Thiopurine drugs in the treatment of childhood leukaemia: the influence of inherited thiopurine methyltransferase activity on drug metabolism and cytotoxicity

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Aims The response to 6-mercaptopurine (6MP) is highly variable. Its antileukaemic effect can be related to drug derived 6-thioguanine nucleotides (TGNs). The inherited level of thiopurine methyltransferase (TPMT) activity may be a major factor in the clinical response to 6MP because TPMT forms methylmercaptopurine metabolites (MeMPs) at the expense of TGNs. The aim of this study was to explore the clinical importance of TPMT phenotype.

Methods Thiopurine metabolism was studied in a consecutive cohort of children with acute lymphoblastic leukaemia (ALL) treated according to the Medical Research Council trial UK ALL XI. TPMT phenotype was measured in 38 children at diagnosis, and thiopurine metabolites were measured at defined times during 2 years treatment in 29 of these children.

Results TPMT activities at diagnosis ranged from 5.5 to 18.5 units ml^{-1} packed RBCs, no different from the range of activities reported in healthy children. TGNs and MeMPs measured during the first 6MP cycle at 75 mg m^{-2} ranged from 187 to 594 pmol 6TGNs, median 327, and 0.5 to 22.0 nmol MeMPs, median 4.5, per 8×10^8 RBCs. TPMT activity was not significantly related to the generation of MeMPs ($r_s = 0.06$), but was negatively correlated to 6TGNs ($r_s = -0.44$, $P < 0.025$, $n = 29$). TGNs were related to neutropenia at the point of dose reduction ($r_s = -0.5$, $P < 0.01$). TPMT activity was also inversely related to the duration of cytopenia driven 6MP withdrawal ($r_s = -0.41$, $P < 0.05$).

Conclusions These findings support the suggestion that the inherited activity of TPMT in a given individual can modulate the cytotoxic effect of 6MP, and this information may help in clinical management.

Keywords: 6-mercaptopurine metabolism, thiopurine methyltransferase, thioguanine nucleotides, pharmacogenetics

Introduction

The purine analogue drug 6-mercaptopurine (6MP) is widely used in continuing chemotherapy protocols for standard risk lymphoblastic leukaemia (ALL) in children. This phase of treatment, using low dose oral antimetabolites, is an important component of successful therapy [1]. There is enormous variability in 6MP cytotoxicity in children given a standard prescribed dose [2] and this variability is difficult to control or eliminate because it is multifactorial. To exert its cytotoxic properties the oral therapy must be taken, absorbed and extensively metabolized to produce a variety of intracellular thionucleotides. Competing with thionucleotide formation are the oxidative and *S*-methylation pathways of 6MP metabolism, both of which show individual variation. Human liver xanthine oxidase activity varies four-fold [3], but TPMT catalysed *S*-methylation can vary over 40-fold [4]. TPMT forms methylmercaptopurine and methylmercaptopurine nucleotide metabolites (MeMPs) at the expense of 6MP derived 6-thioguanine nucleotides (TGNs) [3, 6].

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The activity of TPMT, in all cells and tissues, is regulated by a common genetic polymorphism [7, 8]. In a Caucasian population, 89% have high enzyme activity and are homozygous for the $TPMT^H$ allele, whilst 11% are heterozygotes with an intermediate enzyme activity. Very low enzyme activity occurs in 1 in 300 individuals. Such subjects are homozygous $TPMT^L$ and lack functional TPMT activity. In the majority of subjects two point mutations in the wild-type allele are responsible for the production of $TPMT^L$, but both or either mutation results in decreased expression of TPMT [9]. Whilst TPMT deficiency is associated with grossly elevated TGN concentrations and profound myelosuppression [10], the converse is also true for subjects with very high TPMT. The latter sub-group can be identified as at a higher risk of disease relapse [5].

TPMT activity in leukaemic blasts taken from children with ALL at diagnosis is significantly correlated with the TPMT activity in their red blood cells (RBCs) [11].

Measurement of TPMT activities at diagnosis would be of immediate benefit to those children who lack functionally active enzyme, and it could also identify those children with very high activities who are at a potential risk of suboptimal treatment. These two groups of children are at the opposite ends of the TPMT spectrum. The former would develop profound, life-threatening myelosuppression at standard 6MP dosages whilst the latter may form inadequate amounts of cytotoxic TGN metabolites. The aim of this study was to investigate the clinical importance of RBC TPMT activities at diagnosis in terms of any effect on thiopurine metabolism and toxicity during continuing chemotherapy.

