Improving the treatment of childhood acute lymphoblastic leukemia by optimizing the use of 70-year-old drugs

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In the current issue of *Haematologica*, Larsen, Schmiegelow and colleagues (1) have provided clear and convincing evidence that the addition of a relatively low dose of thioguanine (TG; $\leq$12.5 mg/m^2/day) can significantly increase the amount of thioguanine nucleotides (TGN) incorporated into DNA of normal leukocytes in patients with ALL, when compared to treatment with only mercaptopurine (MP) in historical controls or in the same patient prior to the addition of low-dose TG. As depicted in **Figure 1**, MP requires intracellular metabolism by multiple enzymes to produce thioguanine nucleotides, whereas TG is converted directly to thioguanine nucleotides. The deoxy thioguanine triphosphates are then available for incorporation into DNA, which is thought to be the principal mechanism of MP's antileukemic effects.

Mercaptopurine is a mainstay of combination chemotherapy for the treatment of acute lymphoblastic leukemia (ALL), which is curative for over 90% of children and ~70% of adults, whereas TG is not widely used to treat ALL. MP was the first antileukemic agent for which pharmacogenomics was shown to be an important determinant of the optimal dosage, with those inheriting non-function variants of thiopurine methyltransferase (TPMT) more likely to develop dose-limiting hematological toxicity, if treated with conventional MP doses (75 mg/m^2/day) (2, 3). In the 1990s, MP became one of the first medications for which preemptive genotyping for common variants (in *TPMT)* were used to determine the optimal dosage(2, 3), and twenty years later this strategy was expanded to include testing for inactivating variants in *NUDT15* (nucleotide diphosphatase nudix hydrolase 15) (4, 5). Non-functional alleles of *TPMT* are the primary determinants of MP toxicity in people of European and African ancestry, whereas *NUDT15* variants are the primary determinants in people of Asian and Native American ancestry (6). Patients who inherit two non-functional alleles for either of these enzymes must be treated with only 5-10% of the conventional dose of MP to avoid toxicity, whereas for patients who are heterozygous it is recommended to reduce the starting dose by about 50%. (4) Even with these dose reductions, these enzyme-deficient patients maintain higher average erythrocyte TGN levels than homozygous wildtype patients treated with full

doses of MP and have comparable cure rates. It is unclear whether TPMT deficient or heterozygous patients require supplemental doses of TG to achieve DNA-TG in the target range, and if so, what dosage of supplemental TG should be given.

As a complement to preemptive genotyping, monitoring the concentration of thioguanine nucleotides (TGN) in erythrocytes is commonly used to identify patients who accumulate excessive levels of TGN or patients who have low TGN levels due to non-compliance with daily oral MP therapy. Although measuring TGNs in erythrocytes is clinically useful, this is not measuring the active drug in the target tissue (leukemia cells) nor active drug at the presumed site of action (thioguanine incorporated into the DNA). It will be important to determine in a large prospective clinical trial whether measuring TG incorporated into DNA is indeed a better metric of MP treatment than measuring TGN in erythrocytes, because measuring TG in DNA requires a more complex assay, which may not be widely available. Larsen et al report that such a clinical trial (ALLTogether-1) is ongoing. It is interesting that in the current report, Larsen et al did not find any correlation between median erythrocyte TGN and median TG incorporated into leukocyte DNA (supplemental Figure 6Sc).

Although it is not known how closely TG incorporated into DNA of normal leukocytes reflects TG incorporated into DNA of primary ALL cells in patients, it is reasonable to assume this is a better surrogate than measuring TGNs in the cytosol of erythrocytes, in part because only the trinucleotide is incorporated into DNA, whereas inactive mono- and di-phosphate nucleotides are measured in erythrocytes. Measuring TG incorporated into the DNA of primary leukemia cells in patients would be the ideal metric, but this is not feasible because patients are generally in complete remission before MP therapy is initiated and thus there are no leukemia cells to assess. It is also not known whether the incorporation of TG into DNA of normal leukocytes has a uniform relation to TG incorporated into DNA of leukemia cells of different molecular and lineage subtypes of ALL.

