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Mercaptopurine/Methotrexate Maintenance Therapy of Childhood Acute Lymphoblastic Leukemia: Clinical Facts and Fiction

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Summary: The antileukemic mechanisms of 6-mercaptopurine (6MP) and methotrexate (MTX) maintenance therapy are poorly understood, but the benefits of several years of myelosuppressive maintenance therapy for acute lymphoblastic leukemia are well proven. Currently, there is no international consensus on drug dosing. Because of significant interindividual and intraindividual variations in drug disposition and pharmacodynamics, vigorous dose adjustments are needed to obtain a target degree of myelosuppression. As the normal white blood cell counts vary by patients' ages and ethnicity, and also within age groups, identical white blood cell levels for 2 patients may not reflect the same treatment intensity. Measurements of intracellular levels of cytotoxic metabolites of 6MP and MTX can identify nonadherent patients, but therapeutic target levels remains to be established. A rise in serum aminotransferase levels during maintenance therapy is common and often related to high levels of methylated 6MP metabolites. However, except for episodes of hypoglycemia, serious liver dysfunction is rare, the risk of permanent liver damage is low, and aminotransferase levels usually normalize within a few weeks after discontinuation of therapy. 6MP and MTX dose increments should lead to either leukopenia or a rise in aminotransferases, and if neither is experienced, poor treatment adherence should be considered. The many genetic polymorphisms that determine 6MP and MTX disposition, efficacy, and toxicity have precluded implementation of pharmacogenomics into treatment, the sole exception being dramatic 6MP dose reductions in patients who are homozygous deficient for thiopurine methyltransferase, the enzyme that methylates 6MP and several of its metabolites. In conclusion, maintenance therapy is as important as the more intensive and toxic earlier treatment phases, and often more challenging. Ongoing research address the applicability of drug metabolite measurements for dose adjustments, extensive host genome profiling to understand diversity in treatment efficacy and toxicity, and alternative thiopurine dosing regimens to improve therapy for the individual patient.

Key Words: leukemia, acute, lymphoblastic, maintenance therapy, 6-mercaptopurine, methotrexate, pharmacology, drug metabolism, pharmacokinetics, adherence

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The folate analog methotrexate (MTX) and the thio-substituted purine analog 6-mercaptopurine (6MP) became pioneering anticancer agents more than half a century ago, when first Farber et al[1] and then Burchenal[2,3] demonstrated that such drugs can induce temporary remissions in childhood leukemia. Soon cure became the goal,[4,5] and through a series of trials it was shown that the chance of long-term remission of childhood acute lymphoblastic leukemia (ALL) was significantly improved, when patients received several years of postremission maintenance therapy with daily 6MP and weekly MTX.[4–8] Today, ALL protocols include an induction regimen with 3 or 4 antileukemic drugs followed by several months of consolidation therapy.[9–15] Then oral 6MP/MTX maintenance therapy is given until 2 to 3 years from diagnosis, the longer duration being reserved for boys due to their inferior prognosis with shorter therapy.[16–20] Despite its long history, the antileukemic mechanisms of maintenance therapy remain to be revealed. Recent studies of *NT5C2* mutations in relapsed ALL clones emerging during maintenance therapy[21,22] support a direct antileukemic effect of maintenance therapy.[23,24] ALL stem cells may be uniquely sensitive to inhibition of de novo pathways in nucleotide synthesis crucial for DNA repair, methylation, and mitotic duplication.[25] In addition, maintenance therapy could modulate apoptotic pathways,[26] or induce changes in the microenvironment of the leukemic stem cells,[27–30] for example, by impeding antiangiogenesis.[31,32]

IS YEARS OF 6MP/MTX THERAPY NEEDED FOR ALL PATIENTS?

Maintenance therapy seems to be important for most ALL subsets, including T-cell ALL[21] and other patients with hyperleukocytosis at diagnosis,[33] adolescents,[34] and Down syndrome patients with ALL.[35] Observational studies support that 6MP/MTX maintenance therapy is superior to other drug combinations,[33] and that poor physician compliance or poor patient adherence significantly increase the risk of relapse.[36–39] As intensification of induction and consolidation therapy have improved cure rates of ALL, currently being $>80\%$,[9,15,40] the necessity of

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several years of the less toxic maintenance therapy has been questioned. A systematic review of 42 randomized studies with 12,000 childhood ALL cases indicated that longer maintenance therapy gave a slightly (although statistically significantly) lower risk of relapse, but with no difference in survival due to a higher risk of death in remission.[19] Furthermore, longer duration of maintenance therapy as well as higher 6MP/MTX drug doses have in 3 recent studies been associated with an increased risk of second malignancies,[41–43] but so far the subset of patients at risk of developing second cancers has not been identified. Shortening ALL therapy from 24 to 18 months significantly reduces the probability of event-free survival (pEFS),[44] and if all chemotherapy is truncated at 52 weeks from diagnosis, the $pEFS_{5y}$ may be as low as 60%, even for non–high-risk ALL patients.[45]

The fact that some children with ALL are cured after only a few months of chemotherapy[7,45] is not surprising, as monitoring of minimal residual disease (MRD) has shown that patients may have $< 10^{-5}$ leukemic cells in the bone marrow already at the end of induction therapy.[46,47] Unfortunately, no randomized studies of therapy duration have included MRD monitoring.

DRUG DOSAGE

Maintenance therapy of childhood ALL is a challenging exception to the golden standard of body size–based drug dosing in cancer chemotherapy. Currently, there is no international consensus on administration of maintenance therapy, and protocols reflect tradition rather than empirical evidence.[48] In most of continental Europe the starting dose of oral 6MP is 50 mg/m^2/d, whereas in the United Kingdom, the Nordic countries, and most of the United States it is 75 mg/m^2/d.[48] For MTX, the starting dose varies from 20 to 40 mg/m^2/d given orally, or sometimes parenterally to secure medication adherence and increase systemic drug exposure,[48,49] although this route of administration has not been shown to be more efficacious and may even be more neurotoxic.[48–51] In contrast, escalating intravenous MTX doses without leucovorin rescue during interim maintenance therapy seems to provide a better pEFS than oral MTX.[52] However, without reliable pharmacological endpoints it is difficult to determine differences in the pharmacokinetic and pharmacodynamic aspects of these approaches.

