Effects of allopurinol on 6-mercaptopurine metabolism in unselected patients with pediatric acute lymphoblastic leukemia: a prospective phase II study

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Abstract

Allopurinol can be used in maintenance therapy (MT) for pediatric acute lymphoblastic leukemia (ALL) to mitigate hepatic toxicity in patients with skewed 6-mercaptopurine metabolism. Allopurinol increases the erythrocyte levels of thioguanine nucleotides (e-TGN), which is the proposed main mediator of the antileukemic effect and decreases methyl mercaptopurine (e-MeMP) levels, associated with hepatotoxicity. We investigated the effects of allopurinol in thiopurine methyltransferase (TPMT) wild-type patients without previous clinical signs of skewed 6-mercaptopurine metabolism. Fifty-one patients from Sweden and Finland were enrolled in this prospective before-after trial during ALL MT. Mean e-TGN increased from 280 nmol/mmol hemoglobin (Hb) after 12 weeks of standard MT to 440 after 12 weeks of MT with addition of allopurinol 50 mg/m^2 (P<0.001). Mean e-MeMP decreased simultaneously from 9,481 nmol/mmol Hb to 2,791 (P<0.001) and mean alanine aminotransferase declined by almost 50%. Primary endpoint, defined as e-TGN >200 nmol/mmol Hb, was reached for 91% of the patients after 12 weeks of allopurinol (week 25) compared to 67% before (week 13) (P<0.001). This level was chosen as the median e-TGN in a previous NOPHO ALL-2008 study was just below 200 nmol/mmol Hb. During weeks on allopurinol a slightly higher proportion of the patients had a white blood cell count within target 1.5-3.0×10^9/L. Allopurinol did not increase severe adverse events and no life-threatening events were reported. In conclusion, allopurinol add-on treatment is safe and leads to increased e-TGN and reduced e-MeMP also in ALL-patients without previous signs of skewed thiopurine metabolism and is a promising approach to increase antileukemic effect and reduce toxicity.

Introduction

Allopurinol has a favorable impact on 6-mercaptopurine (6MP) metabolite balance in pediatric patients with acute lymphoblastic leukemia (ALL) with skewed 6MP metabolism presenting with difficulty in achieving target white blood cell (WBC) or absolute neutrophil count (ANC) levels and/or liver toxicity or other adverse reactions. However, this is only described in small studies and case reports.[1-4]

Maintenance therapy (MT) with oral 6MP and methotrexate (MTX) is an essential part of treatment for ALL and constitutes the greater part of the treatment period. Many decades of research still leaves unanswered how to achieve the best antileukemic effect[5-8] while avoiding side effects that for many patients are severe and may compromise treatment efficacy.[1,8,9]

Purine metabolism is complex and exhibits a large inter- and intraindividual variation.[10] 6MP dosage therefore requires frequent adjustments to avoid excessive myelosuppression while simultaneously achieving the therapy intensity required for effective treatment. Steering of maintenance therapy is often challenging, particularly in patients not reaching target blood count levels despite high MT doses or experiencing hepatic side effects such as nausea and hypoglycemia that significantly limit the tolerated dose.

It has long been known that event-free survival (EFS) correlates with WBC[11] and ANC[12] during MT and these parameters are used to steer MT in most treatment protocols. In

the Nordic Society of Pediatric Hematology and Oncology (NOPHO) ALL-2008-based protocols the dosage of MTX and 6MP was titrated to a target WBC of 1.5-3.0×10^9/L (see *Online Supplementary Appendix* for details).

Thioguanine nucleotides (TGN) are considered to be the main mediators of the antileukemic effect of 6MP and relapse risk has been negatively correlated with the level of TGN in erythrocytes (e-TGN)[13] or the product of erythrocyte levels of TGN and MTX (e-TGN × e-MTX).[14] More recent studies have shown DNA-incorporated TGN to be better correlated to outcome.[5] Other 6MP metabolites, such as 6-methylmercaptopurine in erythrocytes (e-MeMP), are associated with liver toxicity[15,16] and hypoglycemia.[17]

In a subgroup of ALL patients, it is difficult to reach the desired level of myelosuppression despite high 6MP doses resulting in hepatic toxicity. These patients often have high levels of e-MeMP and low e-TGN.[9] Similar skewed purine metabolism is seen in some patients with inflammatory bowel disease (IBD) on thiopurine therapy. Adding a low dose of the xantine oxidase inhibitor allopurinol can increase levels of e-TGN and decrease levels of e-MeMP leading to reduced liver toxicity and increased efficacy of IBD treatment.[18] Allopurinol has been used in selected ALL patients suffering from 6MP-induced induced hepatic toxicity during MT, with the same effects on 6MP metabolite balance and decreased hepatic toxicity, so far published only in case reports or small retrospective studies.[19]

We wanted to investigate if allopurinol has the same impact on the 6MP metabolism in patients without previous signs of skewed metabolism. The rationale is that it seems reasonable to increase the e-TGN levels also in these patients, since the antileukemic effects of 6MP are believed to be mediated mainly by TGN, and allopurinol could potentially lead to higher e-TGN without increasing the side effects and myelotoxicity that an increased 6MP dose would lead to.