Methods

Chemotherapy

The children studied were taking continuing chemotherapy according to the Medical Research Council trial UK ALL XI (Figure 1) [12]. 6MP dosages in the UK ALL XI trial are titrated to the limits of tolerance as determined by myelosuppression. The daily dose of 6MP is titrated to toxicity, dosages are *reduced* to 50% of the starting dose ($75\ mg\ m^{-2} = 100\%$ dosage) in response to a neutrophil count $< 1.0 \times 10^9\ l^{-1}$ or a platelet count $< 100 \times 10^9\ l^{-1}$, and withdrawn if the neutrophil count falls $< 0.5 \times 10^9\ l^{-1}$ or the platelet count $< 50 \times 10^9\ l^{-1}$. When the blood counts recover 6MP dosages are *increased* in a protocol directed stepwise manner. 50% 6MP dosage is introduced at a neutrophil count of $> 1.0 \times 10^9\ l^{-1}$ and maintained until the blood counts recover to within the normal range. 6MP dosages are then increased to 75% for 2 weeks, and if tolerated, up to 100%. If the protocol standard dose is tolerated for at least 1 month the daily 6MP dose is escalated by 25% (125% dosage). If this dose is tolerated for 4 weeks the dosage is increased again by 25% (dosage 150%), and likewise every 4 weeks until the blood count drops. Cell counts are measured at 7 or 14 day intervals and the 6MP dose adjusted accordingly. A weekly dose of methotrexate ($20\ mg\ m^{-2}$) is adjusted in parallel with the 6MP. Monthly single intravenous vincristine ($1.5\ mg\ m^{-2}$) and oral prednisolone ($40\ mg\ m^{-2}$, daily × 5) are given to all children irrespective of blood cell counts. Unless interrupted by intensive blocks, the cycle of daily 6MP, weekly methotrexate and monthly vincristine and steroids repeats until the end of treatment, approximately 2 years from diagnosis.

Two 5 day blocks of intensive therapy are given during weeks 5 and 20. In addition to oral 6-thioguanine (6TG; $80\ mg\ m^{-2}$ daily × 5) this block contains intravenous etoposide ($100\ mg\ m^{-2}$ daily × 5), cytarabine ($100\ mg\ m^{-2}$, 12 hourly for 5 days) and daunorubicin ($45\ mg\ m^{-2}$ on days 1 and 2) plus the monthly vincristine and oral prednisolone. A third intensive block may be given between protocol weeks 35 to 42, this is randomized. This block consists of 4 weeks asparaginase/vincristine/dexamethasone followed by 4 weeks cyclophosphamide/cytarabine/thioguanine.

Study design

TPMT phenotype was measured at diagnosis using a pre-transfusion blood sample. RBC TGNs generated from 6TG were measured daily during the two 5 day blocks of intensive therapy. The oral dose of 6TG was given before breakfast and the blood sample obtained 12 h post dose via a central line, immediately prior to access for cytarabine therapy. TGNs and MeMPs were measured during 6MP based therapy in blood samples taken immediately prior to the monthly vincristine injection. 6MP metabolism was investigated (a) at the start of continuing chemotherapy and (b) after the second block of intensive therapy (6 months post diagnosis) at 100% protocol dose ($75\ mg\ m^{-2}$). Blood samples were taken after at least 7 days at 100% 6MP, but not within 6 weeks of a blood transfusion or intensive block. The blood samples were taken on an out-patient basis approximately 6 h post-dose. For the first 6MP metabolite assay this corresponded to a sample taken at the start of protocol week 12, 16 or 20, and for the second 6MP assay a blood sample taken at or after week 27. For