The importance of 6MP/MTX dose intensity was first demonstrated in the 1960s, when children randomized to receive maintenance therapy with 6MP (50 mg/m^2/d), MTX (20 mg/m^2/wk), vincristine, and cyclophosphamide had longer remission duration than patients who only received half doses of these drugs to reduce toxicity.[53]

Because of large interindividual variations in 6MP/MTX bioavailability and cellular pharmacokinetics, patients receiving identical doses per body surface area may experience very different systemic and intracellular drug exposures.[54–60] The doses of 6MP and MTX doses are poorly related to their AUC,[60] and drug doses are not significantly related to relapse rates in multivariate analyses.[61] Accordingly, the recommended 6MP and MTX doses in ALL protocols should only be regarded as starting doses that are to be individually adjusted according to myelotoxicity (Fig. 1, and Supplemental Fig. 1, Supplemental Digital Content 1, http://links.lww.com/JPHO/A72). The average 6MP and MTX doses prescribed during maintenance therapy and adjusted by WBC vary widely (Figs. 1, 2). Importantly, patients tolerant to the starting doses of 6MP and MTX, but for whom maintenance therapy is not intensified, have a poorer outcome compared both with patients who receive reduced drug doses due to leukopenia and with those who are upward dose adjusted to obtain target myelosuppression.[36–38,62,63] Furthermore, recurrent unwarranted treatment interruptions due to a rise in aminotransferases are also an adverse factor for risk of relapse.[37] In a more recent study, the cumulative duration of treatment interruptions, likely to be primarily determined by bone marrow toxicity and infections, did not seem to be related to an increased relapse rate in multivariate Cox regression analysis.[61]

6MP AND MTX PHARMACOKINETICS AND PHARMACODYNAMICS

6MP

The absorption of 6MP is rapid, and the elimination half-life is short (1 to 2 h). Even though small studies (somewhat surprisingly) have been able to associate plasma 6MP concentrations to relapse rates,[64,65] such measurements cannot be used for 6MP dose adjustments, due to very large (up to 70-fold) interindividual and intraindividual variations in bioavailability.[54,60,66]

6MP has 3 major metabolic pathways (Fig. 3). First, a substantial and highly varying fraction of 6MP is converted to inactive 6-thiouric acid by the enzyme xanthine oxidase in first pass metabolism.[54,55,67] Xanthine oxidase and thiopurine methyltransferase (TPMT) can be inhibited by allopurinol,[68–70] and coadministration of allopurinol thus requires 6MP dose reductions, as these interactions increases 6MP bioavailability and skews the metabolism of 6MP toward 6-thioguanine nucleotide (6TGN) production (Fig. 3). However, for childhood ALL this drug combination has not been explored beyond case reports.[71]

Second, 6MP is a prodrug that through a multistep process, involving hypoxanthine guanine phophoribosyl transferase mediated coupling of 6MP with phosphoribosyl pyrophosphate, base modification, and further phosphorylation to form 6TGN (Fig. 3). The deoxy form of 6TGN is then incorporated into DNA (DNA-TG) in nucleated cells,[72–74] which may activate postreplication mismatch repair systems that lead to DNA strand breaks and apoptosis.[24,75]

A third metabolic pathway is thiomethylation of 6MP and some of its metabolites catalyzed by TPMT, thus reducing 6TGN formation[76] (Fig. 3). Previously, methylated 6MP metabolites were considered largely insignificant for 6MP pharmacodynamics. However, some methylated metabolites, not least methylthioinosine monophosphates (MeMP), are strong inhibitors of purine de novo synthesis.[77] As the purine salvage pathway is low in lymphoblasts that primarily depend on purine de novo synthesis,[78] the reduced levels of endogenous nucleotides and the resulting enhanced DNA-TG incorporation in the presence of MeMP is likely to play a clinical role.[74,79] Still, the impact of these pharmacodynamic interactions on relapse rates and toxicities remains undetermined, partly as a sufficiently sensitive and reliable assay for routine measurements of DNA-TG in nucleated blood has only recently become available.[80]

After a few weeks of oral 6MP therapy a steady state level in Ery-6TGN level is obtained.[81,82] Early studies showed Ery-6TGN levels to be associated with both myelotoxicity and remission duration,[83–85] even though the

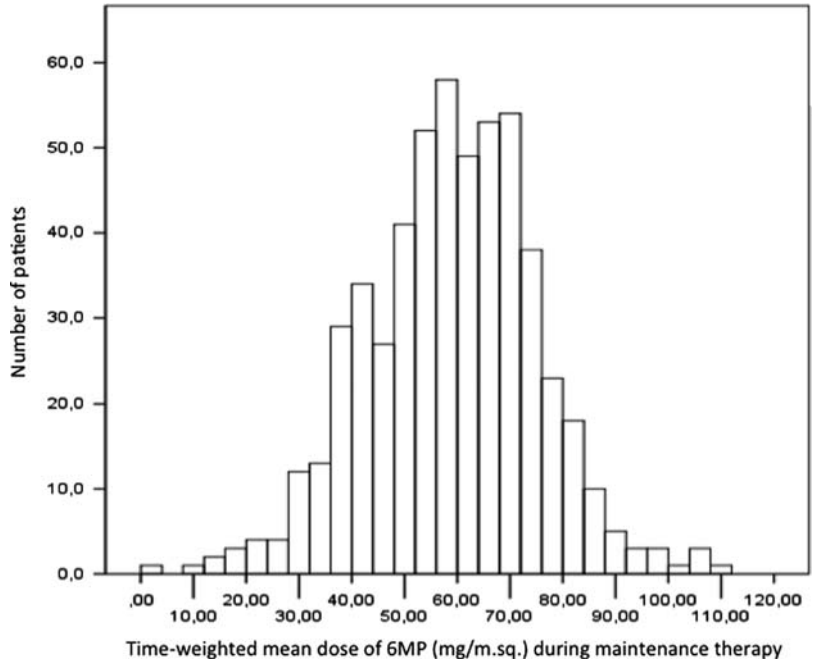

FIGURE 1. Distribution of mean prescribed 6-mercaptopurine (m6MP) doses during maintenance therapy (protocol starting oral dose: 75 mg/m^2/d) for 538 patients included in the NOPHO ALL-92 maintenance therapy study.[61] Means are based on a total of >28,000 registered drug doses and calculated by weighting each registered dose according to the time interval to the next measurement. The median m6MP dose for all patients is 59.4 mg/m^2/d.

