

ORIGINAL ARTICLE

***Pneumocystis jiroveci* pneumonia prophylaxis during maintenance therapy influences methotrexate/6-mercaptopurine dosing but not event-free survival for childhood acute lymphoblastic leukemia**

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Abstract

Trimethoprim-sulfamethoxazole (TMP/SMX) is used in children with acute lymphoblastic leukemia (ALL) to prevent *Pneumocystis pneumonia* (PCP). We explored to which extent TMP/SMX influenced methotrexate (MTX)/6-mercaptopurine (6MP) dosage, myelosuppression, and event-free survival (EFS) during maintenance therapy. Of 447 study patients treated by the NOPHO ALL92 protocol, 120 patients received TMP/SMX continuously for 2–7 d/wk (TMP/SMX_{2–7}) and 287 patients never received TMP/SMX (TMP/SMX_{never}). Ten patients (all TMP/SMX_{never}) developed PCP, eight of which occurred within 7 months from the start of maintenance therapy. The TMP/SMX_{2–7} group received lower oral 6MP doses than TMP/SMX_{never} patients (50.6 vs. 63.9 mg/m²/d; $P < 0.001$) but had lower absolute neutrophil counts (ANC) (median 1.7 vs. 2.0×10^9 /L; $P < 0.001$). In Cox multivariate analysis, higher ANC levels ($P = 0.04$) and male gender ($P = 0.06$) were related to reduced EFS. ANC had no effect on EFS among TMP/SMX_{2–7} patients ($P = 0.40$) but did for TMP/SMX_{never} patients ($P = 0.02$). The difference in the effect on EFS between TMP/SMX_{2–7} and TMP/SMX_{never} patients was not significant ($P = 0.46$). EFS did not differ between TMP/SMX_{2–7} and TMP/SMX_{never} patients (0.83 vs. 0.83; $P = 0.82$). These results suggest that TMP/SMX is effective in preventing PCP and may have an antileukemic effect. TMP/SMX should be given the entire duration of maintenance therapy.

Key words childhood acute lymphoblastic leukemia; treatment; infection

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The treatment of childhood leukemia is a delicate balance between the antileukemic efficacy and the risk of unacceptable toxicities. The intensification of antileukemic treatment has been feasible owing to improved supportive care. The combination of trimethoprim and sulfamethoxazole (TMP/SMX) is frequently used in

children with acute lymphoblastic leukemia (ALL) to prevent *Pneumocystis pneumonia* (PCP) (Table 1), caused by *Pneumocystis jiroveci*, formerly known as *Pneumocystis carinii*. Most healthy individuals are colonized in the lung alveoli during early life (1, 2), and 75% of all children have antibodies to *P. jiroveci* by the age

Table 1 Current administration of trimethoprim-sulfamethoxazole treatment during maintenance therapy for childhood acute lymphoblastic leukemia (ALL) according to study group

Study group	Duration of trimethoprim-sulfamethoxazole treatment	Total dose/d ¹	No. of doses/d	No. of d/wk	Consecutive days recommended	Comments
AIEOP	Throughout	5	2	1	n/a	
BFM Austria	Throughout	5	2	3	Yes	
BFM Germany	Throughout	5	2	2	Yes	
COALL	Throughout	5	2	2	No	Treatment continued until 6 wk after cessation of MT
COG	Throughout	5	2	3	Yes	
CPH	Throughout	5	2	2	Yes	
DCOG	Throughout	3	n/a	3	n/a	
DFCI	Throughout	5	1–2	3	n/a	Treatment continued until 6 months after cessation of MT
EORTC	Throughout	5	2	3	n/a	
FRALLE	Throughout	5	1	3	No	Treatment continued after cessation of MT until lymphocyte count above 1×10^9
INS	Throughout	5	n/a	3	n/a	
JPLSG	Throughout	5	2	3	n/a	The guideline for 2011, currently no uniform guideline among groups in Japan
NOPHO	First months of MT	5	2	2–3	No	Specifically stated not to be given during MT except for the first few months ²
SJCRH	Throughout	150 ³	2	3	Yes	
TPOG	Throughout	5	2	3	No	Treatment continued until 3 (standard or low risk) or 6 months (other patients) after cessation of MT
UK CCLG	Throughout	80/120/160 ⁴	2	2	Yes	

