Allopurinol increases DNA-thioguanine nucleotides during maintenance therapy in pediatric acute lymphoblastic leukemia

Allopurinol can be used during acute lymphoblastic leukemia (ALL) maintenance therapy (MT) to mitigate hepatotoxicity and enable adequate myelosuppression in patients with unfavorable, high-methylating 6-mercaptopurine (6MP) metabolism.[1] We report a prospective, before-after trial on 51 pediatric ALL patients without prior signs of unfavorable 6MP metabolism, with data suggesting that allopurinol may enhance efficacy in ALL MT.

6MP is a prodrug that is metabolized in various ways, with thioguanine nucleotides (TGN) considered as the key intermediate metabolites mediating the antileukemic effect through incorporation into the DNA-strand.[2] Levels of DNA-incorporated TGN (DNA-TG) correlate more strongly with event-free survival (EFS) than erythrocyte levels of TGN (e-TGN).[2,3] Methylated mercaptopurine metabolites (MeMP) are associated with liver toxicity, including transaminitis, nausea, and hypoglycemia.[4] Allopurinol is sometimes used in ALL MT in patients with unfavorable, high-methylating 6MP metabolism,[1,5] clinically characterized by hepatotoxicity and difficulty achieving targeted myelosuppression. In these patients, allopurinol reduces e-MeMP and increases e-TGN. In a prospective study we showed that addition of allopurinol to MT leads to higher e-TGN and lower e-MeMP even in patients without prior severe liver toxicity.[6] In the same study cohort, we now aimed to assess whether allopurinol could improve the efficacy of ALL MT by determining the effect on DNA-TG.

The Allopurinol study included 51 pediatric ALL patients from Sweden and Finland with thiopurine methyltransferase (TPMT) wild-type and without prior significant hepatotoxicity (*Online Supplementary Figure S1*), treated according to NOPHO ALL-2008 based protocols. Patient characteristics and outcome are shown in *Online Supplementary Table S1*. 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*).

The study comprised three phases: 12 weeks of standard MT, followed by 12 weeks of MT with addition of allopurinol 50 mg/m², and finally four weeks of MT without allopurinol.[6] To prevent excessive myelosuppression, the 6MP dose was halved at the start of the allopurinol phase.[5] 6MP dose was thereafter titrated according to standard protocol guidelines.

6MP metabolites (e-TGN, e-MeMP and DNA-TG) were measured at nine predefined timepoints and analyzed at Bonkolab, Copenhagen. For DNA-TG quantification 1–2 microgram DNA was purified from whole blood and thioguanine measured with ultra-performance liquid chromatography tandem mass spectrometry, as detailed by Jacobsen *et al.*[7] A total of 375 DNA-TG measurements were analyzed during the standard MT phase (N=166), during the allopurinol+MT phase (N=169), and four weeks after allopurinol was discontinued (N=40). Mean DNA-TG levels increased rapidly after addition of allopurinol and decreased when allopurinol was discontinued (Table 1, Figure 1). Intraindividual DNA-TG varied substantially, with mean co-efficient of variation 32% during standard MT and 38% during the allopurinol phase. We, therefore, calculated each patient´s median DNA-TG for the different study phases and found a mean increase by 398 fmol / microgram DNA (paired analysis, 95% Confidence Interval [CI]; 257-539, P<0.001) when allopurinol was added, followed by a mean decrease by 543 (95%CI: 393-692, P<0.001) when discontinued. Compared to historical data from the NOPHO ALL-2008 MT substudy (492 fmol/microgram DNA) and the TEAM-pilot study (530-764 fmol/microgram DNA),[8] the DNA-TG values were higher in our cohort even before adding allopurinol (748) and yet increased to 1,251 when allopurinol was added (Table 2). The reason for the high baseline levels in our study is unclear but may partly be explained by the exclusion of patients with previous severe hepatotoxicity possibly indicating unfavorable 6MP metabolism.

The increase in mean e-TGN was more rapid than for DNA-TG, reaching a plateau within four weeks after addition of allopurinol (Figure 1). In the same period, e-MeMP showed a rapid and persistent decrease.