We therefore conducted this prospective study as a proof of principle, to investigate if allopurinol shifts 6MP metabolites in the desired direction (higher e-TGN and lower e-MeMP) also in ALL patients without previous signs of skewed metabolism and evaluate if allopurinol is safe to use in this context.

Methods

Patients

The trial was conducted in all six childhood cancer centers in Sweden and two centers in Finland. Patients with precursor B-cell and T-cell ALL at the age of 0-18 years with treatment according to NOPHO ALL-2008 or NOPHO ALL 2014-Infant standard- or intermediate-risk protocols and thiopurine methyltransferase (TPMT) wild-type were eligible for inclusion.

Patients with unknown TPMT status or presence of TPMT mutation (both heterozygous and homozygous), major organ failure and severe liver toxicity were excluded.

Study design

This was an interventional phase II, non-randomized, open label study with a before-after design carried out in the beginning of the final maintenance phase. With this design every patient served as their own control enabling comparison of parameters during MT with or without allopurinol, diminishing the effect of interindividual variation of thiopurine metabolism.

TPMT heterozygotes were excluded since they already have an increased e-TGN/e-MeMP ratio compared to patients with wild-type TPMT.[20]

All participants started with 1-3 months of normal MT to avoid interference from delayed toxicity from the previous treatment phase. The study stretched over 28 weeks starting with 12 weeks of conventional MT followed by 12 weeks with addition of allopurinol and finally 4 weeks of MT without allopurinol but with continued monitoring.

At the start of allopurinol treatment the dose of 6MP was reduced by 50% to prevent excessive myelosuppression.[9] Subsequent dose increments and reductions were thereafter done according to standard protocol guidelines (*Online Supplementary Appendix*).

Clinical examination was done biweekly, except for the first 5 weeks with allopurinol when examination was done weekly. At these time points blood samples for hematological values (Hb, WBC, ANC, lymphocytes), alanine aminotransferase (ALT), bilirubin and creatinine were analyzed. Every 4 weeks more extensive blood sampling including drug metabolites was done (see *Online Supplementary Appendix* for details). At every visit a case report form was filled out registering adverse effects of special interest including fever, antibiotic treatment, skin rash, clinical signs of liver toxicity, nausea, vomiting, transfusions and days with MT and/or allopurinol on hold. Severe adverse events were reported to the study center within 24 hours in accordance with Good Clinical Practice standards.

Allopurinol dose

All participants started week 13 on allopurinol 50 mg/m^2 daily, but for those patients who did not reach e-TGN 200 nmol/mmol Hb after 4 weeks (w) of allopurinol treatment (w17), allopurinol dose was increased to 100 mg/m^2 w19-24.

Ethics

The study was approved by the Ethics Committee (Dnr 913-16 in Sweden and EETTMK: 52 /2018 in Finland) and the Medical Products Agency of the participating countries (EudraCT-number 2016-003409-33 and *clinicaltrials gov. Identifier: NCT03022747*).

Metabolite assessment

6MP metabolites were analyzed at Bonkolab in Copenhagen with high performance liquid chromatography as detailed by Nielsen *et al.*[5,21] Other laboratory analyses were performed at the study sites.

Data analysis and statistics

The primary endpoint, comparing the fraction of patients with e-TGN >200 nmol/mmol Hb w13 and w25, was analyzed using Pearson's χ^2 test. When comparing other parameters, during standard maintenance therapy and maintenance with addition of allopurinol, mean differences were analyzed using paired *t* test.

Results

Study population

The target population of 60 patients was not reached, as only 51 patients were enrolled before the NOPHO ALL 2008-protocol was closed. The patient characteristics are summarized in Table 1. The first patient entered the study in January 2017 and the last one finished in February 2022. Fifty-four patients were recruited for the study, three patients withdrew their consent prior to study start, 51 commenced and 48 completed the study (Figure 1). Eleven patients were recruited from Finland and 40 from Sweden, of whom 28 from Gothenburg.

Withdrawal from the study

Three patients discontinued participation during the study. The first patient discontinued w17 by parental decision due to the extra blood samples required for the study.
The second patient withdrew from the study w19 by parental decision due to nausea and vomiting during the last study week. The patient was found to be positive for adenovirus at the time of withdrawal.
The third patient withdrew from the study w21 due to parental concerns over elevated ALT.