AIEOP indicates Associazione Italiana di Ematologia ed Oncologia Pediatrica; BFM, Berlin–Frankfurt–Münster ALL Study Group; COALL, Cooperative ALL Study Group; COG, Children's Oncology Group; CPH, Working Group for Pediatric Hematology in the Czech Republic; DCOG, Dutch Childhood Oncology Group; DFCI, Dana–Farber Cancer Institute ALL Consortium; EORTC, European Organisation for Research and Treatment of Cancer; FRALLE, French Acute Lymphoblastic Leukemia Pediatric group; INS, Israeli National Studies of childhood ALL; JPLSG, Japanese Pediatric Leukemia/Lymphoma Study Group; NOPHO, Nordic Society of Paediatric Haematology and Oncology; SJCRH, St Jude Children's Research Hospital; TPOG, Taiwan Pediatric Oncology Group; UK CCLG, United Kingdom Children's Cancer and Leukaemia Group; MT, maintenance therapy; n/a, not available.

¹mg of trimethoprim/kg/d.

²Since July 2008. Based on the data from Poulsen *et al.* (28) and Rees *et al.* (13).

³mg of trimethoprim/m².

⁴mg of trimethoprim/0.5–0.75 m²/0.75–1.0 m²/above 1.0 m².

of 4 (3). The primary infection is nearly always asymptomatic (4). However, *P. jiroveci* is a frequent clinical disease in immunocompromised patients with PCP as the most common manifestation (1). PCP is a potentially fatal infection, but it can be treated successfully with TMP/SMX (1, 5). TMP/SMX is presently the drug of choice for both prevention and treatment of PCP in children with ALL (1, 2, 4). For prophylaxis, it seems as effective given 3 d a week as when given daily (1). However, in children with ALL, there is no international consensus regarding PCP prophylaxis (1). Although a study from 1980 demonstrated that without PCP prophylaxis the vast majority of PCP cases occur within the first months of therapy (6), there is little published data from contemporary protocols on the incidence of PCP during maintenance therapy in children with ALL.

Several studies have shown an improved cure rate, when the doses of MTX and 6MP during childhood ALL maintenance therapy are titrated to a low white blood cell count (WBC) (7–10). However, as TMP/SMX inhibits two sequential steps in the folic acid synthesis, and TMP/SMX can block DNA replication and cause myelosuppression (7, 11, 12), it could interfere with WBC-targeted dose titration of methotrexate (MTX)/6-mercaptopurine (6MP) maintenance therapy (13). So far only one randomized US study has tested the effect of TMP/SMX during maintenance therapy on outcome. It showed no effect on the risk of relapse among 126 patients. However, the study showed an overall low cure rate and the compliance with MTX/6MP dose titration was not analyzed (14). Accordingly, we explored the influence of TMP/SMX prophylaxis on (i) the association

between MTX/6MP drug doses, drug metabolite levels, and degree of myelosuppression and (ii) event-free survival (EFS) among 447 non-Down children enrolled in the NOPHO ALL92 maintenance therapy study (15).

Patients and methods

Patients

Patients were eligible for the present study, if they were included in the NOPHO ALL92 maintenance therapy study (15) and had their use of TMP/SMX during maintenance therapy registered. Patients included in the NOPHO ALL92 maintenance therapy study were (i) diagnosed with B-cell precursor ALL in the Nordic countries (Denmark, Finland, Iceland, Norway, and Sweden) between January 1992 and December 1996; (ii) between 1.0 and 14.9 yr of age at diagnosis; and (iii) in first remission after induction and consolidation therapy. Of the 538 patients that entered the ALL92 maintenance therapy study, the six patients with Down syndrome were excluded from the present study.