Since e-MeMP has been suggested to inhibit purine synthesis, thereby reducing guanine levels, which compete with TGN for DNA incorporation,[9,10] there was initial concern that a substantial reduction in e-MeMP following addition of allopurinol might compromise DNA incorporation of TGN. We, therefore, calculated the DNA-TG/e-TGN ratio for each patient (Table 1). A low ratio would indicate a reduced DNA incorporation. Although we did see a slightly lower DNA-TG/e-TGN ratio the first weeks after addition of allopurinol, we believe that this merely reflects a natural delay caused by the time required for the metabolic process of integrating TGN into DNA, rather than inhibition of DNA incorporation due to low e-MeMP. This is supported both by the gradually increasing ratio during the allopurinol phase (to the same levels as before allopurinol, despite e-MeMP remaining low) and by the even higher ratio four weeks after truncation

Table 1. 6MP metabolite levels, hematologic parameters and ALT across all study weeks.

	Std MT				MT + allopurinol				Std MT
Study week	**w1**	**w5**	**w9**	**w13**	**w17**	**w19**	**w21**	**w25**	**w29**
DNA-TG Mean (95% CI)	735 (521-987)	980 (818-1,261)	798 (676-1,121)	963 (718-1,143)	1,216 (921-1,707)	1,233 (927-1,364)	1,301 (1,082-1,795)	1,288 (1,143-1,678)	713 (510-833)
e-TGN Mean (95% CI)	254 (209-300)	282 (247-318)	251 (217-285)	280 (235-325)	485 (424-546)	447 (383-511)	462 (383-541)	440 (379-501)	185 (161-210)
DNA-TG/e-TGN-ratio Mean (95% CI) *P* value compared to w13	3.2 (2.7-3.7) -	3.7 (3.2-4.2) -	3.3 (2.9-3.8) -	3.6 (3.1-4.2) -	2.9 (2.1-3.6) 0.04	3.1 (2.5-3.6) 0.05	3.5 (2.7-4.3) 0.76	3.3 (2.7-3.9) 0.27	3.8 (3.3-4.3) -
e-MeMP Mean (95% CI)	6,993 (4,904-9,082)	9,830 (7,718-11,943)	9,753 (7,726-11,780)	9,481 (7,346-11,616)	3,395 (2,397-4,393)	2,459 (1,634-3,283)	2,230 (1,455-3,005)	2,791 (1,868-3,714)	3,459 (2,324-4,595)
WBC 1.5-3.0x10^9/L Mean (95% CI) Values within target 1.5-3.0 N/total (%)	3.3 (3.0-3.6) 18/48 (38)	3.2 (2.7-3.6) 25/49 (51)	2.7 (2.5-3.0) 32/48 (67)	3.7 (2.7-4.7) 28/49 (57)	2.3 (2.0-2.7) 34/45 (76)	2.4 (2.2-2.7) 31/49 (63)	2.7 (2.4-3.1) 30/47 (64)	2.5 (2.2-2.7) 33/45 (73)	3.7 (3.1-4.3) 19/45 (42)
ANC x10^9/L Mean (95% CI)	1.8 (1.6-2.1)	1.8 (1.4-2.1)	1.4 (1.2-1.6)	1.9 (1.5-2.3)	1.2 (0.9-1.5)	1.2 (1.0-1.4)	1.5 (1.2-1.8)	1.3 (1.1-1.5)	2.2 (1.6-2.8)
ALT IU/L Mean (95% CI)	130 (86-175)	125 (82-167)	170 (101-240)	184 (124-245)	132 (96-169)	129 (80-177)	112 (65-160)	99 (56-141)	110 (79-140)

