Chapter 5
Pharmacokinetics, Pharmacodynamics and Pharmacogenetics of Antileukemic Drugs

Kjeld Schmiegelow and Inge van der Sluis

5.1 Introduction

At least 85% of children with acute lymphoblastic leukemia (ALL) can be cured by the best contemporary therapy, but it is uncertain which of the multiple effector mechanisms of the antileukemic agents that are responsible for the efficacy (Fig. 5.1 and Table 5.1). This contrasts the modern era of targeted therapy, where molecular mapping of chemoresistant cancer cells has led to development of drugs that specifically target aberrant pathways (see Chap. 9).

Antileukemic chemotherapy [1, 2] has its roots in the late 1940s, when Sidney Farber and coworkers demonstrated that antifolates could induce remission in childhood ALL [3]. A few years later Joseph Burchenal and coworkers obtained similar results with thiopurines [4]. Soon Vincristine (VCR) and glucocorticosteroids (Steroid) and even adrenocorticotropic hormone were shown to be most effective (and least toxic) for inducing morphologic bone-marrow remission (<5% leukemic blasts), while a combination of daily oral 6-mercaptopurine (6MP) and weekly oral methotrexate (MTX) was superior for remission maintenance. By the late 1960s all the currently used, so-called *traditional*, antileukemic drugs were available, i.e.

K. Schmiegelow, MD, DrMedSci. (✉)
Department of Pediatrics and Adolescent Medicine, University Hospital Rigshospitalet, Copenhagen, Denmark

Institute of Clinical Medicine, University of Copenhagen, København, Denmark

Department of Pediatrics and Adolescent Medicine, JMC-4072, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark
e-mail: kschmiegelow@rh.dk

I. van der Sluis, MD, PhD
Department of Pediatric Hematology-Oncology, Erasmus Medical Center – Sophia Children's Hospital, Rotterdam, The Netherlands

The Princess Maxima Center for Pediatric Oncology, Utrecht, The Netherlands

A. Vora (ed.), *Childhood Acute Lymphoblastic Leukemia*,
DOI 10.1007/978-3-319-39708-5_5

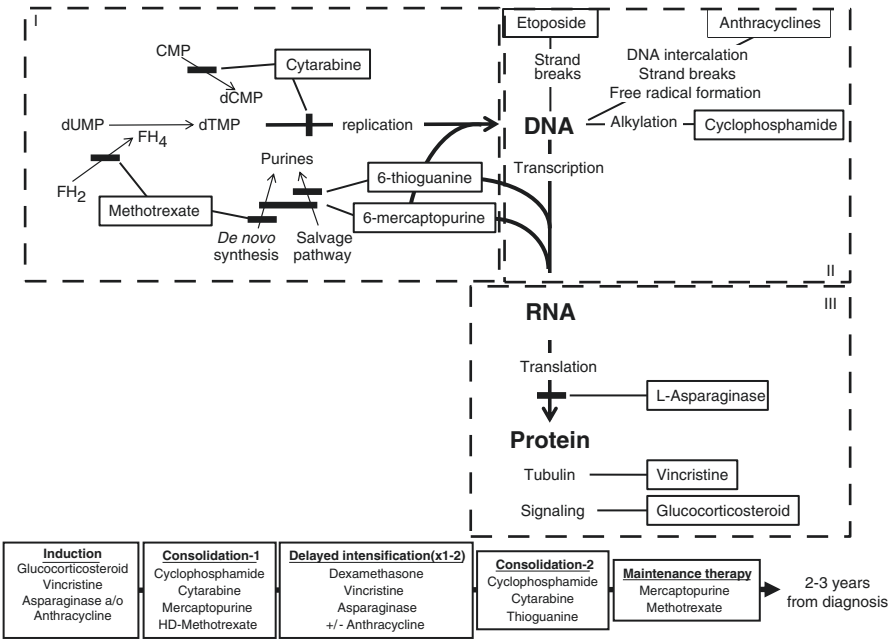

Fig. 5.1 Primary sites of action of *traditional* antileukemic drugs. Their use during antileukemic treatment program is outlined at the bottom. The antileukemic agents can roughly be divided into 3 groups. Goups II drugs interact with DNA. They generally cause profound acute nausea, hair loss, mucositis and myelo-/immunosuppression. They may also cause serious late effects, e.g. cardiotoxicity, urothelium damage, and second cancer. Group I, the antimetabolites, are analogues of normal folate or nucleotide precursors that interferes with DNA synthesis. Group III are the post-translational drugs. The acute toxicities of groups I and III are relatively limited compared to group II, including less nausea, hair loss, mucositis, myelo- and immunosuppression (can be significant for high dose methotrexate/cytarabine). In addition, each drug may be associated with specific toxicities (see text). Groups I and III drugs rarely lead to serious late effects, unless severe acute toxicities have occurred. CMP = cytidine monophosphate; dCMP = deoxycytidine monophosphate; FH_2 = dihydrofolate; FH_4 = tetrahydrofolate; dTMP = deoxythymidine monophosphate; dUMP = deoxyuridine monophosphate

L-asparaginase (Asp), thioguanine (6TG), cyclophosphamide, and cytosine arabinoside (AraC) in the 1950s, and ifosfamide and epipodophylloxins in the 1960s. Improved understanding of their pharmacokinetics (PK) and pharmacodynamics (PD) has made childhood ALL therapy one of the most impressive successes of modern medicine. This was not least due to (i) introduction of central nervous system (CNS) directed therapy in the 1960s, i.e. intrathecal (i.t.) chemotherapy, high-dose MTX and AraC (HD-MTX, HD-AraC) and irradiation [5, 6], (ii) introduction of intensive post-induction consolidation therapy and delayed intensification in the 1970s [7], (iii) observational studies linking biologically defined subsets of ALL patients to specific treatment requirements (e.g. lower propensity for MTX polyglutamation by T-lineage leukemia necessitating HD-MTX [8]), (iv) implementation of precise quantification of minimal residual disease (MRD) for risk/treatment

Table 5.1 Pharmacokinetics of traditional antileukemic drugs

Class of drug	Drug	Cell cycle specific	CNS penetration	Plasma half-life	Metabolism (primarily)	Route of elimination	Protein binding in plasma	Therapeutic drug monitoring	Toxicities
Glucocorti-costeroids	Prednisolone	No	Yes	2–4 h	Liver (CYP3A4, conjugation)	Urine	Yes	No	Hypertension, hyperglycemia, hepatotoxicity, osteonecrosis, neurobehavioural side effects
Glucocorti-costeroids	Dexamethasone	No	Yes, 4–5 times higher than prednisolone	3–6 h	Liver (CYP3A4, conjugation)	Urine	Yes	No	See prednisolone
Vinca alkaloid	Vincristine	Yes	No	Rapid steep decrease; subsequently terminal T½ of 15–155 h	Liver (CYP3A4)	Urine (10%) Bile (80%)	Yes	No	Neurotoxicity (peripheral/autonomic) Constipation SIADH (rare)
Anthracycline	Doxorubicin Daunorubicin	No	No (CSF/plasma ratio very low)	T1/2 10–30 min T1/2 30–50 h	Liver (GSTs and conjugation)	Bile Urine (10%) Large proportion of the active drug is immediately bound to tissue DNA	Yes 75%	No	Nausea/vomiting, hair loss, immuno−/myelosuppression, mucositis, cardiotoxicity

(continued)

Table 5.1 (continued)

Class of drug	Drug	Cell cycle specific	CNS penetration	Plasma half-life	Metabolism (primarily)	Route of elimination	Protein binding in plasma	Therapeutic drug monitoring	Toxicities
Asparaginase	E.coli asparaginase	No	No (<1%)[a]	1.24 ± 0.17 days	Reticuloendothelial system	–	No?	Asp activity	Hypersensitivity Allergic reaction Silent inactivation Thrombosis Pancreatitis Increased liver enzymes Hyperammonaemia Hypoalbuminaemia Hyperglycemia
Asparaginase	Pegylated E.coli asparaginase	No	No[a]	5.73 ± 3.24 days (Non-linear, time-dose dependent clearance $T_{1/2}$ varies from 11.8 days at day 1 to 2.4 days at day 20)	Reticuloendothelial system	–	No?	Asp activity	See E.coli asp , less immunogenic through pegylation
Asparaginase	Erwinia asparaginase	No	No[a]	0.65 ± 0.13 days	Reticuloendothelial system	–	No?	Asp activity	See E.coli asp, but immunologically different
Thiopurine	6-mercap-topurine	Yes	Yes (limited)	50 min	Inactive parent compound Xanthine oxidase in gut and liver, TPMT, NUDT15	Hepatic metabolism, urine	Yes (<20%)	Ery-TGN/ MeMP DNA-TGN	Immuno−/ myelosuppression, hepatotoxicity, second cancer

Thiopurine	6-thioguanine	Yes	Yes (limited)	90 min Terminal $T_{½,l}$ 5–9 h	Inactive parent compound. Xanthine oxidase in gut and liver, TPMT	Hepatic metabolism, urine	Yes	Ery-TGN/ MeMP DNA-TGN	Myelosuppression Hepatotoxicity Nausea/vomiting
Folate-analogue	Methotrexate oral dose <40 mg/m^2	Yes	No	3–10 h	Liver (storage)	Renal (90%) Bile (10%)	Yes (50%)	Ery-MTXpg	Myelosuppression Hepatotoxicity
Folate-analogue	Methotrexate high dose 1–5 g/m^2	Yes	No	8–15 h	Liver (storage)	Renal (90%) Bile (10%)	Yes (50%)	p-MTX	Myelosuppression Hepatotoxicity Renal toxicity Neurotoxicity
Arabinoside-cytidine	Arabinoside-cytidine	Yes	Yes	7–20 min	Inactivation by deamination	Metabolism (deamination in liver, plasma and tissue) renal	Limited (13%)	No	Myelosuppression, mucositis neurotoxicity, conjunctivitis
Oxazaphos-phorines	Cyclophos-phamide	No	Yes	4–8 h	Inactive parent compound. Activation by CYP450 enzymes (most important enzymes CYP2B6, CYP2C19 and CYP3A4)	Renal (<15% of the parent drug, 80% inactive drug)	Yes 20%, metabolites up to 60%	No	Nausea/vomiting, hair loss, immuno-/ myelosuppression, mucositis, hemorarrhagic cystitis, second cancer
					Detoxification mainly GSTs and alcohol dehydrogenase (ALDH1, ALDH3), but also by CYP3A4				

(continued)

Table 5.1 (continued)

Class of drug	Drug	Cell cycle specific	CNS penetration	Plasma half-life	Metabolism (primarily)	Route of elimination	Protein binding in plasma	Therapeutic drug monitoring	Toxicities
Oxazaphosphorines	Ifosfamide	No	Yes	7–15 h	Inactive parent compound Activation by CYP450 enzymes (slower rate than cyclophosphamide)	Renal	Limited	No	Nausea/vomiting, hair loss, immuno–/myelosuppression, mucositis, hemorarrhagic cystitis, second cancer
Epipodophyllotoxins	Etoposide	No	Yes (poorly)	Distribution $T_{1/2}$ 1.5 h Terminal $T_{1/2}$ 4–11 h	Hepatic (CYP3A4/5) Hepatic metabolism (glutathione/glucuronide conjugation)	Renal (10–70% unchanged drug) Biliary excretion (minor)	Yes (95%)	No	Myelosuppression Hepatotoxicity Nausea/vomiting Hypersensitivity Second cancer

[a]But asparagine markedly reduced

group stratification [9–13], and (v) exploration for acquired mutations allowing precision medicine approaches [14] (see Chaps. 7 and 9).