Risk grouping

Details of the NOPHO ALL92 protocol have been described previously (15, 16). The risk group assignment was based on age and WBC at diagnosis (standard risk, SR-ALL: age 2.0–9.9 yr and $WBC < 10.0 \times 10^9/L$; intermediate risk, IR-ALL: age 1.0–1.9 or ≥ 10.0 yr and/or $WBC 10\text{--}49.9 \times 10^9/L$) and presence of higher risk features, HR-ALL: $WBC \geq 50.0 \times 10^9/L$, T-lineage ALL, the presence of CNS or testicular leukemia, translocations t(9;22)(q34;q11) or t(4;11)(q21;q23), lymphomatous leukemia or mediastinal lymphoma, and a poor treatment response ($\geq 25\%$ leukemic blasts in the bone marrow at day 15 or $\geq 5\%$ day 29) (16). Only G-band karyotyping was mandatory in the NOPHO ALL92 protocol. All cytogenetic results were scrutinized annually by the NOPHO cytogenetic working group and described according to ISCN 1995 (17).

Therapy

As induction therapy, all patients received prednisolone ($60 \text{ mg/m}^2/\text{d}$ on days 1–36, then tapered), weekly vincristine (VCR; 2.0 mg/m^2 six times, maximum 2.0 mg), doxorubicin [40 mg/m^2 times three (SR- and IR-ALL) or four (HR-ALL)], *Erwinia* asparaginase ($30\,000 \text{ U/m}^2$ daily on days 37–46), and intrathecal (i.t.) MTX on four occasions.

Consolidation therapy for SR-ALL included three courses of high-dose MTX (HD-MTX; $5 \text{ g/m}^2/24 \text{ h}$ with i.t. MTX and leucovorin rescue), whereas patients with

IR- and HR-ALL received alternating series of (i) i.v. cyclophosphamide (total cumulative dose, 3 g/m^2) with low-dose cytarabine (AraC) and either oral 6MP or 6-thioguanine; (ii) HD-MTX (IR-ALL, 5 g/m^2 and HR-ALL, 8 g/m^2 , both administered four times) with either (IR-ALL only) concurrent oral 6MP ($25 \text{ mg/m}^2/\text{d}$) or (HR-ALL only) alternating high-dose AraC (12 g/m^2 four times) with two 2-month intervening periods of oral weekly MTX and daily 6MP with two VCR/prednisolone reinductions per period; and (iii) 4 wk of delayed intensification with dexamethasone ($10 \text{ mg/m}^2/\text{d}$ for 3 wk, then tapered), weekly VCR (2.0 mg/m^2 four times), weekly anthracycline [$30 \text{ mg/m}^2/\text{d}$ doxorubicin three times (HR-ALL) or daunorubicin four times (IR-ALL)], and *Erwinia* asparaginase ($30\,000 \text{ U/m}^2$ four times) (Table S1) (16).

MTX/6MP maintenance therapy was initiated at treatment weeks 13 (SR-ALL), 32 (IR-ALL), or 63 (HR-ALL) and continued until 2 (IR- and HR-ALL) or 2½ yr (SR-ALL) after diagnosis. The starting dose of 6MP was $75 \text{ mg/m}^2/\text{d}$, and starting dose of MTX was $20 \text{ mg/m}^2/\text{wk}$. During the first year of maintenance therapy, patients with SR- or IR-ALL received alternate pulses at 4-wk intervals of (i) VCR (2.0 mg/m^2 once) and prednisolone ($60 \text{ mg/m}^2/\text{d}$ for 1 wk) and (ii) HD-MTX $5 \text{ g/m}^2/24 \text{ h}$ with i.t. MTX and leucovorin rescue until five courses of HD-MTX had been given. Every 8 wk throughout maintenance therapy, HR-ALL patients received reinductions of VCR (1.5 mg/m^2 once) and prednisolone ($40 \text{ mg/m}^2/\text{d}$ for 5 d) with i.t. MTX.

Maintenance therapy randomization in NOPHO ALL92

The ALL92 study explored the prognostic impact of pharmacologically based monitoring and dose adjustments of oral MTX/6MP maintenance therapy by erythrocyte levels of MTX and 6-thioguanine nucleotides (6TGN). In total, 538 patients were randomized to two different dose-adjustment strategies (control and pharmacology group). This included >97% of all eligible patients during the study period. The dose-adjustment strategies have previously been published in detail (15). In short, the control and pharmacology group had their dosing of oral 6MP and MTX targeted to a WBC of $1.5\text{--}3.5 \times 10^9/L$. Unless the WBC was $< 1.5 \times 10^9/L$, the pharmacology group had in addition the doses of 6MP and/or MTX increased in steps of 20%, if Ery-6TGN*MTX was $< 1350 \text{ (nmol/mmol Hb)}^2$ and such dose increments were regarded as tolerable by the treating physician. As part of that study, all 28 580 data sets of blood counts as well as 6MP and MTX doses available for the 538 patients were prospectively registered. Furthermore, erythrocyte levels of the cytotoxic metabolites of 6MP (i.e., Ery-6TGN) and of MTX (i.e., MTX polyglutamates, Ery-MTX) were repeatedly analyzed

