Thioguanine pharmacokinetics in induction therapy of children with acute myeloid leukemia

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We studied the pharmacokinetics of 6-thioguanine (6TG) in 50 children treated for newly diagnosed acute myeloid leukemia, four of them with Down syndrome (DS). They received oral 6TG 100 mg/m^2 body surface area twice daily for 4 days. Etoposide, 100 mg/m^2/24 h, and cytarabine, 200 mg/m^2/24 h, were administered concomitantly by intravenous infusion. On day 5, doxorubicin 75 mg/m^2 was given as an 8-h infusion. The concentration of thioguanine nucleotides (TGN) in erythrocytes, the active metabolites of 6TG, was determined by high-performance liquid chromatography. The mean TGN concentration from 72, 95, and 106-h samples was used as a measure of drug exposure for each individual. The median TGN concentration in non-DS children above 2 years of age was 2.30 μmol/mmol Hb (range 0.57–25.3). The TGN concentrations varied widely (30-fold) also after dose normalization. We found no correlation with demographic, clinical, or biochemical parameters, and differences in bioavailability might be the most important explanation to interpatient variability. Children with high TGN concentration tended to have longer treatment interval to the next course, but we found no correlation with our predefined parameters for clinical response, that is, remission and relapse rate. Therefore, 6TG does not seem to be a candidate for therapeutic drug monitoring by TGN measurement, at least not in the setting of short multidrug treatment courses. Children with DS had significantly higher TGN concentrations, indicating that dose reduction might be considered to reach the same drug exposure as in non-DS children. *Anti-Cancer Drugs* **20:7–14 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Introduction

Since four decades, the thiopurines 6-thioguanine (6TG) and 6-mercaptopurine (6MP) have been important drugs in the treatment of childhood acute lymphoblastic (ALL) and myeloid leukemia (AML) [1]. Both thiopurines are prodrugs that require intracellular conversion to thioguanine nucleotides (TGN) for cytotoxic activity. The TGN are incorporated into DNA replacing the endogenous guanine, and the resulting DNA strand-breaks and chromatid damage are the main documented mechanisms of cytotoxicity. For 6TG the conversion to TGN requires only one enzymatic step, whereas for 6MP the conversion requires three steps [2,3]. Several studies have been performed comparing the effect of 6TG and 6MP with the main question whether or not 6MP should be replaced by 6TG in ALL maintenance treatment [4–7]. The conclusions drawn from these studies were that 6TG is more cytotoxic and penetrates better into CNS, but that toxic side effects such as myelotoxicity, veno-occlusive disease, and portal hypertension still make 6MP the drug of choice in maintenance therapy of ALL. Today, 6TG is used mainly in combination with other cytotoxic agents as part of intensive treatment blocks, both in AML and ALL [1,8]. In a series of protocols used in the Nordic countries for the treatment of childhood AML, 6TG has been part of the induction treatment [9].

6TG is usually administrated orally, which gives a low and highly variable bioavailability on account of the extensive first-pass metabolism in the intestinal mucosa and liver [3,10,11]. The plasma concentration of 6TG varies markedly during the dose interval, whereas TGN gradually accumulate in erythrocytes and reach a steady state, which makes the erythrocyte TGN concentration a more suitable parameter for pharmacokinetic studies [3,12]. A correlation between erythrocyte TGN levels and neutropenia has been well documented during maintenance treatment of ALL, both for 6MP and 6TG [12–14]. However, the role of 6TG in block therapy is much more difficult to evaluate, and no studies of pharmacokinetics and effect in this context are known to us. As the use of 6TG in induction therapy of childhood

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AML is based on clinical experience rather than studies of pharmacokinetics and dynamics, the dosing of 6TG is empirical. The lack of evidence-based dosing rules is even more evident when it comes to the treatment of subgroups of children, for example infants below 2 years of age and children with Down syndrome (DS). DS children have a higher morbidity than non-DS children after treatment with some anticancer drugs [15]. This might be partly because of differences in drug distribution or elimination, but for 6TG no such data have been published so far, though DS children constitute 5–15% of children diagnosed with AML.

In this study we have measured erythrocyte TGN levels during induction therapy in children with AML, treated according to a common Nordic protocol. Pharmacokinetic data were correlated to clinical effect, estimated by bone marrow morphology after remission induction therapy and by long-term clinical follow-up. In a subgroup of patients, pharmacokinetic data for doxorubicin and etoposide were available, allowing an effort to correlate data for all three drugs.

Patients and methods

Patients

Between March 1995 and October 2000, 50 children were successfully included in the study at nine Nordic centres for pediatric oncology: Copenhagen, Göteborg, Helsinki, Linköping, Lund, Tampere, Ullevål, Umeå, and Uppsala. During this time period, 87 children were diagnosed with AML, that is, our patient group represents 57% of the patient population at these centers. Reasons for not including patients were mostly practical difficulties, such as lack of extra venous access or lack of staff to handle research samples, or sometimes refusal of patients or parents to participate.