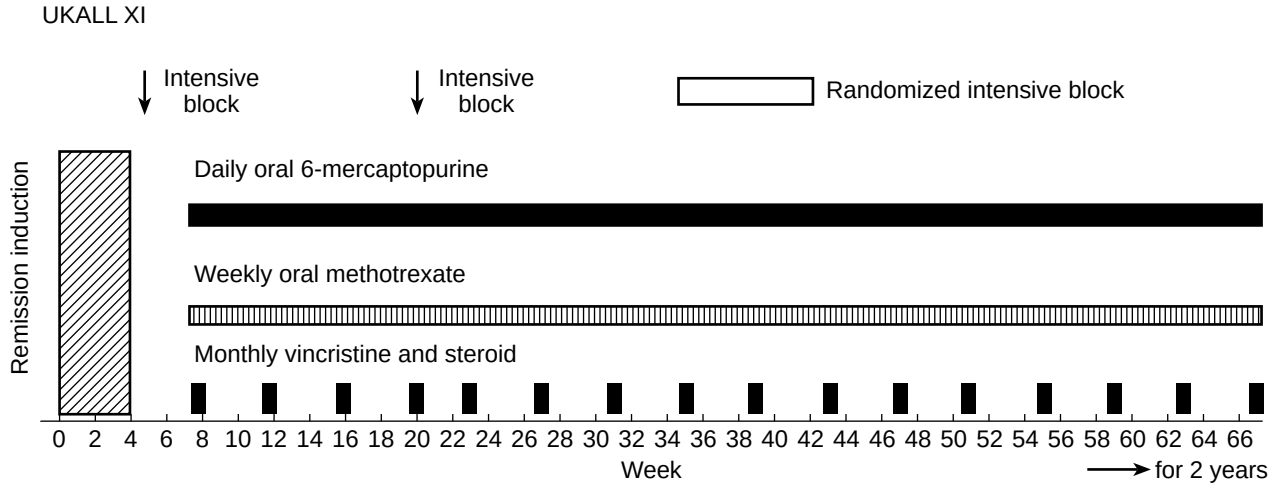

Figure 1 UKALL XI continuing chemotherapy. The arrows depict the week 5 and week 20 6TG containing intensive blocks in which blood samples were obtained to study 6TG metabolism. Continuing chemotherapy starts at week 8. The oblongs depicted on the x axis indicate the time of the monthly vincristine injection. These correspond to the time of blood sampling for the study of 6MP metabolism, but not if within 6 weeks of an intensive block. The full details of blood sampling are in the text. The cycle of daily 6MP, weekly methotrexate and monthly vincristine and steroids repeats until the end of treatment, approximately 2 years from diagnosis.

the comparison of drug metabolism during year 1 and year 2 the assay values used were those obtained from blood samples taken under identical dosing conditions. All patient procedures were approved by the local Ethics Committee.

Assays and statistics

RBC TPMT activities were measured as previously described, the lower limit of quantitation was 0.74 units TPMT ml^{-1} packed RBCs [13]. One unit of enzyme activity represented the formation of 1 nmol of methylmercaptopurine per hour of incubation, per ml of packed RBCs. Thiopurine metabolites were measured as previously described, the lower limits of quantitation are 30 pmol and 120 pmol/8×10^8 RBCs for TGNs and MeMPs respectively [14]. RBC TGNs represent the mono- di- and triphosphates of 6-thioguanosine whilst RBC MeMPs represent methylmercaptopurine nucleotide (6-methyl thioinosine 5′monophosphate) [14]. With every TPMT incubation a quality control was included, this was a pooled lysate preparation of a known TPMT activity. With every thiopurine metabolite assay a quality control of RBCs spiked with 300 pmol TGNs and 3 nmol MeMPs/ 8×10^8 RBCs was included. All assays were performed in duplicate. Statistical comparisons were made by the Mann-Whitney test, and for paired data by the Wilcoxon signed-ranks test. Correlations were assessed by the Spearman rank correlation coefficient (r_s). Two-tailed P values are quoted for all statistical analysis.

Results

Study group

From a potential cohort of 48 children with ALL, 38 (24 boys, 14 girls) had pre-transfusion blood samples taken at diagnosis, and 29 (21 boys, 8 girls) had all the required blood samples taken during chemotherapy. Twelve of these 29 children received a third intensive block. The children studied were aged between 1 to 10 years (median 4).

Assays

The interassay coefficient of variation for the TPMT quality control of 11.7 units ml^{-1} packed RBCs was 5.06% for five assays over a 3 month period. The interassay coefficients of variation for the metabolite quality control sample of 300 pmol TGNs and 3 nmol MeMPs/8×10^8 RBCs through 51 assays over a 2 year period were 5.4% and 6.08% respectively.

TPMT activities

TPMT activities at diagnosis ranged from 5.5 to 18.5 units ml^{-1} packed RBCs, no different to the range of TPMT activities previously reported for healthy children (4.6 to 18.8 units ml^{-1} packed RBCs for 110 children with intermediate and high enzyme activities [5] (Mann-Whitney $P > 0.25$, Figure 2).