Table 2 Patient characteristics and event rate in relation to type of trimethoprim-sulfamethoxazole treatment

TMP/SMX prophylaxis	Never	Intermittent	Continuously 2–3 d/w	Continuously 7 d/w	P ¹
No. of patients	287	40 ²	61	59	
DK/SF/N/S/I	65/2/77/136/7	18/2/3/17/0	17/31/1/11/1	5/54/0/0/0	
SR/IR/HR	151/112/24	19/10/11	23/30/8	30/29/0	
Male/female	156/131	20/20	35/26	26/33	
Age at diagnosis (yr) ³	4.0 (2.9–6.0)	4.1 (2.6–5.2)	4.0 (3.1–8.8)	4.0 (2.7–6.2)	
WBC at diagnosis ($\times 10^9/L$) ³	6.0 (3.0–16.0)	7.5 (5.0–48.5)	6.0 (2.0–19.0)	8.0 (3.0–14.0)	
WBC ($\times 10^9/L$) ³	3.4 (3.0–3.9) ⁴	3.3 (3.0–4.1)	3.2 (2.9–3.7)	2.9 (2.5–3.5)	<0.001
ANC ($\times 10^9/L$) ³	2.0 (1.7–2.4) ⁵	1.9 (1.7–2.3)	1.8 (1.5–2.1)	1.6 (1.4–1.9)	<0.001
6MP (mg/m ² /d) ³	63.9 (55.3–72.1) ⁴	58.7 (51.5–68.7)	46.1 (38.2–59.3)	53.0 (44.1–65.9)	<0.001
Ery-6TGN (nmol/mmol Hb) ³	181 (143–228) ⁶	178 (134–216)	150 (105–204)	137 (114–195)	<0.001
MTX (mg/m ² /wk) ³	16.3 (14.0–18.1) ⁴	14.6 (12.3–18.1)	12.6 (9.3–16.1)	14.1 (10.9–16.7)	<0.001
Ery-MTX (nmol/mmol Hb) ³	5.6 (4.6–6.7) ⁷	5.2 (4.5–6.7)	5.7 (4.3–7.0)	5.5 (4.8–7.2)	0.64
Pharmacology ⁸ /control group ⁹	146/141	22/18	27/34	31/28	
Dead in CR1	0	0	0	1	
SMN	5	1	1	0	
Relapse	44	4	7	12	
BM/CNS/testis ¹⁰	37/6/9	4/0/1	6/3/2	10/4/0	
pEFS at 12 yr (\pm SE)	0.83 (2.3)	0.87 (5.3)	0.87 (4.3)	0.78 (5.4)	

d/w indicates days per week; DK/SF/N/S/I, Denmark, Finland, Norway, Sweden, Iceland; SR/IR/HR, standard/intermediate/high risk of acute lymphoblastic leukemia; WBC, white blood cell count; ANC, absolute neutrophil count; 6MP, 6-mercaptopurine; Ery-6TGN, erythrocyte levels of 6-thioguanine nucleotides; MTX, methotrexate; Ery-MTX, erythrocyte levels of methotrexate polyglutamates; dead in CR1, dead in first complete remission; SMN, second malignant neoplasm; BM, bone marrow; CNS, central nervous system; pEFS, probability of event-free survival; SE, standard error.

¹Cuzick test for trend across all groups according to TMP/SMX prophylaxis administration.

²Including the ten patients who developed *Pneumocystis pneumonia* (all of whom received trimethoprim-sulfamethoxazole only after this diagnosis).

³Median value (50% range).

⁴Data were not available for one patient.

⁵Data were not available for four patients.

⁶Data were not available for two patients.

⁷Data were not available for five patients.

⁸Pharmacology group had dose adjustments by red blood cell levels of MTX and 6MP metabolites.

