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Low-intensity therapy cures over 40% of children with rapid Flow-MRD responding ALL: the ALL-MB 2008 trial results

A. Popov[1], G. Henze[2], Yu. Roumiantseva[1], O. Budanov[1, 3], M. Belevtsev[3], T. Verzhbitskaya[4, 5], E. Boyakova[6], L. Movchan[3], G. Tsaur[4, 5], M. Fadeeva[1], S. Lagoyko[1], L. Zharikova[1], N. Myakova[1], D. Litvinov[1], O. Khlebnikova[4], O. Streneva[4, 5], E. Stolyarova[3], N. Ponomareva[7], G. Novichkova[1], L. Fechina[4, 5], O. Aleinikova[3], A. Karachunskiy[1]

[1]*Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology of Ministry of Healthcare of the Russian Federation, Moscow, Russian Federation*
[2]*Department of Pediatric Oncology Hematology, Charité – Universitätsmedizin Berlin, Berlin, Germany*
[3]*Belarusian Research Center for Pediatric Oncology, Hematology and Immunology, Borovlyany, Minsk District, Belarus*
[4]*Regional Children's Hospital, Ekaterinburg, Russian Federation*
[5]*Research Institute of Medical Cell Technologies, Ekaterinburg, Russian Federation*
[6]*Moscow City Blood Center named after O.K. Gavrilov, Moscow, Russian Federation*
[7]*Republican Children's Hospital, Moscow, Russian Federation*

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Correspondence:
Alexander M. Popov,
MD, PhD, head of Leukemia immunophenotyping laboratory, Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology of Ministry of Healthcare of the Russian Federation
Address: 1 Samory Mashela St., Moscow 117997, Russia
E-mail: uralcytometry@gmail.com

Serious side effects occur during therapy for childhood acute lymphoblastic leukemia (ALL), and survivors can experience long-term consequences. This study aimed at identifying patients who can be successfully treated with low treatment intensity combining clinical parameters and minimal residual disease (MRD) measurements. The study was approved by the Independent Ethics Committee and the Scientific Council of the Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology. ALL-MB studies used reduced-intensity therapy from the beginning, for standard risk (SR) patients no cyclophosphamide, a very low daunorubicin dose, no high dose of methotrexate, no cranial irradiation. In the ALL-MB 2008 study, 1702 children (49.1% of all patients) were classified as SR due to favorable initial characteristics. These included 295 patients treated in institutions who took part in a pilot study on MRD measurement using flow cytometry on day 15 and/or at the end of induction (EOI). The most suitable time point for MRD measurement was EOI with threshold 0.1% in 90.5% of the patients with excellent results: event-free survival of 95% and overall survival of 97%, that identified the large proportion of patients (more than 40% of all ALL patients). The outcome of children with slower MRD response was significantly worse. Initial SR characteristics plus one single MRD measurement at EOI identify more than 40% of all children with ALL who can be successfully treated with low-intensity regimens as used in the MB protocols.

Key words: *low-risk acute lymphoblastic leukemia, minimal residual disease, reduced-intensity treatment*

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In the last few decades, increasing therapy intensity has been the most important tool to improve the survival of children with acute lymphoblastic leukemia (ALL) [1–4]. Currently, their survival rate is in the 90% range [3, 4]. Yet, we are dealing with acute treatment related morbidity and mortality and long-term consequences [5–9]. Some of these are serious and others debilitating, affecting not only the quality of life but also the life expectancy of long-term survivors [5–7]. Therefore, it would be desirable to reduce the intensity of the treatment and thus also the frequency of long-term effects. Nevertheless, there is an ongoing controversial discussion about the criteria children had to meet for low-dose therapy. The lack of reliable criteria for selecting patients with an extremely low likelihood of recurrence was seen as a major obstacle to such de-escalation of treatment. In most studies [10–13], the favorable initial clinical parameters (low white blood cell count (WBC), young age, lack of organomegaly and CNS involvement) were helpful in selecting patients who did not require further intensification. But more precise criteria were required to estimate a really low risk of recurrence. Therefore, more precise low risk criteria were needed.

Early response to therapy has become one of the most valuable risk factors in recent years. Initially, the response was determined by the rate of decline in leukemia cells in the blood after one week of prednisone (PRED) monotherapy (plus an intrathecal dose of methotrexate (MTX)) [14] and further by microscopic bone marrow (BM) analysis during and at the end of remission induction. The response to therapy is now mainly assessed by measuring the minimal residual disease (MRD). Molecular genetic techniques (poly-

merase chain reaction) or multicolor flow cytometry (MFC) enable the detection of residual leukemia cells in the range of 10^{-4} to 10^{-5} [15–17]. Measurement and monitoring of MRD has proven to be the strongest outcome predictor [18–24] and is used in many ALL protocols today, sometimes at multiple time points [21, 25, 26], with the aim of moving patients to more intensive or experimental treatment arms [27, 28]. Originally aimed at finding children with a high risk of relapse, later the detection of residual leukemia at an early point in time was also seen as a valuable tool for the selection of groups with a low risk [19, 29]. The possible applicability of the results of MRD monitoring for the de-intensification of treatment in a variable proportion of children with ALL has been proven in several studies [30–33]. Recently, the results of two studies measuring MRD with a rather simple MFC assay were published to identify patients with B-cell precursor ALL (BCP-ALL) at very low risk of relapse (VLR) [34, 35]. These studies showed that approximately 25% of patients with BCP-ALL could be treated with a reduced-intensity protocol, resulting in 5-year event-free survival (EFS) and overall survival (OS) rates greater than 90% and 95%, respectively.