Contemporary treatment programs can roughly be divided into (i) a three to four drugs remission induction phase with VCR, Steroid, and Asp and/or anthracyclines [15], (ii) a consolidation phase with alternating of additional drugs combinations including HD-MTX, (iii) delayed intensification phases using drug classes similar to those used for remission induction followed by a short consolidation phase, (iv) CNS targeted treatment with or without cranial irradiation [6], and (v) maintenance therapy with oral daily 6MP and weekly MTX until 2.0–3.0 years from diagnosis, which in some protocols include VCR/Steroid pulses [16, 17] (see Chap. 8).

5.2 Pharmacokinetics and Pharmacodynamics

PK deals with drug (and metabolite) concentration-time courses in body fluids after administration of a specific dose, whereas PD covers the effects (efficacy and toxicity) resulting from a certain drug concentration (Fig. 5.2) [18]. Thus, PK is what the body does to the drug, and PD is what the drug does to the body. For all antileukemic agents there is a several fold interindividual variation in the so-called LADME parameters, i.e. Liberation (e.g. from a liposomal formulation), Absorption, Distribution, Metabolism and Excretion, where that latter four primarily reflect variations in liver and kidney function as well as patients' age, size and body composition. With few exceptions, PK parameters are not predictable, but need to be

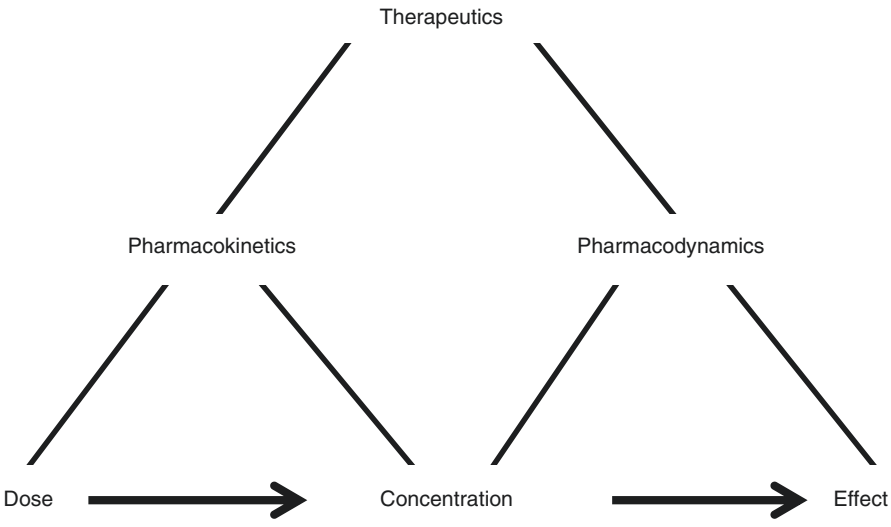

Fig. 5.2 Pharmacokinetics and pharmacodynamics

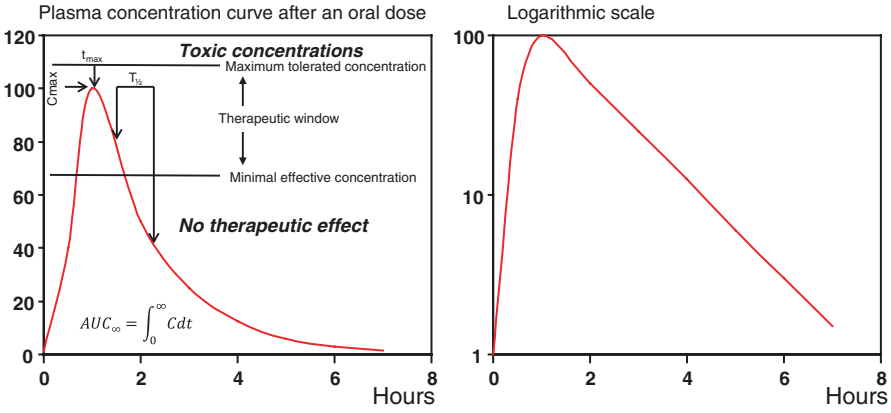

Fig. 5.3 Basic pharmacokinetics. With continuous intravenous administration (or repetitive oral dosing) the steady state concentrations is obtained after 3-5 times the elimination half life (t½). The steady state concentration is inversely correlated to drug clearance and volume of distribution (Vd). The Vd is defined as an apparent volume of distribution that would be needed to contain the total amount of an administered drug at the same concentration as that which is observed in the plasma. The clearance equals ke x Vd, where ke is the elimination rate constant, i.e. the rate at which the drug is eliminated from the body.

$$ke = \frac{\ln(2)}{t\frac{1}{2}} = \frac{\text{clearance}}{\text{Vd}};\ Cleareance = \frac{\text{dose (or absorbed dose fraction)}}{\text{AUC}}$$

AUC = area under the plasma concentration curve; C_{max} = peak plasma concentration after dose administration; t_{max} = time to reach C_{max}; T½ = elimination half life

measured and calculated directly. Such calculations can be performed using non-compartmental or compartmental methods in various PK models. For many drug dosing regimens (e.g. HD-MTX) the PK model can be approximated to a 2-compartment model. Although these are theoretical model compartments, the central compartment from where the maternal drug and its metabolites are eliminated generally represent the circulation, including the liver and kidneys. First order kinetics of elimination of antileukemic agents describe that the volume of blood cleared per time unit is constant (but not the absolute amount cleared). Accordingly, the plasma concentration will be linear on a logarithmic curve (Fig. 5.3). In zero order kinetics the amount eliminated per time unit is constant (e.g. ethanol), since the elimination capacity is saturated. If the rate of clearance of an infused drug, is independent of its concentration (approximately the case for HD-MTX), the steady state concentration (and the areas under the concentration curve, AUC) will change proportional to the dose given. Thus, a reduction of the infused dose by 50% will provide a 50% reduction in the steady-state concentration.

The therapeutic window for most anticancer agents is very narrow, and the *standard* dose of an antileukemic agent is determined by the maximum tolerated dose, i.e. the dose that in phase 1 studies caused unacceptable toxicities in an acceptable

very low percentage of patients [19]. However, due to wide interindividual variations in PK, this *standard* dose will provide insufficient systemic exposure to many patients and a potentially increased risk of relapse (Fig. 5.3).

Except for folinic acid rescue after HD-MTX [20], Asp dosing based on enzyme activity measurements [21, 22], and toxicity-targeted 6MP/MTX-based maintenance therapy [17], individualised drug dosing according to drug concentrations or to the limit of toxicity is not used outside research trials [23–25]. Thus, in spite of huge differences in tissue distribution and drug metabolism virtually all *traditional* antileukemic agents are rigorously dosed by body surface area (BSA). The few exceptions include i.t. chemotherapy (dose based on age) and infants (dose based on body weight). The original Du Bois formula for calculating BSA has nowadays been replaced by the far simpler Mosteller formula [26]: $BSA = \sqrt{\text{weight}(kg) \times \text{height}(cm) / 3600}$. BSA-based drug dosing does not provide equal or even predictable PK for individual patients, but its general use at least allows comparison of treatment intensity across ALL protocols [27, 28].

5.3 Pharmacogenetics

Pharmacogenetics covers genetic variations affecting PK and/or PD, i.e. treatment response phenotypes (review see Davidsen et al. [29] and Dulucq et al. [30]). Recent technological opportunities for low-cost, genome-wide analysis of millions of common host genome variants (primarily single nucleotide polymorphisms (SNPs) and indels) and easy-to-use bioinformatics online tools for data handling have created an expectation that mapping of host genome variants, will allow more precise dosing of anticancer agents [31, 32]. So far pharmacogenomics data has mainly focused on the widely used Steroid, MTX, and thiopurines, or on metabolic pathways and transport mechanisms that are common to several drugs, such as the glutathione S-transferases (GST) and cytochrome P450 enzymes (CYP) [29, 30, 33–35]. Although pharmacogenomic drug dosing may reduce toxicity [36, 37], no prospective studies have so far demonstrated that host genome based dosing of chemotherapy provides better cure rates in childhood ALL than drug dosing by BSA or by toxicity [37], and attempts to replicate genotype-phenotype associations in childhood ALL have often failed [29, 30]. However, it is noteworthy that variants associated with treatment response are frequently associated with PK and PD of the antileukemic drugs [38, 39].

ALL treatment strategies often include more than 10 different antileukemic agents, hundreds of genes, and thousands of common genome variants that can influence PK and PD, which questions the likelihood that single SNPs will have clinically significant impact on response phenotypes [29]. Complex bioinformatics analysis integrating the clinical impact of multiple variants in a pathway is doable, but has so far not been clinically implemented in health care [39, 40].

5.4 Glucocorticosteroid

The Steroids prednisone, prednisolone, and dexamethasone are among the most effective antileukemic agents, and in vitro Steroid sensitivity of leukemic cells are significant predictors of early treatment response and risk of relapse [41, 42]. Accordingly, many continental European groups use the reduction of blast count in peripheral blood after a seven days prednisolone prephase for risk group stratification, since in vivo prednisone poor response correlates with the likelihood of later leukemic recurrence [43].

Steroids are used during induction (prednis(ol)one or dexamethasone) and delayed intensification (generally dexamethasone), and as 5–6 days pulses in combination with VCR during maintenance therapy, although the efficacy of the latter is uncertain [25, 44, 45]. There is plenty of room for adjusting Steroid treatment intensity through choice of drug and dose-intensity. Continuous and discontinuous (1 week on, 1 week off, 1 week on) Steroid during induction phases, reduces the risk of osteonecrosis, but do not seem to interfere with the antileukemic effects [46, 47]. The in vivo antileukemic superiority of dexamethasone compared to prednisolone depends on the treatment intensity, with prednisolone (or prednisone) being less efficacious than dexamethasone, if administered less than six- to sevenfold higher doses, e.g. 60 mg prednisolone per m.sq. vs 10 mg dexamethasone per m.sq. [48, 49].

In vitro the antileukemic potency of dexamethasone is much higher than that of prednisolone [50], but due to the risk of toxicities associated with dexamethasone [46, 51], some collaborative ALL study groups restrict the use of a 10 mg/m^2 dose of dexamethasone during induction therapy to T-ALL, since it provides better event-free survival (EFS) rates as well as overall survival, whereas the latter may not the case for other ALL subsets [52]. Other groups such as the United Kingdom ALL group have used a lower dexamethasone dose (6 mg/m^2) during induction therapy for all non-infant ALL patients with acceptable toxicity rates and excellent cure rates [53, 54].