Four of the children had DS, and because AML in children with DS differs markedly from other forms of AML, the two groups were analyzed separately. Baseline data for DS and non-DS children are displayed in Table 1. All children were treated according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO) AML-93 protocol [16]. As shown in Fig. 1, the induction course included an intrathecal injection of methotrexate on day 1, followed by etoposide, 100 mg/m^2 body surface area (BSA)/24 h, and cytarabine, 200 mg/m^2/24 h, administered concomitantly by continuous infusion over a 96-h period on days 1–4. During the same 96-h period, 100 mg/m^2 of 6TG was administered orally every 12 h to a total dose of 800 mg/m^2. On day 5, doxorubicin 75 mg/m^2 was given as an 8-h infusion. Data on other drugs administered, for example antiemetics, analgesics, and antibiotics, were not available to us. According to the treatment protocol, BSA was calculated by the formula $m2 = \sqrt{}$[height (cm) × weight (kg)/3600] for children ≥ 2 years of age. For children below two years of age all doses were calculated based on body weight according to the formula m^2 = (BSA dose/30) × weight in kg. Accordingly, the dosage of 6TG in children below 2 years of age was 3.3 mg/kg twice daily.

Table 1 Patient characteristics

	Non-DS	DS
No.	46	4
Age (years) median	11.0	1.8
Range	0.5–17.7	1.1–3.3
Sex (*n*)		
Male	18	1
Female	28	3
WBC (109/l) median	11.8	25.6
Range	0.5–190.3	7.1–44.7
FAB (*n*)		
M0	3	0
M1	7	2
M2	10	0
M3	0	0
M4	13	0
M5	9	0
M7	2	2
Other	2	0

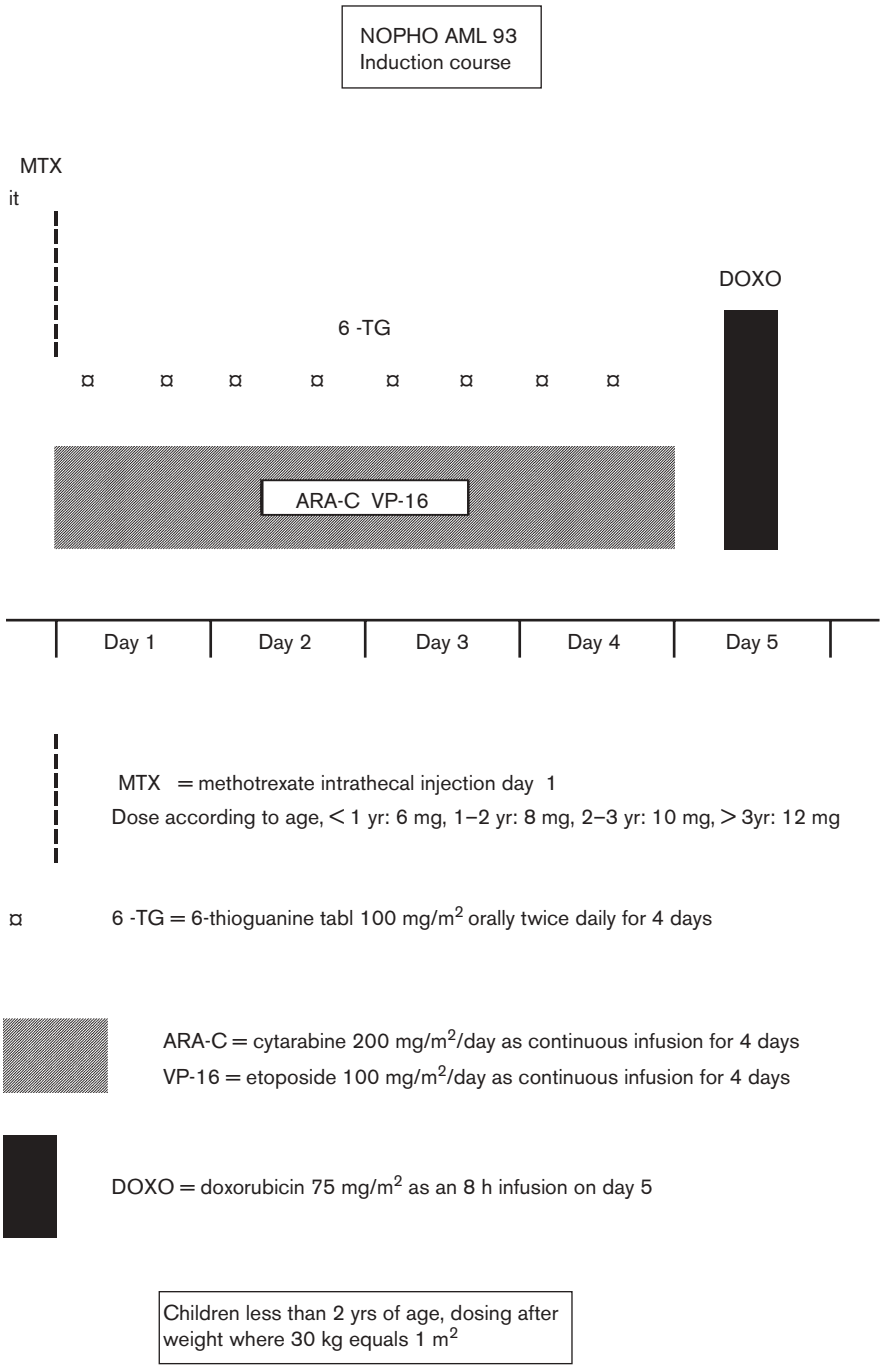
DS, Down syndrome; WBC, white blood cell.

