Oral Methotrexate/6-mercaptopurine may be Superior to a Multidrug LSA$_2$L$_2$ Maintenance Therapy for Higher Risk Childhood Acute Lymphoblastic Leukemia

Results From the NOPHO ALL-92 Study

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Summary: The importance of maintenance therapy for higher risk childhood acute lymphoblastic leukemia (ALL) is uncertain. Between 1992 and 2001 the Nordic Society for Pediatric Haematology/Oncology compared in a nonrandomized study conventional oral methotrexate (MTX)/6-mercaptopurine (6MP) maintenance therapy with a multidrug cyclic LSA$_2$L$_2$ regimen. 135 children with B-lineage ALL and a white blood count $\geq 50 \times 10^9$/L and 98 children with T-lineage ALL were included. Of the 234 patients, the 135 patients who received MTX/6MP maintenance therapy had a lower relapse risk than the 98 patients who received LSA$_2$L$_2$ maintenance therapy, which was the case for both B-lineage (27% ± 5% vs. 45% ± 9%; $P = 0.02$) and T-lineage ALL (8% ± 5% vs. 21% ± 5%; $P = 0.12$). In multivariate Cox regression analysis stratified for immune phenotype, a higher white blood count ($P = 0.01$) and administration of LSA$_2$L$_2$ maintenance therapy ($P = 0.04$) were both related to an increased risk of an event (overall P value of the Cox model: 0.003), whereas neither sex, age at diagnosis, administration of central nervous system irradiation, nor presence of a day 15 bone marrow with $\geq 25\%$ versus $< 25\%$ lymphoblasts were of statistical significance. These results indicate that oral MTX/6MP maintenance therapy administered after the first year of remission can improve the cure rates of children with T-lineage or with higher risk B-lineage ALL.

Key Words: acute lymphoblastic leukemia, maintenance therapy, immune phenotype, relapse rate

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The overall cure rate for childhood acute lymphoblastic leukemia (ALL) has reached eighty percent.[1,2] However, for patients with higher risk (HR) features such as T-cell disease and white blood cell count (WBC) $\geq 50 \times 10^9$/L, the increase in event-free survival has been less impressive, and many of the treatment failures occur during therapy.[1] The treatment of childhood ALL consists of 4 treatment elements: (a) remission induction, (b) consolidation and delayed intensification, (c) central nervous system (CNS)-directed treatment, and (d) maintenance therapy, which is continued 2.0-3.0 years from the time of diagnosis.[3] The backbone of most maintenance therapy programs consists of oral daily 6-mercatopurine (6MP) and weekly methotrexate (MTX) with dosages adjusted to keep the WBC below 3.0-3.5×10^9/L.[4] Many randomized studies have supported the necessity of maintenance therapy, at least for children with B-lineage ALL, although its mode of action is uncertain.[5,6] Recently, reduction of maintenance therapy to only 6 months with a total duration of antileukemic therapy of 1 year was tested by the Tokyo ALL group.[7] This strategy gave a 5-year event-free survival (EFS) for nonhigh risk patients (age < 7.0 y and WBC $< 20 \times 10^9$/L) of only 60%, but a survival rate of 91%. The 5-year EFS for the highest risk patients (including T-ALL with WBC $> 50 \times 10^9$/L, and B-lineage ALL with a WBC $> 100 \times 10^9$/L) was 63%, which is rather similar to what has been obtained in historical controls or by other collaborative groups, who offered longer therapy for HR ALL.[2,8–10] That study as well as other clinical observations have questioned whether a treatment duration of 2 years, and more specifically extended MTX/6MP maintenance therapy, is necessary for HR ALL patients.

On the basis of unsatisfactory results of the Nordic Society for Paediatric Haematology and Oncology (NOPHO) ALL-86 protocol for patients with HR ALL,[2,11] the NOPHO tested in a nonrandomized study the efficacy of multidrug, cyclic LSA$_2$L$_2$ remission maintenance therapy[12] compared with oral MTX/6MP maintenance therapy for patients with HR features. The present report analyses the outcome of patients with B-lineage ALL and a WBC $\geq 50 \times 10^9$/L or with T-lineage ALL, who received either of these 2 types of maintenance therapy. The overall results of Nordic HR-ALL patients treated according to the NOPHO ALL-92 protocol have previously been published.[2,13]

