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# Efficacy and toxicity of high-risk therapy of the Dutch Childhood Oncology Group in childhood acute lymphoblastic leukemia

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## Abstract

**Background:** Children with acute lymphoblastic leukemia (ALL) and high-risk (HR) features have a poor outcome and are treated with HR blocks, often followed by allogeneic stem cell transplantation (SCT).

**Procedure:** This article analyses the outcomes of children treated with HR blocks between 2004 and 2017 according to DCOG ALL10/11 protocols. 1297 patients with newly diagnosed ALL were consecutively enrolled, of which 107 met the HR criteria (no complete remission; minimal residual disease (MRD)  $> 10^{-3}$  after consolidation; “MLL-AF4” translocation and in ALL-10 also poor prednisone response). Patients were treated with one induction and consolidation course followed by three HR chemotherapy blocks, after which they received either SCT or further chemotherapy. MRD levels were measured at end of induction, consolidation, and after each HR block.

**Results:** At five years, the event-free survival was 72.8% (95% CI, 64.6–82.0), and the cumulative incidence of relapse was 13.0% (95% CI, 6.3–19.8). Patients with only negative or low-positive MRD levels during HR blocks had a significantly lower five-year cumulative incidence of relapse (CIR) of 2.2% (95% CI, 0–6.6) compared with patients with one or more high-positive MRD levels (CIR 15.4%; 95% CI, 3.9–26.9). During the entire treatment protocol, 11.2% of patients died due to toxicity.

**Conclusions:** The high survival with HR blocks seems favorable compared with other studies. However, the limit of treatment intensification might have been reached as the number of patients dying from leukemia relapse is about equal as the number of patients dying from toxicity. Patients with negative or low MRD levels during HR blocks have lower relapse rates.

## KEYWORDS

acute lymphoblastic leukemia, chemotherapy, minimal residual disease, pediatric, toxicity

**Abbreviations:** ALL, acute Lymphoblastic Leukemia; CID, cumulative incidence of death; CIR, cumulative incidence of relapse; CR, complete remission; CTCAE, Common Terminology Criteria for Adverse Events; DCOG, Dutch Childhood Oncology Group; EFS, event-free-survival; HR, high risk; MRD, minimal residual disease; OS, overall survival; PCR, polymerase chain reaction; PPR, poor prednisone response; SCT, stem cell transplantation; WBC, white blood count.

## 1 | INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. In recent decades, the prognosis of childhood ALL has improved dramatically with overall survival (OS) now exceeding 80%. However, survival is poor for a selected group of patients with a high risk (HR) of relapse.<sup>1-7</sup> Numerous studies have shown that the strongest risk factor for the occurrence of relapse is the assessment of early therapy response by minimal residual disease (MRD) measurement.<sup>8-17</sup> Other known prognostic factors include prednisone response, induction failure, t(4;11) and t(9;22) translocations, older age (> 10 years) at diagnosis, and a high white blood cell count (WBC).<sup>18-20</sup> Recent trials are now using MRD levels at the end of induction therapy, and/or the end of first consolidation therapy, among other known HR features, for the risk stratification of children with ALL, because of its high predictive value.

In nearly all studies, patients with HR features received intensification of post-induction therapy, leading to five-year event-free survival (EFS) between 50.1% and 75.3%.<sup>4,7,8,21-23</sup> The only randomized study conducted was the UK ALL2003 trial, in which subjects with MRD end induction > 0.01% were randomly assigned to receive either standard or more intensive therapy. This led to a significantly better five-year EFS for the latter group.<sup>22</sup> The drawback of therapy intensification for HR patients is high toxicity.<sup>1,5,22</sup>

It remains largely unclear to what extent intensification of treatment is necessary. HR criteria and treatment protocols vary among the various studies and randomized trials are rare. In Europe, so-called HR chemotherapy blocks are used for intensification of post-induction therapy.<sup>1,9,21,22</sup> Controversial opinions exist on the balance of efficacy and toxicity of these HR blocks, because some HR patients may be overtreated with unnecessary toxicity as a result, whereas for another subset of HR patients, current treatment still results in suboptimal survival. This paper describes the toxicity and efficacy of Dutch HR blocks measured by MRD and the long-term outcome of the HR group in detail.

## 2 | METHODS

### 2.1 | Patients

A total of 1297 children, aged 1 to 18 years, with newly diagnosed ALL were consecutively enrolled between November 2004 and April 2017 in two prospective nationwide studies: the DCOG ALL-10 protocol and the first five years of the ALL-11 protocol<sup>1</sup> (trial number ALL-11: EudraCT: 2012-000067-25). Infants younger than one year and patients with mature B-cell ALL or BCR-ABL-positive ALL were excluded. Ethical approval was obtained from the institutional review boards, and informed consent was signed by all patients and/or their parents.