6MP metabolite profiles differ widely between red blood cells and neutrophils.[86] However, more recent studies have failed to confirm a significant association between Ery-6TGN and risk of relapse.[38,61] Ery-6TGN levels reflect adherence to therapy[87] and TPMT activity,[63] but are only weakly, although statistically significantly, related to DNA-TG levels.[74,79] Furthermore, 6MP dose increments to achieve higher Ery-6TGN levels primarily increase the methylated metabolite levels,[88] which may enhance hepatotoxicity.[89] There is a lack of large, prospective studies that explore

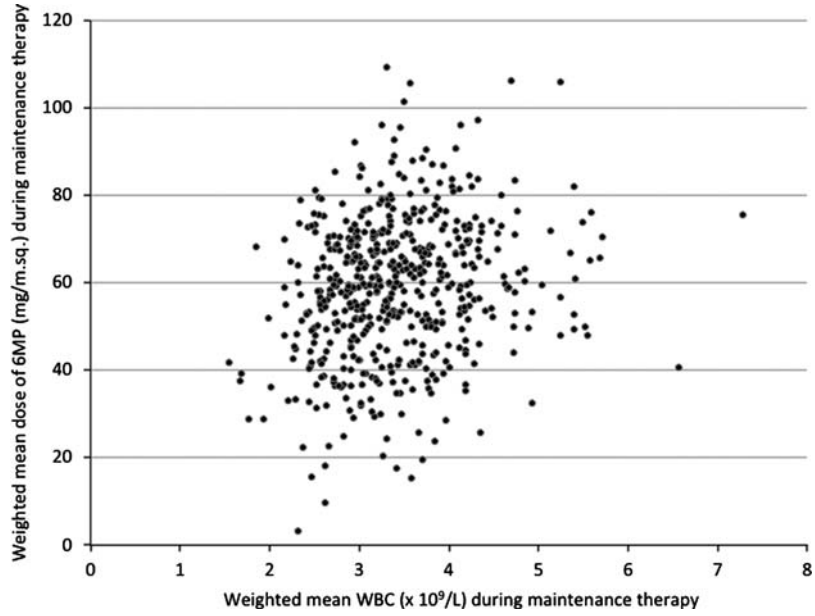

FIGURE 2. Distribution of mean white blood cell count (mWBC) and prescribed mean 6-mercaptopurine (m6MP) doses during maintenance therapy for 538 patients included in the NOPHO ALL-92 maintenance therapy study.[61] Means are based on a total of >28,000 registered drug doses and blood counts and calculated by weighting each registration according to the time interval to the next registration. The median m6MP (59.4 mg/m^2/d) and median mWBC (3.3×10^9/L) are significantly correlated ($r_S = 0.20$; $P < 0.001$).