Allopurinol was administered throughout study weeks (w) 13-24. Samples collected weeks 17–25 are considered being influenced by allopurinol. 1.5-3.0x10^9/L is the target level of white blood cells (WBC) during maintenance therapy (MT) in the NOPHO ALL-2008 protocol. CI: Confidence Interval; N: number of patients with WBC within target 1.5-3.0x10^9/L. Std MT: standard maintenance therapy; Tot: total number of patients with available WBC data for the corresponding week. Units of measurement - DNA incorporated thioguanine nucleotides (DNA-TG): fmol / microgram DNA. Thioguanine nucleotides in erythrocytes (e-TGN): nmol / mmol Hb DNA-TG / e-TGN-ratio: (fmol/microgram) / (nmol/mmol). Methylmercaptopurine nucleotides in erythrocytes (e-MeMP): nmol/mmol Hb. WBC: x10^9/L. Absolute neutrophil count (ANC): x10^9/L. Alanine aminotransferase (ALT): IU/L. 60 IU = 1 μkat.

of allopurinol, caused by a more pronounced decrease in e-TGN compared to DNA-TG. The DNA-TG/e-TGN ratio was surprisingly high in our study, with mean 3.3 for the whole study period, which is approximately 50% higher than in the whole NOPHO ALL-2008 cohort.[8] The reason why the ratios are higher in our cohort is unclear, but the maintained high ratios indicate that allopurinol does not significantly inhibit the DNA incorporation of TGN, despite lower e-MeMP.

Acute lymphoblastic leukemia MT treatment is traditionally targeted to a controlled myelosuppression, measured with white blood cell (WBC) or absolute neutrophil count (ANC) values.[11] NOPHO ALL-2008 used WBC for dose titration. As previously published,[6] the mean of all patients' median ANC within each phase declined from 1.6 to 1.3x10^9/L (95%CI: 1.4-1.8 *vs*. 1.2-1.5, *P*=0.007) and WBC declined from 3.0 to 2.5x10^9/L (95%CI: 2.8-3.2 *vs*. 2.3-2.7, *P*<0.001) when allopurinol was added, whereas the proportion of WBC analyses within the therapeutic target 1.5-3.0x10^9/L increased from 54% to 65% (95%CI: 47-62% *vs*. 58-72%, *P*=0.01) (Table 1). Despite lower mean ANC, the frequency of severe adverse events (SAE) (mainly febrile neutropenia) did not increase. This, however, must be interpreted with caution due to the short intervention period. The total number of transfusions were low with no significant difference in the rate of transfusions between the study phases.

The mean administered 6MP dose, as reported in the patient diary, was 27 mg/m^2/day (95%CI: 23-30) during the allopurinol phase, precisely half the reported dose during standard MT (54 mg/m^2/day; 95%CI: 48-60). There were more treatment interruptions during the allopurinol phase, affecting 26 patients (34 events) during the allopurinol phase compared to 13 patients (*P*=0.03) (20 events, *P*<0.01) during the standard MT phase. The number of days with therapy on hold was also higher with allopurinol at 200 out of 4,158 (4.8%) patient-days compared to 92/4,284 (2.1%) without (*P*<0.01).