⁹Control group, see text and Schmiegelow *et al.* (15).

¹⁰Any relapse involving that site.

throughout maintenance therapy (15). Importantly, neither TMP nor SMX at concentration levels routinely seen during TMP/SMX prophylaxis (18, 19) interferes with the Ery-MTX analysis (data not shown).

PCP prophylaxis

The NOPHO ALL92 protocol included no recommendations on prophylactic treatment with TMP/SMX. Thus, the use of TMP/SMX was determined by guidelines at the local departments of pediatric oncology (56 departments administered maintenance therapy dose adjustments). Of the 532 non-Down syndrome patients who started therapy according to the ALL92 maintenance therapy study, data on use of TMP/SMX prophylaxis (given/not given) during maintenance therapy were obtained for 447 patients. Information regarding the use of TMP/SMX was obtained as an additional registration of the prospective registrations in the ALL92 maintenance

therapy study. One hundred and twenty patients registered to receive TMP/SMX prophylaxis in all registrations were classified as continuously receiving TMP/SMX prophylaxis, and 287 patients registered not to receive TMP/SMX prophylaxis in any registrations were classified as never receiving TMP/SMX prophylaxis. Twelve patients with a registered use of TMP/SMX between 6 and 19 d (median 13.5 d) were included in the group never receiving prophylaxis. The remaining 40 patients were registered as intermittent. The actual dose of TMP/SMX administered to the individual patients was not registered prospectively. Instead, information on weekly dosage of TMP/SMX prophylaxis and all episodes of PCP during maintenance therapy (available for 387 patients) was retrieved through retrospective questionnaires sent to physicians at the local departments during 2010.

The clinical characteristics and treatment assignments of the eligible 237 boys and 210 girls are given in

Table 2. The 447 study patients did not differ significantly from the 85 non-included patients with respect to gender ($P = 0.60$) or age ($P = 0.98$) but differed with respect to WBC at diagnosis (median WBC: 6.0 vs. $13.0 \times 10^9/L$; $P = 0.001$) and probability of event-free survival (pEFS_{12Y} $0.83 \pm 1.8\%$ vs. $0.73 \pm 4.7\%$; $P = 0.01$). However, there was no significant difference between study patients and non-included patients when comparing WBC at diagnosis in each risk group ($P > 0.09$ in all subanalysis). Neither did EFS differ significantly between study patients and non-included patients when analysis was stratified by risk group ($P = 0.21$).

Finally, the current guidelines for TMP/SMX prophylaxis during maintenance therapy were retrieved from major childhood ALL collaborative groups in Europe, the US, and Japan through a survey (Table 1).

Statistics

Nonparametric methods were applied to compare the distribution of parameters between subgroups and to compare the correlation between parameters ($r_s =$ Spearman's correlation coefficient) (20). Cox proportional hazard regression analyses were carried out to detect prognostic factors (21, 22). As the background event intensity differed among the risk groups, Cox analyses were stratified for risk groups assuming the same effect (i.e., hazard coefficient) on possible prognostic factors on the basic event risk across risk groups (21). Where relevant, the covariates were analyzed as time-dependent continuous parameters with recalculations of the weighted means of these variables every time a patient treatment failed using as weight the interval between the sample in question and the next blood sample (10).

Survival analyses were carried out with a basic time scale defined by the date of diagnosis with delayed entry of patients at the start of their maintenance therapy. The duration of EFS was defined as the time from diagnosis until the date of relapse, death, the diagnosis of a secondary cancer (whichever first), or the last registered follow-up for event-free survivors. The Kaplan–Meier method was applied for the estimation of remission duration, for calculations of cumulated risks, and for the generation of survival curves (23). Subgroups were compared with the log-rank test, stratified where needed (24). Two-sided P -values < 0.05 were regarded as significant. Statistical analyses were performed with the SAS statistical software (SAS Institute Inc., Cary, NC, USA) and the SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA).

This study has not been registered at the public trial registries. The ALL92 protocol was approved by the ethical committee of Copenhagen (no. V.200.2080/91) as

well as by the local ethical committees, and participants gave informed consent according to the Helsinki Declaration.