These limitations notwithstanding, measuring TG incorporated into DNA (DNA-TG) of normal leukocytes offers a potential advance for optimizing MP treatment of ALL. A major unknown is what level of TG incorporation into DNA is indicative of optimal treatment with MP, which will require assessment of the relation between TG in leukocyte DNA and event free survival in a large enough cohort of uniformly treated patients so that all relevant covariates can be incorporated into a multivariate analysis.

It is also not known whether the level of increase in DNA-TG documented by Larsen et al, translates into an improvement in event free survival, although Larsen *et al* speculate that this could reduce the relapse hazard rate by as much as 59%, based on their prior research reporting a relapse hazard ratio of 0.81 per 100 fmol/µg DNA increase, (95% CI 0·67–0·98; p=0·029) (7). A final issue that will need further study in a larger cohort of patients, is whether the addition of this small dose of TG is associated with additional toxicity during ALL therapy, as TG has been associated with veno-occlusive disease in ~20% of children with ALL (8), and the risk with low-dose TG when given concomitantly with MP is unknown.

Today, we are faced with the challenge of pushing the cure rate of childhood ALL beyond 90% while also improving the quality of life for those we cure. Improving the cure rate from 20% to 90% over the past six decades has been largely achieved by optimizing the use of conventional chemotherapy like MP, not by the development of new antileukemic agents. Most of these improvements have been incremental in nature, but their cumulative effects have produced remarkable progress (9, 10). The work of Schmiegelow and colleagues may offer yet another small step toward maximizing the effects of medications that we have been using for many decades. Much hope and hype have been raised around the development of more targeted therapy for cancer, yet when available these targeted agents, such as tyrosine kinase inhibitors, are being added to and not replacing conventional chemotherapy in treating ALL. Its wise that we not abandon efforts to further improve the use of these older anticancer agents and avoid placing all our hope on so called “targeted chemotherapy”. And it should not go unnoticed that we continue to expand our knowledge of how best to use anticancer agents developed 70 years ago, suggesting that in the coming decades we may still be optimizing the use of both targeted and conventional chemotherapy as we work to push the ALL cure rate closer to 100%.