### 2.2 | HR features

Patients were stratified into the HR group of the ALL-10 and ALL-11 studies based on at least one of the following criteria: No complete remission (CR) at day 33; presence of t(4;11) translocation or the corresponding fusion gene "MLL-AF4"; MRD level of  $\geq 10^{-3}$  or unknown at day 33 and MRD level of  $\geq 10^{-3}$  at day 79. In the ALL-10 protocol also, patients with a poor prednisone response (PPR) (patients with  $\geq 1000$  leukemic blast/ $\mu$ L blood after seven days of prednisone therapy and one dose of intrathecal methotrexate) were included in the HR group. Children with Down syndrome who fulfilled one of these criteria were assigned to the medium risk group and therefore not included in the study.

### 2.3 | Treatment protocol

All patients with intention to treat according to the DCOG ALL-10 or ALL-11 HR protocol were included. All patients received seven days of prephase treatment with prednisone and a single dose of intrathecal methotrexate, followed by induction protocol IA and consolidation protocol IB (Supporting Information Table S1). Thereafter, HR patients received three courses of chemotherapy followed by SCT or underwent six courses of chemotherapy followed by protocol II and oral maintenance chemotherapy for a total duration of two years (Supporting Information Table S1). HR patients with MRD level of  $\geq 10^{-3}$  at days 33 and 79 or patients with T-cell ALL and no CR at day 33 were eligible for allogeneic SCT. Furthermore, in the ALL-10 protocol, patients with t(4;11); no CR on day 33 or a PPR in combination with T-cell ALL, pro-B-ALL or a white blood cell count  $> 100 \times 10^9/L$  also received SCT if a suitable donor was available.

### 2.4 | MRD assessment

MRD was measured by real-time polymerase chain reaction (PCR) for immunoglobulin and/or T-cell receptor gene rearrangements in bone marrow samples<sup>24</sup> and was evaluated according to EURO-MRD guidelines<sup>24,25</sup> as described previously.<sup>26</sup> Based on the junctional region of the identified rearrangements, patient-specific primers were designed and tested for sensitivity and specificity in a real-time quantitative PCR assay.<sup>25</sup> MRD levels were measured at day 33 (end of induction) and day 79 (end of consolidation) to determine the risk stratification. Moreover, MRD levels were detected during HR intensification before each HR chemotherapy block and pre SCT, allowing to measure the MRD response for each block of chemotherapy.

### 2.5 | Treatment-related outcome definitions and statistics

CR at the end of induction (day 33) was defined on morphological grounds by the presence of < 5% leukemic blasts and by regenerating

hematopoiesis without documented extramedullary leukemia. Relapse was defined as the presence of > 5% leukemic cells in the bone marrow or the presence of leukemic blast in the peripheral blood or central spinal fluid or leukemic infiltration elsewhere, after CR was achieved. Toxicity was graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) III guidelines.<sup>27</sup>

The principal endpoints in the analysis were EFS, OS, cumulative incidence of relapse (CIR), and cumulative incidence of death in remission (CID). EFS was defined as the time from diagnosis to first event. Relapse, secondary malignancy, nonresponder, or death in remission were classified as events. OS was defined as the time elapsing from diagnosis until death due to any cause. A competing risk model since CR with relapse and death as competing events was used to estimate the CIR and CID.<sup>28</sup> If no events occurred, the observation time was censored at last follow-up. EFS and OS were estimated according to Kaplan-Meier's methodology. Comparisons between survival outcomes were performed with the log-rank test. A landmark analysis from chemotherapy block 3 was performed to estimate the survival between patients who received SCT or chemotherapy.<sup>29</sup> This means that only patients alive at landmark time were included in the analysis. In some patients major treatment modifications were made due to clinician choice or severe treatment-related toxicity. These patients did not follow HR treatment according to protocol but were not excluded based on an intention-to-treat analysis.

MRD response was measured after each HR block. The patients for whom at least two MRD measurements were known during the HR blocks were divided in two groups. Group 1 consisted of patients with only negative and low-positive MRD measurements during HR therapy ( $\text{MRD} < 1 \times 10^{-4}$ ). Group 2 consisted of patients with at least one high-positive MRD measurement ( $\geq 1 \times 10^{-4}$ ). EFS, CIR, and CID were estimated for these two MRD groups, for the entire HR cohort and for the different ALL protocols. To assess the difference between cumulative incidence estimated for different MRD levels and between the two ALL protocols, the Gray test was employed.<sup>28</sup> All statistical analyses were conducted on the base of an intention-to-treat analysis. Analyses were carried out in SPSS 21.0 software (SPSS Inc. Chicago, IL, USA) and in R. All competing risk analyses were performed with the mstate library in R environment.<sup>29,30,31</sup>

## 3 | RESULTS

### 3.1 | HR treatment

Of the 1297 consecutively enrolled, newly diagnosed ALL patients, 107 (8.2%) patients were classified as HR and eligible to start HR treatment (Figure 1). However, eight patients did not start HR chemotherapy according to protocol because of major treatment modifications made due to severe treatment-related toxicity ( $n = 3$ ), clinicians' choice ( $n = 4$ ) or relapse ( $n = 1$ ). After starting with HR treatment, another 17 patients did not reach chemotherapy block 3, because of death in CR ( $n = 7$ ) or because of major treatment modifications made due to severe treatment-related toxicity ( $n = 5$ ) or clinician choice ( $n = 5$ ). Patients

who did not follow HR protocol due to major treatment modifications were not excluded based on an intention-to-treat analysis. Patients fulfilling three HR blocks received either three extra-chemotherapy blocks followed by protocol II and oral maintenance chemotherapy ( $n = 22$ ) or SCT ( $n = 60$ ) depending on the fulfillment of the SCT criteria and the availability of a suitable donor. Most of the patients were classified as HR based on their MRD levels (46%) or PPR only (41%).