Results

Of the 447 patients, 287 never received TMP/SMX during maintenance therapy, 40 received TMP/SMX intermittently, and 120 received TMP/SMX continuously for 2–3 d/wk ($n = 61$) or for 7 d/wk ($n = 59$) at a median dose of 5 mg/kg/d of trimethoprim. The 40 patients with an intermittent TMP/SMX administration are presented in detail in Table 2 only.

A total of ten patients (one of which subsequently relapsed) developed PCP during maintenance therapy (five of 38 HR-ALL, two of 163 IR-ALL, and three of 186 SR-ALL patients). All cases of PCP occurred in patients not receiving PCP prophylaxis, and none of the ten children died because of PCP. The cumulative incidence of PCP was 4.0% (10/250) for patients not receiving PCP prophylaxis and 0% (0/112) for patients continuously receiving PCP prophylaxis ($P = 0.03$). The cumulative risk of PCP during maintenance therapy among patients never treated with TMP/SMX prophylaxis was significantly related to the risk group being 19% for HR-ALL, 2% for IR-ALL, and 2% for SR-ALL patients ($P < 0.001$; Fig. 1). For the nine patients for whom it was available, the median lymphocyte count within the last month prior to PCP was $0.4 \times 10^9/L$ (75% range, 0.2 – $0.9 \times 10^9/L$) compared to a median lymphocyte count of $1.0 \times 10^9/L$ (75% range, 0.6 – $1.4 \times 10^9/L$) among all 538 Nordic children included in the ALL92 study. Apart from two cases of PCP in SR-ALL patients occurring 10 and 17 months from start of maintenance therapy, all other cases of PCP were

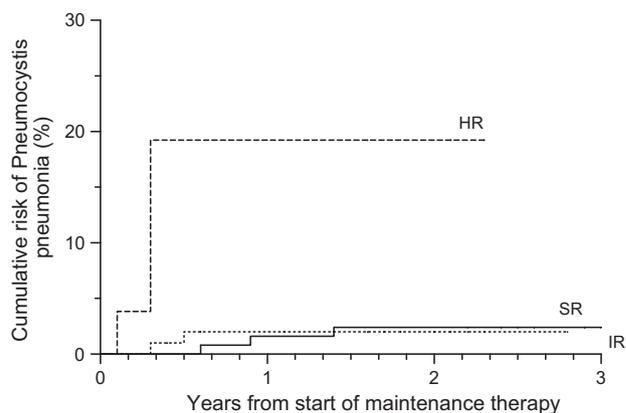


Figure 1 Cumulative risk of developing Pneumocystis pneumonia by risk group during maintenance therapy. The cumulative risk for children with standard risk (SR-ALL) was 2% (3/124); for intermediate risk (IR-ALL) 2% (2/100); and for high risk (HR-ALL) 19% (5/26), $P < 0.001$. ALL, acute lymphoblastic leukemia.

diagnosed within 7 months (median 4 months) after the start of maintenance therapy. The median lymphocyte counts within the last month prior to PCP were 2.1 and $0.6 \times 10^9/L$, respectively, for the two patients diagnosed with PCP 10 and 17 months from the start of maintenance therapy.

Patients treated throughout maintenance therapy with TMP/SMX for 2–7 d/wk (TMP/SMX_{2–7}) and patients never treated with TMP/SMX (TMP/SMX_{never}) differed on several MTX/6MP dose-adjustment parameters (Table 2). Thus, the TMP/SMX_{2–7} patients had lower average absolute neutrophil counts (ANC) and WBC during maintenance therapy compared to those never treated with TMP/SMX (median ANC: 1.7 vs. $2.0 \times 10^9/L$; $P < 0.001$; median WBC: 3.1 vs. $3.4 \times 10^9/L$; $P < 0.001$). The higher degree of myelosuppression among the TMP/SMX_{2–7} patients was achieved in spite of lower prescribed average 6MP and MTX doses during maintenance therapy compared to the TMP/SMX_{never} patients (median 6MP: 50.6 vs. 63.9 mg/m²/d; $P < 0.001$; median MTX: 13.2 vs. 16.3 mg/m²/wk; $P < 0.001$). Patients receiving TMP/SMX 2–3 d/wk were given lower 6MP doses, however of borderline significance, compared to patients receiving TMP/SMX 7 d/wk (median: 46.1 vs. 53.0 mg/m²/d; $P = 0.049$). There was no significant difference in the prescribed MTX dose between patients receiving TMP/SMX 2–3 d/wk and patients receiving TMP/SMX 7 d/wk (median: 12.6 vs. 14.1 mg/m²/wk; $P = 0.14$). Additionally, the TMP/SMX_{2–7} patients achieved a lower Ery-6TGN level compared to the TMP/SMX_{never} patients (median: 145 vs. 181 nmol/mmol Hb; $P < 0.001$), whereas the Ery-MTX level did not differ significantly (median: 5.6 vs. 5.6 nmol/mmol Hb; $P = 0.67$).