The lipophilic Steroids passively diffuse intracellularly, where they bind to the Steroid receptor (GR or NR3C1), which becomes activated by dissociation from the protein complex it is otherwise bound to [29, 55]. The Steroid-receptor complex is then translocated to the nucleus, where it binds to glucocorticosteroid responsive elements (GRE), which then up- or downregulates specific gene transcriptions leading to apoptosis of Steroid-sensitive leukemic blasts. Prednisolone and dexamethasone seem to regulate the same genes [56]. The Steroid-GR–mediated response can be modified by interactions between the inactive GR and several other proteins such as heat shock proteins, polymorphic hormone receptors and cytokines, including tumor necrosis factor (TNF) and interleukins (ILs). The binding of Steroid to the GR receptors up-regulates the expression of CYP3A and IL-10, but decreases expression of TNF[30]. Conversely TNFs reduces and IL10 increases the number of GRs and thus modulate Steroid sensitivity. Currently, there is little knowledge on how to score the combined interactions of these cytokines in the individual patient to further optimise glucocorticosteroid therapy [57].

Oral Steroids have almost complete bioavailability [58, 59]. After absorption, prednisone is rapidly converted to prednisolone in the liver, and these two drugs have similar PK [60]. The highly polymorphic genes encoding GSTs and CYP3A are involved in Steroid elimination, and Steroid are in themselves inducers of CYP3A enzymes, which subsequently may enhance the metabolism of others drugs, including VCR, epipodophyllotoxins, and cyclophosphamide [29]. Dexamethasone has better CNS penetration than prednisolone, a longer half-life in cerebrospinal fluid (CSF), but is also associated with a higher risk of neurotoxicity [51, 61]. Adding a physiologic dose of hydrocortisone to dexamethasone treatment may compensate for dexamethasone induced deficiency of cerebral mineralocorticosteroid signalling, and thus reduce the occurrence of serious neuropsychological adverse effects and sleep-related difficulties [62].

Multiple host genome variants influence Steroid ligands, receptors, and downstream effectors, and candidate gene studies have, although inconsistently, been associated with risk of hyperbilirubinemia, gastrointestinal toxicity, osteonecrosis, and risk of CNS relapse in high-risk ALL [29, 63]. However, due to the complexity of the Steroid responses, the power of the pharmacogenetics studies, and the diversity of results, no SNPs involved in Steroid PK, pharmacodynamics (PD) or downstream pathways have so far found a clinical role in the care of childhood ALL patients [57]. Patients with poor prednisone response, who are heterozygous for TNF -308G>A have been shown to have a significantly increased risk of relapse compared with the wild-type patients [64]. In addition, deletions of *GSTM1* and *GSTT1* has been associated with increased risk of prednisone poor response [34, 65]. Finally, a SNP in the *IL10* promoter region (IL-10 -1082A>G), leads to elevated plasma levels of IL-10 in patients homozygous for the G allele, and they are less likely to be prednisone poor responders, although this may not lead to an overall reduction in risk of relapse [29, 64].

5.5 Vincristine

The Vinca alkaloid VCR binds to β-tubulin and disrupts the mitotic spindle necessary for chromosome separation, which ultimately leads to apoptosis [66]. VCR is used in induction, consolidation, delayed intensification phases, and as reinduction pulses together with Steroid during maintenance therapy [45]. Doses vary from 1.5 to 2.0 mg/m^2, and are generally capped at a maximum dose of 2.0 mg to limit the risk of serious neurotoxicity, although some groups have capped the dose at 2.5 mg[1]. It is most commonly given as an intravenous (iv) bolus injection or as a brief diluted infusion to prevent the risk of accidental and fatal i.t. administration [67]. Extravasation of VCR results in local tissue damage, although less severe than with anthracyclines.

VCR is metabolised in the liver by CYP3A4 and CYP3A5 [68]. Fifty percent of excreted products are metabolites, the biliary system being the primary route of elimination [69]. Only 10% are excreted in the urine. *CYP3A4* can be induced by

multiple agents, including Steroid, VCR itself, phenytoin, and carbamazepine, thus reducing VCR exposure and potentially increasing the risk of relapse [70, 71]. On the other hand, inhibitors of CYP3A activity, such as the antifungal azoles, as well as significantly reduced liver or biliary function can decrease clearance of VCR, and thus increase the side effects of vincristine, but the effects in the individual patient is unpredictable and useful dosing guidelines are lacking. If clinically feasible, the administration of concomitant strong *CYP3A* inhibitors, such as azoles, should be interrupted, not least when VCR is administered weekly.

The inter- and even intraindividual variations in PK are large and unpredictable [72], which may explain their lack of correlation with in vivo antileukemic effect in some [73], although not all, studies [74]. Prolonged infusion seems to result in less neurotoxicity, but unchanged antileukemic efficacy. Recent use of slow release liposomal VCR supports that the risk of neurotoxicity, but not efficacy, is associated with high peak concentrations [75].

Peripheral neuropathy caused by interference with axonal microtubules is the primary and dose-limiting side effect giving VCR a narrow therapeutic index [76, 77]. The symptoms are generally symmetric and include sensory-motor polyneuropathy such as neuropathic pain, loss of tendon reflexes, motor dysfunction, foot/wrist drop, and paralysis [78]. The very rare occurrence of paresis of the vocal cords starts with hoarseness, but may result in severe airway obstruction. Autonomic neurotoxicity may cause constipation, abdominal pain and ileus, and prophylactic administration of laxatives and/or gut motility promotors should be used. Although VCR passes very poorly across the blood-brain-barrier, it can in rare cases affect the hypothalamic/pituitary axis directly and cause *syndrome of inappropriate secretion of antidiuretic hormone* (SIADH) with profound hyponatremia and convulsions. In a few patients neuropathy is very severe and indicates exploration for Charcot-Marie-Tooth syndrome [79], but common germline SNPs may also markedly increase the risk of dose-limiting neuropathy [77, 80].

5.6 Anthracyclines

The anthracycline antibiotics doxorubicin and daunorubicin are among the most effective antileukemic drugs, but may cause serious toxicities, not least cardiotoxicity [81]. They are generally used for remission induction and/or during intensification phases, including in high risk blocks. Due to risk of cardiotoxicity and severe myelo-/immunosuppression, the use of anthracyclines has been reduced or even abrogated in very low-risk patients as defined by younger age and low white blood cell count (WBC) at diagnosis, good prognosis karyotypes, and low MRD at the end of induction therapy. In contrast, it remains to be determined which subsets of higher risk ALL patients that similarly can be cured without anthracyclines [82, 83].

Anthracyclines mediate their cytotoxicity through free radical formation, inhibition of topoisomerase II, disturbance of helicase function, DNA intercalation, modification of signal transduction, and ultimately induction of apoptosis. The primary

effector mechanism is still not clarified, but most likely reflects its induction of DNA breakage, whereas free-radical formation is probably of less importance.

Doxorubicin and daunorubicin are usually administered at iv doses of 30 mg/m^2 per week or as 60 mg/m^2 every 3 weeks as prolonged infusion (1 or more hours) with caution to avoid extravasation of anthracyclines, since this results in severe local tissue damage.

The PK of anthracyclines is very variable [84, 85], but doxorubicin and daunorubicin display very similar PK. Liposomal and conventional daunorubicin have comparable plasma PK, but the liposomal formulation provide lower levels of the metabolite daunorubicinol and seems associated with a lower risk of later cardiotoxicity [86, 87]. In the blood 75% of doxorubicin and daunorubicin are bound to plasma proteins. Due to rapid binding to tissue DNA, the plasma concentration drops rapidly, but the terminal half-life is long. Neither the plasma concentrations of daunorubicin nor of daunorubicinol seem associated with outcome in ALL, whereas a higher intracellular AUCs rather than peak levels are associated with efficacy [88].

Doxorubicin and daunorubicin are inactivated by GSTs and by the conjugating enzyme NAD(P)H quinone oxidoreductase (NQO1), which make them more water-soluble and suitable for excretion, and deletions of *GSTM1* and *GSTT1* have been associated with reduced risk of relapse [29, 34]. The 13-hydroxylated metabolites doxorubicinol and daunorubicinol have only 5–10% of the cytotoxic activity of doxorubicin and daunorubicin, but may be more cardiotoxic [89]. Fifty percent of a dose is eliminated by hepatic aldo/ketoreductases and excreted by the biliary system, and only 10% by renal excretion. Systemic clearance of the anthracyclines is reduced in patients with decreased liver function and hyperbilirubinaemia. Accordingly, dose reduction may be indicated in patients with severe hepatic or biliary impairment or with exposure to drugs that diminish hepatic reduced glutathione pools (e.g. acetaminophen), but clear guidelines are not available.

The most studied *NQO1* polymorphism is the *NQO1* 609C>T, which reduces the enzymatic activity to only 2% of the wild-type protein [29]. Although this should increase anthracycline exposure and efficacy, a reduced leukemic relapse rate has not been reported [90].

Common adverse reactions include nausea, vomiting, mucositis, myelo- and immunosuppression with risk of serious infections. The risk of cardiomyopathy is associated with female gender, young age, higher cumulative doses and shorter infusion time. It has been suspected that longer infusion time (≥4 h) would reduce cardiotoxicity without compromising the antileukemic effect, but only the latter seems true [91]. Recent studies have identified host genome variants that may be associated with anthracycline-induced cardiotoxicity, but this awaits further validation [92].

5.7 Asparaginase

Asp has been part of childhood ALL treatment protocols for decades, but its optimal administration has only been clarified within the last 10–15 years.

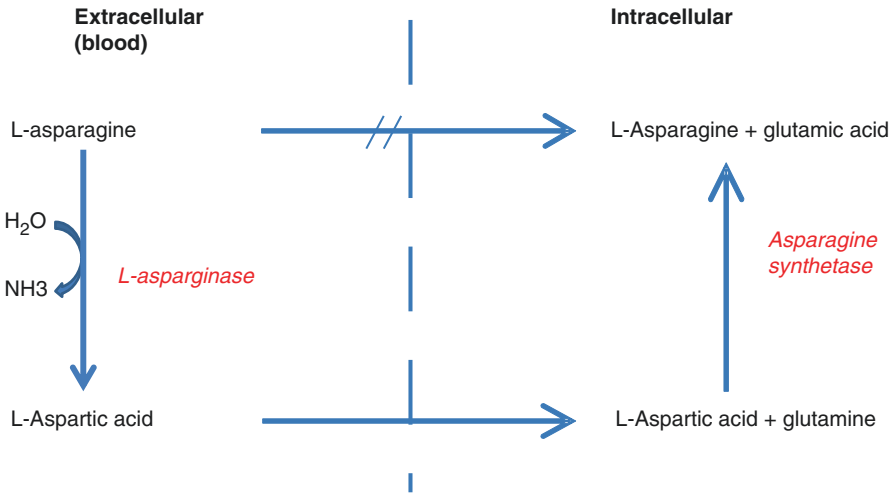

Fig. 5.4 L-asparaginase hydrolyses serum asparagine, but also has a low glutaminase activity. Most normal cells can synthetize L-asparagine from aspartic acid and glutamine and are therefore less susceptible to asparaginase than leukemic blasts. Leukemic blasts are restricted in their ability to up-regulate asparagine synthetase and have a higher need of asparagine due to enhanced proliferation rate

Asp can be extracted from two bacteriae, *Erwinia chrysanthemi* and *Escherichia coli*. To prolong half-life and decrease immunogenicity, *E-coli Asp* has been modified by covalent binding of polyethylene glycol (Peg-Asp). Peg-Asp has become the drug of choice in most first-line ALL treatment protocols. The various Asp preparations and recombinant analogs do not differ in their mode of action, but only in their biologic half-lives (shortest for the Erwinia preparation and longest for PEG-Asp due to reduced uptake in the reticuloendothelial system) [93, 94] and in their immunogenicity (lowest for PEG-Asp).