According to the protocol, a bone marrow sample was drawn 3 weeks after the start of the induction course (median 24 days, range 13–42 days) to evaluate treatment response. Less than 5% blast cells in a stained smear of a nonhypoplastic bone marrow was the main criterion for complete remission (CR). When the first bone marrow was too hypoplastic to determine remission, bone marrow samples were to be obtained at weekly intervals until normal hemopoiesis or regrowth of malignant cells emerged. Thirty-six out of the 50 patients reached CR after the initial course (three with DS), and received a second course of treatment, identical to the one given upfront. Repeated sampling for pharmacokinetic analysis was successful in 18 of the 36 patients who received two identical treatment courses (one of them with DS). Patients not in CR after the first course received treatment with cytarabine and mitoxantrone and intrathecal methotrexate. After two induction courses, all patients who had reached CR received a total of four consolidation blocks, which consisted of intrathecal methotrexate and high-dose intravenous cytarabine x (6–18 g/m^2), combined with etoposide (two blocks) or mitoxantrone (one block) [16]. Children with a matched related donor were candidates for allogeneic stem cell transplantation (SCT) in first CR.

Patient characteristics and clinical follow-up data were obtained from annual reports submitted from the treating clinicians to the Nordic registry at the Childhood Cancer Research Unit in Stockholm, and the last day of follow-up was January 2008. Toxicity was not routinely reported to the registry.

Local ethics committees approved the study.

Fig. 1



Induction course of the Nordic Society of Paediatric Haematology and Oncology (NOPHO AML-93) protocol.

Samples

Blood samples were drawn before and 48, 72, 95, and 106 h after the start of the treatment. Blood was drawn from a venous line not used for drug infusion and collected in tubes containing EDTA. The sample was immediately put into ice water and centrifugated within 60 min. Plasma was then removed and plasma and erythrocytes stored separately at −70°C until analysis.

Patient data (body weight, height, actual dose administered), as well as times for drug administration and for blood sampling, were noted. Data on exact dose and

time for administration of 6TG were missing in six cases. Serum concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), albumine, Hb, and white blood cell (WBC) count determined before the start of the induction course, were also recorded.

Analytical procedure

The thioguanine nucleotides in erythrocytes were determined by reversed-phase high-performance liquid chromatography as purine bases after acid hydrolysis and an extraction procedure, as described by Lennard and Singleton [17]. The metabolites were quantified by absorbance at 330 nm. Calibrators were prepared by adding 6-TG from a stock solution to drug-free red blood cell concentrates. The limit of quantification for TGN was 13.3×10^{-3} μmol/mmol Hb. At this concentration, the interassay coefficient of variation was 18% ($n = 11$). The standard curve was linear over a 50-fold concentration range.

For 35 patients, the etoposide plasma level was measured on days 2–4 during the 4-day constant infusion, and in 36 patients the doxorubicin plasma concentration was measured during the doxorubicin infusion on day 5. For both drugs, the steady-state concentration and total body clearance were calculated as reported in earlier publications [18,19]. Data for all three drugs were available for 28 children.

Pharmacokinetic evaluation and statistics

On the basis of recorded data for body weight and height, we recalculated the BSA of all patients by using the formula $m^2 = \sqrt{[\text{height (cm)} \times \text{weight (kg)}/3600]}$. Body mass index was calculated as weight/(height)^2. Dose-normalized TGN concentration was calculated by using the formula TGN concentration × (target dose/administered dose) where the target dose was set at 200 mg/m^2/24 h.

The Spearman's rank test (two sided) was used to examine correlations, the Mann–Whitney *U* test to compare values from two groups, the Kruskall–Wallis test to examine differences among three or more groups, the Wilcoxon's signed-rank test to compare two related samples, the Friedman test to examine several related samples, and logistic regression analysis to test the probability of a defined event. The SPSS 15.0 software package (SPSS Inc., Chicago, Illinois, USA) was used for the calculations. A *P* value of less than 0.05 was considered as statistically significant.

