71 (1.39 to 5.26)	.003	82.2 (5.1)	3.28 (1.55 to 6.97)	.002	12.7 (4.5)	3.21 (1.31 to 7.88)	.011
WBC count										
< 25	554	87.1 (1.5)	1.00 (Ref)		92.6 (1.1)	1.00 (Ref)		8.1 (1.2)	1.00 (Ref)	
25-50	80	93.7 (2.7)	0.44 (0.18 to 1.09)	.77	95.0 (2.4)	0.60 (0.22 to 1.67)	.33	1.3 (1.3)	0.14 (0.02 to 1.02)	.053
> 50	142	82.9 (3.2)	1.35 (0.86 to 2.10)	.19	87.2 (2.8)	1.60 (0.94 to 2.73)	.084	12.9 (2.8)	1.66 (0.98 to 2.81)	.059
Genetics										
ETV6-RUNX1	168	95.1 (1.7)	1.00 (Ref)		98.7 (0.9)	1.00 (Ref)		4.9 (1.7)	1.00 (Ref)	
DS	40	66.1 (7.8)	8.60 (3.86 to 19.2)	< .001	65.2 (8.0)	36.8 (8.31 to 163.3)	< .001	6.3 (4.3)	2.17 (0.70 to 6.75)	.18
E2A-PBX1	19	100.0*	0.00*		100.0*	0.00*		0.0*	0.00*	
HD > 50	182	87.9 (2.5)	2.28 (1.08 to 4.79)	.030	93.3 (1.9)	7.84 (1.80 to 34.1)	.006	8.9 (2.2)	1.57 (0.72 to 3.45)	.26
Other T-lineage	110	80.7 (3.8)	3.93 (1.87 to 8.26)	< .001	85.4 (3.4)	14.0 (3.24 to 60.8)	< .001	8.5 (2.7)	1.80 (0.76 to 4.25)	.18
Other B-lineage	255	86.2 (2.2)	2.62 (1.30 to 5.27)	.007	92.8 (1.6)	7.33 (1.72 to 31.2)	.007	10.8 (2.0)	1.97 (0.96 to 4.04)	.06
DNA index										
< 1.16	544	86.6 (1.5)	1.00 (Ref)		91.6 (1.2)	1.00 (Ref)		8.7 (1.2)	1.00 (Ref)	
≥ 1.16	169	89.9 (2.4)	0.78 (0.47 to 1.29)	.33	95.2 (1.7)	0.65 (0.33 to 1.27)	.21	7.2 (2.1)	0.80 (0.44 to 1.46)	.47
Phenotype										
B-lineage	661	88.4 (1.3)	1.00 (Ref)		93.3 (1.0)	1.00 (Ref)		8.1 (1.1)	1.00 (Ref)	
T-lineage	116	80.0 (3.7)	1.83 (1.17 to 2.87)	.008	84.4 (3.4)	2.25 (1.33 to 3.82)	.003	9.0 (2.7)	1.22 (0.65 to 2.03)	.52
CNS status†										
CNS1	330	89.3 (1.7)	1.00 (Ref)		92.5 (1.5)	1.00 (Ref)		7.4 (1.5)	1.00 (Ref)	
CNS2	328	87.8 (1.8)	1.19 (0.77 to 1.83)	.44	92.9 (1.4)	1.14 (0.66 to 1.95)	.64	8.4 (1.6)	1.19 (0.70 to 2.00)	.53
CNS3	8	50.0 (17.7)	6.91 (2.46 to 19.4)	< .001	62.5 (17.1)	6.60 (1.99 to 21.9)	.002	14.3 (13.2)	2.10 (0.25 to 17.9)	.50
TLP+	80	81.0 (4.4)	1.96 (1.11 to 3.47)	.021	90.0 (3.4)	1.68 (0.81 to 3.50)	.16	10.4 (3.5)	1.64 (0.80 to 3.38)	.18
TLP−	20	75.0 (9.7)	2.47 (0.97 to 6.28)	.057	85.0 (8.0)	2.13 (0.64 to 7.06)	.22	15.8 (8.4)	2.19 (0.64 to 7.50)	.21
Prednisone response										
Good	715	88.1 (1.2)	1.00 (Ref)		93.0 (1.0)	1.00 (Ref)		7.9 (1.0)	1.00 (Ref)	
Poor	59	72.9 (5.8)	2.54 (1.52 to 4.27)	< .001	78.0 (5.4)	3.28 (1.82 to 5.88)	< .001	13.6 (4.5)	1.89 (0.92 to 3.88)	.086
MRD‡										
SR	198	93.2 (1.8)	1.00 (Ref)		99.0 (0.7)	1.00 (Ref)		6.2 (1.8)	1.00 (Ref)	
MR	460	87.7 (1.6)	1.57 (0.92 to 2.70)	.099	92.3 (1.3)	4.29 (1.53-12.0)	.006	8.5 (1.4)	1.24 (0.69 to 2.23)	.18
HR	28	78.4 (7.8)	3.38 (1.40 to 8.14)	.007	82.1 (7.2)	12.3 (3.46-43.4)	< .001	14.4 (6.7)	2.56 (0.91 to 7.20)	.19

Abbreviations: CIR, cumulative incidence of relapse; CSF, cerebrospinal fluid; DS, Down syndrome; EFS, event-free survival; HD, hyperdiploid; HR, high risk; MR, medium risk; MRD, minimal residual disease; OS, overall survival; Ref, reference; SR, standard risk; TLP, traumatic lumbar puncture.

*No events in these subgroups.

†CNS1: nontraumatic puncture, ≤ 5 WBC/μL CSF without leukemic cells after cytocentrifugation; CNS2: nontraumatic puncture, ≤ 5 WBC/μL CSF with identifiable leukemic cells; CNS3: nontraumatic puncture, > 5 WBC/μL CSF with identifiable leukemic cells; TLP+: traumatic lumbar puncture with leukemic cells; TLP−: traumatic lumbar puncture without leukemic cells.

‡Landmark analysis; only patients with known MRD stratification at day 79.

and four SCT related. Five patients (0.6%) had a second malignancy (acute myeloid leukemia, osteosarcoma, leptomeningeal gliomatosis, melanoma, and histiocytic sarcoma); 69 patients (8.9%) relapsed.

The 5-year EFS and OS rates were 87.0% (SE 1.2%) and 91.9% (SE 1.0%), respectively. The 5-year CIR of all relapses was 8.3% (SE 1.0%), of which isolated CNS relapse was 1.4% (SE 0.4%).

Figure 3 displays overall EFS, OS, and CIR for ALL10 intention-to-treat patients.

Down Syndrome (DS)

Appendix Table A3 (online only) presents outcome data of DS versus non–DS patients. Because seven of 40 patients with DS (18%) died during induction, only 33 were stratified: 7 SR and 26 MR (among whom two MRD-HR were assigned to MR according to protocol). Four of 40 patients died during remission (10%), four relapsed (10%), and 25 are in CCR (63%). The 5-year EFS and OS rates for DS-ALL cases were 66.1% (SE 7.8%) and 65.2% (SE 8.0%), respectively, versus 88.1% (SE 1.2%) and 93.3% (SE 0.9%) for non–DS-ALL cases. The protocol was amended for DS by deleting anthracyclines during induction. Numbers were too low to analyze the effect.