Primary endpoint - fraction of patients with thioguanine nucleotides in erythrocytes >200 nmol/mmol hemoglobin

The primary endpoint was the fraction of patients with e-TGN >200 nmol/mmol Hb after 12 weeks of allopurinol compared to before. This level was chosen as the mean e-TGN in a previous NOPHO ALL-2008 study was around 200 nmol/mmol Hb.[21]
e-TGN values were reported for 46 patients w13 (before allopurinol) and 45 patients w25 (with allopurinol). Thirty-one of 46 (67%) had e-TGN >200 nmol/mmol Hb w13, compared to 41 of 45 (91%) w25 (*P*<0.001) (Figure 2).

Erythrocyte level of thioguanine nucleotides and given dose 6-mercaptopurine

Mean e-TGN w13 increased from 280 nmol/mmol Hb to 440 nmol/mmol Hb w25. Paired analysis showed that mean e-TGN was 187 (95% confidence interval (CI): 119-255) nmol/mmol Hb higher w25 (*P*<0.001). The increase in e-TGN was seen already at the first sample after starting on allopurinol (w17) and was maintained throughout the phase (Figure 3).

In w29, 4 weeks after allopurinol was discontinued, mean e-TGN decreased to 185 nmol/mmol Hb (P<0.001) with only 18 of 44 (41%) patients above 200 nmol/mmol Hb (Table 2). Mean given dose 6MP was 354 mg/week (95% CI: 297-411) for the weeks before allopurinol, 170 mg/week (95% CI:

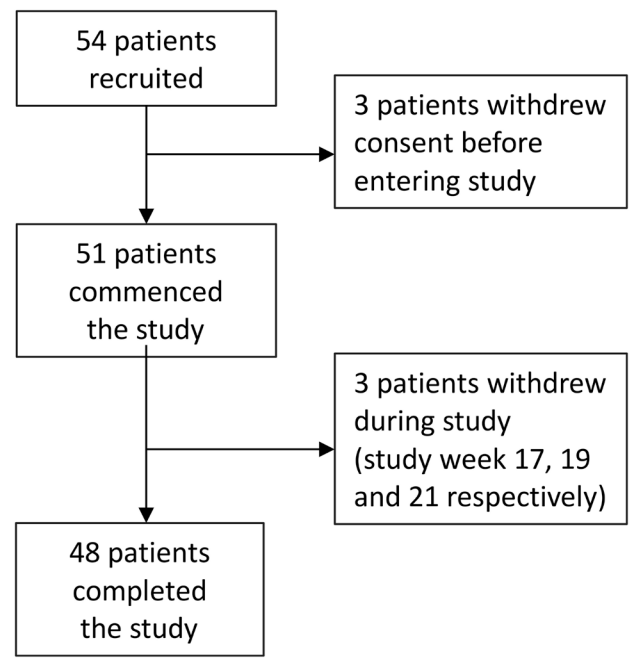


Figure 1. Consort diagram. Fifty-four patients were recruited. Three of them withdrew consent before commencing the study. The remaining 51 patients constitute the allopurinol study cohort. Three of these withdrew during the study for reasons described in the results section.

Table 1. Patient characteristics.

	N	%	Median	Min-Max
Age in years at diagnosis	-	-	4	0-15
WBC x10^9/L at diagnosis	-	-	8.0	0.8-265
Sex				
Female	24	47	-	-
Male	27	53	-	-
Immunophenotype				
preB	46	90	-	-
T	5	10	-	-
NCI risk group				
Low risk	38	75	-	-
High risk	13	25	-	-
Protocol risk group				
NOPHO ALL-2008				
Standard risk	33	65	-	-
Intermediate risk	17	33	-	-
NOPHO ALL 2014-Infant				
Standard risk	-	-	-	-
Intermediate risk	1	2	-	-

Characteristics of the 51 patients that commenced the allopurinol study. Protocol risk group stratification is based on immunophenotype and minimal residual disease (MRD). Patients with pre B cell (preB) and MRD <0.1% day 29 are stratified to standard risk. The distributions of sex, age, immunophenotype and risk group are consistent with the entire Nordic Society of Pediatric Hematology and Oncology (NOPHO) acute lymphoblastic leukemia (ALL)-2008 population. NCI: National Cancer Institute; WBC: white blood cell count; T: T cell; Min-Max: minimum-maximum.