The cooperative Moscow–Berlin ALL studies initially were aimed at achieving favorable results with comparatively moderate treatment intensity, which could be carried out under Russian conditions [36]. Since the expected goal of reducing acute side effects and the need for supportive therapy were achieved in the first study ALL-MB 91, one of the guiding principles of the following MB studies was the possibility of further therapy de-escalation [37, 38]. In study ALL-MB-2008, MFC was used for the MRD measurement in parallel to treatment [39, 40], initially without drawing any consequences from these results with regard to patient stratification. Here, we describe a very simple procedure, based on the MFC-MRD measurement of early response to therapy, to identify initially standard risk (SR) children with a particularly favorable prognosis who were curatively treated with a low-dose therapy regimen.

MATERIALS AND METHODS

The study was approved by the Independent Ethics Committee and the Scientific Council of the Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology.

Patients

Between February 2008 and November 2014, 3466 consecutive pediatric patients (age 1 to 18 years) with ALL in Russia and Belarus were enrolled in the Moscow–Berlin group study ALL-MB 2008 (NCT01953770). Patients were assigned to the SR group if they met the following criteria [41]: BCP-ALL with an initial WBC count below 30 × 10^9/L, spleen enlargement less than 4 cm below the costal margin, no CNS3-status, no translocation t(4;11)(q21;q23)/*KMT2A/AFF1* or t(9;22)(q34;q11)/*BCR-ABL*, and achievement of hematological remission at the end of induction (EOI; day 36).

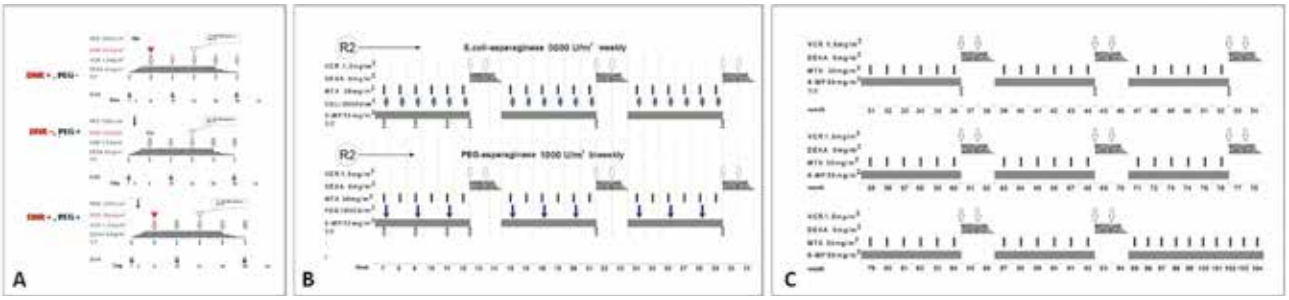
Treatment protocol

The treatment design is shown in *figure 1*. SR patients were randomly assigned to one of three arms for induction therapy. In all patients, induction therapy began with increasing doses of dexamethasone (DEXA) during the first week up to 6 mg/m^2 per day plus one triple intrathecal injection of MTX/PRED/ARA-C repeated weekly up to 5 doses, and all patients received weekly 1.5 mg/m^2 vincristine (VCR) for 5 doses starting on day 8.

In randomization arm 1, daunorubicin (DNR) was administered on day 8 at a dose of 45 mg/m^2; in arm 2, the patients received PEG asparaginase (PEG) 1000 U/m^2 on day 3 and no DNR; in arm 3, DNR was administered at a dose of 45 mg/m^2 plus PEG 1000 U/m^2 on day 3. Patients were given a second dose of DNR only if they had more than 10% leukemia cells in the BM on day 15.

After induction, patients received three 6-week consolidation courses consisting of 50 mg/m^2 oral

Figure 1
Schematic description of SR group therapy in ALL-MB 2008 trial. Panel A shows induction therapy with randomization to three arms; panel B depicts consolidation therapy with two branches of randomization; on panel C maintenance is shown. Coli – Coli-asparaginase; TIT – triple intrathecal injection of MTX/(PRED/Cytarabine (ARA-C). Microscope sign indicates time-points for cytomorphologic BM investigation



mercaptopurine daily, and 30 mg/m^2 intramuscular MTX weekly (the intramuscular route was chosen to ensure that the drug was actually administered). In addition, asparaginase was randomly administered with either 5000 U/m^2 intramuscularly weekly, native *E. coli* asparaginase, or 1000 U/m^2 PEG intravenously.