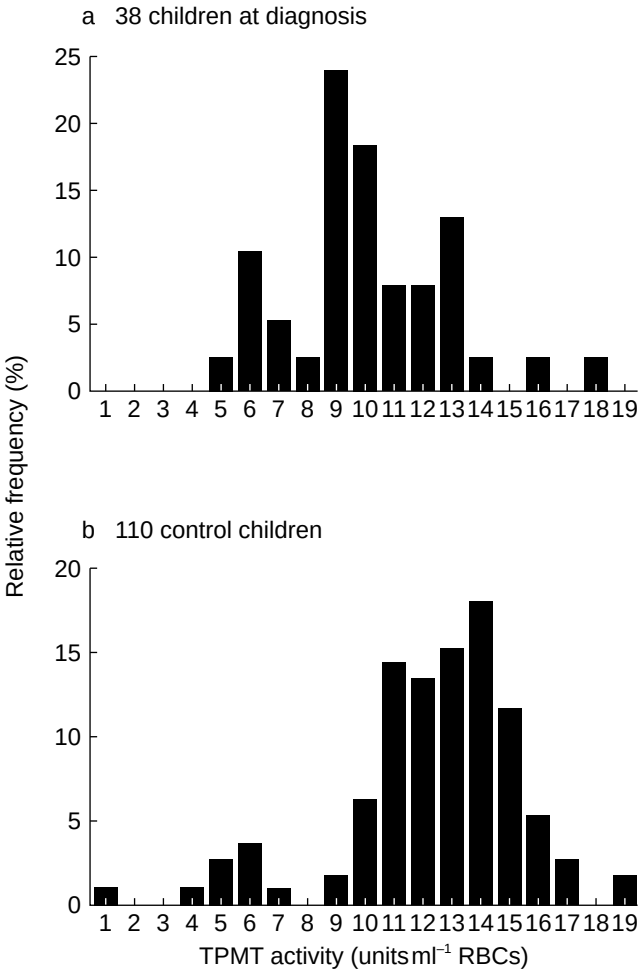

Figure 2 RBC TPMT activities at diagnosis in 38 children with ALL a) compared with those previously reported in 110 healthy children b). Figure 2b modified from Lennard *et al.* (1990) [5].

Thiopurine metabolism

In an attempt to define the earliest point in therapy that TPMT activities at diagnosis could be related to RBC TGN production, thiopurine metabolism was studied during the week 5 and week 20 blocks of 6TG containing intensive therapy, and during the intervening 6MP continuing chemotherapy. To investigate the variability of drug metabolism over time 6MP metabolites were also measured after 6 months therapy and during the second year of continuing chemotherapy.

6TG intensification blocks The TGNs generated from 6TG during the protocol week 5 multi-agent intensification block were not related to the at diagnosis TPMT activity, but 25 of the 29 children studied (86%) had received post diagnosis red cell transfusions, 14% receiving additional transfusions within 3 weeks of the study.

The formation of TGNs from 6TG was again studied during the week 20 intensification block. No child had received a red cell transfusion within 6 weeks of this block. The initial rate of TGN accumulation varied from 75 to 449 pmol/8×10^8 RBC.day (median 190), and showed a negative relationship to the at diagnosis TPMT activity ($r_s = -0.601$, $P < 0.005$) (Figure 3).

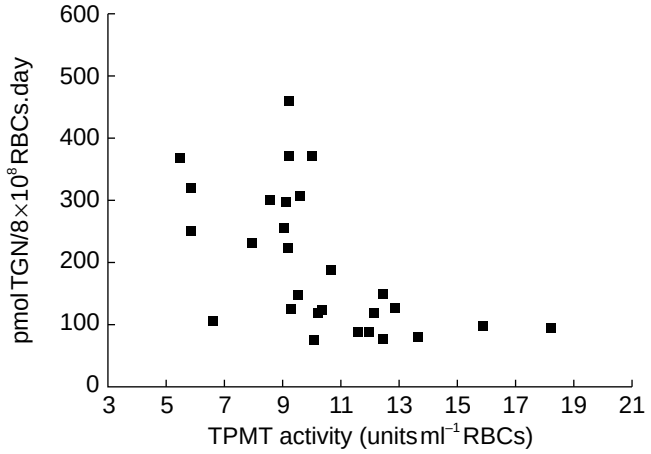

Figure 3 Correlation between RBC TPMT activity at diagnosis and the rate of RBC TGN accumulation in 29 children with ALL during 6TG therapy in the week 20 intensive block ($r_s = -0.601$, $P < 0.005$).

6MP continuing chemotherapy The duration of 75 mg m^{-2} (100%) 6MP at the first metabolite assay ranged from 2 to 4 weeks, median 4. 6MP metabolite concentrations ranged from 187 to 594 pmol TGNs, median 327, and 0.5 to 22.0 nmol MeMPs, median 4.5, per 8×10^8 RBCs. TPMT activity at diagnosis was not significantly related to the generation of MeMPs ($r_s = 0.06$), but was negatively correlated to TGNs ($r_s = -0.44$, $P < 0.025$, $n = 29$) (Figure 4). Twenty-one children had TGN assays at the point of protocol directed 6MP dose reduction. TGN concentrations were negatively correlated to the neutrophil count ($r_s = -0.5$, $P < 0.01$) (Figure 5).