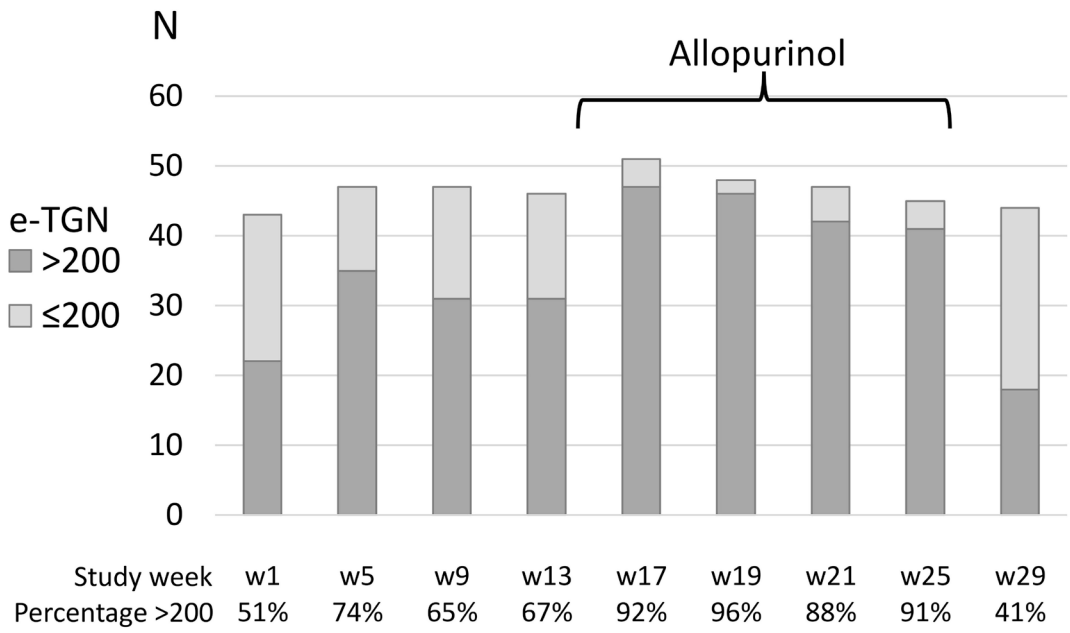
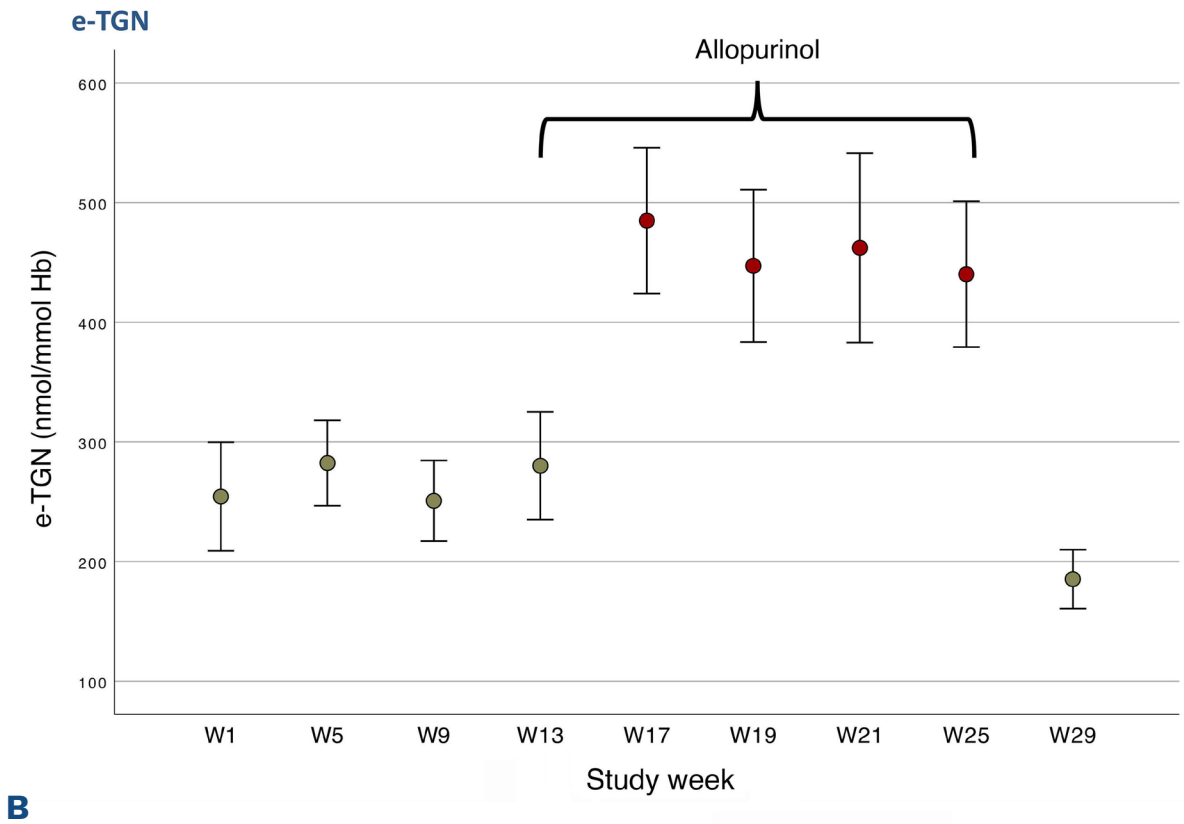