Between the consolidation blocks, three two-week reinductions with DEXA (6 mg/m^2) and two doses of VCR (1.5 mg/m^2) were administered. Thereafter, maintenance therapy was given up for a total of 2 years. Cyclophosphamide, cytarabine, high dose MTX and CNS irradiation were not part of the treatment plan.

Samples

An assessment of the MRD for all participating institutions and patients in the ALL-MB 2008 study was unrealistic for logistical reasons. The MFC-MRD pilot study was therefore carried out in institutions that are connected to the MFC laboratories of the Moscow–Berlin group Flow-network [2, 3]. BM samples for MFC-MRD monitoring were collected on day 15 and/or EOI. In total, samples from day 15 were examined in 287 cases and EOI samples in 273 cases.

MRD investigation

MRD was assessed by MFC in three laboratories (two in Russia and one in Belarus) according to well-harmonized approach [40] based on AIEOP-BFM-ALL-MRD-Flow study group guidelines [42], as previously described [40]. All three laboratories use the MFC methodology based on standard analyses, and had participated in AIEOP-BFM QA [43] system as well as in intragroup proficiency tests [40]. 4–9-color MFC was used to evaluate the expression of antigens commonly used for MRD detection in BCP-ALL: CD19, CD10, CD34, CD45, CD20, CD38, CD58, and CD11a [40]. The MRD values were expressed as percentage of leukemia cells among all nucleated BM cells which were defined by positivity for nucleic acid staining (Syto16 or Syto41 dye). MRD negativity was defined as < 0.01%. Despite the increasing number of colors in use, the basic principles of MFC-MRD detection had not changed over time. This sensitivity was thus achievable with high reliability over the entire study period.

Statistical analysis

EFS was defined as the time from diagnosis to the first event, i. e., non-response, relapse, death from any cause, or second malignant neoplasm, whichever comes first. Observation periods were censored at the time of last contact if no events were reported. OS was assessed from diagnosis to death from any cause. EFS and OS curves were generated using the Kaplan–Meier method [44] and standard errors were calculated according to Greenwood. Differences in outcome between groups were compared using the log-rank test. Cumulative incidence of relapse (CIR) curves were estimated adjusting for competing risks of the other pertinent events and compared by Gray's test [45]. All tests were two sided. Analyses were performed using R-statistics v3.4.2.

RESULTS

1702 were assigned to the SR group because they met the respective criteria (see above). 295 of these 1702 patients were treated in clinics that participated in a pilot study on MRD measurement. The patient characteristics (examined for MRD versus others) are listed in *table 1*. With a median follow-up time of 8.4 years, the following results were obtained for 295 studied children initially classified as SR: 5 children died during induction, 21 had a relapse, all others

Table 1
Characteristics of patients included in the current MRD study compared with those from the SR group of the ALL-MB 2008 trial not having been studied for MFC-MRD. All patients met the initial SR criteria: BCP-ALL with an initial WBC count below 30 × 10^9/L, spleen enlargement less than 4cm below the costal margin, no CNS3-status, no translocation t(4;11)(q21;q23)/*KMT2A/AFF1* or t(9;22)(q34;q11)/*BCR-ABL*, and achievement of hematological remission at the EOI (day 36)

Parameter	Studied for MFC-MRD		Not studied for MFC-MRD		*p**
	n	%	*n*	%	
Total	295	100	1407	100	ND
Sex					
Male	150	50.8	755	53.7	0.3787
Female	145	49.2	652	46.3	
Age					
< 10 y.o.	247	83.7	1216	86.4	0.2255
≥ 10 y.o.	48	16.3	191	13.6	0.2321
Initial WBC count					
< 10 × 10^9/L	202	68.5	1013	72.0	0.2235
≥ 10 × 10^9/L	93	31.5	394	28.0	
Steroid response**					
Good	285	99.3	1310	98.3	0.1997
Poor	2	0.7	23	1.7	
Day 15 BM response (by cytomorphology)‡					
M1	247	83.7	1013	72.0	0.013
M2	36	12.2	224	15.9	
M3	12	4.1	96	6.8	
ND	0	0	74	5.3	
t(12;21)(p13;q22)/ETV6-RUNX1⁂					
Present	81	27.5	259	18.4	0.0004
Absent	214	72.5	1148	81.6	

*Note. * – patients' distributions were compared with two-sided chi-square test; ** – poor glucocorticoid response: blast count in peripheral blood ≥ 1000 cells per μL on day 8; ‡ – M1 – BM status was defined as leukemia cells < 5%; M2 – leukemia cells ≥ 5–25%; M3 – leukemia cells ≥ 25%. ND – no data. ⁂ – the differences in the proportion of ETV6-RUNX1-positive patients can probably be explained by an underestimation in clinics that did not take part in the MRD study. In the ALL-MB 2008 study there were no obligatory centralized laboratory investigation; therefore, standardized cytogenetic and molecular genetic studies were not available in all participating clinics. In the next trial ALL-MB 2015 centralized laboratory diagnostics was implemented and the incidence of t(12;21)(p13;q22)/ETV6-RUNX1 has increased to the appropriate values.*

are in complete remission for a median duration of 8.6 years (7 to 12.5 years). The 10-year EFS ± SE for all SR patients was 90.9 ± 1.7%, OS – 94.3 ± 1.4%, and the cumulative incidence of relapses (CIR ± SE) was 7.4 ± 1.6%. The results did not differ between the patients who were assigned to the three randomization arms (see treatment) (*figure 2*). Data for MRD measurement on day 15 were available from 287 patients, for MRD at EOI from 273 children (*table 2*).