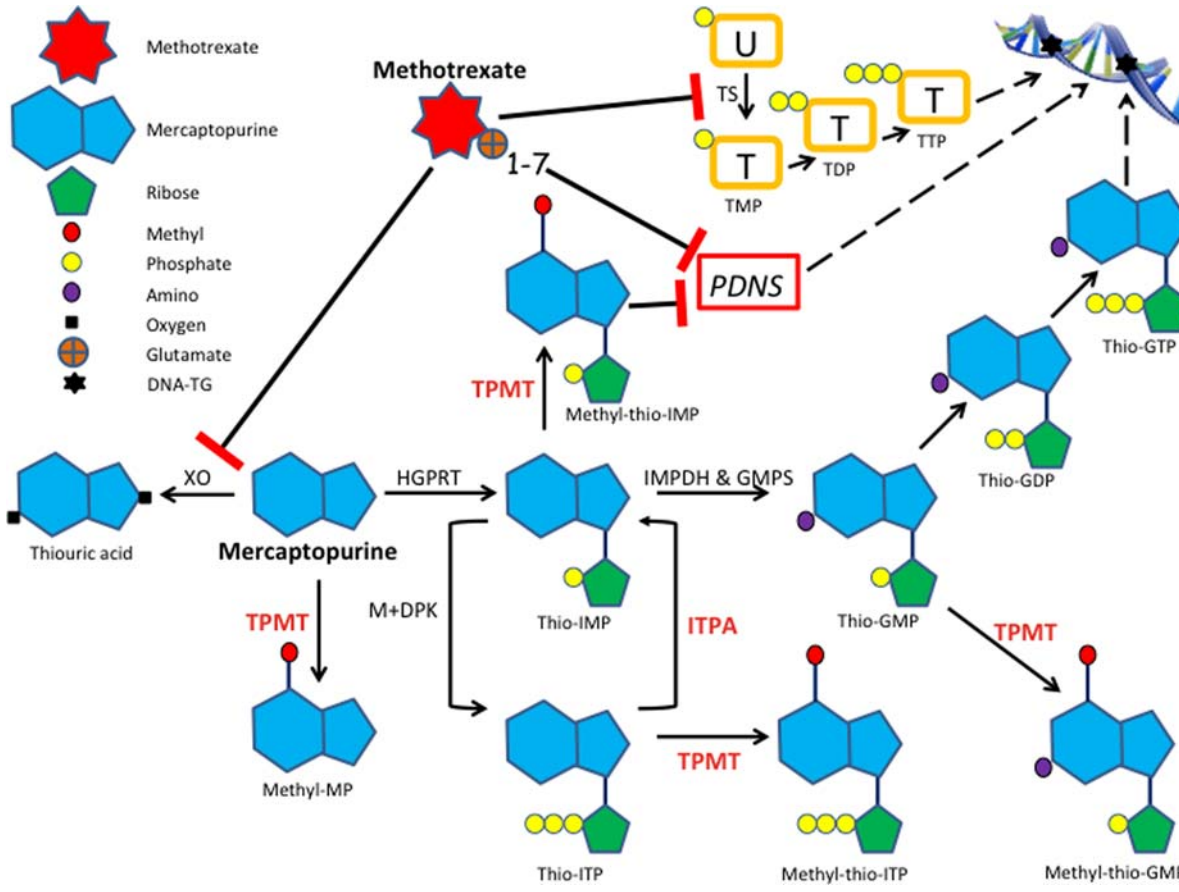

FIGURE 3. Simplified draft of 6-mercaptopurine (6MP) metabolism and methotrexate (MTX)-6MP interactions. DNA-TG indicates thioguanine nucleotides incorporated into DNA; GDP, guanosine diphosphate; GMP, guanosine monophosphate; GMPS, guanosine monophosphate synthetase; GTP, guanosine triphosphate: HGPRT, hypoxanthine guanine phosphoribosyl transferase; IMP, inosine monophosphate; IMPDH, inosine monophosphate dehydrogenase; ITP, inosine triphosphate; ITPA, inosine triphosphate pyrophosphatases; M + DPK, monophosphate and diphosphate kinases; MP, mercaptopurine; PDNS, purine de novo synthesis; TDP, thymidine diphosphate; TMP, thymidine monophosphate; TPMT, thiopurine methyltransferase; TTP, thymidine triphosphate; U, uridine monophosphate; XO, xanthine oxidase.

whether monitoring of Ery-6TGN, Ery-MeMP, and DNA-TG adds dose adjustments advantages compared with adjustments by only myelotoxicity and hepatotoxicity.

As dose increments of 6MP increase the methylated metabolites and their associated toxicities far more than Ery-6TGN, several alternative treatment strategies have been efficacious in improving the 6TGN/MeMP ratio, including coadministration of allopurinol[70,71,90] and splitting the daily 6MP dose in a morning and an evening dose,[91,92] but it remains to be determined whether such approaches increase DNA-TG levels, ease 6MP dose adjustments to obtain target WBC/absolute neutrophil counts (ANC) levels, or reduce relapse rates of childhood ALL.

MTX

Although MTX disposition and pharmacodynamics have been well mapped in cancer cell lines,[23,93,94] far less is known on how to implement such data into maintenance therapy strategies. The folate pathway gene expression profiles vary widely among subsets of ALL, which affects treatment efficacy of MTX.[95] However, current guidelines for MTX dosing do not take into account the diversity of different leukemia subtypes' sensitivity to MTX.

Bioavailability of low dose oral MTX is generally >90%, but is significantly reduced at doses >40 mg/m^2.[57] Similar to natural folates, MTX is converted into MTX polyglutamates (MTX_{PG}, with 2 to 7 glutamyl residues) by the enzyme folylpolyglytamyl transferase, which enhances intracellular retention, inhibition of the target enzymes in purine and pyrimidine de novo synthesis, and treatment efficacy (Fig. 3).[96] The propensity for MTX to undergo polyglutamation is higher for B-cell precursor ALL subtypes (not least the high-hyperdiploid cases) than for T-cell ALL.[95,97,98] Accordingly, many groups offer high-dose MTX at doses of 5 g/m^2/24 h during consolidation therapy to cure T-cell ALL. MTX_{PG} bind tightly to and inhibit dihydrofolate reductase, the enzyme responsible for reducing folates to their bioactive tetrahydrofolate form.[23] During weekly low-dose oral MTX therapy, MTX_{PG} accumulates in red blood cell precursors in the bone marrow, and MTX_{PG} with longer glutamyl chains are then retained in the erythrocytes (Ery-MTX_{PG}) throughout their life span.[99] Steady-state Ery-MTX_{PG} is achieved after 4 to 8 weeks.[100,101] High Ery-MTX_{PG} levels have been associated with increased risk of myelotoxicity,[100,102] but only a single Nordic study has found Ery-MTX_{PG} levels

significantly related to remission duration,[102] and this association could not be confirmed in later studies,[61,103] potentially due to more intensive use of intravenous MTX in these studies. No study has explored the impact on relapse rates of various Ery-MTX polyglutamate chain lengths. At steady state, Ery-MTX_{PG} is both interindividually and intraindividually related to the dose of oral MTX and may thus be used for monitoring treatment adherence.[34,100]

PHARMACOGENETICS

Single nucleotide polymorphisms in genes that affect the disposition of anticancer agents influence the outcome of childhood ALL.[104,105] However, so far only *TPMT* variants have influenced drug dosing,[76,96] and it is poorly explored which host genome variants that ultimately determine the complex metabolism and efficacy of thiopurines and MTX, how this influences the toxicity profiles across ethnic groups,[106–110] and how such data should be applied for dose adjustments.