Figure 2. Thioguanine nucleotides in erythrocytes > or ≤ 200 nmol/mmol hemoglobin. Number of patients with erythrocyte levels of thioguanine nucleotides (e-TGN) > or ≤200 nmol/mmol hemoglobin (Hb) and percentage reaching primary endpoint, e-TGN > 200 nmol/mmol Hb. Exact numbers are presented in Table 2. w: week.

A



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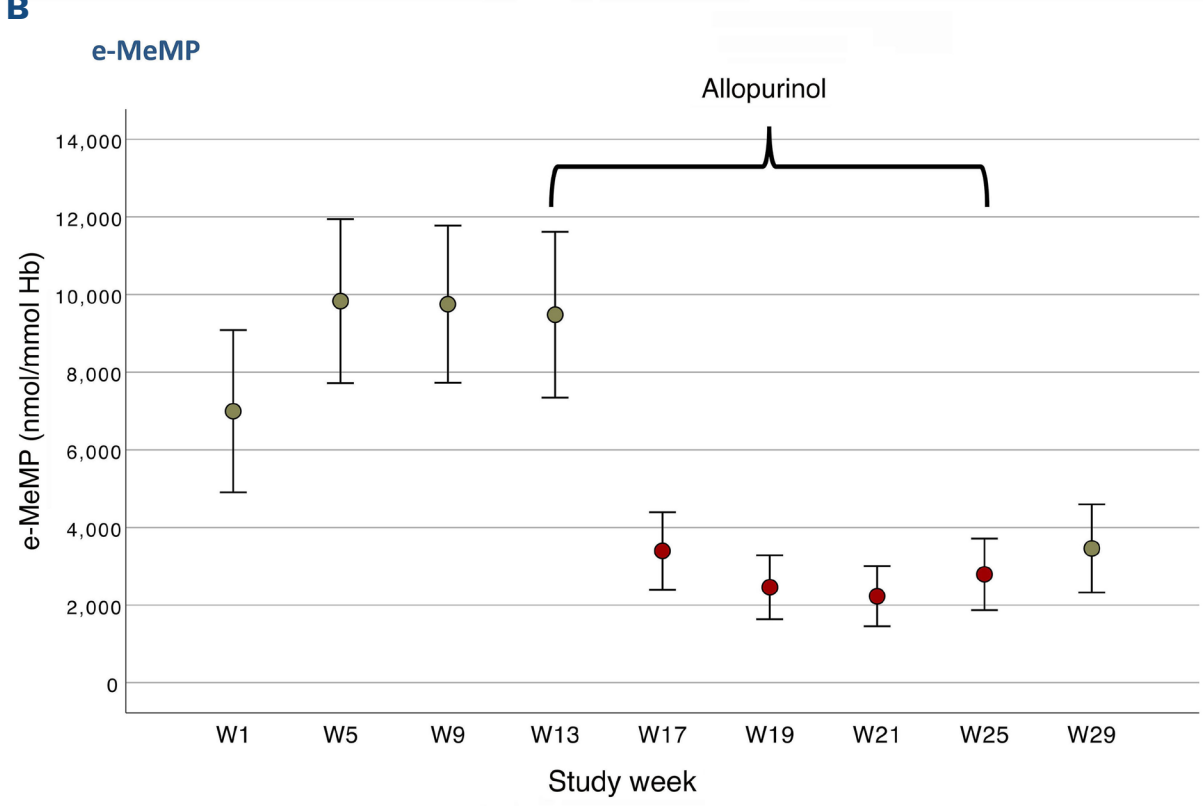

Figure 3. Mean eryhtrocyte levels of thioguanine nucleotides and methylmercaptopurine. Erythrocyte levels (nmol/mmol hemoglobin [Hb]) of thioguanine nucleotides (e-TGN) (A) and methylmercaptopurine (e-MeMP) (B) during the 3 trial phases: week (w)1-12 standard maintenance therapy (MT), w13-24 MT + allopurinol, w25-29 standard MT. Error bars display mean and 95% confidence interval. See Table 2 for exact values.