### 3.2 | Patient characteristics

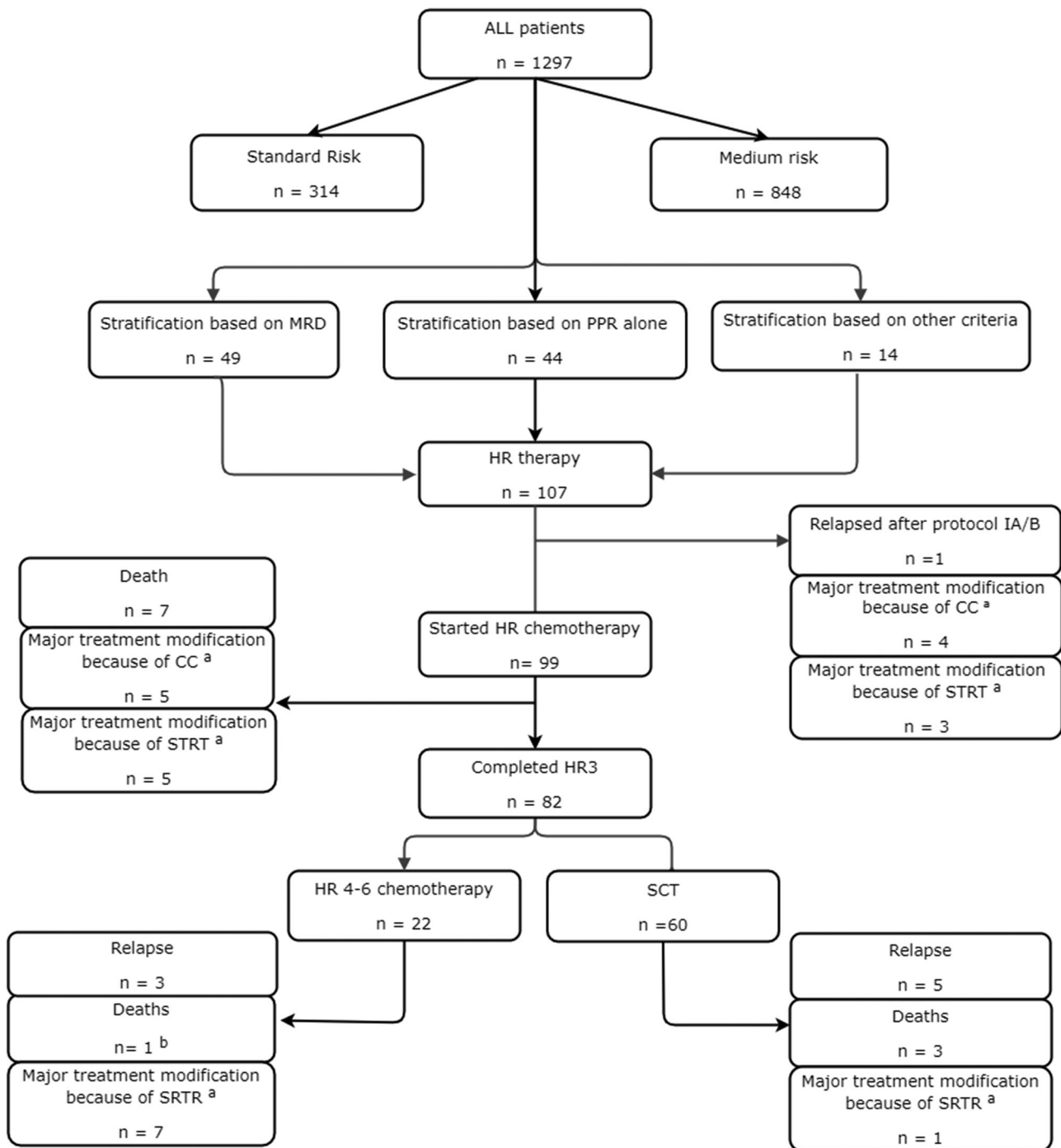
An overview of the characteristics of all HR patients is presented in Table 1. Of the patients, 63.6% were males, and the median age at diagnosis was 7.1 years (IQR 4-14). 46.7% of the patients had T-cell ALL and 40.2% of patients had a WBC of  $> 100 \times 10^9$ . Few HR patients presented with the favorable "ETV6-RUNX" or "TCF3-PBX1" alteration (1.87%). Another favorable prognostic factor, hyperdiploidy ( $> 50$  chromosomes), was found in 12.1%.