Results

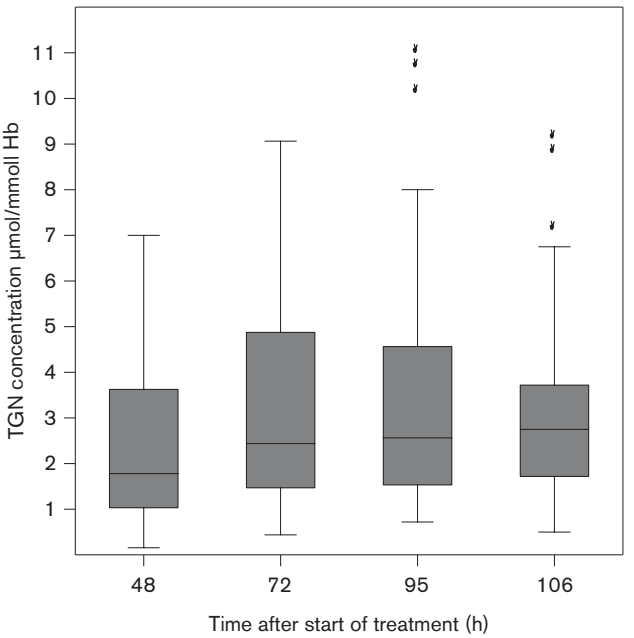
Non-Down syndrome children

Erythrocyte TGN concentrations were measured at 48, 72, 95, and 106 h after the start of treatment. Samples from all four points in time were available for 21 patients. The concentration in the 48-h sample was significantly lower than in the 72, 95, and 106-h samples, ($P < 0.001$), whereas there were no significant differences (NS) among the three other samples, see Fig. 2. In the following calculations we used the mean TGN concentration of the 72, 95, and 106-h samples as a measure of drug exposure for each individual (in about half of the patients only two values were available).

The median TGN concentration in children above 2 years of age was 2.30 μmol/mmol Hb (Table 2). The median 6TG dose received by children above 2 years of age was 199 mg/m^2/24 h, which was very close to the target dose of 200 mg 6TG/m^2/24 h (the exact dose administered was known in 35 of 41 children). The five children below 2 years of age (0.53, 0.61, 1.0, 1.3, and 1.8 years of age, respectively) received 109, 143, 149, 157, and 138 mg 6TG/m^2/24 h resulting in TGN concentrations of 3.04, 2.13, 0.82, 1.57, and 2.98 μmol/mmol Hb, respectively.

To allow a comparison between patients, irrespective of dose, the dose-normalized TGN concentration was calculated. As evident from Table 2, there was a large interindividual variation in erythrocyte TGN levels also after dose normalization (30-fold). The median dose-normalized concentration of TGN in children below

Fig. 2



Thioguanine nucleotides (TGN) concentration in erythrocytes (μmol/mmol Hb) at 48, 72, 95 and 106 h after the start of treatment in the 21 patients for whom samples were analyzed at all four time points. The concentration at 48 h was significantly lower than at the three following time points ($P < 0.001$). The box and whisker plot shows median, first, and third quartiles, and whiskers extend to the highest and lowest value, excluding outliers, which are denoted by circles.

and above 2 years of age was 2.97 and 2.85 μmol/mmol Hb, respectively (NS).

The dose-normalized concentration of TGN was used to explore the correlation with background variables, and all non-DS children were included in this analysis. We did not find any significant correlation with age, weight, height, ALT, AST, albumine, WBC count, or Hb at diagnosis (Table 3). As expected, there were wide variations in WBC count and Hb concentration values (see Table 1), but very few children had amino transferase values outside the normal limits.

No correlation was observed between erythrocyte TGN and plasma etoposide steady-state concentrations ($n = 35$; ρ −0.04, $P = 0.80$) or between erythrocyte TGN and plasma doxorubicin concentrations ($n = 36$; ρ −0.08, $P = 0.64$).

Table 2 Summary of pharmacokinetic parameters in children with or without DS

	Non-DS <2 years	P^a	Non-DS >2 years	P^b	DS
TGN concentration (μmol/mmol Hb)					
No.	5		41		4
Median	2.13	0.49	2.30	0.29	3.76
Range	0.82–3.04		0.57–25.3		1.83–9.29
p 25–75			1.38–4.47		
Dose (mg/m^2/24 h)					
No.[c]	5		35		4
Median	143	0.001	199	0.002	114
Range	109–157		113–233		83–169
p 25–75			189–209		
Dose normalized TGN concentration[d] (μmol/mmol Hb)					
No.	5		35		4
Median	2.97	1.0	2.85	0.04	7.44
Range	1.11–5.58		0.79–23.4		4.17–11.0
p 25–75			1.80–4.58		

p 25–75, 25–75 percentile.
6TG; 6-thioguanine; DS, Down syndrome; TGN, thioguanine nucleotides.
[a]Non-DS <2 years versus >2 years.
[b]DS versus all non-DS children.
[c]Data on the exact dose of 6TG administered were missing in six cases.
[d]Dose normalized TGN concentration was calculated by the formula TGN concentration × (target dose/administered dose) where the target dose was set at 200 mg/m^2/24 h.

Table 3 Analysis of the influence of demographic, clinical, and biochemical variables on the dose normalized concentration of TGN

Variable	*P* value	ρ
Sex	0.24	
Age	0.23	−0.19
Weight	0.15	−0.23
Height	0.40	−0.14
BMI	0.087	−0.27
ALT	0.76	−0.05
AST	0.8	−0.04
Albumine	0.63	−0.08
WBC	0.43	−0.13
Hb	0.40	0.14

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; TGN, thioguanine nucleotides; WBC, white blood cell count at diagnosis. Only children without Down syndrome were included in the analysis.

Children with Down syndrome

The four children with DS were 1.1, 1.7, 1.9, and 3.3 years of age. They received 169, 142, 83, and 87 mg 6TG/m^2/24 h and their TGN concentrations were 9.29, 2.96, 1.83, and 4.57 μmol/mmol Hb, respectively. Their median dose-normalized TGN concentration was 7.44 μmol/mmol Hb, which was significantly higher than for the non-DS children ($P = 0.04$; Table 2).