PATIENTS AND METHODS

Patients

From January 1992 to October 2001 1703 children of 1.0 to 14.9 years of age were diagnosed with B-cell precursor (pre-B) or T-cell ALL in the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) (Fig. 1). Two patients with Down syndrome received no antileukemic therapy, 10 patients were treated according to non-NOPHO protocols, 1 patient was treated according to the previous NOPHO ALL-86 protocol, and 45 patients were treated according to the NOPHO ALL-2000 protocol, before it was officially opened. Of the remaining 1645 patients, who started therapy according to the NOPHO ALL-92 protocol, 22 patients died during induction therapy, and 9 patients had resistant disease and were excluded from further analysis. Finally, 2 patients with HR-ALL received neither MTX/
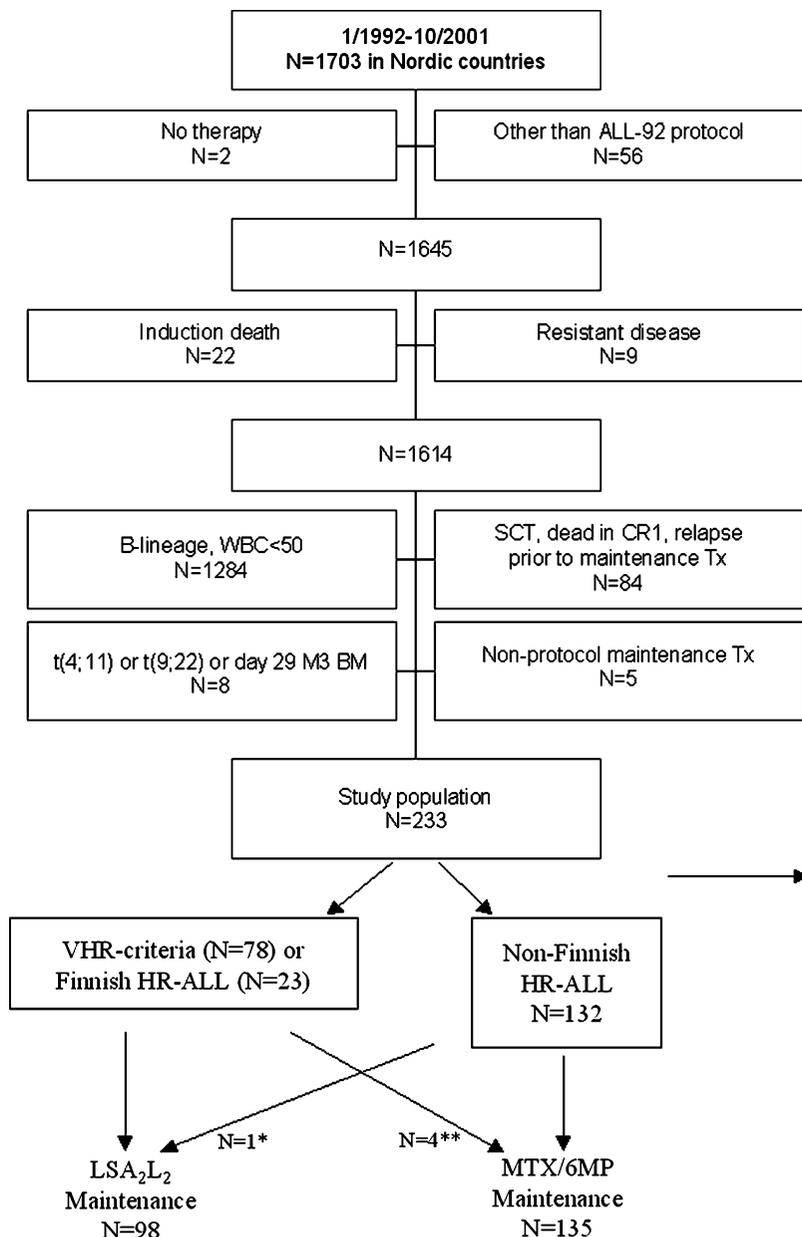

FIGURE 1. Patient inclusion/exclusion chart on all childhood acute lymphoblastic leukemia cases from 1.0-14.9 years of age diagnosed in the Nordic countries between January 1992 and October 2001. WBC count ($\times 10^9$/L). SCT = stem cell transplantation. CR1 = first remission. Day 29 M3 bone marrow = $\geq$ 25% leukemic blasts in the bone marrow at protocol treatment day 29. Tx = therapy. *A 4.9-year-old girl with T-acute lymphoblastic leukemia and WBC>50 $\times 10^9$/L was treated according to the VHR-ALL protocol including CNS irradiation and LSA$_2$L$_2$ maintenance therapy. **Three patients with VHR-ALL criteria (all with WBC >100 $\times 10^9$/L) and 1 Finnish HR-ALL patients were by the treating clinician assigned to HR-ALL protocol and received oral methotrexate/6mercaptopurine maintenance therapy. HR-ALL indicates higher risk acute lymphoblastic leukemia; VHR-ALL, very high-risk acute lymphoblastic leukemia; WBC, white blood cell.

6MP nor LSA_2L_2 maintenance therapy, and 3 HR-ALL patients shifted between these 2 maintenance therapies. None of the latter 5 patients have so far developed a relapse, and they were all excluded from this study.