Prognostic significance of the MFC-MRD level on day 15

In 74 of 287 patients in the study cohort with MFC-MRD-negativity on day 15, the 10-year EFS was 97.3 ± 1.9%, and the CIR 2.7 ± 1.9%. In contrast, the EFS and CIR were 88.4 ± 2.2% and 9.2 ± 2.0% respectively ($p = 0.0293$ and $p = 0.0806$) in 213 MFC-MRD-positive patients (*figure 3A*). Nineteen out of twenty-one relapses (90.5%) occurred in the MFC-MRD-positive group. The alternative cut-off for low-risk identification proposed in the AIEOP-BFM day 15 MFC-MRD risk classification (less than 0.1%) [19] identified 131 patients with also excellent results: EFS 96.9 ± 1.5%, and the CIR 3.1 ± 1.5%, again better compared to 156 children with a higher MFC-MRD level (EFS 85.5 ± 2.9%, CIR 11.3 ± 2.6%; $p = 0.0012$ and $p = 0.0119$, respectively; *figure 3B*). Only 19.0% of the recurrences (4 of 21) were registered in this low-risk group. In addition, using a relatively high threshold of 1% for MFC-MRD results on day 15 identified a relatively large group of patients with very favorable treatment outcomes (*figure 3C*): 215 patients with MFC-MRD less than 1% had an EFS of 94.7 ± 1.5% and a CIR of 4.8 ± 1.5%. In contrast, 72 children with higher MFC-MRD fared significantly worse: EFS 78.6 ± 4.9% and CIR 15.8 ± 4.5% ($p < 0.0001$ and $p = 0.0026$). More than half of the recurrences (11 of 21, 52.4%) occurred in this relatively small group of patients with really high MFC-MRD values (≥ 1%).

Figure 2
EFS (solid lines) and CIR (dashed lines) according to randomization arms in induction for patients in the study group ($n = 288$). Standard errors are shown in parentheses

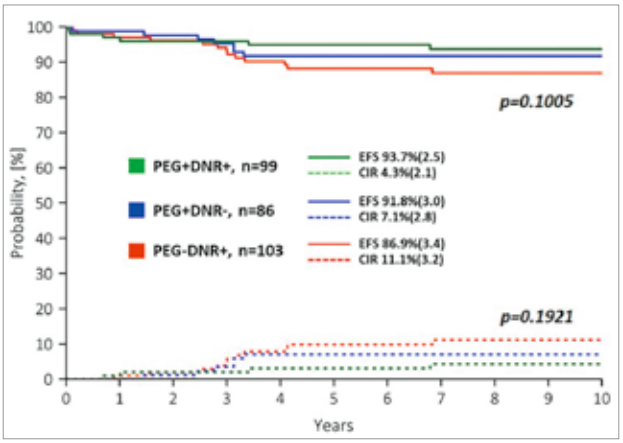


Table 2
Number of patients and samples studied in each laboratory

Laboratory	Patients	Day 15 samples	Day 36 samples
1	72	71	72
2	161	159	143
3	62	57	58
Total	295	287	273