After the second (week 20) intensification block 6MP metabolites were again measured at 100% dosages (duration 2 to 4 weeks, median 4). TGNs ranged from 206 to 604 pmol/8×10^8 RBCs, median 325, and MeMPs from 0.64 to 14.7 nmol/8×10^8 RBCs, median 3.75. There was no significant difference in either TGN or MeMP concentrations when compared with the cycle 1 levels, Wilcoxon median difference for the TGNs = 3 pmol, $P > 0.9$ and for the MeMPs = 0.22 nmol, $P > 0.5$.

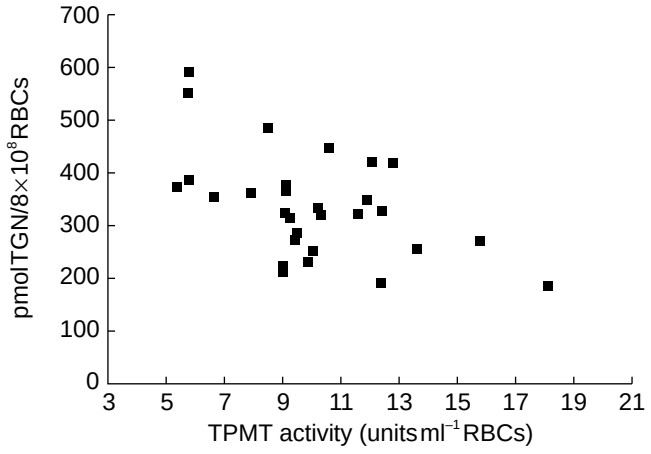

Figure 4 Correlation between RBC TPMT activity at diagnosis and RBC TGN concentrations in 29 children with ALL undergoing 6MP continuing chemotherapy ($r_s = -0.44$, $P < 0.05$).

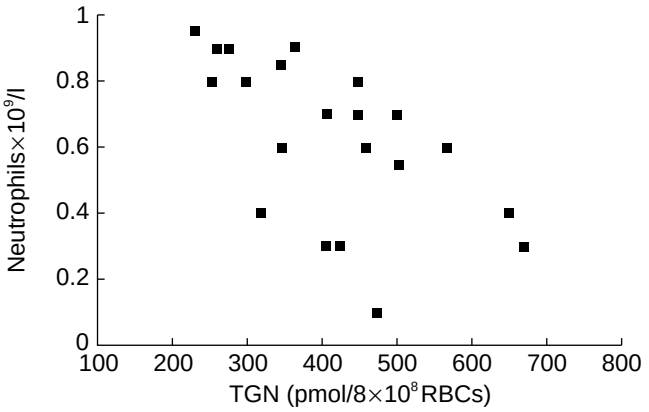

Figure 5 Correlation between RBC TGN concentrations and the neutrophil count at the point of 6MP dose reduction in 21 children with ALL undergoing 6MP continuing chemotherapy ($r_s = -0.5$, $P < 0.02$).

6MP metabolism was measured after identical cycles of dose increments in year 1 *vs* year 2. The cycle was 50% × 1 week, 75% × 2 weeks, 100% × 4 weeks, 125% × 2 weeks (i.e. the protocol directed cycle of dose increments following the introduction of 6MP after cytopenia induced withdrawal), and metabolite data were available for 21 children. At the end of the cycle there was no significant difference between TGN levels in year 1 (199 to 637 pmol, median 384) *vs* year 2 (196 to 578 pmol, median 373; Wilcoxon median difference 30 pmol $P = 0.19$). That was not the case for the MeMPs. At the end of the identical dosage cycle MeMPs ranged from 1.4 to 29 nmol/8×10^8 RBCs, median 6.2 in 1 year and 1.9 to 54.7 nmol/8×10^8 RBCs, median 12.7, in the next (Wilcoxon median difference 8.42 nmol, $P < 0.003$, 95% CI 1.4 to 16.5 nmol). Inspection of the data showed that there were nine children whose MeMPs increased over two-fold in year 2, four of these children were randomized for receipt of a third intensifive block. In the other 12 children (six randomized for a third intensive block) there was no difference between year 1 (1.38 to 14.7 nmol MeMPs, median 6.1) and year 2 (1.93 to 15.3 nmol MeMPs, median 5.6) concentrations (Wilcoxon $P > 0.4$). Comparison of the at diagnosis TPMT activities showed a median difference of 2.85 units ml^{-1} RBCs between the 12 children without and the nine children with grossly increased methyl metabolites, which approached but did not reach significance ($P = 0.08$). In the nine children with increased methylation, MeMPs ranged from 2.3 to 29 nmol, median 9.3, in 1 year compared with 13.8 to 54.7 nmol, median 40, in the next (Wilcoxon median difference 23 nmol, 95% CI 13.6 to 34, $P < 0.01$). The TGN concentrations measured in these same samples were 231 to 567 pmol (median 338) in year 1 and 227 to 578 pmol (median 404) in year 2 (Wilcoxon median difference 17 pmol, $P > 0.8$).