Of the remaining 1609 patients, 325 patients had B-lineage ALL with a WBC $\geq 50 \times 10^9$/L or T-lineage ALL and were treated according the NOPHO HR protocols. Of these 84 patients did not receive maintenance therapy during their first remission owing to stem cell transplantation in first complete remission (CR1, n = 32), death in CR1 (N = 7), or a relapse before the scheduled start of maintenance therapy (n = 45).

Of the remaining patients we furthermore excluded patients allocated to the HR-protocol owing to a t(4;11)(q21:2q23)-translocation (n = 1), a t(9;22)(q34:q11)-translocation (n = 2), or 5% or more leukemic blasts in the bone marrow day 29 (n = 5).

The clinical characteristics and treatment assignment of the remaining eligible 152 boys and 81 girls with 98 cases of T-lineage and 135 B-lineage are given in Table 1. None of the patients had Down syndrome.

Risk Group Assignment

In the NOPHO ALL-92 protocol the HR groups were defined by WBC ($\geq 50 \times 10^9$/L) at diagnosis, T-lineage ALL, the presence of CNS or testicular involvement, translocations t(9;22)(q34:q11) or t(4;11)(q21;q23), lymphomatous leukemia or mediastinal lymphoma, or a poor treatment response (M3 bone marrow at day 15 or M2/M3 at day 29).[2,13] In the NOPHO ALL-92 protocol, patients who had HR features were to be assigned to the very high risk (VHR) treatment arm, if they were at least 5 years of age at diagnosis (owing to the use of cranial irradiation in that protocol arm) and in addition had (1) CNS leukemia, (2) lymphomatous leukemia, (3) HR ALL at diagnosis and a day 15 M3 or a day 29 M2/M3 bone marrow, and/or (4) T-cell disease with 1 or more additional HR-features. In addition to the inclusion of cranial irradiation, the primary therapeutic difference between the HR-protocol and VHR-protocol was the substitution of MTX/6MP (HR) with LSA_2L_2 maintenance therapy (VHR).[2] Finally, to explore whether more intensive cyclic multi-drug maintenance therapy regimen could reduce the relapse rate for patients with HR features, Finnish patients with HR features received LSA_2L_2 maintenance therapy irrespective of whether their induction/consolidation/CNS-directed therapy

had been according to the HR-ALL regimen or VHR-ALL regimen.[14]

Of the 233 patients, 131 patients had HR-ALL and 75 patients had VHR-ALL and were treated accordingly, and 22 Finnish HR-ALL patients were treated according to the HR-protocol, but received LSA_2L_2 maintenance therapy. The last 5 patients did not receive maintenance therapy according to the protocol stratification (Fig. 1). None of these 5 patients have developed an event, and they were included in survival analysis according to the maintenance therapy they actually received. Of the 22 Finnish HR-ALL patients, who were treated with LSA_2L_2 maintenance therapy, all were below 5.0 years of age at diagnosis, and 20 had B-lineage ALL.

Treatment

On the basis of risk group assignments, patients were treated according to the HR or VHR treatment arms[2,13] (Fig. 2).

Induction Therapy

For all patients this consisted of prednisolone (60 mg/m^2/day on days 1 to 36, then tapered), weekly vincristine (VCR, 2.0 mg/m^2 6 times), doxorubicin (40(mg/m^2 4 times, asparaginase (30,000 IU/m^2 daily on days 37 to 46), and intrathecal (IT) MTX on 4 occasions.

Consolidation therapy included alternating series of: (1) intravenous administration of cyclophosphamide (total cumulative dose: 3 g/m^2) with low-dose cytarabine and either oral 6MP or oral 6-thioguanine; (2) HD-MTX 8 g/m^2/24 hours with IT. MTX and leucovorin rescue 3 (VHR) or 4 (HR) times alternating with high-dose cytarabine [12 g/m^2 times 3 (VHR) or 4 (HR)] with 1 (VHR) or 2 (HR) 2-months interval periods of oral weekly MTX and daily 6MP with 2 VCR/prednisolone reinductions per period; (3) 4 weeks of delayed intensification with dexamethasone (10 mg/m^2/day for 3 weeks, then tapered), weekly VCR (2.0 mg/m^2 4 times), weekly daunorubicine (30 mg/m^2/day 3 times), and asparaginase (30,000 IU/m^2 4 times).[2]

CNS Irradiation

In total 76 nontransplanted patients on the VHR-ALL protocol received cranial irradiation in CR1, which included 2 T-ALL patients of 4.5 and 4.9 years at diagnosis of ALL, but above 5 years of age at the time of irradiation. Five patients with CNS leukemia at diagnosis and 4