Outcomes by Patient Characteristics

Table 1 and Appendix Tables A4 and A5 (online only) present outcomes by patient characteristics. Outcomes were not

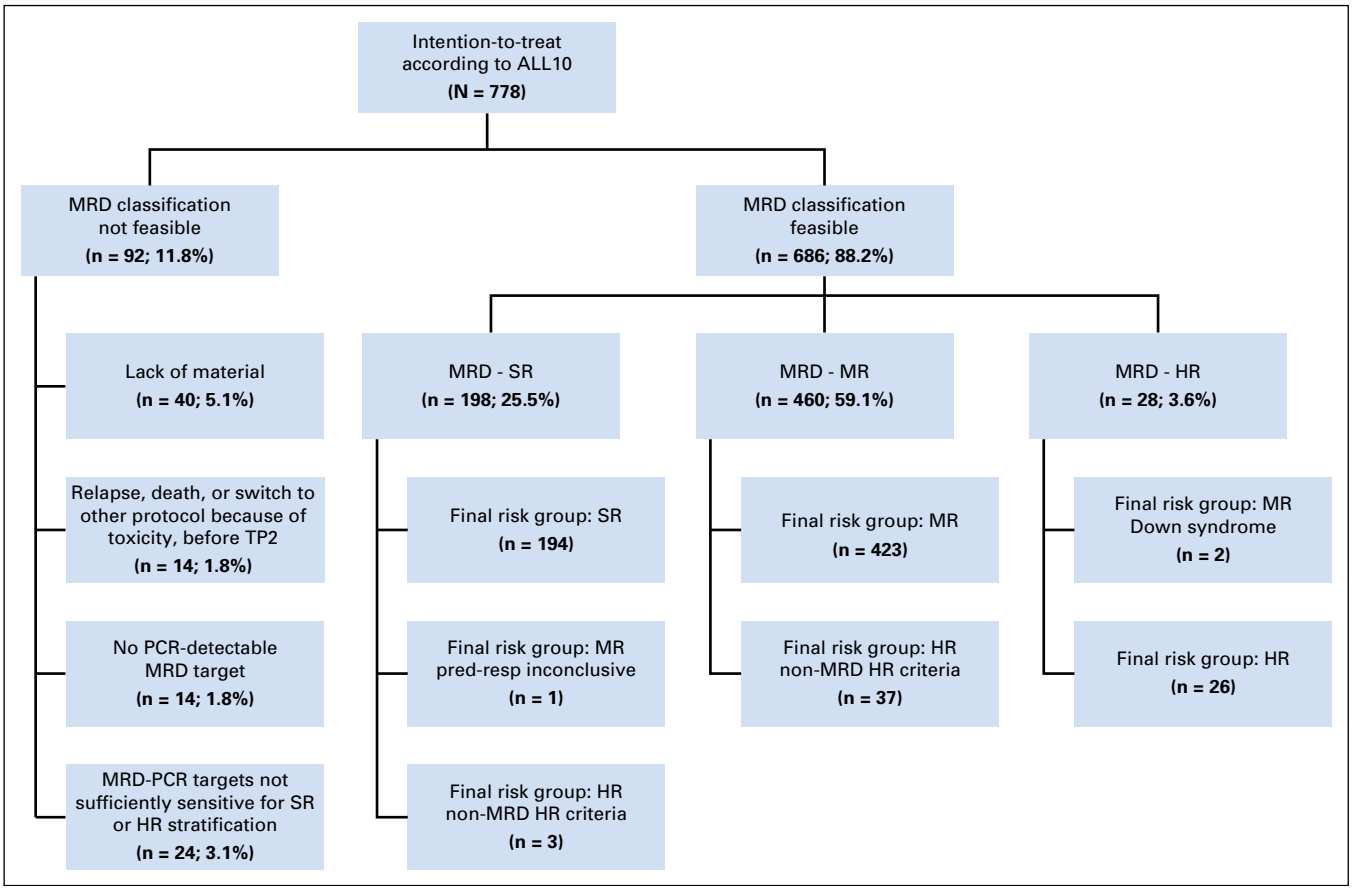

Fig 2. MRD feasibility and MRD-based classification. HR, high risk; MR, medium risk; MRD, minimal residual disease; PCR, polymerase chain reaction; SR, standard risk, TP2, time point 2.

significantly different between boys and girls (EFS 85% *v* 89% and OS 91% *v* 93%, respectively). Children 1 to 4 years of age had a significantly lower 5-year CIR rate of 4.2% ($P < .02$). The percentage of adolescents (15 to 18 years old) in the SR, MR, and HR groups was 3%, 8%, and 15%, respectively. Two of 57 adolescents died before stratification (3.5%), six were SR (10.5%), 37 MR (65%), and 12 HR (21%). Seven adolescents (12%) received alloSCT.

Children with CNS3 or TLP+ had significantly lower EFS than patients with CNS1 (nontraumatic puncture, ≤ 5 WBC/μL CSF without leukemic cells after cytocentrifugation) status ($P < .001$ and $P = .021$, respectively).

T-lineage ALL had a comparable 5-year CIR rate compared with B-lineage ALL (9.0% and 8.1%, respectively) but lower EFS and OS because of more toxic deaths (seven of 116 *v* 13 of 661) and more second malignancies (three of 116 *v* two of 661). Six of seven toxic deaths in T-lineage ALL occurred in the HR group: four after alloSCT and two after HR chemotherapy.

Relapse rates were low in *E2A-PBX1* and *ETV6-RUNX1* ALL, and 5-year OS rates were 100% and 98.7%, respectively. Outcomes in hyperdiploid and other B-lineage ALL cases were slightly worse than in these highly favorable genetic subtypes ($P = .030$ and $P = .007$, respectively). The 5-year EFS and OS rates (90.1% and 93.7%, respectively) of trisomies 4, 10, and 17 were not significantly better than those of other trisomies (82.6% and 91.3%; $P = .19$ and $P = .36$, respectively). Outcomes of trisomies 17 and 18 were not different than those of other trisomies.

Outcome by Risk Group

Figure 4 and Tables 2 and A5 present outcome by risk group. One of 194 SR patients died as a result of chickenpox, one died after a secondary malignancy, and two after relapse. The 5-year EFS rate was 93.1% (SE 1.9%), the 5-year OS rate 99.0% (SE 0.7%), and the 5-year CIR rate 6.4% (SE 1.8%). Fifteen of 194 SR patients relapsed; 12 of these relapses occurred late (32 to 80 months after CR). At the sixth event, 156 SR patients had a cumulative observation time of 478 years (reaching the safe upper limit), and therapy reduction for SR patients was declared safe. Also, at the last update in 2015, the safe upper limit had been reached (17 events in 194 SR patients with a cumulative observation time of 1,305 years). The 5-year EFS rate of MRD-based SR patients (93%, SE 2%, n = 198) was not significantly lower than that of the historical controls (98%, SE 2%, n = 55; $P = .08$).

For MR patients (n = 490), the 5-year EFS, OS, and CIR rates were 88.9% (SE 1.5%), 93.2% (SE 1.2%), and 8.4% (SE 1.3%), respectively. The MRD-based MR group had a higher 5-year EFS rate (88%, SE 2%, n = 460) than historical controls (76%, SE 6%, n = 55; $P = .056$). MR patients with undetectable levels of MRD at TP2 (n = 301) had a higher 5-year EFS rate of 90.9% (SE 1.7%) than those with detectable levels of MRD at TP2 (EFS 84.1%, SE 3.0%; $P = .035$).