Asp reduces the extracellular pool of the non-essential amino acid asparagine by hydrolysing it into L-aspartic acid and ammonia, and to a much lesser extent Asp also catalyses glutamine (Fig. 5.4). The latter is important for de novo synthesis of purines and pyrimidines, but does not seem to be critical for the antileukemic effect. Asp does not enter cells or the CNS, but through depletion of extracellular asparagine Asp deprives these tissues of asparagine. E-coli Asp may give a more complete asparagine depletion in the CNS compared to Peg-Asp, suggesting that small amounts of E-coli Asp might enter the CNS [95, 96]. Asparagine depletion results in decreased protein and nucleic acid synthesis leading to inhibition of leukemic cell proliferation and induction of apoptosis. The specific L-Asp sensitivity of lymphoblasts reflects their restricted ability to up-regulate asparagine synthetase (ASNS) activity, and their higher need of asparagine due to their enhanced proliferation. Accordingly, high expression of ASNS in some ALL subsets or in normal bone marrow stroma may lead to resistance to Asp, and

down-regulation of ASNS can revert this resistance in human leukemia and lymphoma cell lines [97, 98].

Asp is metabolised by the reticuloendothelial system, independent of the hepatic CYP450 enzymes and renal function. Asp can be administered intramuscularly or iv. The intramuscular route results in lower peak levels and may be less immunogenic [97]. The differences in half-lives of the various Asp preparations determine dosing schedules. Due to its short half-life, Erwinia Asp is given three times a week or every other day in a dose of 20,000–25,000 IU/m^2 The dosing schedule of native E-coli Asp is 5000–10,000 IU/m^2 every 3–4 days. Peg-Asp is generally given every other week, at doses that vary from 1000 to 3500 IU/m^2. Real-time measurement of Asp activity level is currently used by several groups and allows dose adjustments to keep Asp activity levels above 100 IU/L to obtain complete and sustained depletion of serum asparagine [21, 22].

The toxicity of Asp can be divided in two major groups; the hypersensitivity reactions and toxicities caused by asparagine depletion. The most serious toxic reactions include hypersensitivity ranging from mild reactions to anaphylactic shock, hyperglycemia, pancreatitis liver toxicity such as hyperbilirubinemia, hypoalbumenia, coagulopathy, hyperammonemia and hypertriglyceridemia [99–103]. Since Asp is a foreign protein, it can cause antibody formation [97, 104]. These antibodies neutralise Asp with or without clinical signs of hypersensitivity. The latter is called silent inactivation, and can only be detected by the measurement of plasma Asp activity levels. In case of clinical allergy, Asp levels will generally be zero irrespective of the severity of the allergy, and these patients may, in addition of their lack of asparagine depletion have enhanced clearance of Steroid [105]. Premedication with glucocorticosteroid and antihistamines and increased infusion time can reduce allergic symptoms, but does not prevent Asp inactivation. Thus, symptoms of hypersensitivity indicate switch from E-coli derived preparations to Erwinia Asp (and vice versa) [106]. HLA-DRB1*07:01 and genetic variations in *GRIA1* on chromosome 5q33 are associated with a higher incidence of hypersensitivity and anti-Asp antibodies [107, 108].

Asp-induced hypoalbuminaemia can decrease the clearance of dexamethasone and other drugs [105], and Asp decreases MTX polyglutamation in a preclinical model, although the clinical significance of this is uncertain [109].

5.8 Thiopurines

The thiopurines 6MP and 6TG are essential drugs in the treatment of childhood ALL. They are included in consolidation therapy (6MP or 6TG in combination with low dose AraC), in combination with HD-MTX (6MP), and during maintenance therapy (6MP in combination with MTX). Although most groups only prescribe 25 mg/m^2 of 6MP, when given in combination with HD-MTX, most patient will tolerate 50 mg/m^2 or some even 75 mg/m^2 [110]. For 6MP/MTX maintenance therapy the starting dose of 6MP is 50–75 mg/m^2 dose of 6MP, which is then adjusted

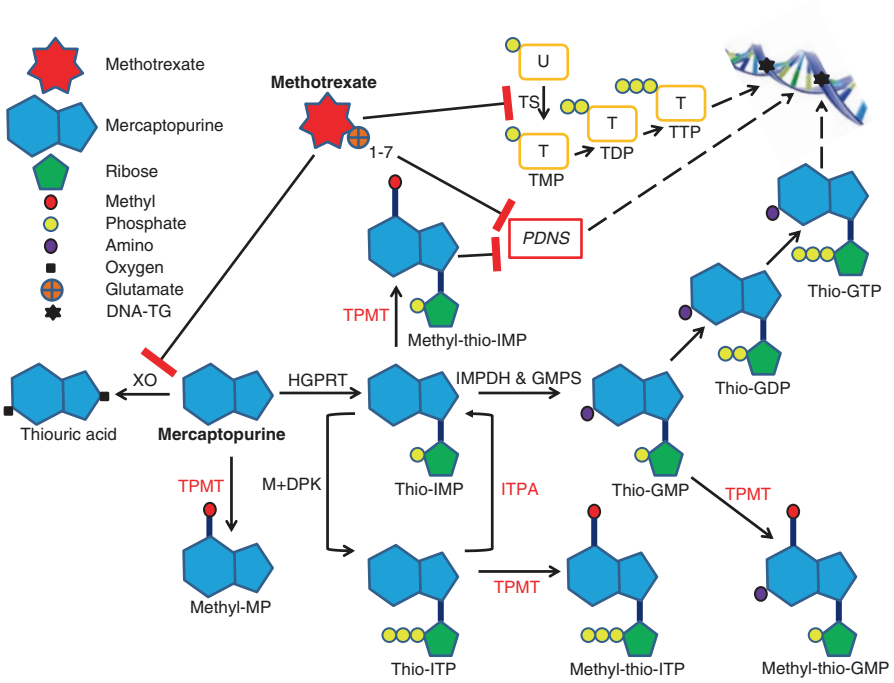

Fig. 5.5 Simplified outline of 6-mercaptopurine (6MP) metabolism, methotrexate (MTX) metabolism and their interactions. DNA-TG = Thioguanine nucleotides incorporated into DNA; GDP = Guanosine diphosphate; GMP = Guanosine monophosphate; GMPS = Guanosine monophosphate synthetase; GTP = Guanosine triphosphate: HGPRT = Hypoxanthine guanine phosphoribosyl transferase; IMP = Inosine monophosphate; IMPDH = Inosine monophosphate dehydrogenase; ITP = Inosine triphosphate; ITPA = Inosine triphosphate pyrophosphatases; M+DPK = mono- and di-phosphate kinases; MP = Mercaptopurine; PDNS = Purine de novo synthesis; TMP = Thymidine monophosphate; TDP = Thymidine diphosphate; TTP = Thymidine triphosphate; TPMT = Thiopurine methyltransferase; U = Uridine monophosphate; XO = Xanthine oxidase

to a set target WBC (usually 1.5–3.0 × 10^9/L) or ANC [16] as this is associated with reduced risk of relapse [17].

Both 6MP and 6TG are prodrugs that exert their cytotoxicity through hypoxanthine guanine phosphoribosyl transferase-mediated conversion into thioinosine monophosphate that are subsequently converted into mono-, di- and triphosphates of 6-thioguanosine (6TGN), which are incorporation into DNA (DNA-TGN) in competition with normal guanine (Fig. 5.5) [111]. During DNA replication and DNA repair, DNA-TGN will reliably match with cytosine. However, DNA-6TGN may become S-methylated which markedly enhance the likelihood of mismatching, specifically with thymidine. Although such mismatching can be recognised by the mismatch repair system, normal DNA sequence repair will be unsuccessful, since the methylated DNA-TGN will continue to mismatch, and the futile repetitive repair attempts will eventually either fail and induce point mutations or the multiple excisions and resynthesis attempts will lead to apoptosis [111].

6TG are more easily converted into 6TGN. 6TG also penetrates better into CSF than 6MP [112] and may be a superior drug for preventing leukemic relapse, but treatment with 6TG as the maintenance therapy thiopurine has in several studies been associated with a 10–20% risk of sinusoidal obstruction syndrome, which in a few patients has led to liver failure and need of liver transplantation [113, 114]. In some patients development of sinusoidal obstruction syndrome during 6TG-based maintenance therapy has been associated with reduced thiopurine methyl transferase (TPMT) activity [115].

Plasma levels of 6MP in children with ALL exert extensive inter- as well as intra-individual variability [116](for review see Schmiegelow et al. [16]). Due to a high first pass effect of xanthine oxidase in gut and liver, the median bioavailability of 6MP is less than 20% [117]. Although variants in the xanthine oxidase and hypoxanthine guanine phosphoribosyl transferase are known, neither have been shown to influence the risk of relapse in childhood ALL.

The conversion of thiopurines to active 6TGN competes with S-methylation of 6MP and several its metabolites mediated by TPMT, and dose increments of 6MP primarily leads to higher concentrations of methylated 6MP metabolites [118]. Methylmercaptopurine cannot be converted into active nucleotides. Methylthioinosine monophosphate is a strong inhibitor of purine *de novo* synthesis [119], and high cytosol 6TGN, methylated 6MP metabolites and MTX-polyglutamates, enhance DNA incorporation of 6TGN [120–122]. TPMT status can be determined by genotyping or phenotyping of erythrocyte TPMT activity. However, TPMT activity will be low at diagnosis, since the red blood cell pool is old, increased during maintenance therapy due to a reduced erythrocyte life span, and confounded in patients who have received allogeneic erythrocyte transfusion [123].

Erythrocyte levels of 6TGN (E-TGN) and methylated metabolites (E-MeMP) have been used to monitor the treatment intensity of 6MP, and although E-TGN initially seemed promising in this respect [124], this parameter tended to lose its significance as intensified 6MP/MTX maintenance therapy gained attention [16, 24]. Still, low levels of both E-6TGN and E-MeMP (or high WBC and lack of elevated alanine aminotransferase levels) in spite of 6MP dose increments can be an indicator of poor treatment adherence [16, 125].

Patients with inherited low TPMT activity will have higher erythrocyte 6TGN levels and be at increased risk of hematopoietic toxicity, and thus tolerate lower doses of 6MP during maintenance therapy [36, 126–128].

Numerous SNPs have been described in the TPMT gene, of which TPMT*2 238G>C, TPMT*3B 460G>A and TPMT*3C 719A>G are the most common variants [129], leading to reduced enzyme activity and tolerance to 6MP and to a lesser extent 6TG [130]. Five to 10% of white are heterozygous for low activity TPMT alleles, and 1 in 300 individuals is TPMT deficient with two low activity alleles. Patients with low activity *TPMT* alleles have more rapid reduction in their MRD [131] and a reduced risk of relapse when treated with 75 mg/m^2 of 6MP [127, 132]. However, the relapse rate for TPMT low activity and wild type patients may be similar, if the maintenance therapy starting dose of 6MP is reduced for the patients with TPMT low activity alleles [37].

The natural substrate for TPMT is unknown, and TPMT deficient patients are otherwise phenotypically normal. So far, TPMT genotype is the only example of routine implementation of pharmacogenetics drug dosing in ALL treatment [36, 37, 130], although most collaborative ALL groups will only test for TPMT variants in patients that demonstrate excessive myelotoxicity [123].