The median follow-up for the 372 patients who remained in first remission was 12.8 yr (50% range: 11.8–13.8 yr). At the end of the study, one patient had died in first complete remission, 67 patients had relapsed 1.1–12.0 yr from diagnosis (median: 3.4 yr), and seven patients had developed a second malignant neoplasm. The projected 12-yr EFS (pEFS_{12y}) and overall survival (pOS_{12y}) of the 447 patients were $0.83 \pm 1.8\%$ and $0.91 \pm 1.4\%$, respectively. With Cox multivariate regression analysis stratified by risk group, we tested the impact on risk of event of gender, age and WBC at diagnosis, randomization group, and ANC. The variable TMP/SMX prophylaxis did not meet the proportionality assumption and was not included in this initial analysis. Four patients (none with event) had to be excluded because they lacked ANC measurements. The only covariate that reached statistical significance was ANC [B (regression coefficient) = 0.50; $P = 0.04$], whereas male gender was of borderline significance ($B = 0.54$; $P = 0.06$). ANC had no effect on EFS among patients continuously receiving TMP/SMX ($B = 0.35$; $P = 0.40$), whereas ANC was significantly related to

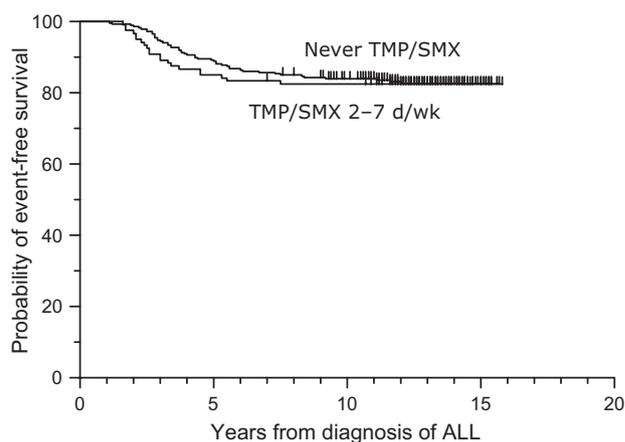


Figure 2 Probability of event-free survival (pEFS) by use of trimethoprim-sulfamethoxazole (TMP/SMX). Children treated continuously with TMP/SMX 2–7 d/wk vs. children never treated with TMP/SMX. pEFS at 12 yr: $0.83 \pm 3.5\%$ vs. $0.83 \pm 2.3\%$, $P = 0.82$.

EFS for patients never receiving TMP/SMX prophylaxis ($B = 0.64$; $P = 0.02$). The difference in the effect of ANC on EFS between TMP/SMX_{2–7} and TMP/SMX_{never} patients was not of statistical significance ($P = 0.46$).

The pEFS_{12y} for the patients treated continuously for 2–7 d/wk did not differ significantly from that of TMP/SMX_{never} patients ($0.83 \pm 3.5\%$ vs. $0.83 \pm 2.3\%$; $P = 0.82$) (see Fig. 2), and this was also the case when analyzed within subtypes defined by sex, karyotype subsets (i) t(12, 21)(ETV6/RUNX1)-translocation or high-hyperdiploidy, (ii) other aberrations, or (iii) normal or missing karyotype, age groups (<5, 5–9.9 or ≥ 10 yr at diagnosis) or randomization groups ($P > 0.75$ for all subanalysis). The difference in the EFS between TMP/SMX_{2–7} and TMP/SMX_{never} patients appears to peak 3–4 yr from diagnosis of ALL; however, the difference in the 3.5-yr EFS was not statistically significant between TMP/SMX_{2–7} and TMP/SMX_{never} patients ($P = 0.08$). Patients receiving TMP/SMX 2–3 d/wk did not significantly better in comparison with both patients receiving TMP/SMX 7 d/wk ($0.87 \pm 4.3\%$ vs. $0.78 \pm 5.4\%$; $P = 0.18$) and patients never receiving TMP/SMX ($0.87 \pm 4.3\%$ vs. $0.83 \pm 2.3\%$; $P = 0.47$).