Repeated courses

Thirty-six out of 50 patients went into CR after the first course and received a second treatment course identical to the first one. The median time interval between the start of the courses was 31 days (range 20–50 days). Patients with high TGN concentrations tended to have a longer interval between treatment courses (ρ 0.31, $P = 0.088$ for non-DS children). The three DS children that went into remission after the first course all had long time intervals between the courses (Fig. 3).

Repeated sampling was successful in 18 of the 36 patients (one with DS) and there was a strong correlation in TGN concentrations between courses, ρ 0.76, *P* value less than 0.001 (Fig. 4).

Thioguanine nucleotides concentration and response in non-Down syndrome patients

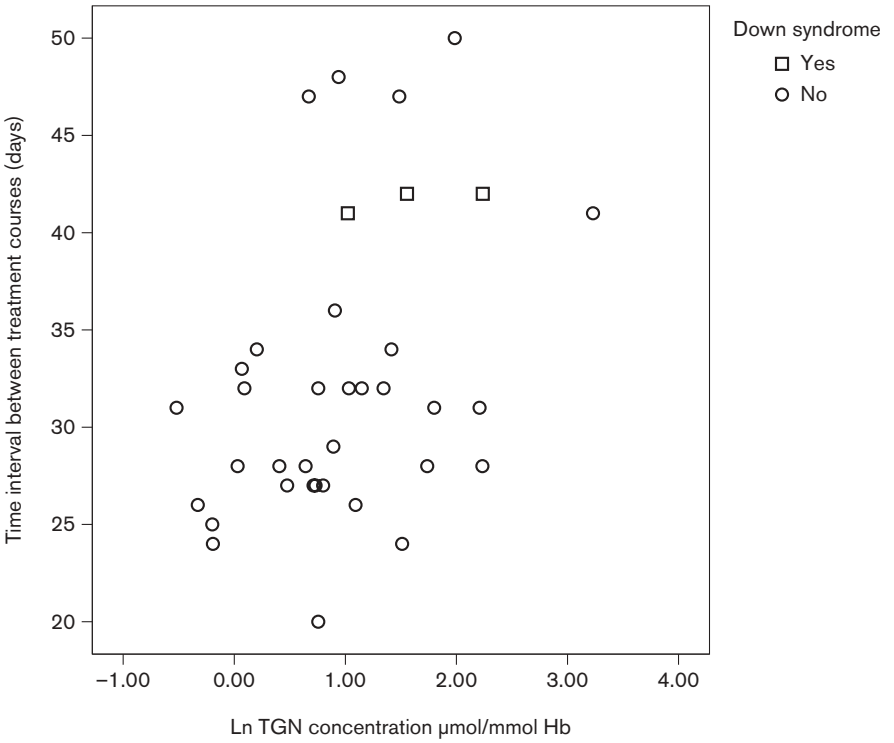
One patient died from aplasia 1 month after the start of treatment, before any evaluation of treatment response, and is therefore not included in the calculations below. We compared the 33 patients who went into CR after the first treatment course with the 12 patients who did not. The median TGN concentration was 2.39 and 2.40 μmol/mmol Hb, respectively (NS). The TGN concentration was not an independent factor for CR in univariate ($P = 0.92$) or multivariate regression analysis including sex, age, and WBC count ($P = 0.92$). Figure 5 shows the predicted probability of CR as a function of TGN concentration in a univariate analysis. In a multivariate analysis including TGN, etoposide, and doxorubicin steady-state concentrations ($n = 25$), none of the drugs was an independent factor for CR.

Twenty-four patients were in continuous CR at the latest follow-up (11 after allogeneic SCT in first CR), with a median follow-up time of 9.2 years (range 7.9–12.7 years), whereas 18 had relapsed (six after allogeneic SCT in first CR). One patient with resistant disease was treated according to another protocol. Two patients died in CR after allogeneic SCT. The median TGN concentration for CR and relapse patients was 2.54 and 2.23 μmol/mmol Hb, respectively (NS).

Discussion

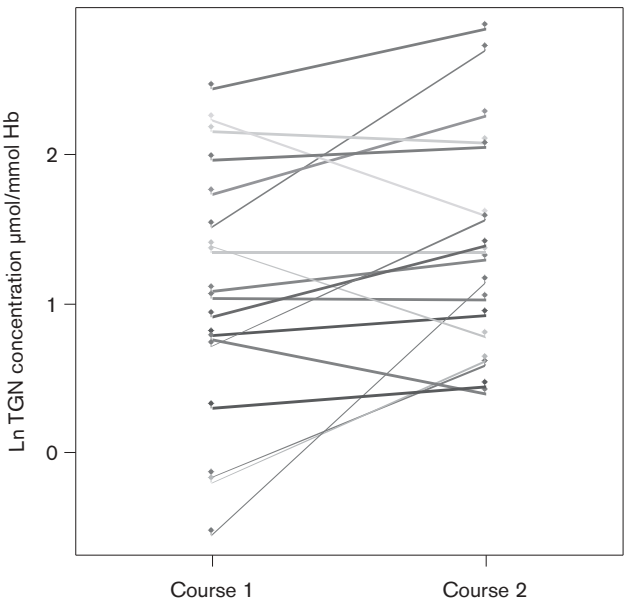
In this study of 6TG pharmacokinetics in 50 Nordic children with AML, we measured the levels of TGN metabolites in erythrocytes, a method that circumvents