During 6MP-based maintenance therapy a median of ~1:8000 DNA nucleotides are replaced by 6TGN in nucleated cells [121]. When children on maintenance therapy are dose adjusted by WBC, *TPMT* wild type and heterozygous patients will differ in their E-6TGN and E-MeMP, but obtain very similar DNA-TGN levels [121]. Low DNA-TGN has recently been associated with an increased risk of relapse [133, but prospective clinical trials are needed to determine, if DNA-TGN can supplement or even replace WBC/ANC as guide for 6MP dose adjustments.

In Asian and South American populations low activity TPMT variants are rare, whereas low activity *NUDT15* variants are common, with allele frequencies up to 20% [134, 135]. NUDT15 mediates dephosphorylation of thioguanine nucleotides, and patients with reduced activity have a phenotype similar to that of TPMT low activity patients, with reduced tolerance to 6MP [135].

Bone marrow suppression is the primary dose-limiting side effect of thiopurines and primarily reflects intracellular TGN levels, whereas methylated metabolites are correlated with hepatotoxicity with a rise in alanine aminotransferase [136, 137]. Patients with low activity alleles of *TPMT* or *NUDT15* experience more myelotoxicity at standard 6MP doses. Thus, the cumulative incidence of 6MP dose reductions during maintenance therapy is highest for TPMT and/or NUDT15 deficient patients (100% of patients), lower for heterozygous, and lowest in wild-type TPMT and NUDT15 patients [135]. Accordingly, some groups reduce the starting doses of 6MP for patients with low activity *TPMT* alleles to reduced toxicity, but similar guidelines for NUDT15 are lacking [123].

Evening dosing of 6MP and MTX was in the 1980'ies associated with a reduced risk of relapse [138, 139], and an evening schedule may provide more favorable PK [140, 141] but with contemporary more effective antileukemic therapy that is no longer the case [142].

Treatment-related second malignant neoplasm (SMN) is a rare toxicity of thiopurine therapy that is associated with longer maintenance therapy and higher 6MP doses and associated with higher 6MP doses and longer maintenance therapy [143, 144]. The risk of treatment-related myeloid neoplasia (t-MN) has been associated with low *TPMT* activity in some [145], but not all studies [132, 146].

5.9 Methotrexate

The folate analogue MTX (4-amino-10-methyl-pteroylglutamic acid) plays a key role in antileukemic therapy. Its complex pharmacology mirrors that of natural folates with a marked interpatient variability in pharmacokinetics, efficacy, and toxicity [20]. In childhood ALL, MTX is administered widely during ALL therapy as (i)

intrathecal (i.t.) therapy in age-adjusted doses, (ii) intravenous escalating doses without folinic acid rescue, (iii) higher intravenous doses (1.0–5.0 g/m^2) necessitating high dose folinic acid rescue, and finally as (iv) oral or parenteral low doses of 20–40 mg/m^2 at 1–2 week intervals as part of the backbone of maintenance therapy.

MTX enters the cell by active transport via the reduced folate carrier 1 (RFC1), coded on chromosome 21, although other influx and efflux mechanisms mediated by ABC transporters (specifically the ABCC1-4) and breast cancer resistance protein also play a role for cellular MTX concentrations [147]. At high MTX doses passive diffusion across the cell membrane also plays a significant role. MTX interferes with the natural folate-homocysteine cycle and inhibits multiple folate-dependent enzymes and pathways, including dihydrofolate reductase (DHFR), thymidylate synthase (TYMS), 5,10-methylene-tetrahydrofolate reductase (MTHFR), and purine de novo synthesis, which leads to lack of reduced folate, inhibition of DNA synthesis, apoptosis, increased adenosine levels and potentially life-threatening toxicities [29, 148]. When at least 95% of DHFR the synthesis of tetrahydrofolate is compromised [149], but folinic acid (reduced folate) can then counteract the effect. Intracellularly, the enzyme folylpolyglutamate synthetase (FPGS) polyglutamates MTX (as well as normal folate) to polyglutamates forms with 2–6 polyglutamate residues (MTXpg), which increase intracellular retention as well as affinity (and thus efficacy) for its target enzymes proportional to glutamyl chain length [150–154]. In contrast, MTXpg hydrolysis by gamma-glutamyl hydrolase will reduce the pool of MTXpg due to efflux of the maternal drug and the short-chained polyglutamates [154].

The ability for MTX polyglutamation is reduced in T-cell leukemia, probably since T-ALL blasts have lower expression of FPGS and higher breast cancer resistance protein and gammaglutamyl hydrolase (GGH) activity, which reverses polyglutamylation process and favorises MTX efflux [147]. Accordingly, most T-ALL require higher HD-MTX doses (5 g/m^2), whereas B-ALL, especially high-hyperdiploids, can do with lower doses [23, 155].

Measurement of plasma MTX concentrations during HD-MTX to adjust dosing of folinic acid rescue was the first example of routine therapeutic drug monitoring in pediatric oncology. Folinic acid rescue is usually postponed until hour 42 after the start of the MTX infusion in order to avoid rescue of leukemic blasts [156, 157], although this is not well documented. Depletion for more than 42–48 h will cause irreversible cytotoxicity to normal tissue [158].

HD-MTX is widely used in childhood ALL therapy, and therapeutic drug monitoring of HD-MTX has been shown to reduce relapse rates of B-cell precursor ALL [23], although its role is yet not completely clarified. Although it enhances cellular uptake and is important for MTX-polyglutamation, a 300-fold increase in MTX dose only leads to a 2.0–2.5 fold difference in intracellular MTX content [20]. In addition, exposure time, including timing and intensity of folinic acid rescue, rather than peak concentrations, are of importance. Thus, 1 g/m^2/24 h with two doses of folinic acid rescue gives 100-fold lower peak concentrations than 12 g/m^2 over 4 h (7.2 μM versus 700 μM), but more toxicity, longer lasting serum MTX concentrations higher than 1.0 μM, and similar event-free and overall survival [159, 160].

When HD-MTX is given, MTX clearance is dependent on a normal renal and liver function. A rapid clearance has been linked to reduced cure rates [23, 161], although not all studies have confirmed this association [157] potentially reflected dosing strategies for both HD-MTX and folinic acid rescue [156]. The liver is responsible for degrading approximately one-third of a dose of 5 g/m^2 to 7-hydroxy-MTX (7-OH-MTX) by hepatic aldehyde oxidase. The plasma concentration of (7-OH-MTX) can exceed that of MTX, and even enhance MTX-induced toxicity including nephrotoxicity [20, 162]. A smaller proportion is metabolised in the liver to the inactive metabolite (4-[[2,4-diamino-6-(pteridinyl)methyl]-methylamino]-benzoic acid) (*DAMPA*), while the remainder is being excreted unmetabolised by the kidneys.

MTX and its metabolites are weak acids that can crystallise in the acidic renal environment and cause acute, although reversible, severe reduction in renal function [163–165]. The acute renal failure cannot be predicted prior to HD-MTX therapy, but can be recognised early by a rise in serum creatinine. Thus, a rise of >50% within 24 h from the baseline value has a sensitivity of 0.32 and a specificity of 0.99 to predict delayed MTX elimination, and 99% of courses with normal clearance have a rise in serum creatinine of less than 50% [166]. The severe acute renal toxicities with significantly delayed MTX clearance most frequently occur after the first or second HD-MTX courses, and rarely recur [167]. When a significant delay in MTX clearance occurs, hydration should be increased from the usual 3000 to 4500 ml/m^2/24 h accompanied by proportional intensification of alkalinisation to increase solubility of MTX in the urine. With very severely delay in MTX clearance and kidney dysfunction the enzyme carboxypeptidase can be administered, since it rapidly degrades MTX to DAMPA and glutamate, although it will not change the time to normalisation of the renal function [168, 169].

The efficacy and toxicity of low-dose MTX may be mediated by different mechanisms. Oral MTX is rapidly absorbed by an active, but saturable, transport mechanism with a bioavailability of 50–95%, a peak concentration of 0.3–2.2 mM within 1.5–2.5 h from intake, and an elimination half-life of 4–6 h, which mainly reflects renal excretion of unmetabolised MTX within 24 h [16, 117]. Thus, parenteral administration at these doses will only increase systemic exposure slightly and has not been shown to reduce the relapse rate [170]. Rheumatologist have routinely supplemented patients with folic acid (5 mg per week) to avoid gastrointestinal toxicity, hepatotoxicity and hyperhomocysteinaemia while preserving efficacy [171]. Although hardly studied in childhood ALL, children who receive folic acid supplementation have higher folate levels and significantly less myelosuppression [172].

Measurements of MTX polyglutamates in erythrocytes (Ery-MTX) has been explored for monitoring of maintenance therapy intensity, since Ery-MTX is related to the dose of MTX in the preceding weeks, the cellular MTX incorporation in the bone-marrow, and the degree of myelosuppression [16, 124]. E-MTX is also strongly associated with DNA-TGN levels during 6MP/MTX maintenance therapy [122, 133]. However, a recent large randomised study could not demonstrate the a benefit of MTX dose adjustments according to Ery-MTX [24].

MTX passes poorly into the CNS, with a concentration ratio in the order of 1%. Accordingly HD-MTX was initially introduced to improve penetration into CNS and testicular tissue and to overcome cellular MTX resistance. However, HD-MTX is costly, requires several days of hospital admissions until the p-MTX is below a set threshold (200 or 400 nM), carries a risk of severe bone-marrow suppression and thus treatment interruptions, and recent meta-analyses have questioned its role for prevention of CNS relapse [173].

Expression patterns in leukemic cells of the multiple genes involved in folate (and MTX) disposition have been strongly associated with leukemia subtypes, and some correlate with MTX response in vivo [174]. However, the gene expression patterns are poorly correlated with host genome polymorphisms of the same genes.

MTX disposition in the individual patient mirrors the many genes involved in the folate-homocysteine pathway, and numerous pharmacogenetic studies have been conducted to explore the impact of host genome variants on MTX PK and PD (Table) (see Davidsen et al. and Schmiegelow et al. for reviews [20, 29]). However, most of the studies are low powered and include only candidate genes, and the few well-powered GWAS studies have, with few exceptions, not been replicated in independent cohorts [32]. Interindividual variations in HD-MTX PK have a genetic component of more than 50%. *SNPs* in *SLC01B1* is one of the strongest and best validated determinators of renal clearance of HD-MTX (2.0–5.0 g/m^2), but still accounts for less than 10% of the interindividual differences in MTX clearance [175, 176]. These and other variants, e.g. thymidylate synthase tandem repeat polymorphism, have been associated with risk of MTX-related toxicities [176].

The RFC1 80G>A is one of the most extensively investigated polymorphism in the *RFC1* gene (also named *SLC19A1*), and several clinical implications of these alleles have been reported. The A allele has a frequency of ~50% and has been associated with better cellular uptake [177], higher end-of-infusion plasma levels of MTX during HD-MTX therapy methotrexate, as well as a reduced relapse rate compared to patients with one or two G alleles among ALL patients repetitively exposed to HD-MTX (5 g/m^2/24 h) [178], but only among patients disomic for chromosome 21 (where RFC1 is coded). The RFC1 80G allele has been associated with hepatotoxicity, including hyperbiliruinemia, and vomiting.