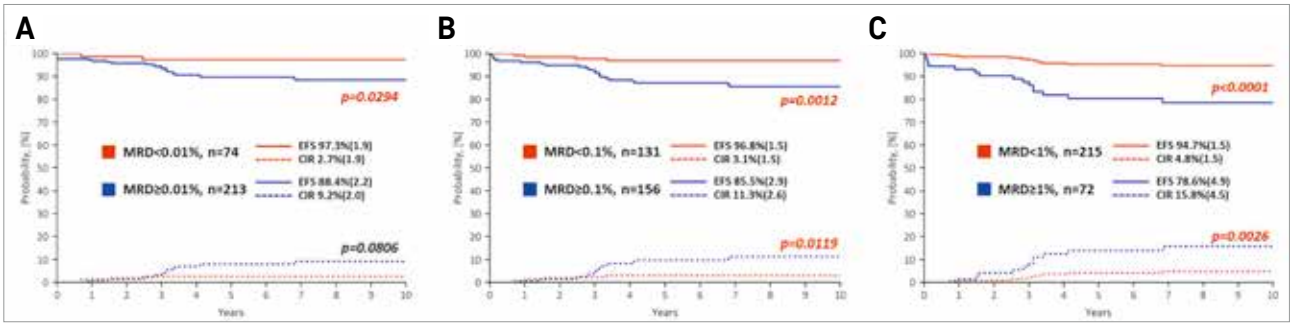
Result of EOI MFC-MRD

Of 273 children with available EOI MFC-MRD data enrolled in the study cohort, 169 (61.9%) had detectable MRD values while the remainder were negative. The outcome of MFC-MRD negative patients (10-year EFS 95.7 ± 1.6% and 10-year CIR 3.7 ± 1.5%) was significantly better than that of 104 MFC-MRD-positive patients (10-year EFS 85.0 ± 3.6% and 10-year CIR 14.1 ± 3.5%; $p = 0.0023$ or $p = 0.0022$; *figure 4A*). With a higher cut-off for MFC-MRD positivity (0.1%), we identified 247 patients, of whom only 11 had a relapse (EFS 95.0 ± 1.6% and CIR 4.6 ± 1.4%). Of the remaining 26 children with EOI MFC-MRD ≥ 0.1%, 9 relapsed (EFS 60.7 ± 9.8% and CIR 35.4 ± 9.8%; $p < 0.0001$ for both comparisons; *figure 4B*).

Selection of the optimal cut-off values for each MFC-MRD time point

Since the main aim of the study was to find a specific time point and threshold for MFC-MRD for

Figure 3
EFS (solid lines) and CIR (dashed lines) according to MFC-MRD values on day 15 with the indicated thresholds: 0.01% (A); 0.1% (B) and 1% (C). The curves for patients with MFC-MRD values below these limit values are shown in red, for patients with higher MFC-MRD values in blue. Standard errors are shown in parentheses



the most accurate assessment of patients for whom low-intensity treatment is sufficiently effective, we compared the patient distribution and results in groups with three different thresholds for MFC-MRD – on day 15 and two for EOI. The results of this comparison are summarized in *table 3*. The outcomes according to all five low-risk criteria were similarly good (EFS 94.7–97.3%, CIR 2.7–4.8%), although the proportion of identified patients was completely different. Apparently, the highest thresholds for both time points (< 1% for day 15 and < 0.1 for EOI) were most effective in assessing children with initial SR characteristics who can be successfully treated with reduced intensity. These cut-offs identify 74.9% and 90.5% of the patients, respectively. Although almost half of the relapses occur in these cohorts a significant proportion of these relapse patients can be cured (OS 97.2% in children with MFC-MRD < 0.1% on day 15 and 97.1% in children with MFC-MRD < 0.1 at EOI). EFS, OS, CIR of the low-risk groups according to MFC-MRD on day 15 (< 1%) and EOI (< 0.1%) are shown in *figure 5* (panels A and B, respectively). Although the outcome in low risk patients as determined by the MFC-MRD assessment at each of these time points was similarly excellent, the EOI was chosen as the most appropriate time point because it captured a higher percentage of low risk patients.

Table 3
Distribution of patients, relapses and outcome data in low-risk groups as defined by MFC-MRD at day 15 and at the EOI using various thresholds

MFC-MRD level	Number of patients identified	% among study group	Number of relapses	% out of all relapses	EFS, %	OS, %	CIR, %
Day 15 (*n* = 287)							
< 0.01%	74	25.8	2	9.5	97.3	97.3	2.7
< 0.1%	131	45.6	4	19.0	96.9	97.7	3.1
< 1%	215	74.9	10	47.6	94.7	97.2	4.8
EOI (*n* = 273)							
<0.01%	169	61.9	6	30.0	95.7	97.0	3.7
<0.1%	247	90.5	11	55.0	95.0	97.1	4.6

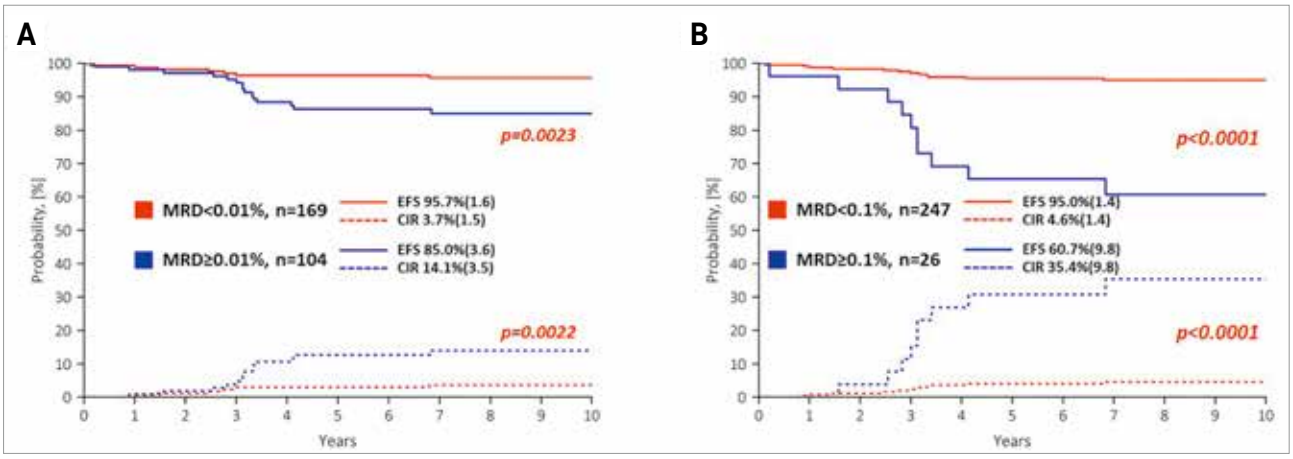
Randomization-related treatment differences and their relationship to MFC-MRD scores