Table 2. Overview of Events and 5-Year Outcome Data in ALL10

Variable	Risk Group Based on Intention-to-Treat				
	SR	MR	HR	Death Before Stratification	Total No. (%)
Intention-to-treat, No. (%)	194 (100%)	490 (100%)	81 (100%)	13	778 (100.0)
Nonresponder, No.	0	0	2 (1*)	0	2 (0.3)
Death during induction, No.	0	0	0	13†	13 (1.7)
CR achieved after induction, No. (%)	194 (100)	490 (100)	79 (98.8)	0	763 (97.9)
Death during CR, No.	1	12	7	0	20 (2.6)
Relapse, No.	15	44	10	0	69 (8.9)
BM alone	11 (1*)	30 (12*)	5 (4*)		46 (5.9)
BM + CNS	0	5 (3*)	2 (2*)		7 (0.9)
BM + other	1 (1*)	0	1 (1*)		2 (0.3)
CNS alone	2	8 (8*)	1		11 (1.4)
Testis alone	1	1	0		2 (0.3)
Other alone	0	0	1		1 (0.1)
Second malignancy	1 (1*)	2 (2*)	2 (1*)	0	5 (0.6)
Alive during CCR, No. (%)	177 (91.2)	432 (88.2)	60 (74.1)	0	669 (86.0)
Survival, No.	194	490	81		778
Cumulative 5-year EFS, % (SE)	93.1 (1.9)	88.9 (1.5)	75.3 (4.8)		87.0 (1.2)
Cumulative 5-year OS, % (SE)	99.0 (0.7)	93.2 (1.2)	81.5 (4.3)		91.9 (1.0)
5-year CIR all relapses, % (SE)	6.4 (1.8)	8.4 (1.3)	12.3 (3.7)		8.3 (1.0)
5-year CIR isolated CNS relapse, % (SE)	1.0 (0.7)	1.6 (0.6)	1.2 (1.2)		1.4 (0.4)
5-year CIR any CNS relapse, % (SE)	1.0 (0.7)	2.5 (0.7)	3.7 (2.1)		2.3 (0.5)

Abbreviations: BM, bone marrow; CCR, continuous complete remission; CR, complete remission; CIR, cumulative incidence of relapse; EFS, event-free survival; HR, high risk; MR, medium risk; OS, overall survival; SR, standard risk.
NOTE: Median follow-up time (assessed by reverse Kaplan-Meier method): 80 months (SE 1.6).
*Death.
†Five of these 13 patients did reach CR during induction but died because of induction-related toxicity.

For HR patients (n = 81), the 5-year EFS, OS, and CIR rates were 75.3% (SE 4.8%), 81.5% (SE 4.3%), and 12.3% (SE 3.7%), respectively. The 5-year EFS rate of MRD-based HR patients (78%, SE 8%, n = 28) was higher compared with historical controls (16%, SE 8%, n = 19; $P < .001$). Outcomes of HR patients, either stratified as HR solely by MRD (n = 26) or solely by prednisone response (n = 44), did not differ.

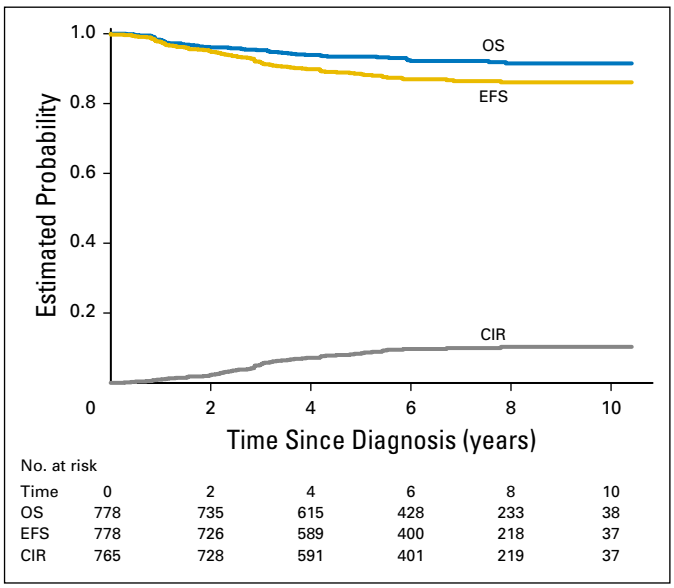

Fig 3. Overall event-free survival (EFS), overall survival (OS), and cumulative incidence of relapse (CIR) for ALL10 intention-to-treat patients (N = 778).

Fifteen of 81 patients (19%) did not reach SCT, 18 (22%) received only chemotherapy per protocol, and 48 (59%) received SCT per protocol. Thus, SCT was performed in 6% of all patients (48 of 778). Outcomes did not differ between alloSCT and chemotherapy only, but the study was not designed to make this comparison. Among 81 HR patients, there were 1 acute undifferentiated leukemia, 40 B-lineage ALL, and 40 T-lineage ALL. In B-lineage ALL, 35% received chemotherapy only, 15% did not reach HR3 per protocol, and 50% underwent transplantation. In T-lineage ALL, these figures were 10%, 20%, and 70% respectively. Five of 778 patients (0.6%) received cranial irradiation.

Outcomes of ALL10 Versus ALL9, ALL8, and ALL7

The overall outcome on ALL10 was compared with DCOG protocols ALL9,[24] ALL8,[7] and ALL7,[25] excluding children older than 15 years, patients with DS, infants, and Philadelphia chromosome–positive ALL because of differences in inclusion. Outcomes improved significantly with each consecutive protocol: the 5-year EFS rates in ALL7, ALL8, ALL9, and ALL10 were 66.1% (SE 3.3%), 75.4% (SE 2.1%), 83.3% (SE 1.3%), and 88.7% (SE 1.2%), respectively ($P < .001$; Table 3).

Toxicity

In SR and MR, infections mainly occurred during IA, IB, and intensification (Table 4). The low intensity of SR intensification phase IV resulted in fewer grade III/IV toxicities, especially infections (4.3%), compared with the MR intensification (45.6%; $P < .001$). HR patients most often had grade III/IV toxicity; approximately two-thirds of patients had infections during HR

Table 3. Outcomes of Consecutive Dutch Childhood Oncology Group Protocols ALL7 (1988 to 1991), ALL8 (1991 to 1997), ALL9 (1997 to 2004), and ALL10 (2004 to 2012)

Outcome	ALL7	ALL8	ALL9	ALL10	P
No.	208	420	767	684	
5-year EFS % (SE)	66.1 (3.3)	75.4 (2.1)	83.3 (1.3)	88.7 (1.2)	ALL10 *v* ALL8: < .001 ALL10 *v* ALL9: = .001
5-year OS % (SE)	80.1 (2.8)	85.4 (1.7)	88.3 (1.2)	93.9 (0.9)	ALL10 *v* ALL8: < .001 ALL10 *v* ALL9: = .002
5-year CIR % (SE)	29.9 (3.2)	22.4 (2.0)	13.1 (1.2)	8.0 (1.1)	ALL10 *v* ALL8: < .001 ALL10 *v* ALL9: = .008

Abbreviations: CIR, cumulative incidence of relapse; EFS, event-free survival; OS, overall survival.

courses. The incidence of osteonecrosis was 1.6% in patients 1 to 4 years of age, 0.7% in patients 5 to 9 years of age, 8% in patients 10 to 14 years of age, and 27% in patients 15 to 18 years of age. Patients who were at least 10 years of age had osteonecrosis more often than younger patients (13.7% *v* 1.4%, respectively; $P < .001$).