### 3.3 | Outcomes for the entire HR cohort

Median follow-up from the date of diagnosis was 74.9 months (range, 19.7 - 121.7 months). In total, 23 patients (21.5%) died, of which 12 were in CR (Table I). One patient died before reaching CR. Two patients (1.87%) were diagnosed with a secondary malignancy (histiocytic sarcoma and a superficial spreading melanoma), of which one died as a result of the malignancy. Relapse occurred in 13 patients (12.1%), of which 10 (77.0%) were detected early, i.e.,  $< 30$  months from diagnosis. Nine of 13 relapsed patients died. At five years, the EFS was 72.8% (95% CI, 64.6-82.0), the OS 79.1% (95% CI, 71.6-87.0), the CIR 13% (95% CI, 6.3-19.8), and the CID 12.3% (95% CI, 6-18.6; Figure 2A). EFS did not differ significantly between different HR subgroups (Figure 2B). When excluding the cases with only a prednisone poor response ( $n = 66$ ) as HR criterion, the five-year EFS is 71.0% (95% CI, 57.1-84.9) and the five-year OS is 81.8% (95% CI, 69.3-94.3). 59.1% of the patients classified as HR based only on a PPR were of T-ALL lineage. EFS did not differ significantly between patients who were treated with SCT compared with HR chemotherapy blocks 4-6 (Figure 2C). However, a significantly higher percentage of patients in the SCT group compared with the chemotherapy group was classified as HR based on MRD levels (respectively, 46.7% vs 9.1%,  $P = 0.01$ ) (Supporting Information Table S2).

### 3.4 | MRD response to chemotherapy blocks

MRD levels at the start of each HR block are displayed in Figure 3. Decreasing MRD levels after each chemotherapy block are observed. The largest MRD reduction was seen after chemotherapy block HR1, with a reduction in MRD levels of  $\geq 1$  log in 59% of the patients compared with, respectively, 24% and 27% after HR blocks 2 and 3. Moreover, the percentage of patients of whom the MRD levels increased



**FIGURE 1** Flowchart of included patients in the HR cohort. Abbreviations: ALL, acute lymphoblastic leukemia; MRD, minimal residual disease; PPR, poor prednisone response; HR, high risk; CC = clinicians choice; STRT = severe treatment-related toxicity; SCT, stem cell transplantation; CR, complete remission;  $n$  = number

<sup>a</sup> All patients in the flowchart that underwent major treatment modifications and therefore did not follow standard HR blocks were not excluded from study based on an intention-to-treat analysis.

<sup>b</sup> This patient died after completion of HR-6 in the maintenance phase of the protocol.

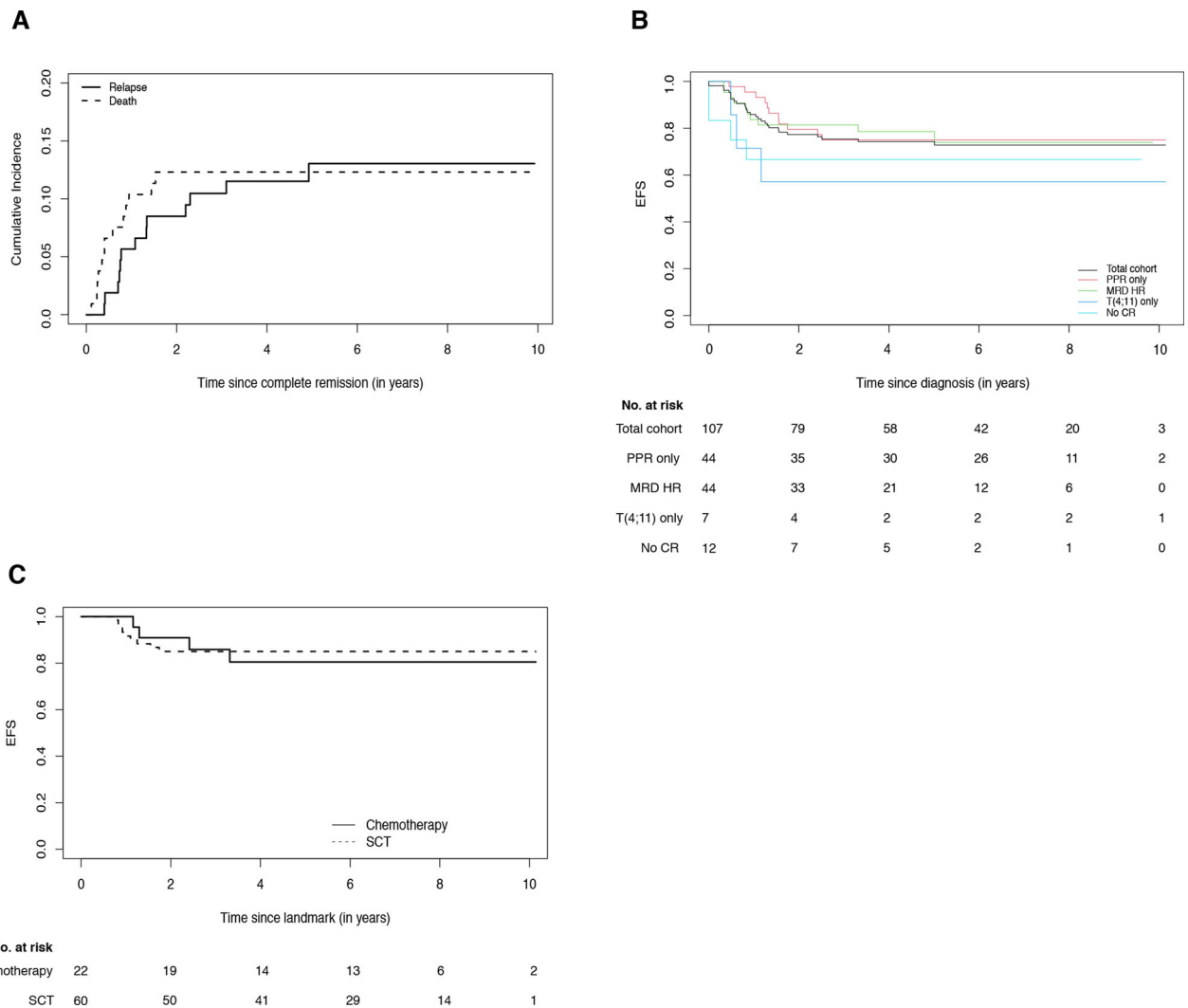
**TABLE 1** Patient characteristics and survival (%)