Methyltetrahydrofolate reductase (MTHFR) is also an important enzyme in the folate-homocysteine cycle, and two SNPs in the gene encoding MTHFR have been extensively studied: MTHFR 677C>T and MTHFR 1298A>C, both of which reduce the enzyme activity [179]. In a study of 520 patients with childhood ALL, the T allele of MTHFR 677C>T was shown to be associated with an increased risk of relapse [180], but only some studies have been able to confirm this [29]. In contrast, the data linking *MTHFR* polymorphisms to hepatotoxicity, myelosuppression, oral mucositis, gastrointestinal and skin toxicity are more solid [179].

Trimetroprim-sulfamethoxazole (TMP/SMX) is generally used as Pneumocystis jiroveci prophylaxis during ALL therapy [181, 182] and there has been a worry that

it could interfere with MTX pharmacokinetics and/or efficacy. However, it seem to interfere with neither low dose [183] or HD-MTX PK [184], and although is does reduce tolerance to oral MTX-based maintenance therapy, this does not influence relapse rate [185].

When oral 6MP is given concurrently with HD-MTX, it seems to be the primary mediator of bone-marrow suppression [186, 187]. This interaction is biochemically and clinically well supported, since MTX increases the bioavailability of 6MP [189, 190], inhibits de novo purine synthesis with increased intracellular levels of phosphoribosyl pyrophosphate, and thus increased formation of 6-thioguanine nucleotides (the primary mediator of 6MP cytotoxicity)(Fig. 5.5) [119]. When HD-MTX (5.0 g/m^2/24 h with folinic acid rescue) is given together with oral 6MP (75 mg/m^2), approximately 40% of the patients will experience treatment interruption of a median of 10 days due to severe myelotoxicity [186]. This myelosuppression can be avoided by reductions of the dose of 6MP 1–2 weeks before and after HD-MTX [190].

5.10 Cytosine Arabinoside

AraC is used either as a low dose 4-days schedule together with a thiopurine, or as HD-AraC for subsets of ALL patients with a significantly increased risk of relapse. The PK of AraC varies widely and the half-life is short with a median T½ of minutes.

Nucleoside transporters, primarily the human equilibrative nucleoside transporter 1 (hENT1 or SLC29A1), play a major role in uptake of AraC by leukemic cells, and a decrease in hENT1 expression is associated with AraC resistance [191]. Intracellularly, AraC undergoes phosphorylation, mediated by deoxycytidine kinase (dCK), to arabinoside-cytidine monophosphate (ara-CMP), which by other kinases is converted into the cytotoxic form ara-CTP, which then competes with natural deoxycytidine triphosphate (dCTP) for incorporation into DNA leading to inhibition of DNA polymerase, blocking of DNA synthesis and repair, and eventually apoptosis. Alternatively, AraC may undergo deamination to the nontoxic uridine arabinoside (ara-U) by cytidine deaminase (CDA), and high levels of CDA correlate with in vitro and in vivo AraC resistance.

Several SNPs in the hENT1 gene and the promoter region of dCK, and some of these have been associated with increased promoter activity and a better outcome for myeloid leukemia patients [29]. However, for both hENT1 and dCK clinical pharmacogenetic studies in childhood ALL are lacking. High levels of 5NT enzyme activity have been associated with a higher relapse rate in childhood ALL [192]. Several SNPs in the CDA gene may also affect expression levels, activity, and risk of toxicity.

The primary dose-limiting toxic effects of AraC are myelosuppression, mucositis, and in addition a risk of encephalopathy when HD-AraC is given.

5.11 Cyclophosphamide and Ifosfamide

The nitrogen mustards, especially cyclophosphamide, are the most commonly used alkylating agents in ALL treatment [193], and applied during consolidation (cyclophosphamide) and as part of intensive blocks for high risk patients by some collaborative groups (both cyclophosphamide and ifosfamide). Although oral dosing is feasible, cyclophosphamide is generally given iv at doses of 500–1000 mg/m^2.

The active metabolites of cyclophosphamide attach an alkyl group to the guanine base of DNA, which interferes with DNA replication by forming irreversible intra- and inter-strand DNA crosslinks, thus inhibiting DNA replication, which eventually leads to apoptosis.

There are large interindividual variations in the PK and metabolism of cyclophosphamide, ifosfamide and their cytotoxic metabolites. Cyclophosphamide is a prodrug that becomes active after metabolic transformation by 4-hydroxylation activation to 4-hydroxycyclophosphamide, which exists in equilibrium with its tautomer aldophosphamide that is spontaneously hydrolysed to phosphoramide mustard and acrolein. The former is an active alkylating agent, while the latter causes hemorrhagis cystis. The conversion of cyclophosphamide to its active metabolites is mediated by several CYP enzymes with CYP2B6 playing the major role, since it has higher affinity for cyclophosphamide and higher metabolic capacity than the other CYP activators CYP2A6, CYP2C9, CYP2C19, CYP3A4 and CYP3A5 [29]. The metabolites are highly protein bound and distributed to all tissues. Detoxification of 4-hydroxycyclophosphamide is mainly by GSTA1 and GSTP1, whereas aldophosphamide, in addition to spontaneous elimination, can be oxidised to inactive carboxyphosphamide by aldehyde dehydrogenase variants ALDH1 and ALDH3. ALDH1 is the most efficient gene variant, and overexpression of ALDH1 has been shown to induce cyclophosphamide resistance in vitro.

No childhood ALL studies have explored in detail the clinical impact of host genome variants in the CYP and ALDH genes on the effect of cyclophosphamide therapy. However, several SNPs have been associated with increased transcriptional activity in vitro and PK of cyclophosphamide in other cancers, including CYP2B6 -82T>C, CYP2B6 516G>T, CYP2B6 785A>G, and CYP2C19*2 681G>A. It remains unclear to what extent GST polymorphisms can be correlated to effects of cyclophosphamide treatment and prognosis in childhood ALL [33, 34].

Drugs inducing hepatic P450 enzyme activity may result in accelerated metabolism of cyclophosphamide to its active metabolites, increasing both efficacy and toxicity of the drug. In contrast, drugs that inhibit hepatic enzymes (table, e.g. corticosteroid and azoles) and severe hepatic impairment result in reduced effect of cyclophosphamide. Cyclophosphamide and its metabolites are primarily excreted in the urine, and the dose should be reduced in patients with impaired renal function.

Cyclophosphamide causes nausea and vomiting, bone-marrow suppression, hemorarrhagic cystitis, and the two latter toxicities are the primary dose-limiting factors [193]. During and for at least 8 h after the administration, adequate iv

amounts of fluid (3000 mL/m^2) and mesna should be administered to reduce the risk of urinary tract toxicity. The most serious long-term toxicity is an increased risk of developing secondary cancer [143].

5.12 Epipodophyllotoxins

Among the epipodophyllotoxins, etoposide (VP16) is the primarily used antileukemic agent, but is currently only used for high risk patients during consolidation blocks.

DNA topoisomerase I and II are essential for DNA replication, transcription, chromosomal segregation, and recombination, and epipodophyllotoxins stabilises cleavable topoisomerase II/DNA complexes, thus preventing re-ligation of DNA strands and causing DNA strand breaks and apoptosis.

The interindividual variability in PK of epipodophyllotoxins in children is significant, and this may play a role for efficacy and toxicity [194–197]. The median bioavailability of oral VP16 is only 50% and oral VP16 is not used in ALL therapy. In plasma, VP16 is more than 95% bound to proteins, and albumin infusion prior to VP16 should be considered in patients with profound hypoalbuminemia to avoid excessive bone-marrow suppression. Ten to seventy percent are excreted unmetabolised in the urine [198].

Penetration into the CSF is quite limited (0.5%), but it will be far less bound to proteins in CSF and may have antileukemic effects [199].

The main non-renal elimination route is hepatic metabolism, and VP16 is a substrate for CYP3A4 and CYP3A5. Mediated by GSTT1/GSTP1 and UGT1A1, respectively, glutathione and glucuronide conjugation can inactivate VP16 and several of its metabolites. VP16 and its metabolites are mainly excreted by the kidney, while biliary excretion plays a minor role. In case of kidney and liver dysfunction the dose of VP16 should be reduced proportionate to the creatinine clearance and hyperbilirubinaemia.

The efficacy of epipodophyllotoxins and other topoisomerase II targeting antineoplastic agents (e.g. anthracyclines) may vary according to polymorphisms in the topoisomerase II genes, but few studies have explored this, even though many candidate genes and SNPs have been identified [29](Table). Epipodophyllotoxins are substrates for both GSTs and CYP enzymes (primarily CYP3A4, but also CYP3A5) and low-activity G alleles of GSTP1 313A>G and of CYP3A5*3 has been association with a higher clearance of etoposide, whereas no significant effect of CYP3A4*1B polymorphism have been demonstrated. UDP-glucuronosyltransferase 1 (UGT1A1) glucuronidates VP16, making it more water-soluble and more suitable for excretion. A polymorphism with 7 (TA) repeats in the promoter region of UGT1A1 (UGT1A1*28) reduces expression of UGT1A1 compared with the wild type with six repeats (6TA), and is associated with lower VP16 clearance in children with ALL. Furthermore, UGT1A1*28 has been reported to be a strong predictor of

hyperbilirubinemia in children with ALL. Finally, VP16 is a substrate for the multiple drug resistance gene P-glycoprotein, and high activity CC genotype of MDR1 3435C>T has been associated with higher VP16 clearance. However, the impact of these polymorphisms on cure rates remains to be demonstrated, and none are currently integrated into clinical care of children with ALL.

Concomitant administration of CYP3A4/5 inducers (e.g. Steroid) can increase clearance of VP16 and potentially reduced efficacy. However, preemptive dose adjustments are not routinely recommended. Drugs that inhibit CYP3A4/CYPA5, such as azoles might also interfere with VP16 metabolism.

The most frequent adverse reactions are nausea and vomiting, mucositis with stomatitis and diarrhea, myelosuppression, hepatotoxicity and allergy-like reactions. The latter can be avoided by giving VP16 as a slow infusion of 30–60 min to prevent unspecific mast cell activation, hypotension and/or bronchospasm. Epipodophyllotoxin associated second myeloid malignancy is a rare toxicity in childhood ALL that frequently involves the *MLL* gene [143].

5.13 Intrathecal Chemotherapy and Central Nervous System Leukemia

When CNS-targeted therapy was not provided in the 1950s and 1960s, 80% of all patients relapsed in the CNS, and although the overall risk of CNS relapse is low with contemporary antileukemic therapy, 30–40% of all relapses still involve the CNS. At diagnosis of ALL leukemic blasts in CSF with leukocyte levels $\geq 5 \times 10^6$/l can be demonstrated by cytospin preparations (so called CNS3) in a few percent of all children with ALL at diagnosis, and these patients have an increased risk of relapse. But if CSF is explored by sensitive methods (e.g. flow cytometry) or morphologically explored before cells decay in CSF, at least 30% have CNS involvement, although at levels far below 5×10^6/L (so called CNS2) [200]. The clinical significance of such limited CNS involvement for risk of later relapse is yet to be determined. However, these findings all underscore the necessity of CNS-targeted therapy. Until recently this included cranial irradiation, but with the improvements of both systemic and i.t. chemotherapy, many groups currently have substituted irradiation with i.t. chemotherapy to reduce the risk of neurotoxicity (see Chap. 12) [6, 201].