DISCUSSION

Our study illustrates that chemotherapy can be substantially reduced without jeopardizing outcome in one-quarter of patients with ALL, ie, those with undetectable PCR-based MRD after the first two chemotherapy courses. SR patients have a 5-year survival rate of 99% and received only nine intrathecal injections, a mild intensification (2 weeks dexamethasone, VCR, and one PEG-asparaginase dose), followed by oral mercaptopurine/MTX maintenance. Relapses in SR usually occur late and three-quarters of these cases can be rescued, because little chemotherapy was administered at first diagnosis.

Others[26,27] have also shown that therapy reduction can be done safely in patients with favorable MRD. These protocols have different accents. The DCOG ALL10-SR protocol contains significantly less dexamethasone (200/200 *v* 1,050/1,600 mg/m^2 for girls/boys), VCR (9 *v* 45 to 64 mg/m^2), and asparaginase (5 weeks of coverage *v* 8 weeks) but more anthracyclines (120 *v* 75 mg/m^2) and cyclophosphamide (2,000 *v* 1,000 mg/m^2) than the UKALL 2003-SR protocol. The first months of SR therapy are more intensive in DCOG ALL10 than in UKALL 2003, whereas the reverse is true for the latter phases.

The Malaysia-Singapore 2003-SR protocol contains slightly fewer anthracyclines (60 mg/m^2) but more dexamethasone (560 mg/m^2) and VCR (30 mg/m^2). Graubner et al[28] showed that reduction of the intensification significantly reduced the infection rate. Even before the use of MRD, good outcomes were achieved with relatively modest therapy, for instance, among National Cancer Institute SR patients.[29] However, all of the ALL subtypes are heterogeneous, and MRD stratification allows patients not fulfilling National Cancer Institute standard criteria (such as teenagers, T-lineage ALL cases, and cases with high cell counts) into the SR group.

The percentage of patients in SR was not different than in the total group for *E2A-PBX1* (3% of SR patients *v* 2% of all cases), hyperdiploid > 50 (23% *v* 23%), and other B-lineage ALL (30% *v* 33%). The percentage of *ETV6-RUNX1* cases was higher in SR (34%) than in the total group (22%) and lower for T-lineage ALL (7% *v* 14%, respectively), reflecting the relative chemosensitivity of *ETV6-RUNX1* ALL and chemoresistance of T-lineage ALL.[30-32] In future studies, further reductions in anthracyclines and cyclophosphamide may be of benefit, especially in *ETV6-RUNX1* ALL.

Outcomes of MR patients improved by using more asparaginase and dexamethasone/VCR pulses. Several studies showed that intensification by asparaginase improved outcome.[33] Others showed that patients with MRD levels $\geq 10^{-2}$ after induction benefitted from postremission intensification with asparaginase, VCR, and MTX.[34] The benefit of dexamethasone/VCR pulses during maintenance remains questionable.[35-37]

Outcomes of HR patients improved significantly with intensive chemotherapy courses and, in most cases, also with alloSCT. We previously showed the efficacy of ANZCHOG-HR courses.[12] Our HR courses seem to be more effective than the AIEOP-HR courses (5-year EFS rate 75% *v* 59% and 5-year OS rate 81% *v* 69%, respectively).[38]

Borowitz et al[39] showed that intensification of therapy in patients with moderately high MRD after induction delayed the occurrence of relapses, whereas our data point to lower relapse rates by therapy intensification guided by MRD in MR and HR patients. Relapses in the HR group occurred earlier than in SR and MR patients (Fig 4C). SR and MR patients showed an identical pattern of relapses; the difference between these two groups is the result of toxic deaths and secondary malignancies.

The current study provided additional interesting findings in biologic subsets of ALL. First, outcomes were not significantly different between boys and girls, although the trend toward inferior EFS/OS for boys might have been statistically significant with larger numbers of patients.

Second, the low relapse rate in T-lineage ALL is probably the result of better stratification of T-lineage ALL cases by MRD, as well as intensive asparaginase treatment in MR and intensive chemotherapy and alloSCT in HR; 53% and 35% of T-lineage ALL patients were in the MR and HR groups, respectively. T-lineage ALL is more drug resistant than B-lineage ALL,[32] which explains the different distributions of T-lineage ALL and B-lineage ALL among MRD risk groups, as has been shown by others.[3,4] The toxic death rate approaches the relapse rate in T-lineage ALL, illustrating that the limit of treatment intensity has been reached. Schrappe et al[4] used identical stratification criteria and showed that 5-year EFS rates were 91%, 81%, and 50% for SR, MR, and HR T-lineage ALL cases, respectively. Our

Table 4. Toxicity Grades III to V by Protocol Phase

	No. of Patients (%)																							
	At Diagnosis	Protocol IA	Protocol IB	Protocol M	Protocol IV	SR Weeks 1-20	SR Weeks 21-40	SR Weeks 41-60	SR Weeks 61-81	MR Weeks 1-19	MR Weeks 20-35	MR Weeks 36-51	MR Weeks 52-67	MR Weeks 68-84	HR1	HR2	HR3	HR4	HR5	HR6	Protocol IIA	Protocol IIB	HR Weeks 1-19	HR Weeks 20-37	SCT
No. of patients evaluable*	778 (100)	766 (100)	737 (100)	674 (100)	188	188	188	189	185	456	447	435	427	421	72	71	64	16	16	11	10	9	9	9	47
Infections	1,72 (22.1)	285 (37.2)	299 (40.6)	102 (15.1)	8 (4.3)	29 (15.4)	22 (11.7)	20 (10.6)	17 (9.2)	208 (45.6)	235 (52.6)	113 (26.0)	102 (23.9)	91 (21.6)	45 (62.5)	51 (71.8)	46 (71.9)	6 (37.5)	15 (93.8)	8 (72.7)	4 (40.0)	3 (33.3)	2 (22.2)	1 (11.1)	41 (87.2)
GI toxicity	41 (5.3)	376 (49.1)	199 (27.0)	132 (19.6)	11 (5.9)	31 (16.5)	50 (26.6)	43 (22.8)	44 (23.8)	167 (36.6)	165 (36.9)	131 (30.1)	79 (18.5)	60 (14.3)	43 (59.7)	38 (53.5)	28 (43.8)	4 (25.0)	6 (37.5)	6 (54.5)	1 (10.0)	3 (33.3)	4 (44.4)	3 (33.3)	30 (63.8)
Central neurotoxicity	2 (0.3)	23 (3.0)	7 (0.9)	2 (0.3)	2 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	12 (2.6)	4 (0.9)	0 (0.0)	1 (0.2)	3 (0.7)	0 (0.0)	2 (2.8)	0 (0.0)	0 (0.0)	1 (6.2)	0 (0.0)	2 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)
Thrombosis	1 (0.1)	28 (3.7)	21 (2.8)	13 (1.9)	2 (1.1)	2 (1.1)	2 (1.1)	1 (0.5)	0 (0.0)	26 (5.7)	20 (4.5)	6 (1.4)	8 (1.9)	9 (2.1)	2 (2.8)	3 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.1)
Osteonecrosis	4 (0.5)	3 (0.4)	2 (0.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	1 (0.5)	5 (1.1)	4 (0.9)	7 (1.6)	7 (1.6)	13 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Glucose	0 (0.0)	53 (6.9)	3 (0.4)	3 (0.4)	6 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	21 (4.6)	27 (6.0)	5 (1.1)	8 (1.9)	4 (1.0)	1 (1.4)	3 (4.2)	0 (0.0)	1 (6.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.3)

Abbreviations: HR, high risk; MR, medium risk; SCT, stem cell transplantation; SR, standard risk.
*Data are not corrected for toxicities that continue from the preceding protocol phase.