	ALL-10	ALL-11	Total
<b>Total HR cohort</b>	81	26	107
<b>Gender</b>			
Male	54 (67%)	14 (54%)	68 (63.6%)
Female	27 (33%)	12 (46%)	39 (36.4%)
<b>Age groups</b>			
< 10 years	52 (64%)	12 (46%)	64 (59.8%)
≥ 10 years	29 (36%)	14 (54%)	43 (40.2%)
<b>WBC count</b>			
< 50 × 10 <sup>9</sup> /l	37 (46%)	12 (46%)	49 (45.8%)
50-100 × 10 <sup>9</sup> /l	11 (14%)	3 (12%)	14 (13.1%)
≥ 100 × 10 <sup>9</sup> /l	32 (40%)	11 (42%)	43 (40.2%)
<b>Lineage</b>			
Pre-B-cell	40 (49%)	16 (62%)	56 (52.3%)
T-cell	40 (49%)	10 (39%)	50 (46.7%)
<b>Cytogenetics</b>			
TEL/AML1	2 (2.5%)	0	2 (1.87%)
t(4;11)	3 (3.7%)	4 (15%)	7 (6.54%)
t(1;19)	1 (1.2%)	0	1 (0.93%)
Hyperdiploid (> 50)	12 (15%)	1 (3.8%)	13 (12.1%)
MLL	NA	2 (7.7%)	2 (1.87%)
<b>Prednisone response<sup>a</sup></b>			
Poor	58 (72%)	NA	58 (71.6%)
Good	23 (28%)	NA	23 (28.4%)
<b>MRD at days 33 and 79<sup>b</sup></b>			
MRD ≥ 10 <sup>-3</sup>	26 (32%)	19 (73%)	45 (42.1%)
MRD < 10 <sup>-3</sup>	55 (68%)	5 (19%)	60 (56.1%)
MRD not done	0	2 (7.7%)	2 (1.9%)
<b>CR at day 33</b>			
No	13 (16%)	10 (39%)	23 (21.4%)
Yes	68 (84%)	16 (61%)	84 (78.5%)
<b>SCT or chemotherapy</b>			
HR4-HR6	18 (22%)	4 (15%)	22 (20.6%)
SCT	48 (59%)	12 (46%)	60 (56.1%)
<b>Relapse</b>	11 (14%)	2 (7.6%)	13 (12.1%)
<b>Death</b>	16 (20%)	7 (27%)	23 (21.4%)
Before complete remission	1 (1.2%)	0	1 (0.93%)
In complete remission	7 (8.6%)	5 (19%)	12 (11.2%)
Due to relapse	7 (8.6%)	2 (7.6%)	9 (8.41%)
Due to secondary malignancy	1 (1.2%)	0	1 (0.93%)
<b>Five-year OS (95% CI)</b>	81.5 (73.1 - 89.9)	72.0 (54.4 - 89.6)	79.1 (71.6 - 87.0)
<b>Five-year EFS (95% CI)</b>	75.3 (65.9 - 84.7)	72.0 (54.4 - 89.6)	72.8 (64.6 - 82.0)
<b>Five-year CIR (95% CI)</b>	13.9 (6-21.6)	8 (0-18.9)	13 (6.3-19.8)
<b>Five-year CID (95% CI)</b>	10 (3.3-16.4)	20 (3.9-36)	12.3 (6-18.6)
<b>Median follow-up duration</b>	84.6 (39.8-121.7)	40.9 (13.3-60.0)	74.9 (19.7 - 121.7)

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; CID, cumulative incidence of death in remission; CIR, cumulative incidence of relapse; CR, complete remission; EFS, event-free survival; HR, high risk; MRD, minimal residual disease; OS, overall survival; SCT, stem cell transplantation; WBC, white blood cell.

<sup>a</sup>Prednisone response was not measured within the ALL-11 protocol. Data on survival are therefore only calculated for ALL-10.

<sup>b</sup>The cutoff point of MRD ≥ 10<sup>-3</sup> at days 33 (end of induction) and 79 (end of consolidation) was chosen, because MRD ≥ 10<sup>-3</sup> at these timepoints is a criterion for HR stratification in the DCOG ALL protocols.