Clinical and laboratory features which could have corresponded with a sudden metabolite increase were investigated. These included febrile or infectious episodes, anaemia, neutropenia, thrombocytopenia, time from intensive block, abnormal liver function tests, abnormal RBC mean cell volume or mean cell haemoglobin concentration. No consistent features were found. The children were well and

receiving standard out-patient care at the time of elevated MeMP measurements.

6MP dosage

During continuing chemotherapy myelosuppression is used as a surrogate measure of the response to 6MP chemotherapy. The amount of time that the drug was withdrawn in response to cytopenias (neutrophils <0.5 and/or platelets $<50\times10^{9}\,l^{-1}$) was expressed as a percentage of the total time 6MP could have been prescribed. Full dosage data was available for 32 of the 38 children and the duration of cytopenia driven drug withdrawal ranged from 1 to 39% of the time, median 11%. This was inversely related to the at diagnosis TPMT activity ($r_s=-0.41$, $P<0.05$) (Figure 6).

Discussion

Despite the fact that 6MP has been available for the treatment of leukaemias for over 40 years it has lately become the focus of renewed interest as more information has become available about its metabolism and mode of cytotoxicity. TGNs are important cytotoxic metabolites [2, 5, 10]. This study has confirmed previous findings that RBC TGN concentrations are inversely related to neutropenia, and after a standard dose of 6MP the interpatient variation in the production of RBC TGNs are high [2, 5]. However, these studies also show that intrapatient variations in TGN production, following identical dosage cycles, are insignificant. This latter finding is new and indicates that a child's ability to metabolize a standard dose of 6MP to TGNs shows very little variation throughout 2 years continuing chemotherapy. In addition, this study has shown that the TPMT activity at diagnosis was inversely related to both the production of TGN active metabolites and the duration of cytopenia driven drug withdrawal during continuing chemotherapy—an index of the degree of 6MP cytotoxicity. This indicates that a measurement of TPMT activity at diagnosis might be helpful in the clinical management of children with ALL.

Measurement of TPMT activity at diagnosis will not necessarily be predictive of 6MP metabolism in all children.

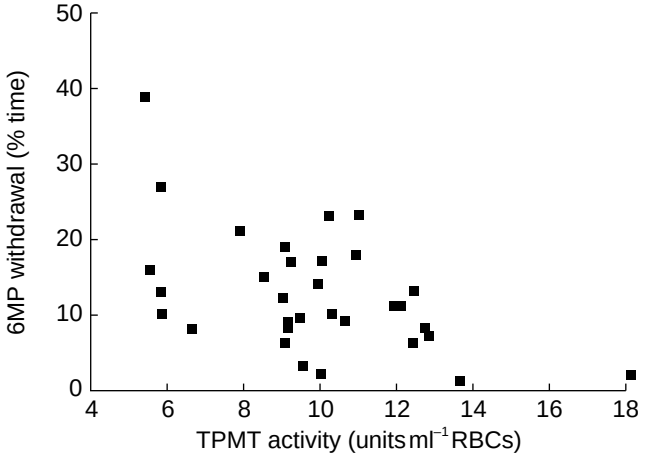

Figure 6 Correlation between TPMT activity at diagnosis and the duration of cytopenia driven drug withdrawal during continuing chemotherapy, ($r_s=-0.41$, $P<0.05$, $n=32$).

Apart from the obvious problem of noncompliance, the influence of TPMT on 6MP metabolism is further confounded by the fact that TPMT activities are increased over and above the basal inherited levels during 6MP chemotherapy [5]. The amount of this increase cannot be predicted, although once increased RBC TPMT activities are relatively stable throughout chemotherapy [11]. A comparison of TPMT activities during and after the completion of chemotherapy indicates that a child with an activity at the lower end of the homozygous high range may develop very high activities during chemotherapy [5]. How chemotherapy effects TPMT activities in other cells and tissues is not known, and the complex mechanisms underlying the accumulation of both RBC TGNs and MeMPs are poorly understood [15]. Additional studies will be required to define the biochemical basis of significantly elevated MeMP accumulation, accompanied by an insignificant movement in the TGNs, in those children studied under identical dosing conditions during year 1 and 2 of their continuing chemotherapy.