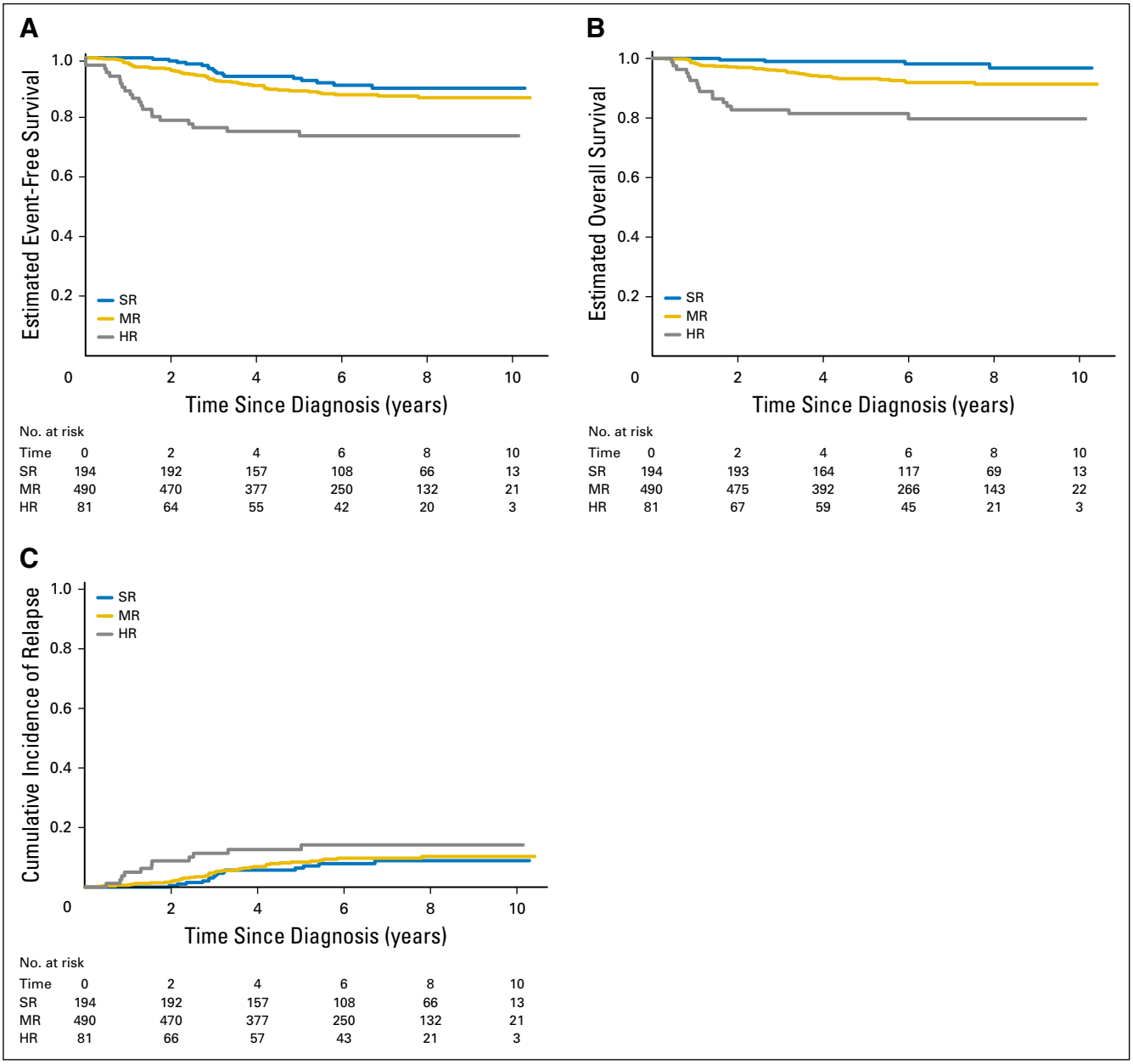

Fig 4. (A) Event-free survival, (B) overall survival, and (C) cumulative incidence of relapse for stratified ALL10 patients by risk group. Standard risk (SR), N = 194; medium risk (MR), N = 490; and high risk (HR), N = 81.

study suggests a slightly better outcome for T-lineage ALL, with 5-year EFS rates of 93%, 88%, and 68% for SR, MR, and HR cases, respectively.

Third, children with *E2A-PBX1*–positive and *ETV6-RUNX1*–positive ALL have excellent 5-year OS rates of 99% to 100%. Hyperdiploid ALL and B-other ALL have 5-year EFS rates of approximately 87% and OS rates of approximately 93%. Hyperdiploid ALL cases are particularly sensitive to the antimetabolites mercaptopurine and MTX;[40,41] prolonged asparaginase treatment in MR patients may have caused myelosuppression, compromising adequate use of antimetabolites. The B-other ALL group includes patients with gene expression profiles that mimic the unfavorable *BCR-ABL*–positive ALL.[42] We and others have shown that the *BCR-ABL*–like group carries a poor outcome and is characterized by abnormalities in B-cell differentiation genes such as *IKZF1*, *CRLF2*, and *JAK*.[42-46]

Fourth, treatment appeared too toxic for children with DS. Therapy reduction should, however, be done cautiously, because DS-ALL patients rarely have genetically favorable subtypes of ALL[47]; one-third of patients carry *IKZF* deletions.[48] The percentage of DS cases was unexpectedly high in our study, with a slightly negative impact on outcome.

Fifth, older patients were more frequently stratified as HR, reflecting the fact that cells from older ALL patients are relatively chemoresistant.[32] Also, older patients experience more adverse effects, especially osteonecrosis.

The major limitation of the current study is its comparison with historical controls, in which the proportion and composition of risk

groups may partially differ. Nevertheless, we conclude that chemotherapy can be substantially reduced without jeopardizing survival in one-quarter of children with ALL who have undetectable MRD levels after induction. Outcomes of patients with intermediate and high MRD levels were improved by more intensive therapies. Overall, outcomes improved significantly compared with those of patients on earlier DCOG protocols.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 From the Dutch Childhood Oncology Group