TABLE 1. Patient Characteristics and Relapse Rate in Relation to Type of Maintenance Therapy

Clinical Characteristics	MTX/6MP	LSA_2L_2	*P*
Male/female	82/53	70/28	ns
Median age (50% range)	3.8 (2.5-5.9)	8.1 (5.1-12.1)	< 0.001
Median WBC (50% range)	70 (53-113)	91 (58-153)	0.09
B-lineage/T-lineage	99/36	36/62	< 0.001
Projected probability of relapse % (SE)			
Male/female	25 (5)/19 (5)	29 (6)/32 (9)	ns
Age: 1.0-4.9/5.0-9.9/10.0-14.9 y	24 (5)/15 (6)/32 (16)	46 (10)/29 (8)/20 (7)	0.03
B-lineage: WBC: 50-99/$\geq$ 100	21 (6)/38 (8)	49 (11)/40 (13)	0.04
T-lineage: WBC: 0-49/50-99/$\geq$ 100	9 (6)/14 (13)/0	23 (9)/15 (10)/22 (8)	0.11
Relapse (n): BM/isol. CNS (any CNS)/testis/other	25/4 (9)/0/0	23/2 (5)/0/3	

The projected probabilities of relapse are calculated at the time of the latest relapse (10.8 years) as percentages with standard errors (SE) in brackets. *P* values for survival comparisons between MTX/6MP and LSA_2L_2 maintenance therapy are performed with stratification for subsets.

6MP indicates 6-mercaptopurine; BM, any relapse involving bone marrow; isol. CNS, isolated CNS relapse (any relapse involving CNS); MTX, methotrexate; ns, not significant; SE, standard error; testes, isolated testicular relapse; WBC, white blood cell count at diagnosis.
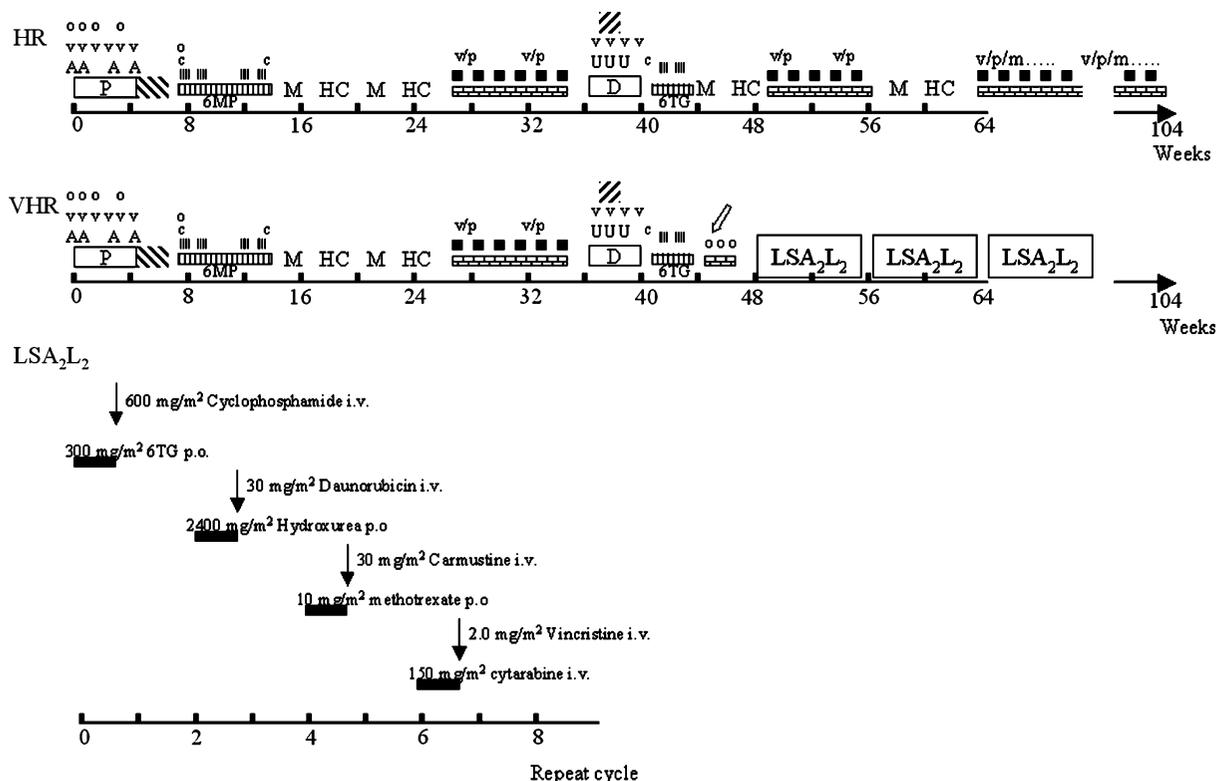
FIGURE 2. Overview of Nordic Society for Paediatric Haematology and Oncology acute lymphoblastic leukemia-92 high risk and very high-risk therapy (text). [P] = oral prednisolone (60 mg/m²/d). V = vincristine (2.0 mg/m²). A = doxorubicin (40 mg/m² IV), = asparaginase (30,000 IU/m² per day for 10 d). o = IT MTX by age. HC = high dose AraC (2.0 g/m² × 6). M = high-dose MTX [5 g/m²/24 h or 8 g/m² (HR only)]. V/P = vincristine (2.0 mg/m² × 1)/prednisolone (60 mg/m²/d for 7 d) reinductions. ■ ■ ■ = MTX 20 mg/m²/wk. = 6MP 75 mg/m²/day. c = cyclo-phosphamide (1 g/m² IV). Low-dose AraC (75 mg/m²). = 6MP or 6TG (thioguanine) 60 mg/m²/d. U = daunorubicin (30 mg/m²), = asparaginase (30,000 IU/m² 2 times weekly for 2 wk). HC = high-dose AraC (2 g/m² twice daily for 3 d). The v/p = vincristine (2.0 mg/m² × 1)/prednisolone (40 mg/m²/d for 7 d) reinductions without or with (/m) methotrexate intrathecally in age-adjusted doses. = cranial irradiation. [LSA₂L₂] = LSA₂L₂ maintenance therapy. Each LS₂A₂ block consisted of 4 courses at 2 weeks intervals with alternating combinations of (1) IT. MTX, 6TG (300 mg/m² × 4), and IV. Cyclophosphamide (600 mg/m² IV × 1), (2) Hydroxyurea (2400 mg/m² for 4 d) and daunorubicin (30 mg/m² × 1; substituted with prednisone and VCR for cycle 5 and 6), (3) oral MTX (10 mg/m² for 4 d) and BCNU (30 mg/m² IV × 1), and (4) IV cytarabine (150 mg/m² IV for 4 d) and VCR (2.0 mg/m² × 1). The scheduled interval between day 1 in each course is 2 weeks, but delayed if absolute neutrophil counts is less than 1.0×10^9/L and/or thrombocyte count is less than 100×10^9/L. AraC indicates Cytarabine; BCNU, Carmustine; HR, higher risk; IV indicates intravenous; IT, intrathecal; MP, mercaptopurine; MTX, methotrexate; 6TG, 6-thioguanine; VCR, vincristine.