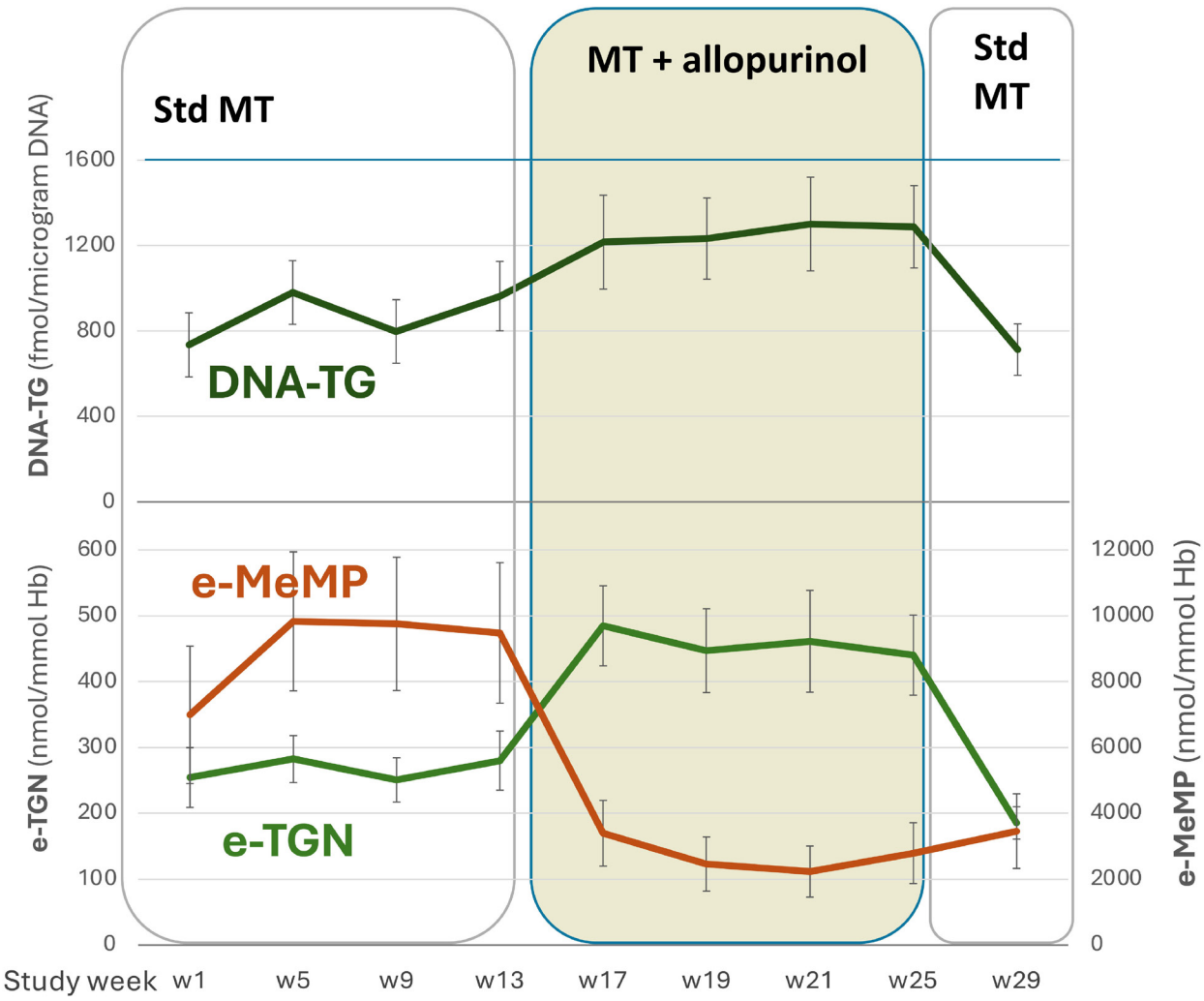

Figure 1. 6-mercaptopurine metabolite levels, means and 95% Confidence Intervals (error bars) across the different study phases. Weeks (w) 1-12: standard maintenance therapy (Std MT) with 6-mercaptopurine and methotrexate. Weeks 13-24: maintenance therapy (MT) with addition of allopurinol. 6-mercaptopurine (6MP) dose reduced by 50% to prevent excessive myelosuppression. Weeks 25-29: Std MT without allopurinol. Weeks 17-25 are considered being influenced by allopurinol as samples were collected at the start of the corresponding study week. DNA-TG: DNA incorporated thioguanine nucleotides; e-MeMP: methylmercaptopurine nucleotides in erythrocytes; e-TGN: thioguanine nucleotides in erythrocytes.

Treatment interruptions due to myelosuppression were most common during the first six weeks on allopurinol (20 of the 34 events). This may reflect the fact that the 50% dose reduction of 6MP when commencing allopurinol is arbitrary and some patients need to reduce their 6MP dose more than 50% when starting on allopurinol. The 12 weeks with allopurinol was not long enough to determine if the number of treatment interruptions would decrease with time.

As previously reported,[6] mean ALT decreased gradually from 184 IU/L week 13 (before allopurinol) to 99 IU/L week 25 (P=0.018) (Table 1) after 12 weeks of allopurinol, indicating reduced hepatotoxicity.