The measurement of MeMPs alongside the TGNs have recently been suggested as compliance indicators [16]. Those children with high TPMT activities have high concentrations MeMPs accompanied by low concentrations of TGNs, whilst those with compliance problems have low concentrations of both metabolites. In the former group the 6MP dose can be safely titrated upwards whereas to do the same in the latter may have disastrous consequences. The dramatic increase in the MeMPs seen in some children reported in this paper does not influence this potential use of MeMPs. The lowest concentration of MeMPs measured was still ten fold higher than the TGN concentration in that same blood sample. 6MP pharmacokinetic studies have shown that taking the blood sample in the 4 h period following drug dosage, and/or failure to process the blood sample within 24 h do not produce elevated MeMPs [17].

The immediate oxidative and methylated products of 6MP (thiouric acid and methylmercaptopurine respectively) are produced in large quantities [18], but are inactive metabolites. Although the nucleotide anabolic pathway results in the eventual formation of the TGNs, the initial nucleotide metabolite of 6MP (thioinosinosine monophosphate) is a good substrate for TPMT [6]. The resultant methylmercaptopurine nucleotides (the MeMPs) are present in the majority of children undergoing 6MP chemotherapy at concentrations far greater than the TGNs [10, 14]. The formation of MeMPs is a detoxification mechanism which is responsible *in vitro* for 'paradoxical cytotoxicity'—a decrease in cytotoxicity with increasing 6MP concentrations. This occurs when the MeMPs are desulphurated, and the endogenous purine nucleotides produced can act as self-rescue agents from 6MP cytotoxicity [19]. *In vitro* studies describe this phenomenon occurring in leukaemic cell lines at 6MP concentrations above 100 μM [19]. Such a mechanism could potentially occur in those children with MeMPs at the upper end of the range recorded in this study. But, prior to desulphuration, the MeMPs are powerful inhibitors of *de novo* purine synthesis, and so the MeMPs decrease the availability of endogenous purine nucleotides. In this role the MeMPs could potentiate the incorporation of any available TGNs into DNA. The MeMPs could also be

acting indirectly by their effects on transmethylation [20]. In short, the possibility of MeMP induced biological effects cannot be ruled out, but the exact roles of the MeMPs are uncertain. It is possible that the TGNs and MeMPs interact in cytotoxicity, TPMT deficient children appear to tolerate much higher concentrations of RBC TGNs [10, 21, 22]. This is also apparent when 6TG, a direct source of the TGNs, is offered as an alternative to 6MP [23].

The elucidation of the molecular basis for the TPMT genetic polymorphism will enable the development of DNA-based diagnostic tests. The measurement of TPMT genotype at diagnosis offers one immediate advantage over RBC phenotype measurements—the former are not influenced by blood transfusions, frequently given before children with ALL can be investigated. A number of variant TPMT alleles have now been described [9, 24–26], and one can expect more to be documented in the near future. *TPMT*3A*, a double mutant, is the most frequently occurring varient allele in white Caucasians [9, 25, 26], but each mutation can occur independently (*TPMT*3B* and *TPMT*3C* [9, 26]). The *TPMT*3A* mutation appears to have no effect on transcription or mRNA stability, but the resultant very low protein levels indicate that post transcriptional events result in the loss of functional TPMT activity [25, 27].

A knowledge of the TPMT phenotype prior to thiopurine treatment could be of immediate benefit to individuals who would be unduly sensitive, and it offers the advantage of indicating the amount of enzyme activity, thus identifying those who may be 'resistant' to standard dose 6MP therapy by virtue of their very high *S*-methylation status. For the latter group, awareness of drug tolerance would encourage titration to toxicity, and for the former the use of attenuated 6MP dosages.

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References

1 Gale RP, Butturini A. Maintenance chemotherapy and cure of childhood acute lymphoblastic leukaemia. *Lancet* 1991; **338**: 1315–1318.

2 Lilleyman JS, Lennard L. Mercaptopurine metabolism and risk of relapse in childhood lymphoblastic leukaemia. *Lancet* 1994; **343**: 1188–1190.

3 Guerciolini R, Szumlanski C, Weinshilboum RM. Human liver xanthine oxidase: nature and extent of individual variation. *Clin Pharmacol Ther* 1991; **50**: 663–672.

4 Szumlanski CL, Honchel R, Scott MC, Weinshilboum RM. Human liver thiopurine methyltransferase pharmacogenetics: biochemical properties, liver-erythrocyte correlation and presence of isozymes. *Pharmacogenetics* 1992; **2**: 148–159.

5 Lennard L, Lilleyman JS, Van Loon JA, Weinshilboum RM. Genetic variation in response to 6-mercaptopurine for childhood acute lymphoblastic leukaemia. *Lancet* 1990; **336**: 225–229.