**FIGURE 2** (A) CIR and death for the HR cohort from complete remission. (B) Event-free survival of the HR cohort and HR subgroups from time of diagnosis. (C) Event-free survival of the chemotherapy and SCT group from HR block 3.

<sup>a</sup> Patients who were both classified as no CR and MRD-HR were assigned to the MRD-HR group in B.

<sup>b</sup> Landmark was set at end of HR chemotherapy block 3.

Abbreviations: HR, high-risk; EFS, event-free survival; CR, complete remission; PPR, poor prednisone reponse; MRD, minimal residual disease; SCT, stem cell transplantation

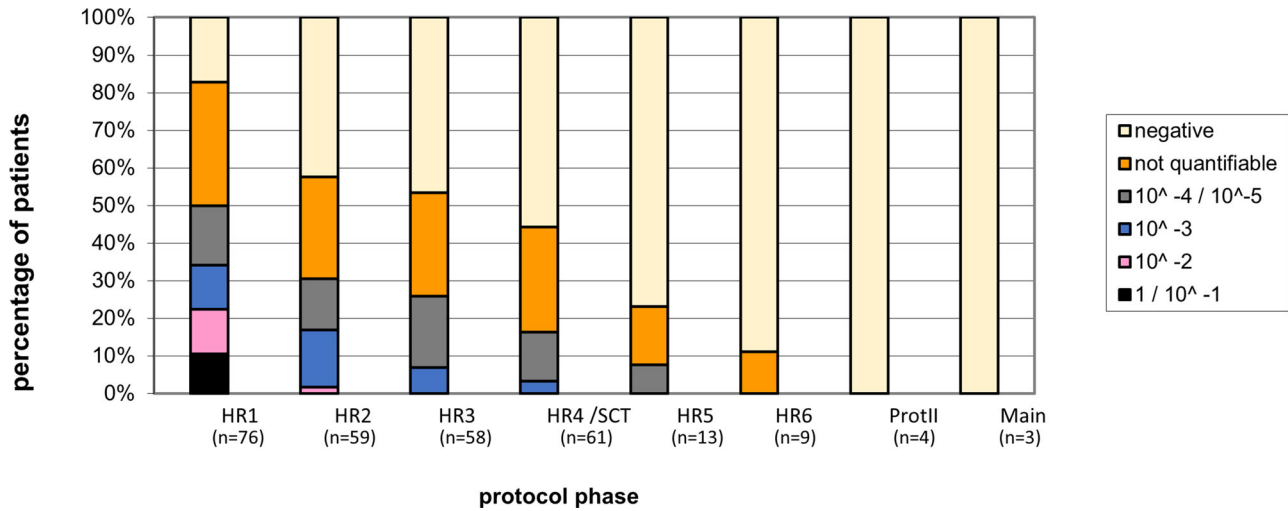
with  $\geq 1$  log was lower after HR block 1 (3.4%) than after chemotherapy blocks 2 and 3 (9.0% and 9.7%, respectively).

To investigate the association between MRD levels during HR blocks and relapses, all patients with more than two MRD measurements during HR therapy were divided into two groups: (1) only negative and low-positive MRD measurements ( $\text{MRD} < 1 \times 10^{-4}$ ) and (2) one or more high MRD measurements ( $\text{MRD} > 1 \times 10^{-4}$ ) (Figure 4). The number of relapses was lower in the MRD 1 group with a five-year CIR of 2.2% (95% CI, 0-6.6) compared with a five-year CIR of 15.4% (95% CI, 3.87-26.9) in the MRD 2 group (Figure 4A). No significant difference was found in CID between the two groups (Figure 4B). In the MRD 1 group, a lower percentage of patients underwent SCT compared with the MRD 2 group, respectively, 64% versus 90% ( $P = 0.01$ ).

### 3.5 | Toxicity

Almost all patients experienced at least one grade III-IV toxicity according to the CTCAE criteria per HR chemotherapy block (Table 2). The number of patients who experienced three or more grade III-IV toxicities in one HR block ranged from 24% in HR block 4 to 79% after SCT. Severe myelosuppression and infections were the most common toxicities reported. In chemotherapy block 4, lower rates of infections were witnessed than in the preceding and following HR blocks. During the entire treatment protocol, 12 patients (11.2%) died due to toxicity. Nine of the 12 toxic deaths (9.3%) were reported during HR blocks or after SCT (cause of death was graft-versus-host disease in two patients, infection in four patients, hemorrhage in one patient, and unknown in