DNA-incorporated thioguanine nucleotides have been correlated to reduced relapse rates in several studies and meta-analyses,[3,12] with adjusted hazard ratio (aHR) as low as 0.72 per 100 fmol / microgram increase in DNA-TG in patients minimal residual disease (MRD)-positive at the end of induction (95%CI: 0.57-0.91).[12] The 398 fmol / microgram DNA mean increase in DNA-TG achieved in our study suggests that allopurinol could substantially improve EFS. Compared to the TEAM strategy,[8] aiming to enhance MT efficacy by adding incremental doses of 6-thioguanine, the allopurinol study achieved higher DNA-TG levels and a greater increase. Rather than adding another cytotoxic agent, this strategy modulates thiopurine metabolism, allowing a 50% reduction of the 6MP dose. The approach is also simpler, using a fixed allopurinol dose and standard titration rules for 6MP and methotrexate (MTX).

In a recent retrospective report by Mo *et al.*,[13] allopurinol was shown to be an independent prognostic factor for improved EFS in patients who received allopurinol during maintenance therapy compared to those who did not (aHR = 0.29; 95%CI: 0.10-0.80).[13] In that study, allopurinol was administered to patients with laboratory signs of hepatotoxicity during MT. Despite methodological weaknesses, including selection bias and immortal time bias, the findings raise the possibility of an association between allopurinol use and improved outcome.

In this transformative era for ALL therapy, when intense chemotherapy phases are being replaced by targeted antibodies with reduced toxicity,[14] we still believe that MT will remain essential for achieving long-term cure. Allopurinol add-on therapy may both improve tolerability and contribute to an increased efficacy of MT.

Our previously published results, with reduced laboratory

Table 2. Mean of patients' median values, in comparison with the TEAM pilot study and the NOPHO ALL-2008 maintenance therapy substudy.[8]

	Allopurinol study		TEAM pilot study		NOPHO ALL-2008
	Std MT	MT+AP	Std MT	MT+TG	MT substudy
DNA-TG fmol/mikrog DNA Mean (95% CI)	847 (728-966)	1,251 (1,095-1,407)	530 (157-1,279)	764 (273-1,402)	492 (21-1,104)
e-TGN nmol/mmol Hb Mean (95% CI)	264 (231-297)	458 (404-512)	240 (100-485)	721 (339-1,396)	231 (8-608)
e-MeMP nmol/mmol Hb Mean (95% CI)	8,878 (7,169-10,587)	2,366 (1,741-2,990)	8,462 (177-21,520)	5,931 (142-14,385)	12,032* (4,577-17,383)*
WBC $\times 10^9$/L Mean (95% CI) Values within target 1.5-3.0	3.1 (2.8-3.2) 54%	2.5 (2.3-2.7) 65%	3.1 (1.9-5.7) -	3.2 (2.2-5.5) -	2.9 (1.7-4.0) -
ANC $\times 10^9$/L Mean (95% CI)	1.6 (1.4-1.8)	1.3 (1.2-1.5)	1.8 (0.7-3.7)	1.9 (1.0-3.7)	1.6 (0.5-2.7)
ALT IU/L Mean (95% CI)	136 (97-175)	101 (77-125)	139 (26-373)	118 (20-265)	- -
6MP dose mg/m^2/day Mean (95% CI)	54 (48-60)	27 (23-30)	53 (6-121)	45 (7-79)	- -

6MP: 6-mercaptopurine; ALL: acute lymphoblastic leukemia; ANC: absolute neutrophil count; ALT: alanine aminotransferase; CI: Confidence Interval; DNA-TG: DNA incorporated thioguanine nucleotides; e-MeMP: methylmercaptopurine nucleotides in erythrocytes; e-TGN: thioguanine nucleotides in erythrocytes; MT+AP: maintenance therapy with addition of allopurinol; MT+TG: maintenance therapy with addition of thioguanine; NOPHO: The Nordic Society of Paediatric Haematology and Oncology; Std MT: standard maintenance therapy; TEAM: thiopurine enhanced ALL maintenance therapy; WBC: white blood cells. *Median of the patients' medians and interquartile range (see TEAM-pilot).[8]

signs of liver toxicity, decreased e-MeMP and no increase in SAE frequency, suggest that allopurinol add-on treatment is safe and may reduce toxicity, whereas increased e-TGN and better controlled WBC and ANC point towards enhanced treatment efficacy.[11] The increase in DNA-TG levels reported here provides further evidence suggesting that allopurinol may lead to a more effective leukemia treatment. This should be evaluated in a future randomized study with endpoints including EFS and quality-of-life measures.

