Does allopurinol enhance efficacy of acute lymphoblastic leukemia maintenance therapy?

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Received: March 3, 2025.
Accepted: March 12, 2025.
Early view: March 20, 2025.

https://doi.org/10.3324/haematol.2024.287274

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Thiopurine-based maintenance therapy, mostly including the purine analog 6-mercaptopurine (6-MP), is an essential part of acute lymphoblastic leukemia (ALL) therapy. Incorporation of thioguanine nucleotides (TGN) into the DNA double strand (as DNA-TG) in competition with natural guanine, with a median of approximately 1 in 6,000 nucleotides being thioguanine (TG) substituted, is the primary cytotoxic mechanism (Figure 1).[1] In addition, accumulation of methylated 6-MP metabolites (MeMP), mediated by thiopurine S-methyltransferase (encoded by *TPMT*), is associated with hepatotoxicity. Five to 10% of patients have at least one TPMT low-activity allele, and they experience lower levels of MeMP (and thus less hepatotoxicity), but more myelosuppression. Likewise, loss of function variants in nudix hydrolase 15 (encoded by *NUDT15*) confer higher TGN and more myelosuppression. Due to large inter-individual variations in 6-MP bioavailability and metabolism, the treatment guidelines usually recommend dose adjustments by the degree of myelosuppression (e.g., white blood cells [WBC] or absolute neutrophil counts [ANC] targets between 1.5-3.0 or 0.75-1.5 x10^9/L, respectively), although it only partly reflects treatment intensity.[2] Higher levels of DNA-TG have been associated with a lower relapse hazard, and various strategies have been proposed to (i) enhance efficacy by increasing DNA-TG, and/or (ii) reduce hepatotoxicity by lowering MeMP levels.[3-5]

Co-administration of allopurinol with 6-MP has been widely used in inflammatory bowel disease and to a much lesser extent in ALL therapy.[5,6] Allopurinol not only inhibits xanthine oxidase, thus increasing 6-MP bioavailability, but more importantly shifts the metabolism of 6-MP away from MeMP towards TGN, thus inducing a TPMT heterozygous phenotype. In the last decade studies on small series of ALL patients have shown that adding allopurinol to 6-MP-based maintenance therapy increased TGN levels making it easier to obtain the protocol WBC or ANC target, while reducing MeMP levels and the degree of hepatotoxicity.

In this issue Yixiao Mo *et al.*[7] report that a cohort of 149 patients who received low-dose allopurinol during 6-MP-based maintenance therapy could be treated with lower doses of 6-MP, experienced less liver toxicity, had longer periods of myelosuppression within the set target, and seemingly had a significantly better event-free survival compared to a control group in a retrospective study. The results were somewhat corroborated by a patient-derived xenograft study, which showed that mice receiving allopurinol and half dose of 6-MP had survival comparable to mice receiving the full dose of 6-MP as well as a significant shift in thiopurine metabolism with higher DNA-TG and reduced MeMP levels. The findings are challenged by several methodological flaws and weaknesses, to a great extent recognized in the paper, which call for caution in the interpretation of the results. Besides selection bias and survival bias, choice of treatment and doses at treating physician's discretion, the addition of allopurinol took place in only 149 of 752 patients, and in some of them even towards the end maintenance therapy, based on specific liver and hematological criteria. Importantly, with data analyzed without a landmark, early events occurred mostly in the control arms, thus amplifying the gap between the allopurinol arm and control arm curves. Although the ALL cohort reported is by far the largest treated with allopurinol, these data can't be considered solid, and we thus agree with the authors that the verdict on the potential benefit of adding allopurinol to 6-MP-based maintenance therapy will require a sufficiently powered randomized trial.

This approach is strongly needed not only for correct statistical assessment but also for the biological context. Of note, thiopurine metabolism, as shown in Figure 1, is complex and it may not be easily related to efficacy. A large Nordic study comparing patients classified according to their TPMT genotype and phenotype demonstrated that TPMT low activity patients had significantly higher TGN and DNA-TG levels and lower MeMP levels, but not a superior