The PCP prophylaxis survey revealed that all major childhood ALL collaborative groups prescribe TMP/SMX, and all groups except NOPHO use this prophylaxis throughout maintenance therapy. Furthermore, all but two groups give approximately the same daily dose (3–5 mg of trimethoprim/kg divided in two doses), although St Jude Children's Research Hospital and United Kingdom Children's Cancer and Leukaemia Group prescribe TMP/SMX per body surface area. However, as TMP/SMX is recommended to be given 1, 2, or 3 d a week, the weekly total TMP/SMX dose varies threefold among the groups (Table 1).

Discussion

The most effective contemporary protocols for childhood ALL achieve cure rates of 80% or more because of improved risk grouping and more intensive combination chemotherapy. This has increased the significance of supportive care, including PCP prophylaxis. Even though only one patient has died from PCP infection during the last two NOPHO ALL protocols including 2735 patients in total (25), PCP should still be regarded as a life-threatening infection (1). Although PCP prophylaxis may interfere with maintenance therapy, the present study indicates that it is still needed during maintenance therapy. Both this and previous studies have shown that lymphocytopenia, not least T-cell depletion, is a risk factor for developing PCP (26–29). The high incidence of PCP among HR-ALL patients suggests that the longer lasting consolidation chemotherapy for this group has resulted in a more reduced T-cell function than for the patients with SR- and IR-ALL.

All collaborative ALL protocols recommend doses of MTX and 6MP during maintenance therapy to be adjusted to a target WBC or ANC level (30), ANC being the primary marker of the degree of myelosuppression, because several studies have shown that low WBC or ANC levels during maintenance therapy are linked to a reduced risk of relapse (7–10, 15, 31–33). This study indicates that TMP/SMX enhances myelosuppression during maintenance therapy, which is in accordance with previous reports (7, 12). However, among the TMP/SMX-treated patients in the present study, ANC was not predictive for EFS. The reason for this loss of association between the degree of myelosuppression and the risk of event is unresolved.

This study clearly demonstrates that when using TMP/SMX prophylaxis, patients receive lower doses of 6MP and MTX. Potentially, this could have compromised the cure rates, as studies support that increased MTX/6MP dose intensity is an important determinant of EFS in ALL (9, 34, 35). However, the pEFS_{12Y} for patients treated continuously with TMP/SMX did not differ significantly from that of patients never treated with TMP/SMX, which suggests that TMP/SMX not only suppresses the normal bone marrow function but may in itself exert an antileukemic effect. TMP binds the enzyme dihydrofolate reductase in a manner analogous to MTX (19), but with less cytotoxic efficacy compared to MTX (36). Furthermore, TMP/SMX suppresses cell replication in leukemic cell lines (18, 19, 37).

All study groups in the survey acknowledge that children on chemotherapy have a significantly increased risk of PCP and accordingly offer TMP/SMX prophylaxis. However, the present study is the first that in detail explore the impact of PCP prophylaxis on maintenance

therapy and outcome for ALL patients. In conclusion, TMP/SMX should be given throughout maintenance therapy, and a 2–3 d/wk schedule should be preferred. Although a once a day dosage seems to be safe (38), the vast majority of collaborative ALL study groups recommend a twice daily administration (Table 1).

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Author contribution

K.S. designed the study. K.S., M.L., C.B., and E.W.A. performed the statistical analyses. M.L. was together with the national representatives (A.H-S., O.G.J., J.K., A.L., B.L., and D.S.) responsible for collecting the data. K.S. and M.L. wrote the manuscript. All authors commented and approved the final manuscript.

Conflicts of interest

The authors declare no competing financial interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Treatment protocols - NOPHO ALL92.

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