The distribution of patients by randomization arms and MFC-MRD levels on either day 15 or EOI is shown in *table 4*. The use of PEG on day 3 resulted in a larger group with lower MFC-MRD levels. However, the proportion of relapses remained similar. As in the group of patients included in this MFC-MRD study (*figure 2*) and in the entire study ALL-MB 2008 (manuscript in preparation), the results between the randomization arms were not statistically significantly different.

Only 28 patients received a second dose of DNR because they had more than 10% of blasts in BM on day 15 as evidenced by cytomorphology (see treatment). EOI MFC-MRD data were available for 25 of them. Eighteen patients achieved low MFC-MRD scores (< 0.1%) on EOI and none relapsed. Seven other patients remained highly positive (≥ 0.1%) for MFC-MRD, while one of them eventually relapsed.

In summary, a one-point MFC-MRD measurement at EOI identified approximately 90% of the patients originally categorized as SR with excellent EFS and OS results. Since 50% of BCP-ALL patients are classified in the SR group based on clinical characteristics, 40% of all BCP-ALL patients can achieve EFS and OS rates of 95.0% and 97.1%, respectively, if they receive reduced-intensity therapy as in the MB protocol. Of the 247 patients with FCM-MRD < 0.1 at EOI, none died in CR and none suffered a second malignancy. Seven patients, 2 boys and 5 girls, developed avascular bone necrosis. Their median age was 13.03 years; 3 of them were < 10 years old and 4 girls were > 10 years old.

Figure 4
EFS (solid lines) and CIR (dashed lines) according to MFC-MRD values at the EOI in respect to following thresholds: 0.01% (A) and 0.1% (B). The curves for patients with MFC-MRD values below these cut-offs are shown in red, for patients with higher MFC-MRD in blue. Standard errors are shown in parentheses



DISCUSSION

From the early studies in the 1960s, we learned that around a third of children with ALL were cured with what we saw as very moderately intensive therapy. Induction therapy consisted mainly of VCR and PRED plus/minus asparaginase. Anthracyclines were not used routinely [46, 47].

When looking for the characteristics of the approximately 30% of children who survived in these studies, clinical parameters such as WBC, age, liver and spleen enlargement, CNS involvement, and later also immunology, were used to assess the risk of relapse and assign patients to risk-adapted treatment arms.

It was later found that the response to therapy was a very strong predictor of outcome. Originally measured as the clearance of blasts in peripheral blood during the glucocorticoid prophase, response to treatment is now assessed by the much more sensitive measurement of MRD [16, 17, 19–21, 25]. Sensitive techniques enable the detection of one residual leukemia cell among 10,000–100,000 normal BM elements [17]. The measurement and monitoring of MRD is now used in many ALL protocols, sometimes at multiple time points, with the aim of assigning patients to more intensive or experimental treatment arms [27, 28].

Table 4
Distribution of patients and relapses according to MFC-MRD thresholds at day 15 and at the EOI in respect to randomization arms

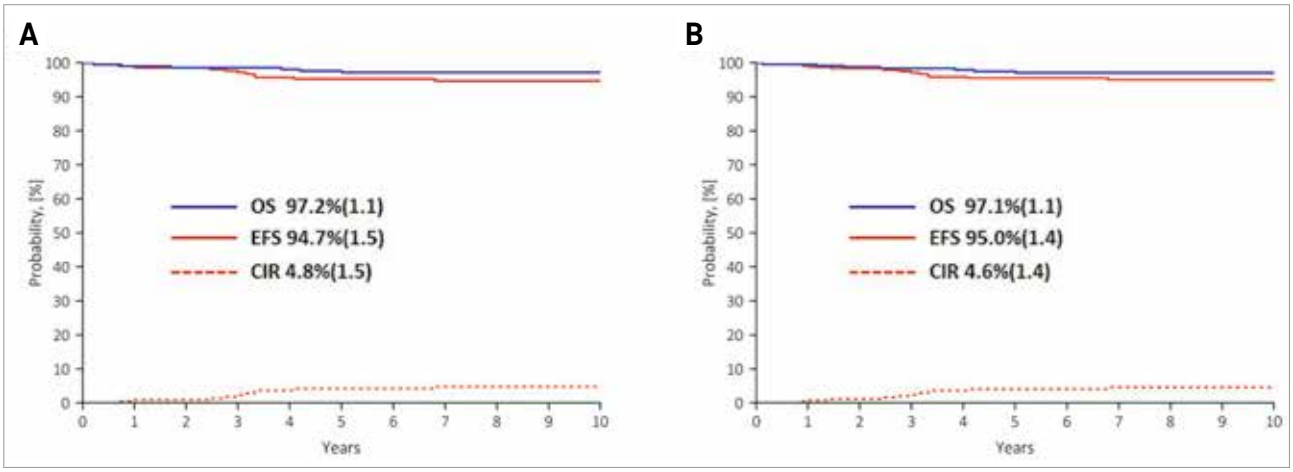
MFC-MRD	PEG–DNR+		PEG+DNR–		PEG+DNR+	
	n (%)	relapses	*n* (%)	relapses	*n* (%)	relapses
Day 15 (*n* = 280)						
< 1%	57 (57)	4	68 (80)	3	85 (89)	3
≥ 1%	43 (43)	7	17 (20)	3	10 (11)	1
EOI (*n* = 266)						
< 0.1%	76 (82)	5	76 (94)	4	88 (96)	2
≥ 0.1%	17 (18)	6	5 (6)	2	4 (4)	1