Fig. 3



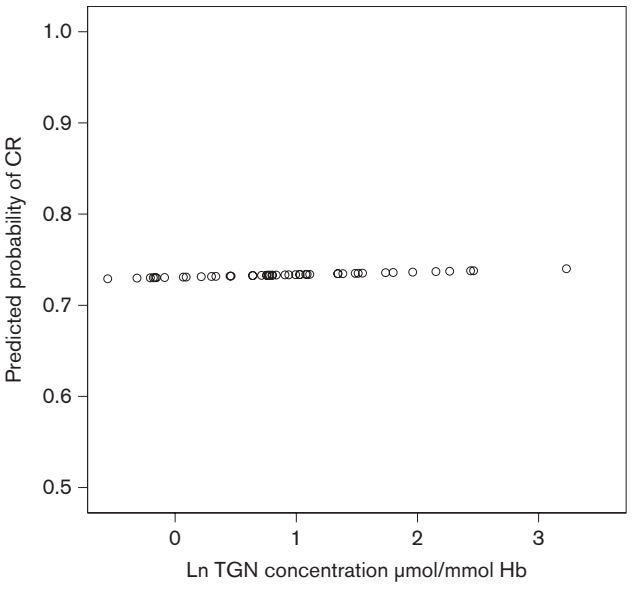
Relationship between the natural log-transformed thioguanine nucleotides (TGN) concentration during the first treatment course and time interval between course 1 and 2. Circles denote children with, and triangles children without Down syndrome.

Fig. 4



Thioguanine nucleotides (TGN) concentrations in 18 patients who received two identical treatment courses. Lines connect individual values.

Fig. 5



Predicted probability of complete remission (CR) after induction therapy as a function of thioguanine nucleotides (TGN) concentration ($n=45$). Children with Down syndrome are excluded. The plot illustrates the lack of a significant concentration–effect relationship within the concentration interval studied.

the problem with widely fluctuating plasma levels of the parent drug during dose intervals. After repeated oral intake, TGN gradually accumulates in the erythrocytes. Patients receiving 40 mg 6TG/m^2 once daily during maintenance therapy reached steady-state concentrations of 0.1–0.3 μmol/mmol Hb after 1–2 weeks [20]. Our patients received 100 mg 6TG/m^2 twice daily, and their TGN levels reached a plateau already after 72 h of treatment, with a median concentration of 2.3 μmol/mmol Hb. Thus, a five-fold increase in dose resulted in a rapid accumulation of TGN in erythrocytes and an approximately 10-fold increase in TGN concentration. Whether this indicates dose-dependent pharmacokinetics or an influence of other drugs concomitantly administered to our patients cannot be judged. The TGN levels measured at 72, 95, and 106 h were very similar, with a little intraindividual variation, but it is not clear whether this represents a true steady state. In any case, we judged that the mean value of these three samples was the best available measure of drug exposure in our patients.

The present data confirm earlier findings of large interindividual variations in 6TG pharmacokinetics [4,10,20,21]. The reason for this variability is not known. We compared the TGN concentrations with baseline data such as age, weight, length, body mass index, ALT, AST, albumine, WBC count, and Hb at diagnosis, but found no significant correlations. The extensive first-pass metabolism in intestinal mucosa and liver after oral administration, including the enzymatic transformation to TGN, leads to a low and highly variable bioavailability, which seems to be the most probable explanation to the interindividual differences in TGN erythrocyte concentration [10,11,22].

Infants are often treated with reduced doses of antineoplastic drugs. In the NOPHO AML-93 protocol used here, dosage was based on body surface area in children ≥ 2 years of age, and on body weight in younger children. We found that the TGN concentrations, normalized for a dose of 200 mg 6TG/m^2/24 h, did not show any significant difference between children below and above 2 years of age. Thus, our data give no support to dose reduction of 6TG in infants. However, it must be kept in mind that only two children were below the age of 1 year and none below 6 months of age.

Children with DS have an increased risk of developing acute leukemia, especially AML, where they constitute 10–15% of all children with this diagnosis. Several groups, including NOPHO, have reported that DS children with AML have an excellent prognosis when actively treated [23–26]. There is still, however, much uncertainty about the optimal dosing of drugs administered in multiagent treatment courses, because the effect and toxicity of individual drugs are very difficult to evaluate. To our knowledge, data on 6TG pharmacokinetics have not been published for children with DS. The NOPHO AML-93 protocol had no recommendations for dosage of 6TG in DS children, but we found that the DS patients studied here in practice received considerably reduced doses. Still, the median TGN concentration tended to be higher, and after dose normalization it was significantly higher than in non-DS children. Although the small number of patients calls for caution, this supports the idea of dose reduction of 6TG in DS children also in future protocols. The conclusion also takes into account that DS children have a higher morbidity than non-DS children after treatment with anti-cancer drugs, but have a more favourable long-term outcome in AML [23,26].