Although many anticancer agents can be administered i.t., only three antileukemic drugs are used in front-line antileukemic therapy, i.e. MTX, Steroid, and AraC. Most collaborative ALL treatment groups have chosen i.t. MTX as the standard drug, whereas the combination of MTX, Steroid, and AraC (triple intrathecal therapy, TIT) has been reserved for higher risk patients and for patients with CNS3 at diagnosis. For the latter patients, additional doses of TIT are given during induction therapy until the CSF is free of leukemic blasts.

Since the brain and CSF volume grows rapidly during the first years of life, and the CSF approaches adult volume by the age of 3 years, the dosing of i.t.

chemotherapy is by age groups; i.e. <1.0 years, 1.0–1.9 years, 2.0–2.9 years, and ≥3.0 years.

After lumbar administration of an anticancer agent it must diffuse against the normal CSF flow, which goes from the lateral ventricles to the third, then the forth ventricle, and finally to the subarachnoid space. Thus, only 10% of an i.t. dose will reach the lateral ventricles [202]. Furthermore, ATP-binding cassette (ABC) transporters will actively pump MTX out of CSF. I.t. MTX causes more bone-marrow suppression than an oral MTX at a similar dose, which reflects longer systemic exposure above the cytotoxic threshold [203].

Liposomal AraC (Depocyte) has been used in many protocols for second line therapy of ALL, but there is a lack of studies with de novo childhood ALL patients [204]. A small controlled trial indicated that it may provide superior outcome compared with intrathecal TIT, but with higher risk of short term CNS toxicities, not least arachnoiditis [205].

5.14 Patient Adherence and Physician Compliance

Since childhood ALL is highly chemosensitive, interindividual differences in drug disposition as well as physician compliance to dose adjustment guidelines and/or patient adherence to orally prescribed chemotherapy may influence risk of relapse [125, 206]. During maintenance therapy blood counts and aminotransferase levels have been used to target treatment intensity and monitor patient adherence, but this strategy is challenged by wide inter-ethnicity, -age and -gender associated difference in normal blood counts. E-6TGN/MeMP/MTXpg and DNA-TGN can be applied to identify lack of patient adherence, but these are not generally available and guidelines for individual dose adjustments based on such pharmacological measurements are lacking [16].

5.15 Treatment of Infants

In the first year of life, significant changes in PK and PD occur as a consequence of normal development in body composition, organ maturation and their maturation of drug elimination pathways. Although infants differ as much as older children in drug disposition, antileukemic drug dosing in infants are generally adjusted by on age: three-fourths for patients 6–12 months old and two-thirds for patients <6 months, respectively, and furthermore based on body weight (equalising 1 m^2 with 30 kg). The clearance of MTX tends to increase in the first year of life which may affect risk of MTX-related toxicities [207–209], and VCR neurotoxicity seems to be enhanced although infants do not seem to differ in PK from older children [210]. PK studies of Steroid, Daunorubicin, and asparaginase have not indicated dose reductions for these drugs [211, 212].

5.16 Treatment of Adolescents

Adolescents with ALL have generally been reported to have an inferior outcome compared to younger children, but the gap in outcome is being closed [213, 214]. Although they are likely to differ from younger children in PK of some anticancer agents [215] it does not seem to be the case for VCR [216], Steroid [59], Asp [217], and i.t. Depocyte [218], whereas adolescents do seem to have slower clearance of HD-MTX [219] and accumulate higher levels of cytotoxic metabolites of 6MP and MTX [17]. They also more frequently have higher risk features, including T-cell leukemia and higher MRD at the end of induction therapy [214, 220], but not necessarily more toxicity, except for the risk of thrombosis, pancreatitis and osteonecrosis [46, 221, 222]. Furthermore, a poor adherence to oral chemotherapy may be risk factor for relapse [223].

5.17 Treatment of Obese Patients

Worldwide the prevalence of childhood obesity is increasing at an alarming rate, and during ALL treatment it may furthermore increase due to exposure to Steroids. Except for capping the VCR dose at 2.0–2.5 mg, capping the dose of antileukemic agents is not routinely recommend in obese patients, primarily since BSA is a poor measure of body composition and a poor predictor of drug disposition although this has only been studied in few patients [224]. Furthermore, obesity have been associated with decreased EFS, increased relapse rate and unchanged toxicity rates in childhood ALL, potentially either due to cytokines released from adipocytes or due to treatment adherence factors associated with cultural patterns of excessive eating and limited physical activity [225]. PK data in obese children are limited, but liver and kidney function and clearance (per m.sq.) of antileukemic agents would not be expected to change markedly in obese patients, although the role of hepatic steatosis is unexplored [226]. The guidelines of the American Society of Clinical Oncology suggest the use of actual body weight for appropriate dosing of chemotherapy of adult obese cancer patients, but similar guidelines have not been validated for children [227].

5.18 ALL Predisposition Syndromes and Chemotherapy

Approximately 5% of children with ALL harbour germline mutations that strongly predispose them to development of ALL (see Chap. 1). Treating a malignancy in a child with an ALL predisposition syndrome is a challenging balance between efficacy and toxicity, since many of these patients are already burdened by their medical condition and may in addition be at increased risk for chemo- and radiotherapy-induced toxicities [228]. It adds to the problem that such patients are generally excluded from collaborative clinical trials, and with few exceptions little is known with respect to their optimal treatment.

For children with Down syndrome and ALL, both smaller studies and wide international collaborations have shown that 6TG and MTX may have different PK in children with Down syndrome and provided some guidelines for the treatment of these patients [229, 230]. In addition to ALL associated risk factors and PK of antileukemic agents, poor physician compliance to protocol recommendations of dose adjustments may contribute to their increased risk of relapse [206]. Among children with Down syndrome and ALL, HD-MTX PK does not predict the increased risk of MTX-related gastrointestinal toxicity in these patients [231].

For children with ataxia telangiectasia and Nijmegen Breakage Syndrome, a recent study indicated that many of these patients stand a good chance of cure with conventional chemotherapy with acceptable toxicity profiles, and that they should be offered chemotherapy with the intention to cure [232]. Almost half of all patients with low-hypodiploid ALL harbor germline *TP53* mutations, and there may be indication to explore for *TP53* mutations in such patients, not least in case of excessive toxicity [233].

5.19 Conclusions and Future Perspectives

Many childhood ALL patients are at risk of relapse or excessive toxicity due to adverse PK and/or tissue tolerance to chemotherapy, and host genome variants are likely to explain much of these diversity. The costs of performing genome-wide exploration of hundreds of thousands of common germline variants has become low and SNP profiling of large patient cohorts on contemporary ALL protocols are expected to clarify the critical genotype-phenotype interactions relevant for efficacy and toxicity, which eventually may lead to implementation of germline variants into future treatment stratification. In addition to genotyping, this will require deeper phenotyping than currently performed both with respect to PK and acute toxicities [105]. In addition, the benefits of individual dose adjustments based on drug monitoring should be explored further and more systematically, not least for Asp, HD-MTX, and oral 6MP/MTX maintenance therapy.

References

1. Schmiegelow K, Forestier E, Hellebostad M, et al. Long-term results of NOPHO ALL-92 and ALL-2000 studies of childhood acute lymphoblastic leukemia. Leukemia. 2010;24(2):345–54.
2. Pui CH, Yang JJ, Hunger SP, et al. Childhood acute lymphoblastic leukemia: progress through collaboration. J Clin Oncol Off J Am Soc Clin Oncol. 2015;33(27):2938–48.
3. Farber S, Diamond LK. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid. N Engl J Med. 1948;238(23):787–93.
4. Burchenal JH, Murphy ML, Ellison RR, et al. Clinical evaluation of a new antimetabolite, 6-mercaptopurine, in the treatment of leukemia and allied diseases. Blood. 1953;8(11):965–99.
5. Simone J, Pinkel D. Rationale and results of combination chemotherapy and central nervous system irradiation in acute lymphocytic leukemia. Bibl Haematol. 1973;39:1068–73.

6. Vora A, Andreano A, Pui CH, et al. Influence of cranial radiotherapy on outcome in children with acute lymphoblastic leukemia treated with contemporary therapy. J Clin Oncol Off J Am Soc Clin Oncol. 2016;34(9):919–26.
7. Riehm H, Feickert HJ, Schrappe M, Henze G, Schellong G. Therapy results in five ALL-BFM studies since 1970: implications of risk factors for prognosis. Haematol Blood Transf. 1987;30:139–46.
8. Galpin AJ, Schuetz JD, Masson E, et al. Differences in folylpolyglutamate synthetase and dihydrofolate reductase expression in human B-lineage versus T-lineage leukemic lymphoblasts: mechanisms for lineage differences in methotrexate polyglutamylation and cytotoxicity. Mol Pharmacol. 1997;52(1):155–63.
9. Coustan-Smith E, Behm FG, Sanchez J, et al. Immunological detection of minimal residual disease in children with acute lymphoblastic leukaemia. Lancet. 1998;351(9102):550–4.
10. Neale GA, Coustan-Smith E, Pan Q, et al. Tandem application of flow cytometry and polymerase chain reaction for comprehensive detection of minimal residual disease in childhood acute lymphoblastic leukemia. Leukemia. 1999;13(8):1221–6.
11. Nyvold C, Madsen HO, Ryder LP, et al. Competitive PCR for quantification of minimal residual disease in acute lymphoblastic leukaemia. J Immunol Methods. 2000; 233(1–2):107–18.
12. Bruggemann M, Schrauder A, Raff T, et al. Standardized MRD quantification in European ALL trials: proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18–20 September 2008. Leukemia. 2010;24(3):521–35.
13. Pui CH, Pei D, Coustan-Smith E, et al. Clinical utility of sequential minimal residual disease measurements in the context of risk-based therapy in childhood acute lymphoblastic leukaemia: a prospective study. Lancet Oncol. 2015;16(4):465–74.
14. Madhusoodhan PP, Carroll WL, Bhatla T. Progress and prospects in pediatric leukemia. Curr Probl Pediatr Adolesc Health Care. 2016;46(7):229–41.
15. Ronghe M, Burke GA, Lowis SP, Estlin EJ. Remission induction therapy for childhood acute lymphoblastic leukaemia: clinical and cellular pharmacology of vincristine, corticosteroids, L-asparaginase and anthracyclines. Cancer Treat Rev. 2001;27(6):327–37.
16. Schmiegelow K, Nielsen SN, Frandsen TL, Nersting J. Mercaptopurine/methotrexate maintenance therapy of childhood acute lymphoblastic leukemia: clinical facts and fiction. J Pediatr Hematol Oncol. 2014;36(7):503–17.
17. Schmiegelow K, Nersting J, Nielsen SN, et al. Maintenance therapy of childhood acute lymphoblastic leukemia revisited – should drug doses be adjusted by white blood cell, neutrophil, or lymphocyte counts? Pediatr Blood Cancer. 2016;63:2104.
18. Meibohm B, Derendorf H. Basic concepts of pharmacokinetic/pharmacodynamic (PK/PD) modelling. Int J Clin Pharmacol Ther. 1997;35(10):401–13.
19. Evans WE, Rodman JH, Relling MV, et al. Concept of maximum tolerated systemic exposure and its application to phase I-II studies of anticancer drugs. Med Pediatr Oncol. 1991; 19(3):153–9.
20. Schmiegelow K. Advances in individual prediction of methotrexate toxicity: a review. Br J Haematol. 2009;146(5):489–503.
21. Boos J. Pharmacokinetics and drug monitoring of L-asparaginase treatment. Int J Clin Pharmacol Ther. 1997;35(3):96–8.
22. Asselin B, Rizzari C. Asparaginase pharmacokinetics and implications of therapeutic drug monitoring. Leuk Lymphoma. 2015;56(8):2273–80.
23. Evans WE, Relling MV, Rodman JH, Crom WR, Boyett JM, Pui CH. Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. N Engl J Med. 1998;338(8):499–505.
24. Schmiegelow K, Bjork O, Glomstein A, et al. Intensification of mercaptopurine/methotrexate maintenance chemotherapy may increase the risk of relapse for some children with acute lymphoblastic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2003;21(7):1332–9.
25. Vrooman LM, Stevenson KE, Supko JG, et al. Postinduction dexamethasone and individualized dosing of Escherichia Coli L-asparaginase each improve outcome of children and