Multiple measurement of MRD is cumbersome, requires adequate logistics, and, when performed with molecular techniques, is quite expensive. Recently, the results of two studies measuring MRD with a simplified flow cytometric assay were published to identify patients with BCP-ALL at VLR [34, 35]. VLR was defined as age between 1 and 10 years, WBC < 50 × 10^9/L, absence of extramedullary leukemia and MFC-MRD < 0.01% on day 19 of remission induction (one point measurement). These patients, comprising about 20–25% of all BCP-ALL patients, were treated on a reduced-intensity treatment plan and achieved estimated 5-year EFS and OS of 92.0 ± 3.9% and 96.0 ± 2.8%, respectively [34, 35].

Since its inception, the aim of the cooperative Moscow–Berlin ALL studies has been to achieve favorable results with comparatively moderate treatment intensity. In the first study ALL MB 1991, which was carried out in a few better equipped clinics, it could be shown that the results were actually satisfactory and that acute side effects and the need for supportive therapy were significantly lower compared to a slightly modified BFM ALL 90 protocol [36]. The consecutive ALL-MB 2002 study showed that DEXA had a superior effect compared to methylprednisolone, especially in extracompartments [37], and that a single weekly administration of ASP at a dose of 5 kU/m^2 in SR patients was not inferior to 10 kU/m^2 [38]. Since the expected goal of reducing acute side effects with an acceptable rate of recurrences had already been achieved in the first studies, one of the guiding principles of the following MB studies was the possibility of further therapy de-escalation.

The SR definition in the first study was very simple [36]. SR patients were those aged above 1 year with WBC < 50 × 10^9/L, B-lineage immunophenotype, and no CNS involvement. In the study ALL MB 2008, SR criteria were specified: BCP-ALL, age > 1 year, WBC

Figure 5
EFS (solid red line), OS (solid blue line) and CIR (dashed red line) in low-risk groups defined according to MFC-MRD data obtained at day 15 (< 1%, *n* = 215, panel A) or at the EOI (< 0.1%, *n* = 247, panel B). Standard errors are shown in parentheses



< 30 × 10^9/L, enlargement of the spleen < 4 cm below the costal margin, absence of CNS3-status, no translocation t(4;11)(q21;q23)/*KMT2A/AFF1* or t(9;22)(q34;q11)/*BCR-ABL*, and achievement of hematological remission at the EOI. Patients who meet these criteria make about 50% of all ALL, and these patients were the subject of the underlying study.

Although widespread in international ALL protocols at the time [18, 25, 26], in Russia MRD monitoring and stratification of all patients in the multicenter, still growing, collaborative study was completely unrealistic. Therefore, this MFC-MRD pilot study had to be restricted to facilities connected to MFC laboratories of the MB group flow network [40, 48]. The design and availability of the data allowed us to examine response dates at different time points in order to find the most appropriate time point and cut-off to define a group of VLR patients in need of minimally intensive therapy.

Historically, MFC-MRD has not been used as widely for patient stratification as compared to molecular techniques [15, 17]. The PCR-based MRD detection is also carried out at a later time point (usually from the EOI) [22, 25] and thus leaves less room for treatment adjustments. For this reason, measurement of early (day 15 (19) or day 19 (29)) MFC-MRD response was considered a useful tool for early treatment changes. As already shown, patients with very rapid MFC-MRD clearance (less than 0.01% on day 19) could be successfully treated with a low-intensity regimen [34, 35]. The difference in the design of our study was that from the start we used a low-intensity protocol for children who initially met the SR criteria rather than trying to reduce treatment for rapid responders [30, 32, 33, 49]. Therefore, the main goal of the MFC-MRD measurement was to identify those patients for whom an excellent treatment result could be achieved with such a low-intensity protocol. This has allowed us to use a higher threshold for MFC-MRD positivity by day 15 (1%), thus identifying more patients who qualify for the VLR group. In addition, even MFC-MRD's EOI measurement perfectly revealed the ability to distinguish patients with excellent results from their less fortunate counterparts. Finally, it was found that the cut-off, which is an order of magnitude higher than the routine sensitivity of MFC-MRD detection, is the best discriminator for the subsets of SR patients.