TPMT

The normal substrate for TPMT is not known, and, in the absence of thiopurines, TPMT-deficient individuals are clinically and biochemically normal. In white individuals, the most common variants are *3A,*3B, and *3C all involving G460A and/or A719G and accounting for at least 90% of low-activity alleles among white individuals of North European decent.[63,76] Approximately 5% to 10% are *TPMT* heterozygous carrying 1 wild-type and 1 low-activity allele, and 1 in 300 is *TPMT* deficient and at risk of life-threatening myelosuppression at standard 6MP doses.[111,112] Although thiopurine dosing according to the *TPMT* genotype has been implemented by a few ALL study groups,[48,79,113] the benefits of this strategy remain uncertain. Compared with *TPMT* wild-type patients, heterozygous patients experience higher intracellular 6TGN levels, more myelotoxicity, higher cure rates,[63,113,114] but probably also a higher risk of second cancers.[41,115,116] The German BFM group that administered lower starting doses of 6MP (50 mg/m^2) failed to confirm the association with second cancers.[117] It is noteworthy that, a recent study indicated that reduction of oral 6MP starting doses from 75 to 50 $mg/m^2/d$, reflecting these BFM data, did reduce the risk of second cancers among *TPMT* heterozygous patients, but at the same time lead to an increased risk of relapse.[43]

Measuring TPMT activity in erythrocytes is an alternative to genotyping and may also identify rare low-activity variants missed by routine allele testing. However, as TPMT activity is inversely related to the erythrocyte age,[118] the TPMT activity will in general be increased during maintenance therapy when the erythrocyte life span is shortened, and be low at diagnosis of ALL due to reduced erythropoiesis, hampering reliable discrimination of heterozygous and wild-type TPMT phenotypes.

Inosine Triphosphate Pyrophosphatase (ITPA)

Low-activity alleles of ITPA, the enzyme that dephosphorylates thioinosine triphosphate (Fig. 3), may increase methylated thiopurine metabolite levels,[119,120] the risk of hepatotoxicity[121,122] and of bone marrow toxicity[123] with febrile neutropenia,[119,124] and potentially also the risk of relapse.[110] The frequency of ITPA low-activity alleles show wide interethnic variability being 1% to 2% among Hispanics, but almost 20% in Asian populations, which may influence tolerance to thiopurine therapy.[125]

Other 6MP Metabolizing Genes

Other 6MP metabolizing enzymes, such as xanthine oxidase and hypoxanthine guanine phosphoribosyl transferase (HGPRT) may vary among individuals,[67,126] in part determined by genetic polymorphisms, and at least low HGPRT in B-cell precursor ALL has been associated with an inferior cure rate, although this association was not related to increased in vitro thiopurine resistance.[127]

MTX

Several groups have demonstrated that MTX treatment efficacy is associated with polymorphisms in dihydrofolate reductase,[128] thymidylate synthetase,[129,130] reduced folate carrier,[131] 5,10-methylenetetrahydrofolate reductase, and methylenetetrahydrofolate dehydrogenase[132] (for reviews on childhood ALL and rheumatoid arthritis, see Davidsen and colleagues[105,133–135]). However, the results of these studies are often contradictory with some studies demonstrating improved cure rates for a specific genetic polymorphism, whereas others demonstrate the opposite, many of the studies are small, most only address 1 or a few of the many genetic polymorphisms involved in the disposition of MTX, and in general they address responses to high-dose MTX rather than low-dose MTX maintenance therapy. Furthermore, it is impossible to evaluate whether a specific polymorphism exert its modifying effect on relapse rate and/or toxicities directly through changed MTX disposition or indirectly by modifying endogenous folate levels. So far no groups have adjusted their MTX treatment strategies based on polymorphisms in the MTX/folate pathway.

TOXICITY AND RELAPSE RATE

Leukopenia

Dose adjustments guided by toxicity assumes that the individual variations in 6MP/MTX pharmacokinetics and/or pharmacodynamics affect leukemic and normal cells in parallel.[136] For maintenance therapy, 6MP/MTX dosage is targeted to a preset degree of myelosuppression, generally a WBC of 1.5 to 3.0 (or 3.5) $\times 10^9$/L,[48] but randomized studies demonstrating benefits hereof are lacking.[137] Most observational studies have shown low WBC and/or ANC during maintenance therapy to be related to red blood cell levels of cytotoxic 6MP/MTX metabolites and/or to a reduced relapse rate.[38,61,82,100,138–144] However, ANC correlates so closely with WBC, that it is virtually impossible to determine which of these 2 parameters is superior as guidance for dose adjustment (Fig. 4A). In the Nordic Society for Paediatric Haematology and Oncology (NOPHO) ALL92 maintenance therapy study,[61] patients with an average ANC $< 2.0 \times 10^9$/L during maintenance therapy had a significantly better relapse-free survival than patients with higher ANC levels (Fig. 4B), and ANC was somewhat more strongly associated with relapse rates than WBC level, although the latter was the dose adjustment target in that protocol. Nevertheless, several factors challenge 6MP/MTX dose adjustments by the leukocyte counts.

First, physicians may be more inclined to decrease 6MP/MTX drug doses in case of toxicity than to escalate