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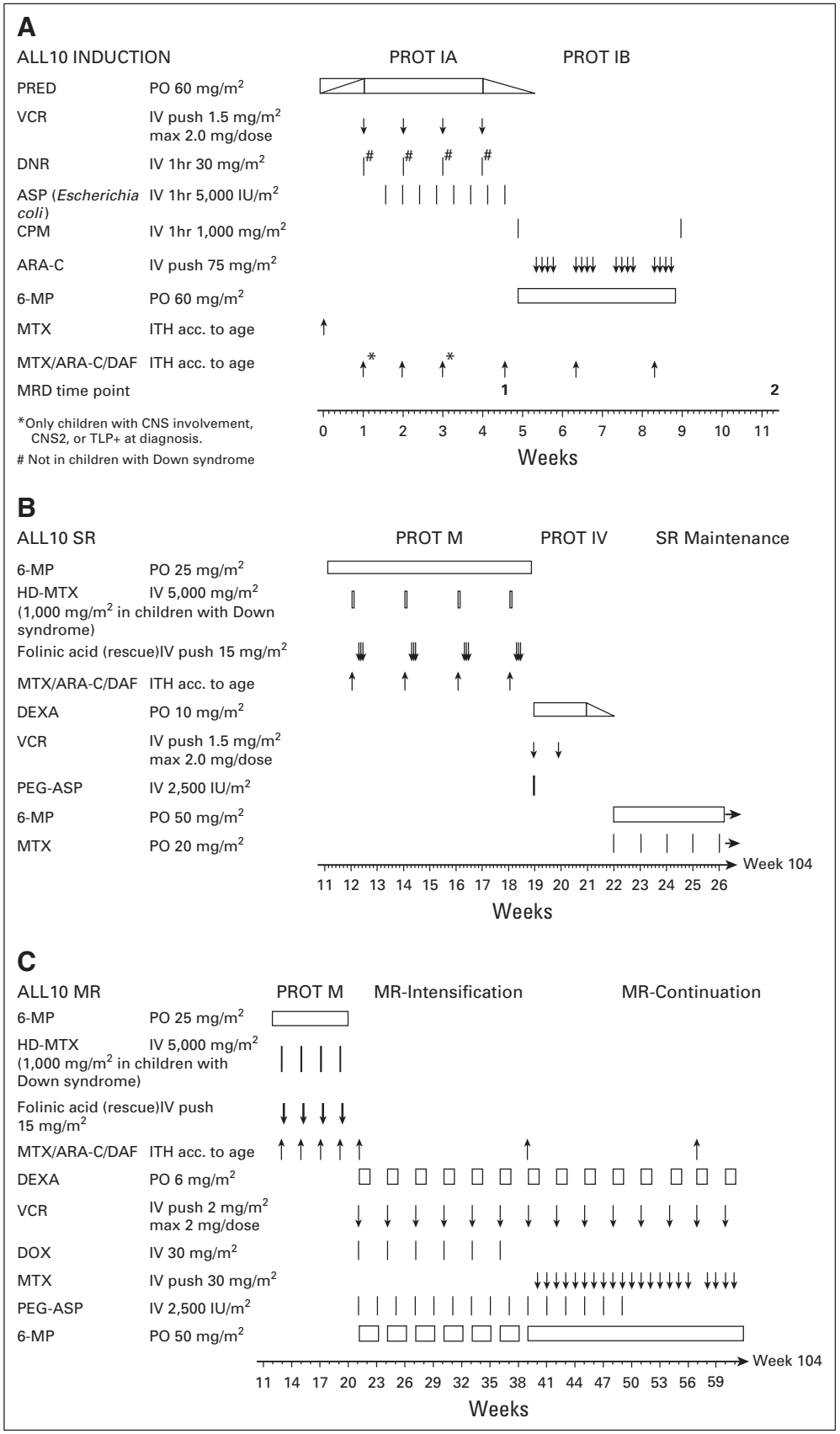
Appendix



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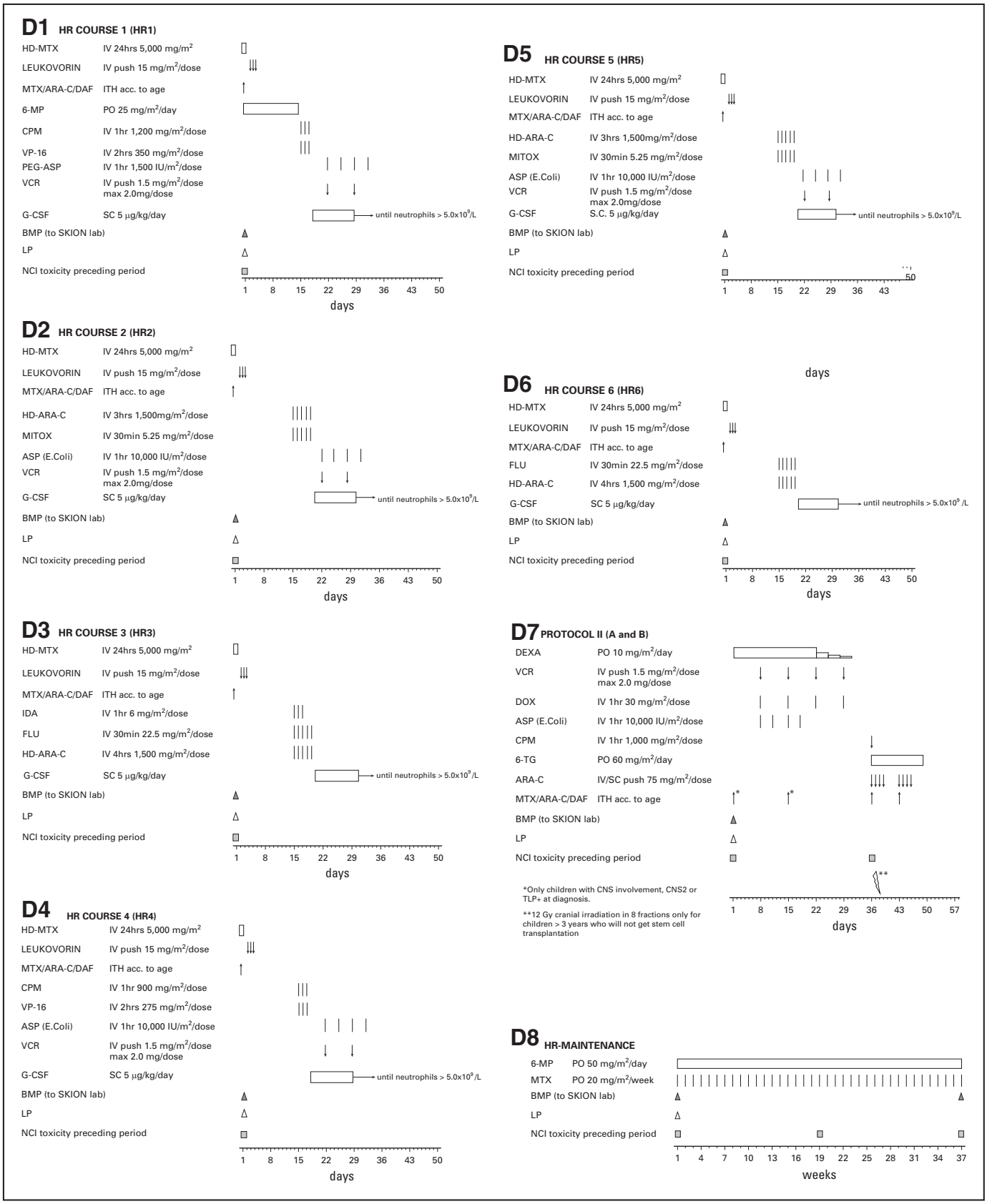
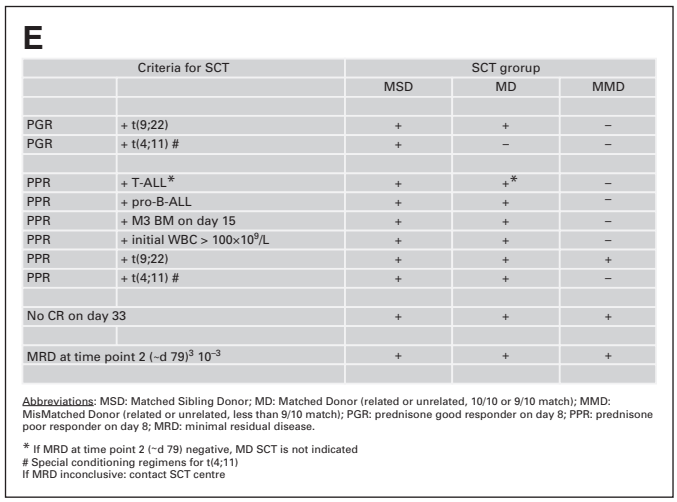


Fig A1. (Continued).