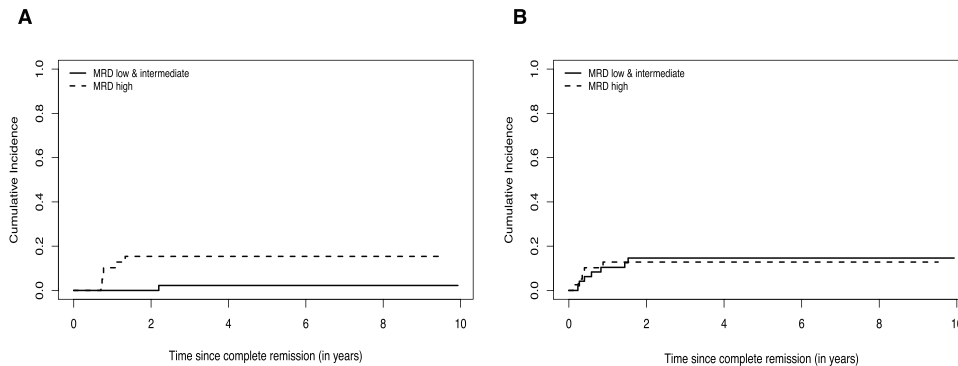




**FIGURE 3** The proportion of patients with detectable MRD levels before each HR chemotherapy block.

<sup>a</sup> We have arbitrarily assigned negative values the value of  $10^{-8}$  for the purpose of this graph.

Abbreviations: MRD, minimal residual disease; HR, high risk; SCT, stem cell transplantation; n, number



**FIGURE 4** (A) CIR by MRD levels after induction and consolidation therapy. (B) Cumulative incidence of death in remission by MRD levels after induction and consolidation therapy.

MRD low and intermediate (group 1) consist of patients with only negative or low-positive MRD levels during HR treatment ( $MDR < 1 \times 10^{-4}$ ); MRD high (group 2) consist of patients with one or more high-positive MRD levels during HR treatment ( $\geq 1 \times 10^{-4}$ )

Abbreviations: CI = confidence interval; n = number; CIR = cumulative incidence rate; CID: cumulative incidence of death in remission; MRD = minimal residual disease

two patients). The cause of death in the four patients who died of infection were septic shock of unknown cause; *e. coli* sepsis; fungal infection and CMV pneumoniae. One toxic death occurred during protocol II and the other two during induction therapy. In 16 patients (15%), major treatment modifications were implemented by the clinician because of severe toxicity. In 13 of the 16 patients' treatment modifications were made during HR chemotherapy blocks (Table 2).

## 4 | DISCUSSION

In the present study, we have reported the treatment results for children with newly diagnosed HR ALL according to the DCOG protocols.

The characteristics of the HR patients were comparable with other published HR cohorts.<sup>18-21,32</sup> The study showed that MRD-based stratification and intensification of treatment with HR blocks, for some followed by SCT, for patients with HR features leads to a five-year OS rate of 79.1% and a low five-year CIR of 13.0%. However, therapy was very intensive, illustrated by a 11% death in remission. Furthermore, a significantly higher CIR was found in patients with high-positive MRD levels during HR blocks than patients with negative or low-positive MRD levels.

The estimated survival and relapse reported in our study look favorable compared with other HR ALL cohorts.<sup>5,9,21,22,33</sup> However, comparison is difficult due to different HR selection criteria and therefore different HR populations. The AIEOP-BFM ALL 2000



**TABLE 2** Severe adverse events (grade III-IV)<sup>a</sup> reported per HR block per category (%)

	HR1	HR2	HR3	HR4	HR5	HR6	SCT
Total number	98	93	82	21	20	14	53
Cytopenias	94 (96%)	91 (98%)	76 (93%)	21 (100%)	19 (95%)	13 (93%)	52 (98%)
Infections	69 (70%)	71 (76%)	57 (70%)	9 (43%)	18 (90%)	10 (71%)	45 (85%)
GI	62 (63%)	48 (52%)	33 (40%)	8 (38%)	9 (45%)	8 (57%)	36 (68%)
Kidney	1 (1.0%)	1 (1.1%)	0	1 (4.8%)	0	1 (7.1%)	2 (3.8%)
Cardiac	3 (3.1%)	5 (5.4%)	2 (2.4%)	2 (9.5%)	1 (5.0%)	0	6 (11%)
Neurological	5 (5.1%)	7 (7.5%)	4 (4.9%)	1 (4.8%)	1 (5.0%)	0	1 (1.9%)
Trombo-hemorrhagic events	13 (13%)	16 (17%)	14 (17%)	2 (9.5%)	4 (20%)	0	16 (30%)
Skin	3 (3.1%)	3 (3.2%)	1 (1.2%)	0	1 (5.0%)	0	1 (1.9%)
Allergic reaction	8 (8.2%)	5 (5.4%)	2 (2.4%)	0	0	0	2 (3.8%)
Metabolic	6 (6.1%)	4 (4.3%)	1 (1.2%)	2 (9.5%)	0	0	2 (3.8%)
≥ 3 toxicities	54 (55%)	52 (56%)	32 (39%)	5 (24%)	10 (50%)	6 (43%)	42 (79%)
Toxic deaths	3 (3.1%)	2 (2.2%)	1 (1.2%)	0	0	0	3 (5.7%)
Major treatment modifications due to toxicity	1 (1.0%)	3 (3.2%)	1 (1.2%)	1 (4.8%)	5 (25%)	1 (7.1%)	1 (1.9%)

Abbreviations: GI, gastrointestinal; HR, high-risk; SCT, stem cell transplantation.