142-198) on allopurinol and 223 mg/week (95% CI: 178-267) for the weeks after allopurinol was discontinued.
Four patients had e-TGN below 200 nmol/mmol Hb w17 and increased allopurinol dose to 100 mg/m^2 w19. Patients 1-3 had MT on hold in accordance with ALL treatment protocol guidelines due to cytopenias the weeks preceding w17. Patients 1 and 2 already had an e-TGN above 200 nmol/mmol Hb w19, before they started on the increased allopurinol dose, while patient 3 had e-TGN 189 nmol/mmol Hb w19, and increased to >200 nmol/mmol Hb after w19.
Patient 4 was an outlier who, due to myelosuppression, tolerated very low doses of 6MP and MTX and had extremely low e-TGN throughout the study with e-TGN 22-42 nmol/mmol Hb before allopurinol, 78 nmol/mmol Hb with allopurinol 50 mg/m^2 and 108 nmol/mmol Hb with allopurinol 100 mg/m^2. e-MeMP was below quantification in samples after increasing the allopurinol dose, but WBC and ANC levels of this patient did not indicate non-compliance (see *Online Supplementary Appendix*).

Erythrocyte level of methyl mercaptopurine in relation to alanine aminotransferase

The e-MeMP levels decreased rapidly after addition of allopurinol from mean 9,481 w13 to 2,791 nmol/mmol Hb w25. In paired analysis e-MeMP was 6,822 lower w25 compared to w13 (95% CI: 4,597-9,047; *P*<0.001) (Figure 3).
There was no significant change in mean ALT during the first 12 weeks without allopurinol nor during the first 8 weeks on allopurinol. A significant decline in mean ALT was seen in w21, i.e., 4 weeks after observing a reduction in e-MeMP. Paired analysis showed that ALT was 88 IU lower w25 compared to w13 (95% CI: 16-160; *P*<0.05).
There was no significant difference between mean ALT w25 and w29 (99 *vs*. 110; *P*=0.57).
The ratio e-MeMP/e-TGN rapidly decreased after addition of allopurinol from mean 47 (95% CI: 29-64) w13 to mean 8 (95% CI: 5-10) w17 and remained at a lower level throughout the allopurinol phase (Table 2).

White blood cells and neutrophils

6MP and MTX doses were adjusted according to WBC. The proportion of all measured WBC values that were within the target 1.5-3.0×10^9/L was 54% during the weeks before allopurinol (w1-13) compared to 65% during the weeks with allopurinol (w14-25) (*P*<0.005) and 42% after allopurinol was discontinued (w29) (*P*<0.001; Table 2). Mean WBC and ANC were 0.7×10^9/L and 0.4×10^9/L lower during allopurinol compared to without (*P*<0.001).

Adverse events and severe toxicity

A total of 27 severe adverse events (SAE) were reported in the study, all of them due to hospitalization with none reported as a life-threatening event or persistent or significant disability/incapacity. The SAE were evenly distributed over the study phases and are outlined in Table 3.
Most SAE were febrile neutropenia (13/27), followed by infection without neutropenia (11/27). The remaining three SAE reported were cutaneous blisters (w10), elevated ALT following a febrile episode (w16) and surgical exploration due to epididymitis (w25).
Creatinine levels were within normal limits throughout the study in all patients with no significant increase during allopurinol treatment. No patient displayed any clinical or laboratory signs of sinusoidal obstructive syndrome/veno-occlusive disease of the liver.

Relapse and second malignant neoplasm

One patient with intrachromosomal amplification of chromosome 21 in the leukemic clone had an early bone marrow relapse 2 months after end of ALL therapy.
One patient developed Hodgkin lymphoma 15 months after end of ALL therapy, 27 months after completing the allopurinol study. This patient had withdrawn from the allopurinol study w17 due to the burden of extra blood sampling.

Discussion

Allopurinol as addition to MT in pediatric ALL can be used to mitigate hepatic toxicity in patients with skewed 6MP metabolism and has been shown to increase e-TGN and lower e-MeMP.[9,22] To our knowledge, this is the first study to investigate if the same effects apply to an unselected cohort of ALL patients without skewed metabolism or previous signs of severe hepatic toxicity or drug intolerance.
Adding allopurinol to ALL maintenance therapy led to a significantly higher proportion of patients reaching e-TGN >200 nmol/mmol Hb, a level above the median e-TGN reported from previous NOPHO ALL studies.[14,21] The effect was rapid with e-TGN reaching a plateau within 4 weeks after initiating allopurinol.
Our cohort had a higher mean e-TGN on normal MT than previously reported from the NOPHO ALL-2008 protocol[21] with 67% having e-TGN >200 nmol/mmol Hb w13 compared to the expected 50%. Nonetheless, we demonstrated a significantly increased proportion when adding allopurinol (91%). The reason why e-TGN was higher even before adding allopurinol can only be speculated upon. Since this study was non-randomized and patients with previous severe liver toxicity were excluded, it is possible that our study had less patients with skewed metabolism. This is supported by a lower mean e-MeMP before allopurinol compared to the study by Nielsen *et al*.[21] Other possible factors could be better adherence from attending physicians, knowing the patient was on a MT-trial, and better compliance from parents reporting every given dose in the patient diary.
Four weeks after allopurinol was discontinued the e-TGN dropped to lower levels than before introducing allopurinol. This is not surprising, since the increase in e-TGN after commencing allopurinol was achieved with a reduction of

Table 2. 6-mercaptopurine metabolites and hematological parameters.