additional patients with T-ALL (with 1 or more additional high risk features) received 24 Gy cranial irradiation, whereas the remaining 65 patients received 18 Gy. No spinal irradiation was given.

The 6MP/MTX maintenance therapy with starting oral 6MP doses of 75 mg/m²/day and oral MTX doses of 20 mg/m²/week was initiated at treatment week 63, titrated to a WBC between 1.5 and 3.5×10^9/L, and continued until 2.0 years after diagnosis. Every 8 weeks throughout maintenance therapy the patients received reinductions of VCR (1.5 mg/m² once) and prednisolone (40 mg/m²/day for 1 week) with IT. MTX is given in age-adjusted doses.

LSA₂L₂ maintenance therapy was given to VHR-patients and to Finnish HR patients and discontinued 2 years from diagnosis irrespective of whether or not the patients had received all 6 scheduled LS₂A₂ courses. Each LS₂A₂ block consisted of 4 courses at 2 weeks intervals with alternating drug combinations (Fig. 2).[2,12] Blood counts, treatment delays, and the number of LS₂A₂ blocks administered to each patient were not registered centrally.

Stem Cell Transplantation

None of the patients received stem cell transplantation in first complete remission after having initiated maintenance therapy.

Statistics

Survival analyses were performed with a basic time scale defined by the date of diagnosis. As events in the EFS analyses, we included relapse, death in remission, or the diagnosis of an SMN, whichever occurred first. Patients who died in first remission or developed a SMN were censored at the time of these events in the analyses of risk factors for a leukemic relapse. Cox proportional hazard regression analyses were performed with the likelihood ratio test for differences in outcome.[15,16] Nonparametric methods were applied to compare the distribution of parameters between subgroups.[17] The Kaplan-Meier method was applied for estimation of remission duration and for the generation of survival curves.[18] Subgroups were compared with the log-rank test,[19] stratified where needed.

Two-sided P values < 0.05 were regarded as significant. Survival analyses were performed with the SAS statistical software. The median follow-up of the 172 patients who did not experience an event was 10.8 years (50% range: 8.2 to 12.5 y).