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Criteria for SCT		SCT grorup		
		MSD	MD	MMD
PGR	+ t(9;22)	+	+	–
PGR	+ t(4;11) #	+	–	–
PPR	+ T-ALL*	+	+*	–
PPR	+ pro-B-ALL	+	+	–
PPR	+ M3 BM on day 15	+	+	–
PPR	+ initial WBC > 100×10^9/L	+	+	–
PPR	+ t(9;22)	+	+	+
PPR	+ t(4;11) #	+	+	–
No CR on day 33		+	+	+
MRD at time point 2 (~d 79)[3] 10^{-3}		+	+	+

Abbreviations: MSD: Matched Sibling Donor; MD: Matched Donor (related or unrelated, 10/10 or 9/10 match); MMD: MisMatched Donor (related or unrelated, less than 9/10 match); PGR: prednisone good responder on day 8; PPR: prednisone poor responder on day 8; MRD: minimal residual disease.

* If MRD at time point 2 (~d 79) negative, MD SCT is not indicated
# Special conditioning regimens for t(4;11)
If MRD inconclusive: contact SCT centre

Fig A1. Overview of ALL10 treatment schedule. (A) Induction. (B) SR. (C) MR. (D) HR. After three HR courses, HR patients with one of the following characteristics are eligible for SCT if a donor is available: a) poor prednisone response in combination with T-ALL or Pro-B-ALL or M3 BM at day 15 or WBC $> 100 \times 10^9$/L; b) no complete remission on day 33; c) time point 2 MRD $\geq 10^{-3}$; d) presence of t(4;11) or *MLL-AF4*; or e) presence of t(9;22) or *BCR-ABL* with no informed consent for EsPhALL protocol. HR patients who are not transplanted and who are older than 3 years will receive 12 Gy in eight fractions prophylactic cranial radiotherapy after protocol II. Patients for whom an SCT is planned will receive prophylactic cranial irradiation in conjunction with the conditioning regimen, which includes TBI for children who are at least 2 years old. (E) SCT. *If MRD at time point 2 (approximately day 79) is negative, MD SCT is not indicated. #Special conditioning regimens for t(4;11). If MRD is inconclusive, contact the SCT center. Abbreviations: 6-MP, mercaptopurine; 6-TG, thioguanine; acc., according; ARA-C, cytarabine; ASP, asparaginase; BM, bone marrow; CNS2, nontraumatic puncture, ≤ 5 WBC/μL CSF with identifiable leukemic cells; CPM, cyclophosphamide; CR, complete remission; DAF, prednisolone; DEXA, dexamethasone; DNR, daunorubicin; DOX, doxorubicin; FLU, fludarabine; G-CSF, granulocyte colony-stimulating factor; HD-ARA-C, high-dose cytarabine; HD-MTX, high-dose methotrexate; hr, hour; HR, high risk; IDA, idarubicin; ITH, intrathecally; IV, intravenously; maint., maintenance; max, maximum; MD, matched donor (related or unrelated, 10/10 or 9/10 match); MITOX, mitoxantrone; MMD, mismatched donor (related or unrelated, $<$ 9/10 match); MR, medium risk; MRD, minimal residual disease; MSD, matched sibling donor; MTX, methotrexate; PGR, prednisone good responder on day 8; PO, orally administered; PPR, prednisone poor responder on day 8; PRED, prednisone; PROT, protocol; SC, subcutaneously administered; SCT, stem cell transplantation; SR, standard risk; TBI, total-body irradiation; TLP+, traumatic lumbar puncture with leukemic cells; VCR, vincristine; VP-16, etoposide.

Table A1. Comparison of Cumulative Doses of Chemotherapy in Dutch Childhood Oncology Group ALL10 With a Contemporary Protocol (BFM-2000) Using the Same Stratifying Criteria

Protocol Phase	Drug	DCOG ALL10	BFM-2000	Additional Information
IA		IA	IA	Identical
IB		IB	IB	Identical
		IV	IIA/IIB	
IIA/IV	Vincristine (mg/m^2)	3	6	
IIA/IV	Doxorubicin (mg/m^2)	0	120	
IA/IIA/IV	Dexamethasone (mg/m^2)	140	140	Plus tapering in both protocols
IA/IIA/IV	Native *Escherichia coli* asparaginase (U/m^2)	40.000	80.000	
	PEG-asparaginase (U/m^2)	2.500	0	
IB/IIB/IV	Cyclophosphamide (mg/m^2)	2.000	3.000	
IB/IIB/IV	Cytosine arabinoside (g/m^2)	1.200	1.800	
IB/IV	Mercaptopurine (mg/m^2)	1.680	1.680	
IIB/IV	Thioguanine (mg/m^2)	0	840	
M		M	M	Identical
Maintenance		Maintenance	Maintenance	Identical

Abbreviation: DCOG, Dutch Childhood Oncology Group.

Table A2. Stopping Rules for ALL10 Standard Risk

No. of Events	Lower Limit	Upper Limit
2	—	307
4	29	388
6	110	469
8	191	550
10	272	631
12	353	712
14	434	793
16	515	875
18	596	956
20	677	1,037
22	758	1,118
24	840	1,199
26	921	1,280
28	1,002	1,361
30	1,083	1,442
32	1,164	1,523
34	1,245	1,604
36	1,326	1,685
38	1,407	1,767
40	1,488	1,848
42	1,569	1,929
44	1,650	2,010
46	1,732	2,091
48	1,813	2,172
50	1,894	2,253

NOTE: For each specific number of events, the corresponding upper and lower limits for the cumulative observation time T has been precalculated. If, for a given number of events, the corresponding T is below the lower limit, the protocol is declared unsafe and therefore must be stopped. On the other hand, if for the same number of events T is above the upper limit, the protocol is declared to be safe. The minimum number of events required to declare the protocol unsafe is four and two to declare the protocol safe.

Table A3. Outcome of Down Syndrome Versus Non-Down Syndrome Patients: Overall and by Risk Group

Outcome	DS ALL, % (SE)	Non-DS ALL, % (SE)	Total, % (SE)
Patients, No.	40	738	778
5-year EFS	66.1 (7.8)	88.1 (1.2)	87.0 (1.2)
5-year OS	65.2 (8.0)	93.3 (0.9)	91.9 (1.0)
5-year EFS SR	100	92.8 (1.9)	93.1 (1.9)
5-year OS SR	100	98.9 (0.8)	99.0 (0.7)
5-year EFS MR	74.6 (9.2)	89.6 (1.5)	88.9 (1.5)
5-year OS MR	73.3 (9.6)	94.2 (1.1)	93.2 (1.2)

Abbreviations: DS, Down syndrome; EFS, event-free survival; MR, medium risk; OS, overall survival; SR, standard risk.