Study week	w1	w5	w9	w13	Allopurinol				w29
					w17	w19	w21	w25	
e-TGN									
Mean	254	282	251	280	485	447	462	440	185
(95% CI)	(209-300)	(247-318)	(217-285)	(235-325)	(424-546)	(383-511)	(383-541)	(379-501)	(161-210)
Min-max	22-786	105-719	37-520	42-797	77-1,065	91-1165	57-1,224	100-978	64-427
>200, N/tot (%)	22/43 (51)	35/47 (74)	31/48 (65)	31/46 (67)	47/51 (92)	46/48 (96)	42/48 (88)	41/45 (91)	18/44 (41)
e-MeMP									
Mean	6,993	9,830	9,753	9,481	3,395	2,459	2,230	2,791	3,459
(95% CI)	(4,904-9,082)	(7,718-11,943)	(7,726-11,780)	(7,346-11,616)	(2,397-4,393)	(1,634-3,283)	(1,455-3,005)	(1,868-3,714)	(2,324-4,595)
Min-max	0-30,962	322-26,277	104-25,382	139-31,503	0-17,511	116-14,173	0-14,545	0-16,348	0-15,430
e-MeMP/e-TGN ratio									
Mean	34	40	45	47	8	6	5	7	18
(95% CI)	(22-45)	(29-50)	(33-57)	(29-64)	(5-10)	(4-8)	(4-7)	(5-10)	(13-22)
Min-max	0-146	2-176	1-217	2-312	0-41	0.2-32	0-25	0-37	0-53
WBC									
Mean	3.3	3.2	2.7	3.7	2.3	2.4	2.7	2.5	3.7
(95% CI)	(3.0-3.6)	(2.7-3.6)	(2.5-3.0)	(2.7-4.7)	(2.0-2.7)	(2.2-2.7)	(2.4-3.1)	(2.2-2.7)	(3.1-4.3)
Min-max	1.5-6.0	1.3–8.0	1.4–5.6	0.9–25.0	1.0–9.3	1.0–4.7	1.3–8.4	1.1–5.2	1.8–14.4
1.5-3.0, N/tot (%)	18/48 (38)	25/49 (51)	32/48 (67)	28/49 (57)	34/45 (76)	31/49 (63)	30/47 (64)	33/45 (73)	19/45 (42)
ANC									
Mean	1.8	1.8	1.4	1.9	1.2	1.2	1.5	1.3	2.2
(95% CI)	(1.6-2.1)	(1.4-2.1)	(1.2-1.6)	(1.5-2.3)	(0.9-1.5)	(1.0-1.4)	(1.2-1.8)	(1.1-1.5)	(1.6-2.8)
Min-max	0.3-4.4	0.2–6.6	0.0–4.0	0.5–8.9	0.1–6.8	0.2–3.2	0.3–6.8	0.4–3.4	0.8–13.2
ALT									
Mean	130	125	170	184	132	129	112	99	110
(95% CI)	(86-175)	(82-167)	(101-240)	(124-245)	(96-169)	(80-177)	(65-160)	(56-141)	(79-140)
Min-max	16-760	13-960	11-1,342	6-1,158	14-528	16-840	16-597	16-780	16-535

Allopurinol is given study weeks (w) 13-25, therefore w17-25 are considered being influenced by allopurinol. Units of measure: thioguanine nucleotides in erythrocytes (e-TGN) and methylmercaptopurine in erythrocytes (e-MeMP) nmol/mmol hemoglobin (Hb), white blood cells (WBC) and absolute neutrophil count (ANC) x10^9/L, alanine aminotransferase (ALT) IU/L, 60 IU=1 μkat. Fraction of patients with e-TGN >200 nmol/mmol Hb after w12 of allopurinol is primary endpoint; 1.5-3.0 is targeted WBC during maintenance therapy in the Nordic Society of Pediatric Hematology and Oncology (NOPHO) acute lymphoblastic leukemia (ALL)-2008 protocol. Min-max: minimum-maximum; CI: confidence interval; tot: total.

the 6MP dose by 50% and the 6MP dose was not immediately increased when discontinuing allopurinol as we had no data on how long the carry over effect would be.