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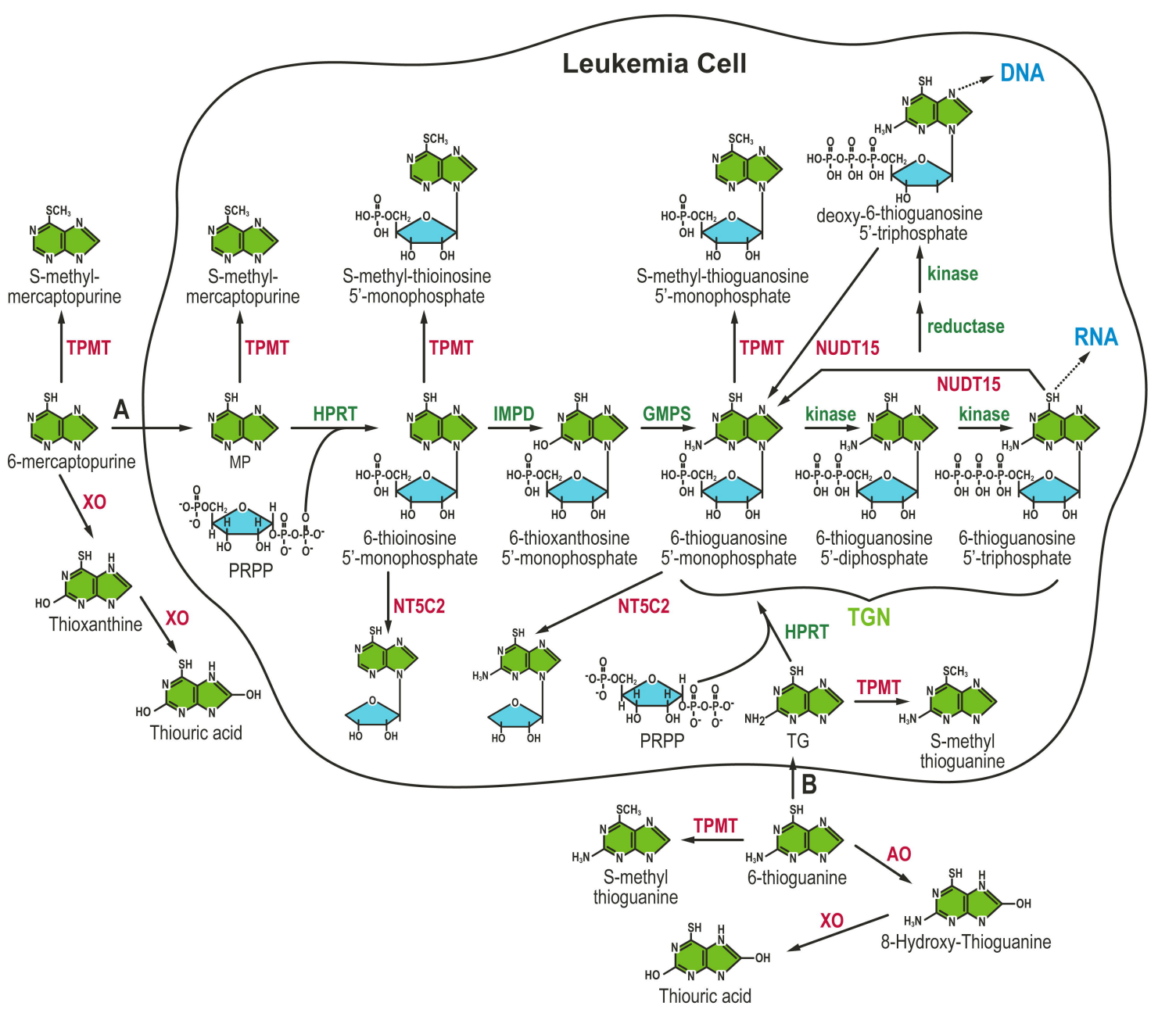
Figure 1 Schematic representation of the metabolism of mercaptopurine (A) and thioguanine (B) and the enzymes involved: HPRT, hypoxanthine phosphoribosyltransferase; IMPD, inosine monophosphate dehydrogenase; GMPS, guanosine monophosphate synthase; kinase, nucleoside kinases; TPMT, thiopurine S-methyltransferase; NUDT15, nucleotide diphosphatase nudix hydrolase 15; NT5C2, 5'-Nucleotidase, Cytosolic II; XO, xanthine oxidase; AO aldehyde oxidase. PRPP, phosphoribosyl pyrophosphate is a substrate in the reaction catalyzed by HPRT to form thiopurine nucleotides. The monophosphate of either the deoxy or ribonucleotide is formed by NUDT15, depending on the substrate. Enzymes shown in red catalyze inactivation of these thiopurine medications whereas those depicted in green are involved in activation to thioguanine nucleotides (TGN), which can be incorporated into DNA and RNA. I thank Mr. Josh Stokes for his many contributions in preparing this Figure.


Leukemia Cell
DNA
RNA
deoxy-6-thioguanosine 5'-triphosphate
kinase
reductase
S-methyl-mercaptopurine
S-methyl-mercaptopurine
S-methyl-thioinosine 5'-monophosphate
S-methyl-thioguanosine 5'-monophosphate
TPMT
TPMT
TPMT
TPMT
NUDT15
NUDT15
6-mercaptopurine
A
MP
HPRT
IMPD
GMPS
kinase
kinase
PRPP
6-thioinosine 5'-monophosphate
6-thioxanthosine 5'-monophosphate
6-thioguanosine 5'-monophosphate
6-thioguanosine 5'-diphosphate
6-thioguanosine 5'-triphosphate
XO
NT5C2
NT5C2
TGN
HPRT
Thioxanthine
XO
Thiouric acid
PRPP
TG
TPMT
S-methyl thioguanine
B
TPMT
AO
S-methyl thioguanine
6-thioguanine
8-Hydroxy-Thioguanine
XO
Thiouric acid