Table A4. Multivariate Survival Analysis (Event-Free Survival, Overall Survival, and Cumulative Incidence of Relapse) by Patient Characteristics

		EFS*		OS*		CIR†	
Variable	No. of Patients	Estimated Hazard Ratio (CI)	P	Estimated Hazard Ratio (CI)	P	Estimated Hazard Ratio (CI)	P
Sex							
Male	377	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Female	327	0.82 (0.54 to 1.25)	.36	0.87 (0.50 to 1.49)	.61	0.80 (0.48 to 1.36)	.42
Age, years							
1-4	328	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
5-9	217	1.65 (0.99 to 2.73)	.053	1.45 (0.76 to 2.77)	.26	3.02 (1.57 to 5.83)	< .005
10-14	112	2.04 (1.14 to 3.66)	.016	1.48 (0.68 to 3.24)	.33	3.70 (1.78 to 7.69)	< .005
15-18	47	1.91 (0.88 to 4.13)	.10	1.88 (0.76 to 4.65)	.17	2.44 (0.84 to 7.07)	.10
WBC count							
< 25	487	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
25-50	77	0.23 (0.070 to 0.73)	.013	0.40 (0.12 to 1.33)	.13	0.00‡	
> 50	140	1.09 (0.60 to 2.00)	.77	1.18 (0.55 to 2.56)	.67	2.15 (1.15 to 4.03)	.02
Genetics							
ETV6-RUNX1	159	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
DS	36	10.31 (4.60 to 23.1)	< .001	43.7 (9.80 to 194.9)	< .001	2.84 (0.87 to 9.25)	.08
E2A-PBX1	19	0.00‡		0.00‡		0.00‡	
HD > 50	167	2.72 (1.15 to 6.40)	.022	10.6 (2.24 to 49.9)	.003	2.29 (0.86 to 6.11)	.10
Other T-lineage	102	1.13 (0.22 to 5.98)	.88	3.15 (0.38 to 26.4)	.29	0.66 (0.06 to 7.63)	.74
Other B-lineage	221	1.92 (0.92 to 4.01)	.084	4.34 (0.97 to 19.5)	.056	1.61 (0.75 to 3.43)	.22
DNA index							
< 1.16	536	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
≥ 1.16	168	0.68 (0.36 to 1.30)	.25	0.49 (0.21 to 1.12)	.091	0.61 (0.26 to 1.42)	.25
Phenotype							
B-lineage	596	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
T-lineage	108	1.93 (0.41 to 9.03)	.40	2.16 (0.42 to 11.2)	.36	1.22 (0.12 to 12.33)	.87
Prednisone response							
Good	648	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Poor	56	1.68 (0.85 to 3.29)	.13	2.30 (1.04 to 5.08)	.040	0.80 (0.33 to 1.90)	.61
MRD§							
SR	179	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
MR	422	1.29 (0.72 to 2.30)	.39	2.57 (0.88 to 7.44)	.083	1.14 (0.61 to 2.13)	.68
HR	26	2.07 (0.78 to 5.49)	.14	4.87 (1.24 to 19.2)	.024	2.15 (0.71 to 6.54)	.18

Abbreviations: CIR, cumulative incidence of relapse; DS, Down syndrome; EFS, event-free survival; HD, hyperdiploid; HR, high risk; MR, medium risk; MRD, minimal residual disease; OS, overall survival; Ref, reference; SR, standard risk.
*Multivariate Cox Regression.
†Multivariate Fine and Gray Model.
‡No events in these subgroups.
§Landmark analysis; only patients with known MRD stratification at day 79.

Table A5. Overview of Patient Characteristics by Final Risk Group and Outcome

Variable	No. of Patients	Early Deaths, No. (%)	SR, Final Risk Group, No. (%)	SR, 5-Year EFS, % (SE)	MR, Final Risk Group, No. (%)	MR, 5-Year EFS, % (SE)	HR, Final Risk Group, No. (%)	HR, 5-Year EFS, % (SE)
Sex								
Male	420	6 (46)	95 (49)	89.2 (3.2)	265 (54)	87.7 (2.1)	54 (67)	75.9 (5.8)
Female	358	7 (54)	99 (51)	97.0 (1.7)	225 (46)	90.3 (2.0)	27 (33)	74.1 (8.4)
Age, years								
1-4	361	5 (39)	99 (51)	96.0 (2.0)	236 (48)	92.4 (1.8)	21 (26)	81.0 (8.6)
5-9	230	2 (15)	67 (35)	90.7 (3.6)	130 (27)	87.9 (2.9)	31 (38)	74.2 (7.9)
10-14	130	4 (31)	22 (11)	86.4 (7.3)	87 (18)	83.0 (4.2)	17 (21)	76.5 (10.3)
15-18	57	2 (15)	6 (3)	100.0	37 (8)	83.8 (6.1)	12 (15)	66.7 (13.6)
WBC count								
< 25	554	10 (77)	159 (82)	93.5 (2.0)	356 (73)	87.6 (1.8)	29 (36)	75.9 (7.9)
25-50	80	3 (23)	14 (7)	92.9 (6.9)	55 (11)	98.2 (1.8)	8 (10)	100.0
> 50	142	0 (0)	21 (11)	90.5 (6.4)	78 (16)	88.0 (3.8)	43 (54)	69.8 (7.0)
Genetics								
ETV6-RUNX1	168	0 (0)	65 (34)	92.2 (3.3)	101 (21)	96.8 (1.8)	2 (3)	100.0
DS	40	7 (54)	7 (4)	100.0	26 (5)	74.6 (9.2)	0 (0)	—
E2A-PBX1	19	0 (0)	6 (3)	100.0	12 (2)	100.0	1 (1)	100.0
HD > 50	182	2 (15)	45 (23)	96.9 (3.1)	123 (25)	89.0 (2.9)	12 (15)	58.3 (14.2)
Other T-lineage	110	2 (15)	13 (7)	92.3 (7.4)	59 (12)	87.8 (4.3)	36 (47)	69.4 (7.7)
Other B-lineage	255	2 (15)	58 (30)	89.6 (4.0)	169 (35)	85.7 (2.8)	26 (34)	88.5 (6.3)
DNA index								
< 1.16	544	10 (91)	128 (72)	92.2 (2.4)	335 (75)	89.3 (1.7)	71 (91)	76.1 (5.1)
≥ 1.16	169	1 (9)	49 (28)	95.2 (3.4)	112 (25)	89.6 (3.0)	7 (9)	71.4 (17.1)
Phenotype								
B-lineage	661	11 (85)	180 (93)	93.1 (1.9)	430 (88)	89.0 (1.6)	40 (50)	85.0 (5.6)
T-lineage	116	2 (15)	14 (7)	92.9 (6.9)	60 (12)	88.0 (4.3)	40 (50)	67.5 (7.4)
CNS status*								
CNS1	330	4 (31)	85 (45)	92.6 (2.9)	216 (45)	90.7 (2.0)	25 (31)	80.0 (8.0)
CNS2	328	6 (46)	81 (43)	95.0 (2.4)	198 (41)	89.9 (2.2)	43 (54)	76.7 (6.4)
CNS3	8	1 (8)	0 (0)	—	6 (1)	66.7 (19.2)	1 (1)	100.0
TLP+	80	1 (8)	18 (9)	88.5 (7.6)	51 (11)	83.9 (5.2)	10 (13)	60.0 (15.5)
TLP−	20	1 (8)	6 (3)	83.3 (15.2)	12 (3)	75.0 (12.5)	1 (1)	0

Abbreviations: CSF, cerebrospinal fluid; DS, Down syndrome; EFS, event-free survival; HD, hyperdiploid; HR, high risk; MR, medium risk; SR, standard risk; TLP, traumatic lumbar puncture.

*CNS1: nontraumatic puncture, ≤ 5 WBC/μL CSF without leukemic cells after cytocentrifugation; CNS2: nontraumatic puncture, ≤ 5 WBC/μL CSF with identifiable leukemic cells; CNS3: nontraumatic puncture, > 5 WBC/μL CSF with identifiable leukemic cells; TLP+: traumatic lumbar puncture with leukemic cells; TLP−: traumatic lumbar puncture without leukemic cells.