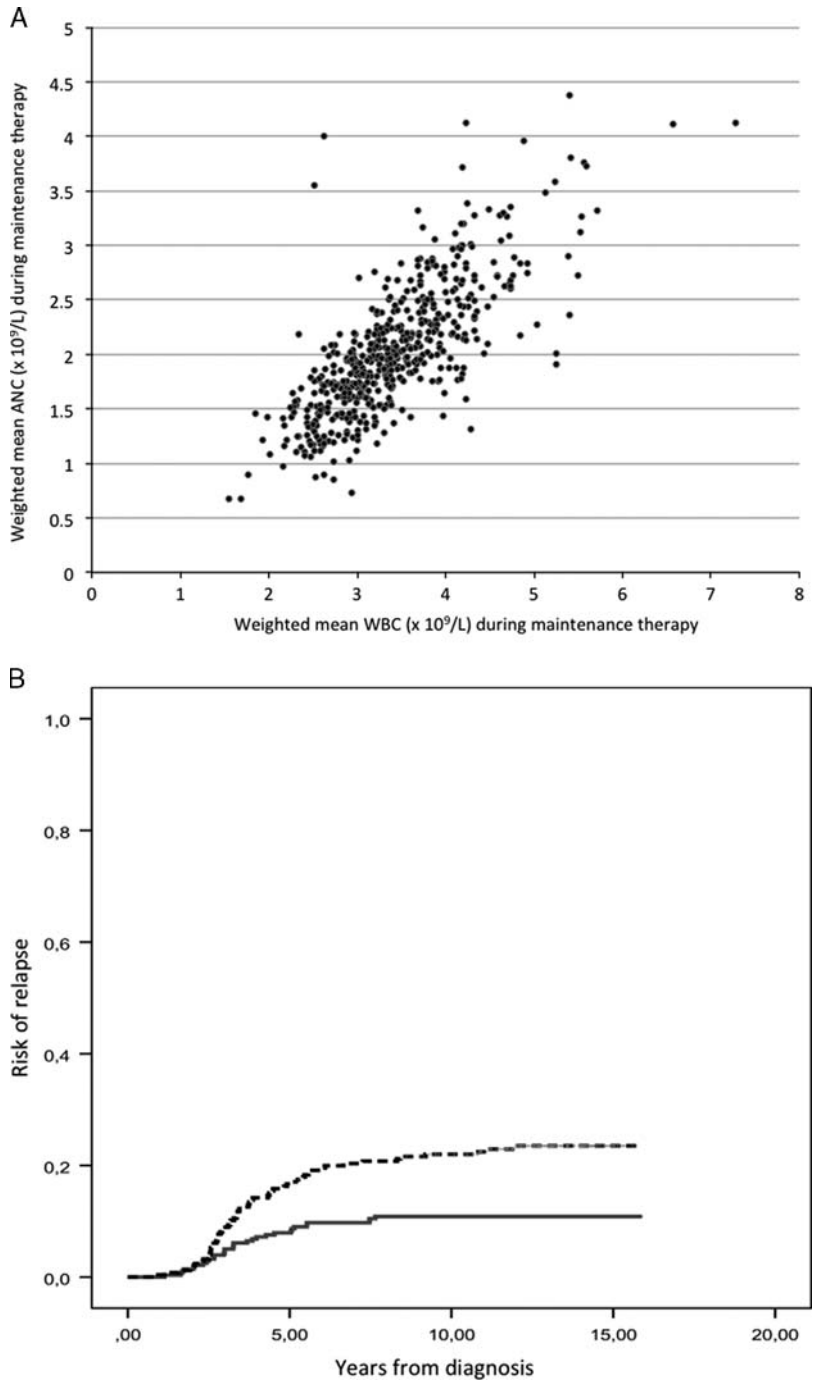

FIGURE 4. A, Mean white blood cell (mWBC) and absolute neutrophil counts (mANC) for 538 patients included in the NOPHO ALL-92 maintenance therapy study.[61] Means are based on a total of >28,000 blood counts and calculated by weighting each measurement according to the time interval to the next measurement. Each dot represents 1 patient. mWBC and mANC are highly correlated ($r_S = 0.77$; $P < 0.001$). The target range for WBC was 1.5 to 3.5×10^9/L in the NOPHO ALL92 protocol from which the data were retrieved. B, Kaplan-Meier relapse risk plots for patients with a mean absolute neutrophil count (mANC) at the end of maintenance therapy above or below 2.0×10^9/L = median mANC for all patients (upper curve, N = 248, relapse risk 23.5% ± 2.7%; lower curve, N = 280, relapse risk 10.9% ± 1.9%; $P < 0.001$). Mean absolute neutrophil counts during maintenance therapy are calculated by weighting each measurement according to the time interval to the next measurement.

doses in patients insufficiently myelosuppressed,[36] requiring different strategies for 6MP dose adjustments, if the 6MP starting dose is 50 versus 75 mg/m^2.

Second, the WBC levels reflect both treatment intensity and the child's normal WBC level, which varies both between and within age groups, and by ethnicity.

Thus, patients with lower WBC levels during therapy also have low WBC after cessation of therapy ($r_S = 0.76$; $P < 0.00001$),[145] and even more important, the best predictor of the rise in WBC after cessation of therapy is not the WBC level during maintenance therapy, but Ery-6TGN and Ery-MTX_{PG}.[102] Thus, an average WBC during therapy of 3.5×10^9/L could reflect more intensive treatment than an average WBC of 3.2×10^9/L, if the patients' off-therapy WBC levels were 8.5 and 4.5×10^9/L, respectively. In support hereof, the red blood cell 6MP/MTX metabolite levels are overall higher in the former patient, and the rise in WBC following cessation of maintenance therapy is a stronger predictor of relapse than the average WBC level during maintenance therapy, associating a high rise in WBC with a reduced relapse rate after cessation of maintenance therapy.[146]

Third, often it is not possible to suppress WBC levels to a target range of 1.5 to 3.0×10^9/L by dose intensification without unacceptable extramedullary toxicity, including hepatotoxicity (see below).

Finally, an aggressive approach with higher 6MP doses and higher treatment intensity to achieve low WBC levels may be counteracted by treatment interruptions,[61] or lead to an increased risk of second cancers.[41,42] Other not yet understood mechanisms such as induction of dormant leukemic stem cells[61] due to inhibition of purine de novo synthesis could also increase the risk of relapse for the dose-intensified patients.

Thrombocytopenia

Thrombocyte counts during and after cessation of maintenance therapy are significantly correlated ($r_S = 0.74$, $P < 0.0001$),[102] but thrombocytopenia is rarely a dose-limiting factor during 6MP/MTX maintenance therapy. However, if 6MP is substituted with the alternative thiopurine, thioguanine (6TG), Ery-6TGN levels become 7-fold higher and severe thrombocytopenia becomes 5- to 10-fold more common.[147] Furthermore, 10% to 15% of 6TG-treated patients develop sinusoidal occlusive disease and/or portal hypertension, which often is accompanied by thrombocytopenia.[148–150] Patients on 6MP with unexplained thrombocytopenia should be explored for hypersplenism and persistent Parvovirus B19 infection.[151]