RESULTS

Of the 233 patients, 1 T-ALL patient on MTX/6MP maintenance therapy died in CR1, 1 T-ALL patient, who had received MTX/6MP maintenance therapy, developed a second malignant neoplasm (acute myeloid leukemia), and 58 patients developed a relapse of ALL 1.2-10.8 years from diagnosis (median: 2.3 y) with a cumulative incidence of relapse of $26\% \pm 3\%$. In total, 36 patients died 1.9 to 6.9 years from diagnosis (median: 3.0 y). The overall projected 11-year event-free survival ($pEFS_{11y}$) and overall survival of the 234 patients was 0.74 ± 0.03 and 0.85 ± 0.02, respectively, with no significant difference between boys and girls.

Overall, patients with T-lineage ALL fared better than those with B-lineage ($pEFS_{11y}$: 0.82 ± 0.04 vs. 0.68 ± 0.04; $P = 0.05$) with a significant difference in risk of relapse ($16\% \pm 4\%$ vs. $32\% \pm 4\%$; $P = 0.02$). For patients with B-lineage ALL, the 10 patients with a WBC $\geq 200 \times 10^9/L$ fared significantly worse than the remaining patients ($pEFS_{11y}$: 0.30 ± 0.15 vs. 0.71 ± 0.04; $P = 0.001$), whereas the WBC at diagnosis did not significantly influence the event risk for those with T-lineage ALL.

Table 1 presents the impact of the 2 types of maintenance therapy by sex, age, immunophenotype, and WBC at diagnosis. In survival analysis stratified by immunophenotype, the patients who received oral MTX/6MP maintenance therapy had a significantly lower relapse risk than the patients who received LSA_2L_2 maintenance therapy ($P = 0.006$), and that was true for both B-lineage ALL (n = 135, $pRelapse_{11y}$: $27\% \pm 5\%$ vs. $45\% \pm 9\%$; $P = 0.02$) and T-lineage ALL (n = 98, $pRelapse_{11y}$: $8\% \pm 5\%$ vs. $21\% \pm 5\%$; $P = 0.12$) (Fig. 3). As relapses occurring after only a few months of maintenance therapy may not be linked to the type of maintenance therapy given, survival analysis with stratification for lineage (B vs. T) were performed with exclusion of patients who had a relapse within 2.0 years from diagnosis. Still, those who received oral MTX/6MP maintenance therapy had a significantly lower relapse risk than the patients who received LSA_2L_2 maintenance therapy ($P = 0.01$).

The 132 HR-ALL patients, who were treated with oral MTX/6MP maintenance therapy, did significantly better than the 23 HR-ALL patients, who were treated according to the LSA_2L_2 protocol ($pEFS_{11y}$: 0.76 ± 0.04 vs. 0.57 ± 0.10; $P = 0.02$). This difference in EFS was also significant for B-lineage HR-ALL patients (n = 118; $pEFS_{11y}$ 0.74 ± 0.05 vs. 0.50 ± 0.11; $P = 0.01$). The difference in $pEFS_{11y}$ between the 2 maintenance therapy groups was as pronounced, if only the 95 HR B-lineage ALL patients below 5.0 years of age were included, as this characterized the B-lineage HR-ALL patients in the Finnish LSA_2L_2 Maintenance Therapy Study ($pEFS_{11y}$: 0.73 ± 0.06 and 0.50 ± 0.11, $P = 0.01$) (Fig. 4). This difference in EFS stayed significant if the B-lineage ALL patients with WBC $\geq 200 \times 10^9/L$ were excluded, or if the impact of the type of maintenance therapy was explored in
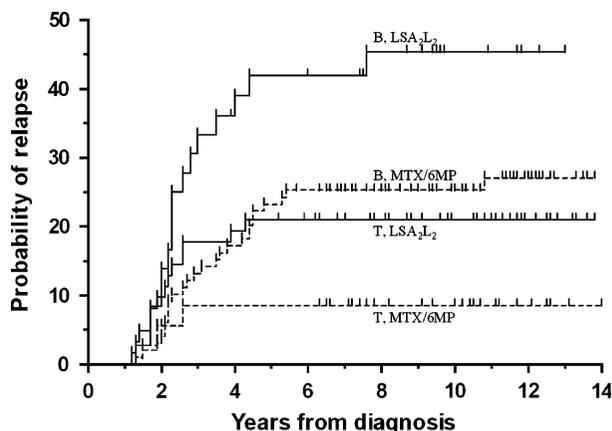

FIGURE 3. Risk of relapse with respect to the type of maintenance therapy. Oral MTX/6MP (lower curves) versus LSA_2L_2 maintenance therapy (upper curves). B-cell precursor ALL: N = 135, probability of relapse $27\% \pm 5\%$ versus $45\% \pm 9\%$; $P = 0.02$. T-lineage ALL: N = 98, probability of relapse $8\% \pm 5\%$ versus $21\% \pm 5\%$; $P = 0.12$. ALL indicates acute lymphoblastic leukemia; MP, mercaptopurine; MTX, methotrexate.