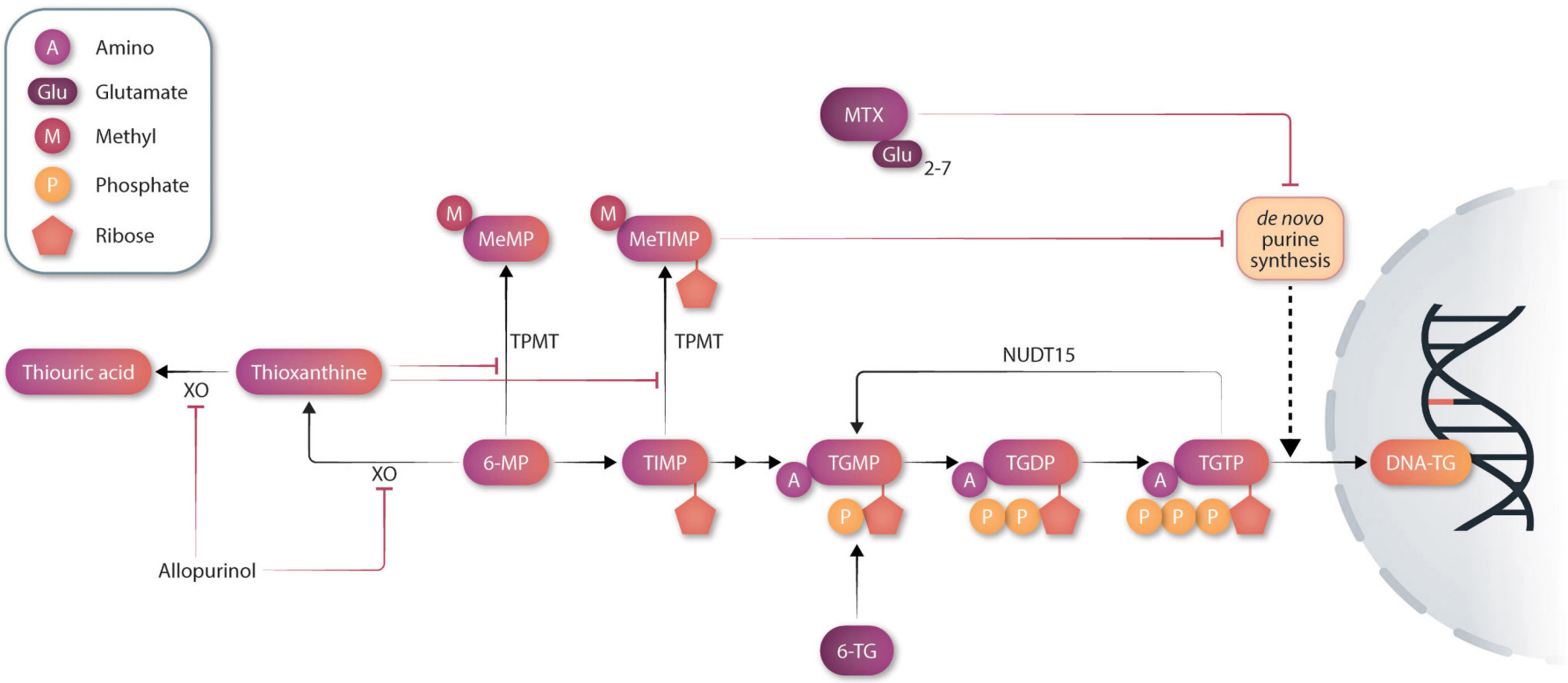

Figure 1. Thiopurine metabolism and mechanisms of action of allopurinol. 6-MP: 6-mercaptopurine; 6-TG: 6-thioguanine; DNA-TG: DNA-incorporated thioguanine; MeMP: methyl-mercaptopurine; MeTIMP: methyl-thioinosine monophosphate; MTX: methotrexate; NUDT15: nudix hydrolase 15; TGDP: thioguanine diphosphate; TGMP: thioguanine monophosphate; TGTP: thioguanine triphosphate; TIMP: thioinosine monophosphate; TPMT: thiopurine S-methyltransferase; XO: xanthine oxidase.

outcome, suggesting that there may be other factors than DNA-TG affecting efficacy in patients who are *TPMT* heterozygous.[8] Both natural DNA-G and DNA-TG can randomly become O^6-methylated (O^6-MeTG) and S^6-methylated (S^6-MeTG), respectively, and then mismatch with T (O^6-MeTG·T; S^6-MeTG·T), as the changed chemical nucleotide structure favors O^6-MeTG·T and S^6-MeTG·T matching. Activation of the mismatch repair (MMR) pathway to correct the S^6-MeTG·T mismatching will be futile, since the polymerase that fills the repair-induced gaps in the DNA strand opposite the modified S^6-MeTG, will continue to generate mismatching, and the repetitive cycles of MMR-induced strand degradation cause replication fork collapse, double strand breaks, and apoptosis through activation of checkpoint signaling networks. The role of TPMT in the S^6-MeTG methylation is unclear but could explain why *TPMT* heterozygous patients do not have a superior outcome. This emphasizes why the potential benefits of allopurinol with respect to relapse rate must be tested in a randomized study, and why other alternatives, such as the addition of low-dose 6-TG to the 6-MP/methotrexate backbone, which also increases DNA-TG levels, could be effective in both *TPMT* wild-type and heterozygous patients. This is currently being tested in a randomized trial in the ALLTogether1 protocol (*clinicaltrials gov. Identifier: NCT04307576*).[9]

Disclosures

No conflicts of interest to disclose.

Contributions

KS, LT and VC conceived and drafted the manuscript. All authors read and acknowledged the final manuscript.

References

1. 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.
2. Nielsen SN, Grell K, Nersting J, Frandsen TL, Hjalgrim LL, Schmiegelow K. Measures of 6-mercaptopurine and methotrexate maintenance therapy intensity in childhood acute lymphoblastic leukemia. Cancer Chemother Pharmacol. 2016;78(5):983-994.
3. 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.
4. Larsen RH, Rank CU, Grell K, et al. Increments in DNA-thioguanine level during thiopurine-enhanced maintenance therapy of acute lymphoblastic leukemia. Haematologica. 2021;106(11):2824-2833.
5. 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.
6. Deswal S, Srivastava A. Role of allopurinol in optimizing

thiopurine therapy in patients with autoimmune hepatitis: a review. J Clin Exp Hepatol. 2017;7(1):55-62.
7. 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.
8. Nielsen SN, Toksvang LN, Grell K, et al. No association between relapse hazard and thiopurine methyltransferase geno- or phenotypes in non-high risk acute lymphoblastic leukemia: a NOPHO ALL2008 sub-study. Cancer Chemother Pharmacol. 2021;88(2):271-279.
9. Toksvang LN, Als-Nielsen B, Bacon C, et al. Thiopurine Enhanced ALL Maintenance (TEAM): study protocol for a randomized study to evaluate the improvement in disease-free survival by adding very low dose 6-thioguanine to 6-mercaptopurine/methotrexate-based maintenance therapy in pediatric and adult. BMC Cancer. 2022;22(1):483.