Hepatotoxicity

6MP and MTX are hepatotoxic and 2-fold elevations or more of serum aminotransferases are frequent,[100,146,152–156] but usually normalize within a few weeks after discontinuation of maintenance therapy.[146,153] Hypoglycemic episodes during fasting[157–160] have been associated with high levels of methylated 6MP metabolites.[161] It can be counteracted by evening meals with slowly absorbed carbohydrates, by administration of rapidly absorbed carbohydrates (eg, apple juice) in case of symptoms, or by shifting to morning dosage. The latter reduces Ery-MeMP levels but the impact on relapse rate is unknown.[161] Few patients develop symptoms of hypoglycemia such as severe nausea, itching, or malaise to a degree that requires dose reductions. A moderate rise in bilirubin or reduced levels of coagulation factors is common, but the risk of serious and/or permanent liver damage seems low.[162–164] Accordingly, most study groups do not recommend dose reductions in case of high aminotransferase levels[48] unless accompanied by biochemical evidence of severe hepatic dysfunction, that is, bilirubin 3 times above the upper normal limit and/or coagulation factor II-VII-X < 0.50 IU/L. Such patients should be explored for other causes, including hepatotropic vira (eg, B or C virus[153,154]), veno-occlusive syndrome (VOD), or Gilbert syndrome with reduced glucuronyltransferase activity and elevated unconjugated bilirubin.

In accordance with the high incidence of hepatotoxicity seen with methylmercaptopurine riboside therapy,[165,166] and the low rate of hepatotoxicity in patients with low TPMT activity,[38,89] most cases of high aminotransferase levels can be related to high levels of methylated 6MP metabolites,[89,167] but have also more rarely been proposed to be associated with high Ery-6TGN[168] or Ery-MTX_{pg},[100] or to accumulation of 6MP in the liver.[169]

A small Danish study from the 1980s linked increased aminotransferases levels during maintenance therapy with a reduced relapse rate.[170] This could reflect reduced first-pass effect on oral 6MP with higher systemic 6MP exposure among developing hepatotoxicity, or higher levels of methylthioinosine monophosphate causing both liver toxicity and inhibition of purine de novo synthesis in leukemic cells,[78,171] which could have increased the incorporation of 6TGN into DNA.[74,79] Alternatively, elevated aminotransferases and reduced relapse risks could merely reflect that the patients were adherent to maintenance therapy. Importantly, patients who continued therapy despite an increase in aminotransferases had a lower relapse rates than patients with treatment interruptions due to hepatotoxicity.[37]

6MP AND MTX INTERACTION

MTX inhibits xanthine oxidase and thereby increases 6MP bioavailability (Fig. 3).[172] In addition, inhibition of purine de novo synthesis can preserve phosphoribosyl pyrophosphate availability, a necessary substrate for 6TGN formation.[171] Cellular depletion of reduced (activated) folates may also lower the availability of S-adenosyl methionine, the methyl donor in TPMT-mediated methylation, and thereby reduce 6MP metabolite methylation. For TPMT wild-type patients (but not for patients with TPMT low-activity alleles), Ery-6TGN and Ery-MTX_{PG} correlate significantly during maintenance therapy (Supplemental Fig. 2, Supplemental Digital Content 2, http://links.lww.com/JPHO/A73), and the longer chained MTX polyglutamates seem most important for enhancing Ery-6TGN accumulation.[173] Because of these synergisms, it is probably better to lower the doses of both 6MP and MTX than to withhold one drug and continue the other in case of dose-limiting toxicity. As the interindividual variation in bioavailability and pharmacokinetics is higher for 6MP than for MTX, above target WBC during maintenance should initially lead to upward dose adjustments in 6MP, followed by dose increments for MTX once the maximum tolerated dose of 6MP has been achieved.

6MP VERSUS 6TG FOR MAINTENANCE THERAPY

In 3 randomized studies by the US CCG, the German COALL, and the British UKALL groups that all compared 6MP with 6TG as the maintenance therapy thiopurine, only males below 10 years of age seemed to have reduced relapse rates with 6TG (OR = 0.70; 95% confidence interval, 0.58-0.84), although with no significant difference in overall survival.[174] The lack of MeMP and the associated inhibition of purine de novo synthesis for patients on 6TG may explain why this thiopurine failed to improve EFS, even though children receiving 6TG had several-fold higher

Ery-6TGN levels.[88,147,174,175] More worrying is that, 10% to 15% of patients on 6TG developed VOD, a few of which were sufficiently severe to require liver transplantation.[148,176–178] It is noteworthy that, the German COALL study[147] did not report 6TG-associated VOD, the only major difference from the other 2 studies being the absence of vincristine/glucocorticosteroid reinductions during maintenance therapy in the German trial. However, the biology behind this association remains uncertain.

PHYSICIAN COMPLIANCE AND PATIENT ADHERENCE TO MAINTENANCE THERAPY

With the complexity of multiple factors influencing therapy (physician compliance, patient adherence, drug disposition, toxicity), there are many potential layers of failure to optimize maintenance therapy. Toxicity-guided dosing relies heavily on physicians' willingness to comply with the protocol guidelines, their experience with maintenance therapy, and their ability to explain the pharmacology, the biology of toxicities, and the importance of treatment adherence to patients and parents. Patient adherence will on the contrary reflect the patient's/family's willingness to accept burdensome toxicities of 6MP and MTX and frequent hospital visits to cure a disease that no longer can be detected. For very young children, not least infants, treatment adherence has been jeopardized by the commercially available 6MP tablets having been developed for adult-sized patients,[179] and not until recently has a liquid formulation of 6MP been marketed (although still not formally tested) in children.[180]

Several groups have reported poor treatment adherence to maintenance therapy in a significant proportion of childhood ALL patients.[39,87,181,182] The reasons for poor medication adherence can be biological, psychological, and social, and they vary across age groups and by ethnicity.[183,184] Various approaches to address this challenge have been proposed, including routine measurements of Ery-6TGN/MeMP/MTX_{PG}, but such analyses are only available in a few centers. If 6MP/MTX metabolite measurements are unavailable, nonadherence should be suspected in *TPMT* heterozygous patients with persistent WBC >3.0 to 3.5×10^9/L despite prescribed 6MP dose increments, and in patients with a *TPMT* wild-type genotype/phenotype, if dose increments do not lead to a rise in aminotransferases.