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Contributions

TE designed the study, obtained necessary approvals in Sweden, and was the data manager of the study; RN was responsible for the approval of the study in Finland and was co-ordinating investigator in Finland; TE and JK extracted and analyzed data, and wrote the manuscript; TE, JK, RN, JF, HV, LK and AC provided material and data; KS and MT were responsible for the thiopurine metabolite analyses. All authors reviewed and approved the final version of the manuscript for publication.

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Data-sharing statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Lines M, Kemper RM, Wallace J, et al. Use of allopurinol to manage skewed 6-mercaptopurine metabolism in pediatric maintenance acute lymphoblastic leukemia treatment. Eur J Haematol. 2024;113(5):584-592.
2. Toksvang LN, Lee SHR, Yang JJ, Schmiegelow K. Maintenance therapy for acute lymphoblastic leukemia: basic science and clinical translations. Leukemia. 2022;36(7):1749-1758.
3. Toksvang LN, Grell K, Nersting J, et al. DNA-thioguanine concentration and relapse risk in children and young adults with acute lymphoblastic leukemia: an IPD meta-analysis. Leukemia. 2022;36(1):33-41.
4. Nygaard U, Toft N, Schmiegelow K. Methylated metabolites of 6-mercaptopurine are associated with hepatotoxicity. Clin Pharmacol Ther. 2004;75(4):274-281.
5. Conneely SE, Cooper SL, Rau RE. Use of allopurinol to mitigate 6-mercaptopurine associated gastrointestinal toxicity in acute lymphoblastic leukemia. Front Oncol. 2020;10:1129.
6. Källström J, Niinimäki R, Fredlund J, et al. Effects of allopurinol on 6-mercaptopurine metabolism in unselected patients with pediatric acute lymphoblastic leukemia: a prospective phase II study. Haematologica. 2024;109(9):2846-2853.
7. Jacobsen JH, Schmiegelow K, Nersting J. Liquid chromatography-tandem mass spectrometry quantification of 6-thioguanine in DNA using endogenous guanine as internal standard. J Chromatogr B Analyt Technol Biomed Life Sci. 2012;881-882:115-118.
8. Larsen RH, Utke Rank C, Grell K, et al. Increments in DNA-thioguanine level during thiopurine-enhanced maintenance therapy of acute lymphoblastic leukemia. Haematologica. 2021;106(11):2824-2833.
9. Hedeland RL, Hvidt K, Nersting J, et al. DNA incorporation of 6-thioguanine nucleotides during maintenance therapy of childhood acute lymphoblastic leukaemia and non-Hodgkin lymphoma. Cancer Chemother Pharmacol. 2010;66(3):485-491.
10. Stanulla M. From niche to blockbuster: a greater role for allopurinol in maintenance treatment of acute lymphoblastic leukemia. Haematologica. 2024;109(9):2764-2766.
11. Schmiegelow K, Nersting J, Nielsen SN, et al. Maintenance therapy of childhood acute lymphoblastic leukemia revisited - Should drug doses be adjusted by white blood cell, neutrophil, or lymphocyte counts? Pediatr Blood Cancer. 2016;63(12):2104-2111.
12. Nielsen SN, Grell K, Nersting J, et al. DNA-thioguanine nucleotide concentration and relapse-free survival during maintenance therapy of childhood acute lymphoblastic leukaemia (NOPHO ALL2008): a prospective substudy of a phase 3 trial. Lancet Oncol. 2017;18(4):515-524.
13. Mo Y, Zhang Q, Wang M, et al. Results from patient-derived xenograft models support co-administration of allopurinol and 6-mercaptopurine to reduce hepatotoxicity and improve event-free survival in pediatric acute lymphoblastic leukemia. Haematologica. 2025;110(7):1610-1615.
14. Gupta S, Rau RE, Kairalla JA, et al. Blinatumomab in standard-risk B-cell acute lymphoblastic leukemia in children. N Engl J Med. 2025;392(9):875-891.