Cox regression analysis with adjustments for the WBC (data not shown).

CNS relapse and CNS irradiation: of the 58 relapses, 48 involved the bone marrow and 14 involved the CNS of which 6 were isolated CNS relapses. The risk of any CNS-involving relapse was $7\% \pm 2\%$ for the patients who received MTX/6MP maintenance therapy and $5\% \pm 2\%$ for the patients who received LSA_2L_2 maintenance therapy ($P = 0.73$). The risk of CNS relapse did not differ significantly between those that did and did not receive CNS irradiation in first remission ($5\% \pm 3\%$ and $7\% \pm 2\%$, respectively; $P = 0.80$). Furthermore, the 76 patients who received CNS irradiation did not differ significantly in their overall risk of relapse from those, who were not irradiated ($24\% \pm 5\%$ vs. $26\% \pm 4\%$, $P = 0.90$) or in the fraction of relapses that involved the
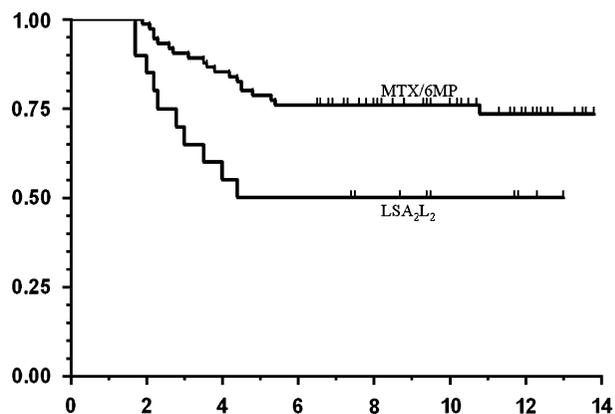

FIGURE 4. Event-free survival curves for high-risk ALL less than 5 years of age. Upper curve: oral methotrexate/6-mercaptopurine maintenance therapy; lower curve: LSA_2L_2 maintenance therapy (text); $pEFS_{11y}$: 0.76 ± 0.04 and 0.57 ± 0.10, $P = 0.02$. ALL indicates acute lymphoblastic leukemia; $pEFS_{11y}$, projected 11-year event-free survival.

CNS (4/18 = 22% vs. 10/40 = 25%). Among the 98 patients who received LSA_2L_2 maintenance therapy, only 18 out of the 76 patients that received cranial irradiation had a relapse compared with 11 out of the 24 patients who did not receive cranial irradiation ($pRelapse_{11y}$: 24% ± 5% vs. 50% ± 11%; $P = 0.03$). However, 21 out of the latter 24 patients who did not receive CNS irradiation had B-lineage ALL, compared with only 16 of the 60 patients that received CNS irradiation. After exclusion of the 76 patients who had received CNS-irradiation, those who received oral MTX/6MP maintenance therapy still had a significantly lower relapse risk than the patients who received LSA_2L_2 maintenance therapy ($P = 0.007$).

Owing to the differences in the distribution of clinical characteristics among the patients that received the 2 types of maintenance therapy, we used Cox multivariate regression model with stratification for the immunophenotype to test the effect on event risk of sex, WBC at diagnosis, age at diagnosis, whether or not CNS irradiation was given, a day 15 bone marrow with ≥ 25% versus < 25% lymphoblasts, and oral MTX/6MP (code = 1) versus LSA_2L_2 maintenance therapy (code = 0). Higher WBC ($P = 0.01$) and administration of LSA_2L_2 maintenance therapy ($P = 0.04$) were both related to an increased risk of an event (overall P value of the Cox model: 0.003). None of the other covariates reached a significance level < 0.05 in any of the backward steps.

As the patients who received LSA_2L_2 maintenance therapy included relatively more boys and more T-lineage leukemias, and had a significantly higher age and a slightly higher WBC at diagnosis (Table 1), we did a Cox regression analysis with stratification by immunophenotype and with forced inclusion of sex, age, and WBC at diagnosis, presence of CNS-leukemia at diagnosis, administration of CNS irradiation, and the type of maintenance therapy given. Even with these adjustments in the Cox model for potential confounders, the patients who received LSA_2L_2 maintenance therapy had a significantly HR of relapse than those who received oral MTX/6MP maintenance therapy ($P = 0.01$).

DISCUSSION

Today the most effective treatment protocols achieve 80% cure rates for children with ALL, which reflects an impressive development in the biologic understanding of the disease and risk grouping, in the intensity of the treatment programs, and in the supportive care.