6 Deininger M, Szumlanski CL, Otterness DM, Van Loon J, Ferber W and Weinshilboum RM. Purine substrates for human thiopurine methyltransferase. *Biochem Pharmacol* 1994; **11**: 2135–2138.

7 Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: Monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *Am J Hum Genet* 1980; **32**: 651–662.

8 Weinshilboum RM, Methylation pharmacogenetics: thiopurine methyltransferase as a model system. *Xenobiotica* 1992; **22**: 1055–1071.

9 Szumlanski C, Otterness D, Her C, *et al*. Thiopurine methyltransferase pharmacogenetics: human gene cloning and characterization of a common genetic polymorphism. *DNA and Cell Biology*, 1996; **15**: 17–30.

10 Lennard L, Gibson BES, Nicole T, Lilleyman JS. Congenital thiopurine methyltransferase deficiency and 6-mercaptopurine toxicity during treatment for acute lymphoblastic leukaemia. *Arch Dis Childh* 1993; **69**: 577–579.

11 McLeod HL, Relling MV, Liu Q, Pui C-H, Evans WE. Polymorphic thiopurine methyltransferase in erythrocytes is indicative of activity in leukemic blasts from children with acute lymphoblastic leukemia. *Blood* 1995; **7**: 1897–1902.

12 Chessells JM, Bailey CC, Richards SM. Intensification of treatment improves survival for all children with lymphoblastic leukaemia: results of MRC UKALL X. *Lancet* 1995; **345**: 143–148.

13 Lennard L, Singleton HJ. High performance liquid chromatographic assay of human red blood cell thiopurine methyltransferase activity. *J Chromatog B* 1994; **661**: 25–33.

14 Lennard L, Singleton H. 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 Chromatog* 1992; **583**: 83–90.

15 Rostrami-Hodjegan A, Lennard L, Lilleyman JS. The accumulation of mercaptopurine metabolites in age fractionated red blood cells. *Br J Clin Pharmacol* 1995; **40**: 217–222.

16 Lennard L, Welch J, Lilleyman JS. Intracellular metabolites of 6-mercaptopurine in children with lymphoblastic leukaemia: a possible indicator of non-compliance. *Br J Cancer* 1995, **72**: 1004–1006.

17 Welch JC, Lennard L, Morton GCA, Lilleyman JS. Pharmacokinetics of mercaptopurine: plasma drug and red cell metabolite concnetrations after an oral dose. *Therap Drug Monit* 1997; **19**: 382–385.

18 Elion GB. The purine path to chemotherapy. *Science* 1989; **244**: 41–47.

19 Adamson PC, Balis FM, Hawkins ME, Murphy RF, Poplack DG. Desulfuration of 6-mercaptopurine. The basis for the paradoxical cytotoxicity of thiopurines in cultured human cells. *Biochem Pharmacol* 1993; **46**: 1627–1636.

20 Keuzenkamp-Jansen CW, De Abreu RA, Blom HJ, Bokkerink JPM, Trijbels JMF. Effects on transmethylation by high-dose 6-mercaptopurine and methotrexate infusions during consolidation treatment of acute lymphoblastic leukaemia. *Biochem Pharmacol* 1996; **51**: 1165–1171.

21 Evans WE, Horner M, Chu YQ, Kalwinsky D, Roberts WM. Altered mercaptopurine metabolism, toxic effects, and dosage requirement in a thiopurine methyltransferase deficient child with acute lymphoblastic leukaemia. *J Pediatr* 1991; **119**: 985–989.

22 McLeod HL, Miller DR, Evans WE. Azathioprine-induced myelosuppression in thiopurine methyltransferase deficient heart transplant recipient. *Lancet* 1993; **341**: 1151.

23 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.

24 Krynetski EY, Schuetz JD, Galpin AJ, Pui C-H, Relling MV, Evans WE, A single point mutation leading to loss of catalytic

activity in human thiopurine *S*-methyltransferase. *Proc Natl Acad Sci USA* 1995; **92**: 949–953.

25 Tai H-L, Krynetski EY, Yates CR, *et al*. Thiopurine S-methyltransferase deficiency: two nucleotide transitions define the most prevalent mutant allele associated with loss of catalytic activity in Caucasians. *Am J Hum Genet* 1996; **58**: 694–702.

26 Otterness D, Szumlanski C, Lennard L, *et al*. Human thiopurine methyltransferase pharmacogenetics: gene sequence polymorphisms. *Clin Pharmacol Ther* 1997; (in press).

27 Krynetski EY, Tai H-L, Yates CR, *et al*. Genetic polymorphism of thiopurine *S*-methyltransferase: clinical importance and molecular mechanisms. *Pharmacogenetics* 1996; **6**: 279–290.

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