The results indicate that the carry-over effect of allopurinol is short and that the 6MP dose needs to be increased more rapidly if allopurinol is discontinued for reasons other than hematological toxicity.

The allopurinol dose of 50 mg/m^2 was chosen as this dose has previously been shown to be effective for modifying thiopurine metabolism and well tolerated both in IBD and ALL patients.[9,23,24] Other studies have used higher or fixed doses[25] which is the rationale for increasing the dose to 100 mg/m^2 for those who did not reach primary endpoint (e-TGN >200 nmol/mmol Hb) after 4 weeks. In our study allopurinol 50 mg/m^2 was sufficient to obtain the desired metabolic shift, supported by our finding that only four of 51 (8%) of our study patients had e-TGN below 200 nmol/mmol Hb w17 and increased their allopurinol dose to 100 mg/m^2. Three of the four patients who increased their allopurinol dose had 6MP on hold the preceding weeks and had e-TGN above or very close to 200 nmol/mmol Hb the week they started on the higher dose (w19). Therefore, it is likely that the low e-TGN w17 was primarily due to interruption of MT and not a result of insufficient effect of the standard allopurinol dose, 50 mg/m^2. The fourth patient had extremely low metabolites throughout the study for reasons we have not understood. With only four patients increasing their allopurinol dose the study cannot deter-

Table 3. Severe adverse events.

SAE category	Before allopurinol N (w1–12)	On allopurinol N (w13–24)	After allopurinol N (w25–29)	All N
Febrile neutropenia	5	8	0	13
Non-neutropenic infection	6	4	1	11
Other	1	1	1	3
Sum	12	13	2	27

All 27 severe adverse events (SAE), occurring in 19 patients, were reported due to hospitalization. No life-threatening event, no persistent or significant disability/incapacity was reported. w: week.

mine whether allopurinol 100 mg/m^2 yields an even better effect on purine metabolites than 50 mg/m^2 but the e-TGN values after w19 do not suggest such benefits.

After adding allopurinol, the levels of e-MeMP decreased within 4 weeks to roughly one fourth of the levels before allopurinol. The ratio e-MeMP/e-TGN dropped after allopurinol in a way that is reminiscent of the 6MP shunters in a descriptive study.[22]

As e-MeMP is associated with hepatic toxicity[26] it could be anticipated that the large reduction of this metabolite would be associated with a lower frequency of hepatic toxicity. Using ALT as a marker for hepatic toxicity, we found a substantial decrease occurring a few weeks after the e-MeMP-levels decreased, with mean ALT reduced by 39% w21, and 47% w25, compared to w13.

With SAE being evenly distributed over the two study phases and no life-threatening events reported the results support that allopurinol is safe to use as add-on treatment for this group of patients. This is in line with previous studies.[25]

The fraction of patients reaching target WBC was higher and the mean ANC and WBC were lower during the weeks on allopurinol compared both to the weeks before and after allopurinol. These parameters reflect MT intensity and have been correlated to EFS.[12,27] Together with higher e-TGN during weeks on allopurinol this raises the question if add-on treatment with allopurinol could have a positive impact on ALL outcome.

An alternative approach to increase e-TGN has been described in a pilot study.[28] It is currently tested in the ALL-together1 protocol substudy TEAM (Thiopurine Enhanced ALL Maintenance therapy, *clinicaltrials gov. Identifier: NCT04307576*) in which incremental doses of 6-thioguanine is added to standard MT. The main endpoint is the 5-year disease-free survival. It would be interesting in the future to perform a larger randomized allopurinol trial with similar clinical end points, to evaluate if allopurinol addition during MT leads to a better outcome without increased toxicity.

In conclusion our study shows that allopurinol is effective for altering thiopurine metabolism towards higher levels of e-TGN and lower e-MeMP for pediatric ALL patients with wild-type TPMT, also for those without previous signs of skewed thiopurine metabolism. The dose 50 mg/m^2 was sufficient to achieve this effect and did not result in more adverse effects or toxicities than normal MT. WBC and ANC were better controlled, and ALT decreased during the phase with allopurinol suggesting that allopurinol add-on treatment might both decrease toxicity and improve efficacy of maintenance therapy for pediatric ALL patents.

Disclosures

No conflicts of interest to disclose.

Contributions

JA and TE were responsible for designing the study, writing the protocol, and obtaining necessary approvals for the study in Sweden. JA was the coordinating investigator for the trial in Sweden. RN was the responsible for the approval of the study in Finland and was coordinating investigator for the trial in Finland. JA, TE and JK were responsible for extracting and analyzing data, interpreting results, and writing the manuscript. All authors provided material and data, reviewed and approved the final version of the manuscript.

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Data-sharing statement

For original data please contact the corresponding author.

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