The use of maintenance therapy is rather specific for ALL. The mode of action of MTX/6MP maintenance therapy could include elimination of persistent preleukemic cells and true leukemic cells,[20] modulation of apoptotic pathways,[21,22] direct antileukemic action,[23,24] induction of differentiation,[25] and/or changes in stromal support[22,26,27] including antiangiogenic mechanisms.[28,29] The metronomic structure of low-dose oral MTX/6MP maintenance therapy may be of significance for several of these modes of action.[30]

Even though maintenance therapy seem to be crucial for cure for a large proportion of childhood ALL cases,[6,31,32] the optimal way to administer MTX/6MP maintenance therapy is yet to be determined, which can explain why the large collaborative groups differ in their guidelines for dose adjustments.[4] Several studies have indicated that the treatment intensity of oral MTX/6MP maintenance therapy is important, whether measured by drug dosage,[33,34] the degree of myelosuppression[35] or hepatotoxicity,[36] or by cytotoxic MTX/6MP metabolite levels.[37,38] However, children with non-HR ALL have dominated most studies on maintenance therapy, and it has been unclear to what extent the conclusions are valid for T-lineage and HR B-lineage ALL. Furthermore, as the complexity of frontline ALL therapy increases, it becomes difficult to evaluate the efficacy and necessity of the different treatment phases outside randomized studies.

Thus, the results of this study should be interpreted cautiously, not least owing to unequal distribution of risk factors among patients on MTX/6MP and LSA_2L_2 maintenance therapy. However, even taking this into account, this nonrandomized study indicates that oral MTX/6MP maintenance therapy is important even for patients with HR features, although the results for T-lineage ALL did not reach statistical significance possibly owing to the lower number of T-ALL patients included. Others have similarly shown that the original United States Childrens' Cancer Group modified LSA_2L_2 treatment is inferior to a more conventional ALL treatment with delayed intensifications and oral MTX/6MP maintenance therapy for patients with poor prognostic features.[12] However, in that study the LSA_2L_2 cycles made up most of the treatment, whereas in the NOPHO ALL-92 study the LSA_2L_2 maintenance therapy was initiated more than 1 year from diagnosis, that is, after the patients had received consolidation therapy, delayed intensification, and CNS-directed therapy with high-dose MTX and high-dose cytarabine with or without CNS irradiation.[2,13] The fact that the relapse rate of the patients even at this late stage of their therapy was significantly influenced by the type of maintenance therapy, is in line with previous publications that demonstrate that the intensity of oral 6MP/MTX maintenance therapy significantly influences the risk of relapse even for patients with high risk features.[35] Similar to the results published by the US Childrens Cancer Group, we found that patients who received both LSA_2L_2 maintenance therapy and cranial irradiation had a significantly lower probability of relapse than those patients who received LSA_2L_2 maintenance therapy but no irradiation.[12]

The reason for the inferiority of the LSA_2L_2 regimen is uncertain. Firstly, the LSA_2L_2 regimen may induce more cytopenic episodes than conventional oral MTX/6MP therapy, which was titrated to avoid both high WBC (target: $< 3.5 \times 10^9/L$) and low WBC (target: $\geq 1.5 \times 10^9/L$). Thus, LSA_2L_2 therapy carries a risk of treatment interruptions, which may allow the regrowth of leukemic cells.[33,34] Whether that played a role for the relapse rate in this study is not known, as treatment delays were not routinely registered. Secondly, the LSA_2L_2 regimen used in the ALL-92 protocol included hydroxyurea and carmustine, which are anticancer agents with little antileukemic efficacy. It has previously been shown that substituting these agents with more efficacious drug combinations and pushing the doses intravenously. MTX to biologic tolerance during the cyclic LSA_2L_2 regimen may yield cure rates as good as, although not superior to, that achieved by MTX/6MP maintenance therapy.[12] No published data support that any other pulsed regimens offer better cure rates than conventional MTX/6MP maintenance therapy. Finally, the patients on the VHR regimen received only 2 courses of high-dose methotrexate and of cytarabine, respectively, compared to

4 courses of high-dose methotrexate and of cytarabine for those 1 the HR regimen. However, this is unlikely to explain the differences in relapse rates, as the Finnish HR-ALL patients that received LSA_2L_2 maintenance therapy did significantly worse than the non-Finnish HR-ALL patients on MTX/6MP maintenance therapy even though these subsets had received the same consolidation and delayed intensification treatment. Noteworthy, when analysing all noninfant children with ALL in the Nordic countries, the Finnish patients had an EFS that did not differ significantly from that of the non-Finnish Nordic patients (data not shown).

In conclusion, these results indicate that oral MTX/6MP maintenance therapy administered after the first year of remission may improve the cure rates of children with T-lineage and with HR B-lineage ALL. Accordingly, in the NOPHO ALL-2008 protocol, oral MTX/6MP maintenance therapy will be given to all risk groups until 2.5 years from diagnosis.

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