A correlation between TGN levels and posttreatment neutropenia and thrombocytopenia has been reported in patients receiving maintenance therapy [5,27]. We found a nonsignificant ($P = 0.088$) correlation between TGN concentrations and time interval to the next treatment course in non-DS children. Although WBC counts during this interval were not available to us, it is reasonable to presume that neutropenia was the major reason for prolonging the treatment interval. The DS children all had marked treatment delays, and part of the explanation might be neutropenia caused by high TGN levels. We did not, however, find any correlation between TGN concentration and our predefined endpoints for clinical effect, that is, bone marrow morphology after induction therapy and long-term clinical follow-up. Several factors might have contributed: 6TG was administered as one of four drugs during the induction course and was followed by consolidation blocks without 6TG, which might 'dilute' or obscure any effect of the drug. Furthermore, it has been reported that TGN concentrations in neutrophils can differ from those in erythrocytes, and therefore erythrocyte TGN may not reflect drug exposure of the target cells as has been presumed [28,29].

We have published data showing that the doxorubicin plasma concentration is an independent factor for CR in children with AML treated according to NOPHO AML-93 [18], but we found no correlation between etoposide pharmacokinetics and remission or relapse rate [19]. Our original plan was to measure the plasma level of all four drugs administered during induction therapy: cytarabine, etoposide, 6TG, and doxorubicin. Cytarabine measurements failed because of the lack of a specific and reproducible method for analysis of the drug. The other drugs were successfully analyzed, but for practical reasons data for all three drugs were not available in all patients (a major factor was a transport accident). No correlation was observed, or any trend to a correlation, between TGN and etoposide steady-state concentrations ($n = 35$), or between TGN and doxorubicin concentrations ($n = 36$). This was not unexpected, because the drugs are metabolized and eliminated by different pathways. In

a multivariate analysis, including TGN, doxorubicin and etoposide concentrations, none of the drugs was an independent factor for CR. However, complete data were only available for 25 non-DS patients.

In summary, we found high and constant concentrations of TGN in erythrocytes 72–106 h after twice-daily oral administration of high-dose 6TG. TGN concentration varied widely, also after dose normalization. We found no correlation with demographic, clinical, or biochemical variables, and literature data indicate that differences in bioavailability might be the most important explanation to interpatient variability. Children with high TGN concentration tended to have longer treatment interval to the next course, but we found no correlation to other parameters for clinical response, that is, remission and relapse rate. Therefore, 6TG does not seem to be a candidate for therapeutic drug monitoring by TGN measurement, at least not in the setting of short multidrug treatment courses. Children with DS had significantly higher TGN concentrations after dose normalization, indicating that dose reduction might be considered to reach the same drug exposure as in non-DS children.