The randomized Brazilian ALL99 study indicated that intermittent oral high-dose 6MP with IV MTX 200 mg/m^2/6 h not only improved adherence but also gave better pEFS than oral 6MP (50 mg/m^2/d) with IM MTX 25 mg/m^2/wk, although only for boys.[185] However, the extent of patient adherence in the oral 6MP arm is difficult to assess, as 6MP/MTX metabolite measurements were not done, and it is also unclear whether the difference in 6MP and/or MTX dosing in the 2 treatment arms caused the difference in EFS.

CIRCADIAN SCHEDULE

The circadian schedule has a strong impact on efficacy and toxicity of a number of anticancer agents.[186] Two maintenance therapy studies from the 1980s and 1990s found that the risk of relapse was several-fold higher for patients who reported taking 6MP and MTX in the morning compared with patients on evening schedule.[187,188] It was speculated that differences in biological activity between malignant lymphoid cells and normal bone marrow cells determined these chronochemotherapeutical findings,[189,190] but whatever the biological mechanism, changing patients from morning to evening schedule seemed a simple procedure to improve outcome, and this has become the general standard.[48] However, a recent large study of 526 children on maintenance therapy with almost 10,000 E-6TGN/MTX_{pg} measurements, found no association between relapse rates and the cumulative duration of evening dosage for the individual patient, when adjusting for 6MP and MTX doses, WBC levels during maintenance therapy, and Ery-6TGN and Ery-MTX_{PG} levels.[191]

FOOD AND MAINTENANCE THERAPY

Several small studies,[56,59,192,193] although not all,[194–196] have demonstrated reduced bioavailability for both MTX and 6MP, when the drugs are administered together with food, and for 6MP specifically with milk due to its content of xanthine oxidase.[197] Accordingly, nearly all study groups recommend 6MP and MTX to be taken without concomitant ingestion of food.[48] Still, titrating the dose of MTX and 6MP by toxicity should counterbalance lower bioavailability, and restricting the individual patients' choices of drug administration could reduce adherence. Only 1 large clinical study has explored the prognostic impact on administering 6MP/MTX with food, and this study demonstrated no significant influence of concomitant food ingestion on relapse rate, this also being the case within subgroups defined by their circadian schedule.[188]

COADMINISTRATION OF OTHER DRUGS

It is unproven that alternative or additive components of maintenance therapy such as intravenous 6MP,[198,199] 6TG, allopurinol, high-dose MTX,[9] vincristine/glucocorticoid,[200,201] or more intensive reinductions[19] significantly reduce relapse rates with contemporary ALL therapy, although they can add to the burden of myelotoxicity[202] and hepatotoxicity, which may necessitate 6MP and MTX dose reductions.[155,203,204] Specifically, vincristine/glucocorticoid pulses during 6MP/MTX have been applied by many groups, but so far most, although not all,[205] randomized studies have failed to demonstrate benefits of such pulses.[200,201,206,207] Folate supplementation has been widely used to counteract MTX-induced toxicity without compromising efficacy in rheumatoid arthritis[208] or post-transplantation.[209] However, folate supplementation should probably be avoided during maintenance therapy, as it has been shown to influence both 6MP metabolism[210] and myelotoxicity.[211] Finally, trimethoprim-sulfamethoxazole given as *Pneumocystis jiroveci* pneumonia prophylaxis[212] interferes with MTX[213] and 6MP pharmacokinetics,[214] and also enhances myelotoxicity leading to lower prescribed 6MP and MTX doses,[215] but in spite hereof does not seem to increase relapse rates,[215] and thus seems safe to prescribe to avoid this life-threatening infection.

RELAPSE DURING MAINTENANCE THERAPY

Several high-risk ALL subsets such as T-cell ALL,[216] patients with hyperleukocytosis,[217] and patients with t(1;19)[E21-PBX1], *MLL* rearrangements,[218] or hypodiploidy[219] nearly always relapse during maintenance therapy. In contrast, the majority of other B-cell precursor ALL relapses occurs within 2 to 3 years after cessation of treatment.[220] Relapse during maintenance therapy has been associated with insufficient 6MP treatment intensity as

indicated by low Ery-6TGN levels,[61] or it may simply reflect poor treatment compliance/adherence.[39,221] Two recent studies demonstrated activating mutations in the *NT5C2* gene, which plays a role in nucleotide homeostasis, in approximately 15% to 20% of both B-cell precursor[22] and T-cell ALL patients[21] that relapse during 6MP/MTX maintenance therapy. It is noteworthy that, such mutations are rare among B-cell precursor ALL patients that relapse off therapy,[22] indicating that such patients relapse rapidly when *NT5C2* mutations emerge, or that the survival advantage for *NT5C2*-mutated clones at the cost of normal hematopoietic cells disappears once 6MP therapy is discontinued. In the future, targeted deep sequencing may allow routine screening for emerging clones with mutations that hamper the efficacy of thiopurines or MTX, which would allow modification of maintenance therapy to counteract such resistance mechanisms.

CONCLUSIONS AND FUTURE DIRECTIONS

During the last decades more attention has been paid to dose titration by myelotoxicity, and some groups even monitor 6MP and MTX metabolites to reveal poor treatment adherence. However, until it has been determined that such therapeutic drug monitoring eases dose adjustments, improves cure rates, and/or reduce toxicity, maintenance therapy should be adjusted according to the WBC, and lack of myelotoxicity and hepatotoxicity regarded as a surrogate marker for nonadherence. Future research should address the applicability of DNA-TG monitoring, extensive host single nucleotide polymorphism profiling, screening methods for resistant leukemic subclones, and alternative thiopurine dosing regimens to improve maintenance therapy for the individual patient.

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