The MFC-MRD assessment at the EOI is known to be controversial if applied together with PCR-based MRD stratification, since the results of both methods are least comparable at this time-point [50–52]. Still, day 36 seems best for a single MRD reading. First, the flow cytometric examination of the BM at the EOI is now regarded as essential for confirming remission [53, 54]. Therefore, in addition to the mandatory MFC-MRD measurements, no further MFC-MRD examinations need to be carried out. In addition, the cellularity in BM samples at EOI is always higher than on day 15 [55], when the BM contains many dead cells. This means that the reproducibility of the MFC-MRD measurement at the EOI is higher than at earlier times. At the same time, the use of the defined cut-off, in contrast to the sensitivity limit of the method, significantly increases the reliability. Even the possible occurrence of normal BCPs [56, 57], which are completely absent on the day 15 [55, 56, 58], cannot be viewed as a major obstacle to the use of EOI-MFC-MRD for stratifying patients. The main criticism of using EOI-MFC-MRD data for stratification is that almost half of recurrences occur in the MRD-negative subgroup [20]. In our study, which used intensity-reduced therapy from the start, nearly half of the relapses occurred in a small group of children with high MFC-MRD scores in patients originally classified as SR.

As shown, a single point measurement at EOI identified 90% of these SR patients as excellent responders with an MFC-MRD < 0.1% and an EFS of 95% and an OS of 97%. These patients, initially (provisionally) classified as SR patients, make up 50% of all ALL patients in the 2008 ALL-MB study (manuscript in preparation). With an induction mortality of 1.5% in this group and the EOI-MFC-MRD data presented here, we come to the conclusion that more than 40% of all children with ALL can be cured with this minimally intensive treatment. This protocol contains no alkylating agents, only 1 dose of DNR for the majority of patients, no ARA-C, no high-dose MTX requiring inpatient treatment, no radiation therapy, and no costly repeated PCR-based MRD measurements.

No child died from treatment-related complications or side effects. The rate of second malignancies was zero, most likely due to the lack of alkylating or mutagenic drugs other than very modestly dosed DNR. Despite the frequent use of DEXA as the sole glucocorticoid, the avascular bone necrosis rate was low and mainly affected girls over 10 years of age. Of interest may be the observation that PEG administered on day 3 of induction therapy resulted in a larger group with lower MFC-MRD levels both on day 15 and at EOI. Although the proportion of recurrences remained the same and the results were not statistically significantly different between the randomization arms, the early use of PEG could reduce the number of patients with high leukemia cell burden and thus improve the outcome of patients with initially favorable characteristics.

It is evident that the treatment outcomes with such a low-intensity regimen are inadequate for a very small group of children with high EOI-MFC-MRD values, even though they are in the SR group by baseline parameters. These patients need more intensive or other

innovative therapies. It is hardly to be expected that intensifying the treatment of slow MRD responders alone will promise success [28]. Presumably, modern, immunotherapeutic treatment approaches are more suitable for such patients [59].

CONCLUSION

The discussion of reduced-intensity protocols is usually aimed at emerging or low-income countries with limited resources. But less intensive treatment means less stress and hardship, a better quality of life and fewer undesirable long-term consequences, especially also fewer secondary malignancies, not only for children in these countries but also for all affected children and their families. Finally, treatment with reduced intensity also contributes to the economization of medicine and is therefore sensible for numerous reasons. Why shouldn't the children here and in other industrialized countries as well as the public health system benefit from such treatment?

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ORCID
Popov A.M. ORCID http://orcid.org/0000-0002-0889-6986
Roumiantseva Yu.V. ORCID: https://orcid.org/0000-0001-9670-3728
Budanov O.I. ORCID: http://orcid.org/0000-0003-3232-2322
Verzhbitskaya T.Yu. ORCID: https://orcid.org/0000-0001-9329-1828
Tsaur G.A. ORCID: https://orcid.org/0000-0002-9881-6221
Lagoiko S.N. ORCID: https://orcid.org/0000-0002-3793-104x
Zharikova L.I. ORCID: https://orcid.org/0000-0002-1105-8676
Myakova N.V. ORCID: http://orcid.org/0000-0002-4779-1896
Litvinov D.V. ORCID: https://orcid.org/0000-0002-7461-0050
Ponomareva N.I. ORCID: https://orcid.org/0000-0002-6499-0977
Novichkova G.A. ORCID: https://orcid.org/0000-0002-2322-5734
Fechina L.G. ORCID: https://orcid.org/0000-0002-1885-3912
Aleinikova O.V. ORCID: https://orcid.org/0000-0003-0143-1921
Karachunskiy A.I. ORCID: http://orcid.org/ 0000-0002-9300-5198

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