Acknowledgements

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References

1 Coulthard S, Hogarth L. The thiopurines: an update. *Invest New Drugs* 2005; **23**:523–532.
2 Adamson PC, Poplack DG, Balis FM. The cytotoxicity of thioguanine vs mercaptopurine in acute lymphoblastic leukemia. *Leuk Res* 1994; **18**: 805–810.
3 Lennard L, Keen D, Lilleyman JS. Oral 6-mercaptopurine in childhood leukemia: parent drug pharmacokinetics and active metabolite concentrations. *Clin Pharmacol Ther* 1986; **40**:287–292.
4 Erb N, Harms DO, Janka-Schaub G. Pharmacokinetics and metabolism of thiopurines in children with acute lymphoblastic leukemia receiving 6-thioguanine versus 6-mercaptopurine. *Cancer Chemother Pharmacol* 1998; **42**:266–272.
5 Harms DO, Gobel U, Spaar HJ, Graubner UB, Jorch N, Gutjahr P, *et al*. Thioguanine offers no advantage over mercaptopurine in maintenance treatment of childhood ALL: results of the randomized trial COALL-92. *Blood* 2003; **102**:2736–2740.
6 Jacobs SS, Stork LC, Bostrom BC, Hutchinson R, Holcenberg J, Reaman GH, *et al*. Substitution of oral and intravenous thioguanine for mercaptopurine in a treatment regimen for children with standard risk acute lymphoblastic leukemia: a collaborative Children's Oncology Group/National Cancer Institute pilot trial (CCG-1942). *Pediatr Blood Cancer* 2007; **49**:250–255.
7 Vora A, Mitchell CD, Lennard L, Eden TO, Kinsey SE, Lilleyman J, *et al*. Toxicity and efficacy of 6-thioguanine versus 6-mercaptopurine in childhood lymphoblastic leukaemia: a randomised trial. *Lancet* 2006; **368**:1339–1348.
8 McCormack J, Johns D. Purine and purine nucleoside antimetabolites. In: Chabner BW, Collins JM, editors. *Cancer chemotherapy; principles and practice*. Philadelphia: J.B. Lippincott; 1990, pp. 234–250.
9 Lie SO, Abrahamsson J, Clausen N, Forestier E, Hasle H, Hovi L, *et al*. Long-term results in children with AML: NOPHO-AML Study Group–report of three consecutive trials. *Leukemia* 2005; **19**:2090–2100.
10 Zimm S, Collins JM, Riccardi R, O'Neill D, Narang PK, Chabner B, *et al*. Variable bioavailability of oral mercaptopurine. Is maintenance chemotherapy in acute lymphoblastic leukemia being optimally delivered? *N Engl J Med* 1983; **308**:1005–1009.
11 Brox LW, Birkett L, Belch A. Clinical pharmacology of oral thioguanine in acute myelogenous leukemia. *Cancer Chemother Pharmacol* 1981; **6**:35–38.
12 Lennard L, Lilleyman JS. Variable mercaptopurine metabolism and treatment outcome in childhood lymphoblastic leukemia. *J Clin Oncol* 1989; **7**:1816–1823.
13 Schmiegelow K, Bruunshuus I. 6-Thioguanine nucleotide accumulation in red blood cells during maintenance chemotherapy for childhood acute lymphoblastic leukemia, and its relation to leukopenia. *Cancer Chemother Pharmacol* 1990; **26**:288–292.
14 Sulh H, Koren G, Whalen C, Soldin S, Zipursky A, Greenberg M. Pharmacokinetic determinants of 6-mercaptopurine myelotoxicity and therapeutic failure in children with acute lymphoblastic leukemia. *Clin Pharmacol Ther* 1986; **40**:604–609.
15 Abildgaard L, Ellebaek E, Gustafsson G, Abrahamsson J, Hovi L, Jonmundsson G, *et al*. Optimal treatment intensity in children with Down syndrome and myeloid leukaemia: data from 56 children treated on NOPHO-AML protocols and a review of the literature. *Ann Hematol* 2006; **85**:275–280.
16 Lie SO, Abrahamsson J, Clausen N, Forestier E, Hasle H, Hovi L, *et al*. Treatment stratification based on initial in vivo response in acute myeloid leukaemia in children without Down's syndrome: results of NOPHO-AML trials. *Br J Haematol* 2003; **122**:217–225.
17 Lennard L, Singleton HJ. High-performance liquid chromatographic assay of the methyl and nucleotide metabolites of 6-mercaptopurine: quantitation of red blood cell 6-thioguanine nucleotide, 6-thioinosinic acid and 6-methylmercaptopurine metabolites in a single sample. *J Chromatogr* 1992; **583**:83–90.
18 Palle J, Frost BM, Peterson C, Gustafsson G, Hellebostad M, Kanerva J, *et al*. Doxorubicin pharmacokinetics is correlated to the effect of induction therapy in children with acute myeloid leukemia. *Anticancer Drugs* 2006; **17**:385–392.
19 Palle J, Britt-Marie F, Goran G, Marit H, Jukka K, Eva L, *et al*. Etoposide pharmacokinetics in children treated for acute myeloid leukemia. *Anticancer Drugs* 2006; **17**:1087–1094.
20 Lancaster DL, Patel N, Lennard L, Lilleyman JS. 6-Thioguanine in children with acute lymphoblastic leukaemia: influence of food on parent drug pharmacokinetics and 6-thioguanine nucleotide concentrations. *Br J Clin Pharmacol* 2001; **51**:531–539.
21 Lennard L, Davies HA, Lilleyman JS. Is 6-thioguanine more appropriate than 6-mercaptopurine for children with acute lymphoblastic leukaemia? *Br J Cancer* 1993; **68**:186–190.
22 Balis FM, Holcenberg JS, Poplack DG, Ge J, Sather HN, Murphy RF, *et al*. Pharmacokinetics and pharmacodynamics of oral methotrexate and mercaptopurine in children with lower risk acute lymphoblastic leukemia: a joint children's cancer group and pediatric oncology branch study. *Blood* 1998; **92**:3569–3577.
23 Creutzig U, Reinhardt D, Diekamp S, Dworzak M, Stary J, Zimmermann M. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. *Leukemia* 2005; **19**:1355–1360.
24 Craze JL, Harrison G, Wheatley K, Hann IM, Chessells JM. Improved outcome of acute myeloid leukaemia in Down's syndrome. *Arch Dis Child* 1999; **81**:32–37.
25 Ravindranath Y, Abella E, Krischer JP, Wiley J, Inoue S, Harris M, *et al*. Acute myeloid leukemia (AML) in Down's syndrome is highly responsive to chemotherapy: experience on Pediatric Oncology Group AML Study 8498. *Blood* 1992; **80**:2210–2214.
26 Zeller B, Gustafsson G, Forestier E, Abrahamsson J, Clausen N, Heldrup J, *et al*. Acute leukaemia in children with Down syndrome: a population-based Nordic study. *Br J Haematol* 2005; **128**:797–804.
27 Lancaster DL, Lennard L, Rowland K, Vora AJ, Lilleyman JS. Thioguanine versus mercaptopurine for therapy of childhood lymphoblastic leukaemia: a comparison of haematological toxicity and drug metabolite concentrations. *Br J Haematol* 1998; **102**:439–443.
28 Lancaster DL, Patel N, Lennard L, Lilleyman JS. Leucocyte versus erythrocyte thioguanine nucleotide concentrations in children taking thiopurines for acute lymphoblastic leukaemia. *Cancer Chemother Pharmacol* 2002; **50**:33–36.
29 Bergan S, Bentdal O, Sodal G, Brun A, Rugstad HE, Stokke O. Patterns of azathioprine metabolites in neutrophils, lymphocytes, reticulocytes, and erythrocytes: relevance to toxicity and monitoring in recipients of renal allografts. *Ther Drug Monit* 1997; **19**:502–509.