<sup>a</sup>Severe adverse events were graded according to the NCI CTCAE III guidelines.

protocol used similar HR criteria to this study. However, there were some differences in treatment regimen. The AIEOP-BFM study used dexamethasone in each HR block but no 6-MP or mitoxantrone. Most other drugs were found in HR courses from the AIEOP-BFM and our HR blocks, although there were slight differences in the combination per HR block. An overview of both treatment regimens is provided in Supporting Information Table S3.<sup>8,9,21</sup> The DCOG HR courses seem to be more effective with a five-year EFS of 72.8% (95%CI 64.6-82.0) compared with a five-year in the AIEOP-BFM trial of 58.9%. Because the AIEOP-BFM trial protocol used the same HR criteria, it can be suggested that the better survival in our study was achieved in a patient group with at least the same risk of relapse and death. This observation is strengthened by the fact that the percentages of patients classified as HR was higher in the AIEOP-BFM trial (15.6% vs 8.0%).<sup>21</sup> When specifically looked at HR T-lineage ALL, the DCOG HR courses also look favorable: a five-year EFS of 68% was found in this study compared with a 7-year EFS of 50% in AIEOP-BFM trial.<sup>8</sup> Nevertheless the different outcome of patients with T-ALL between the two studies must be carefully considered because of the small number of T-ALL patients and the distinctions in HR population: all patients in this subanalysis of the AIEOP-BFM trial were classified as HR based on MRD, whereas in our study 70% of patients with T-ALL were stratified as HR based on other HR criteria.<sup>8</sup> The percentages of patients who underwent SCT was comparable between the two studies. In the UK-ALL 2003 trial, a higher five-year EFS was found of 90% for HR patients in the augmented therapy group.<sup>22</sup> However, almost 25% of the patients in the UK-ALL 2003 trial were classified as HR, probably because classification into HR was based only on MRD levels at end of induction (day 29) and not at end of consolidation (day 79). Therefore, many patients included in the UK-ALL 2003 trial HR group would have classified and treated as medium risk in the DCOG

protocols. This could explain the higher EFS in the UK-ALL 2003 trial.

The DCOG HR treatment in this cohort resulted in considerable toxicity, as illustrated by the high number of toxicities per HR block and 12% of patients who underwent major treatment modifications because of severe toxicity. There was 11% mortality due to toxicity compared with 8% mortality due to relapse, suggesting that the limit of treatment intensification has been reached. This is especially the case when looked at the patients with only negative or low-positive MRD levels during HR treatment. For these patients, the five-year CID was 15% while the five-year CIR was 2%. The spectrum of toxicities, with cytopenias and infections as the most common ones, is similar to other published HR cohorts.<sup>3,11</sup>

The HR chemotherapy blocks 1-3 very effectively decreased MRD levels in patients. No significant reduction in MRD levels was found for the patients who received HR4-6. However, it cannot be stated that these chemotherapy blocks are not effective in decreasing MRD levels, due to the small number of patients of which MRD measurements were known at these time points. It is well established in literature that MRD measurement early in treatment, during induction therapy, is one of the most robust, independent prognostic factors of relapse.<sup>8-17</sup> The results of this study suggest that MRD measured later in the treatment protocol during HR chemotherapy blocks could also be of prognostic value, because there was a strong correlation between high MRD levels during HR blocks and relapse: a five-year CIR of 2.2% (95% CI, 0-6.6) found in patients with only negative or low-positive MRD levels compared with 15.4% (95% CI, 3.87-26.9) in patients with one or more high-positive MRD levels. Several previous investigations demonstrated the potential prognostic value of MRD measurements during intensification therapy. A study of 110 HR patients with a median of five MRD measurements after day 78, showed significant higher occurrence of

relapse in patients with high MRD levels at one of more time points compared with patients with low or without quantifiable MRD levels.<sup>34</sup> Additionally, three studies demonstrated a correlation between the reemerging of MRD during intensification treatment in patients in CR and the occurrence of relapse.<sup>35-37</sup>

In conclusion, the highly selected small group of HR patients have a relatively high survival rate of 79% with HR blocks (for some patients followed by SCT). Survival seems favorable compared with other studies reported in literature. The study further shows that patients with low MRD levels during HR blocks have a very low relapse percentage compared with patients with high MRD during HR blocks. The sequential measurement of MRD during HR treatment could therefore be used as a tool to identify patients in need of more effective preemptive treatment against a potential relapse. Those patients may benefit from new immunotherapeutic approaches such as inotuzumab, blinatumomab, or CAR-T-cell therapy. At the same time, this approach of sequential MRD measurements gives the opportunity to offer less intense treatment to patients with a negative MRD levels during HR treatment and thereby reduce the high burden of toxicity in this group. This is especially important, because this study suggests that the limit of treatment intensification might have been reached as the number of patients dying from leukemia relapse is about equal as the number of patients dying from toxicity. The use of SCT must be carefully weighted due to its burden of late toxicities.

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#### CONFLICTS OF INTEREST

The authors declare no competing financial interests.

#### DATA SHARING STATEMENT

The data that supports the findings of this study are available in the Supporting Information material of this article, and also available from the corresponding author upon reasonable request.

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### SUPPORTING INFORMATION

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