adolescents with newly diagnosed acute lymphoblastic leukemia: results from a randomized study – Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. J Clin Oncol Off J Am Soc Clin Oncol. 2013;31(9):1202–10.
26. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med. 1987;317(17):1098.
27. Canal P, Chatelut E, Guichard S. Practical treatment guide for dose individualisation in cancer chemotherapy. Drugs. 1998;56(6):1019–38.
28. Cheung NV, Heller G. Chemotherapy dose intensity correlates strongly with response, median survival, and median progression-free survival in metastatic neuroblastoma. J Clin Oncol Off J Am Soc Clin Oncol. 1991;9(6):1050–8.
29. Davidsen ML, Dalhoff K, Schmiegelow K. Pharmacogenetics influence treatment efficacy in childhood acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 2008;30(11):831–49.
30. Dulucq S, Laverdiere C, Sinnett D, Krajinovic M. Pharmacogenetic considerations for acute lymphoblastic leukemia therapies. Expert Opin Drug Metab Toxicol. 2014;10(5):699–719.
31. Pui CH, Mullighan CG, Evans WE, Relling MV. Pediatric acute lymphoblastic leukemia: where are we going and how do we get there? Blood. 2012;120(6):1165–74.
32. Moriyama T, Relling MV, Yang JJ. Inherited genetic variation in childhood acute lymphoblastic leukemia. Blood. 2015;125(26):3988–95.
33. Borst L, Wallerek S, Dalhoff K, et al. The impact of CYP3A5*3 on risk and prognosis in childhood acute lymphoblastic leukemia. Eur J Haematol. 2011;86(6):477–83.
34. Borst L, Buchard A, Rosthoj S, et al. Gene dose effects of GSTM1, GSTT1 and GSTP1 polymorphisms on outcome in childhood acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 2012;34(1):38–42.
35. Wahlang B, Falkner KC, Cave MC, Prough RA. Role of cytochrome P450 monooxygenase in carcinogen and chemotherapeutic drug metabolism. Adv Pharmacol. 2015;74:1–33.
36. Relling MV, Hancock ML, Rivera GK, et al. Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. J Natl Cancer Inst. 1999;91(23):2001–8.
37. Levinsen M, Rotevatn EO, Rosthoj S, et al. Pharmacogenetically based dosing of thiopurines in childhood acute lymphoblastic leukemia: influence on cure rates and risk of second cancer. Pediatr Blood Cancer. 2014;61(5):797–802.
38. Yang JJ, Cheng C, Yang W, et al. Genome-wide interrogation of germline genetic variation associated with treatment response in childhood acute lymphoblastic leukemia. JAMA. 2009;301(4):393–403.
39. Wesolowska-Andersen A, Borst L, Dalgaard MD, et al. Genomic profiling of thousands of candidate polymorphisms predicts risk of relapse in 778 Danish and German childhood acute lymphoblastic leukemia patients. Leukemia. 2015;29(2):297–303.
40. Beck T, Gollapudi S, Brunak S, et al. Knowledge engineering for health: a new discipline required to bridge the “ICT gap” between research and healthcare. Hum Mutat. 2012;33(5):797–802.
41. Schmiegelow K, Nyvold C, Seyfarth J, et al. Post-induction residual leukemia in childhood acute lymphoblastic leukemia quantified by PCR correlates with in vitro prednisolone resistance. Leukemia. 2001;15(7):1066–71.
42. Lonnerholm G, Thorn I, Sundstrom C, et al. In vitro cellular drug resistance adds prognostic information to other known risk-factors in childhood acute lymphoblastic leukemia. Leuk Res. 2011;35(4):472–8.
43. Moricke A, Zimmermann M, Reiter A, et al. Long-term results of five consecutive trials in childhood acute lymphoblastic leukemia performed by the ALL-BFM study group from 1981 to 2000. Leukemia. 2010;24(2):265–84.
44. Conter V, Valsecchi MG, Silvestri D, et al. Pulses of vincristine and dexamethasone in addition to intensive chemotherapy for children with intermediate-risk acute lymphoblastic leukaemia: a multicentre randomised trial. Lancet. 2007;369(9556):123–31.
45. Eden T, Pieters R, Richards S. Childhood acute lymphoblastic leukaemia collaborative G. Systematic review of the addition of vincristine plus steroid pulses in maintenance

treatment for childhood acute lymphoblastic leukaemia – an individual patient data meta-analysis involving 5,659 children. Br J Haematol. 2010;149(5):722–33.
46. Mattano Jr LA, Devidas M, Nachman JB, et al. Effect of alternate-week versus continuous dexamethasone scheduling on the risk of osteonecrosis in paediatric patients with acute lymphoblastic leukaemia: results from the CCG-1961 randomised cohort trial. Lancet Oncol. 2012;13(9):906–15.
47. Ramsey LB, Janke LJ, Payton MA, et al. Antileukemic efficacy of continuous vs discontinuous dexamethasone in murine models of acute lymphoblastic leukemia. PloS One. 2015;10(8):e0135134.
48. Inaba H, Pui CH. Glucocorticoid use in acute lymphoblastic leukaemia. Lancet Oncol. 2010;11(11):1096–106.
49. Domenech C, Suciu S, De Moerloose B, et al. Dexamethasone (6 mg/m2/day) and prednisolone (60 mg/m2/day) were equally effective as induction therapy for childhood acute lymphoblastic leukemia in the EORTC CLG 58951 randomized trial. Haematologica. 2014;99(7):1220–7.
50. Ito C, Evans WE, McNinch L, et al. Comparative cytotoxicity of dexamethasone and prednisolone in childhood acute lymphoblastic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 1996;14(8):2370–6.
51. Gaynon PS, Carrel AL. Glucocorticosteroid therapy in childhood acute lymphoblastic leukemia. Adv Exp Med Biol. 1999;457:593–605.
52. Moricke A, Zimmermann M, Valsecchi MG, et al. Dexamethasone vs prednisone in induction treatment of pediatric ALL: results of the randomized trial AIEOP-BFM ALL 2000. Blood. 2016;127(17):2101–12.
53. O'Connor D, Bate J, Wade R, et al. Infection-related mortality in children with acute lymphoblastic leukemia: an analysis of infectious deaths on UKALL2003. Blood. 2014;124(7):1056–61.
54. O'Connor D, et al. Use of minimal residual disease assessment to redefine induction failure in pediatric acute lymphoblastic leukemia. J Clin Oncol. 2017;35(6):660–7. PMID: 28045622.
55. Distelhorst CW. Recent insights into the mechanism of glucocorticosteroid-induced apoptosis. Cell Death Differ. 2002;9(1):6–19.
56. Bindreither D, Ecker S, Gschirr B, Kofler A, Kofler R, Rainer J. The synthetic glucocorticoids prednisolone and dexamethasone regulate the same genes in acute lymphoblastic leukemia cells. BMC Genomics. 2014;15:662.
57. Jackson RK, Irving JA, Veal GJ. Personalization of dexamethasone therapy in childhood acute lymphoblastic leukaemia. Br J Haematol. 2016;173(1):13–24.
58. Choonara I, Wheeldon J, Rayner P, Blackburn M, Lewis I. Pharmacokinetics of prednisolone in children with acute lymphoblastic leukaemia. Cancer Chemother Pharmacol. 1989;23(6):392–4.
59. Petersen KB, Jusko WJ, Rasmussen M, Schmiegelow K. Population pharmacokinetics of prednisolone in children with acute lymphoblastic leukemia. Cancer Chemother Pharmacol. 2003;51(6):465–73.
60. Gambertoglio JG, Frey FJ, Holford NH, et al. Prednisone and prednisolone bioavailability in renal transplant patients. Kidney Int. 1982;21(4):621–6.
61. Balis FM, Lester CM, Chrousos GP, Heideman RL, Poplack DG. Differences in cerebrospinal fluid penetration of corticosteroids: possible relationship to the prevention of meningeal leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 1987;5(2):202–7.
62. Warris LT, van den Heuvel-Eibrink MM, Aarsen FK, et al. Hydrocortisone as an intervention for dexamethasone-induced adverse effects in pediatric patients with acute lymphoblastic leukemia: results of a double-blind, randomized controlled trial. J Clin Oncol Off J Am Soc Clin Oncol. 2016;34(19):2287–93.
63. Rocha JC, Cheng C, Liu W, et al. Pharmacogenetics of outcome in children with acute lymphoblastic leukemia. Blood. 2005;105(12):4752–8.

64. Lauten M, Matthias T, Stanulla M, Beger C, Welte K, Schrappe M. Association of initial response to prednisone treatment in childhood acute lymphoblastic leukaemia and polymorphisms within the tumour necrosis factor and the interleukin-10 genes. Leukemia. 2002;16(8):1437–42.
65. Meissner B, Stanulla M, Ludwig WD, et al. The GSTT1 deletion polymorphism is associated with initial response to glucocorticoids in childhood acute lymphoblastic leukemia. Leukemia. 2004;18(11):1920–3.
66. Jordan MA, Wilson L. Microtubules as a target for anticancer drugs. Nat Rev Cancer. 2004;4(4):253–65.
67. Stefanou A, Dooley M. Simple method to eliminate the risk of inadvertent intrathecal vincristine administration. J Clin Oncol Off J Am Soc Clin Oncol. 2003;21(10):2044. author reply 244
68. Dennison JB, Mohutsky MA, Barbuch RJ, Wrighton SA, Hall SD. Apparent high CYP3A5 expression is required for significant metabolism of vincristine by human cryopreserved hepatocytes. J Pharmacol Exp Ther. 2008;327(1):248–57.
69. Jackson Jr DV, Castle MC, Bender RA. Biliary excretion of vincristine. Clin Pharmacol Ther. 1978;24(1):101–7.
70. Villikka K, Kivisto KT, Maenpaa H, Joensuu H, Neuvonen PJ. Cytochrome P450-inducing antiepileptics increase the clearance of vincristine in patients with brain tumors. Clin Pharmacol Ther. 1999;66(6):589–93.
71. Relling MV, Pui CH, Sandlund JT, et al. Adverse effect of anticonvulsants on efficacy of chemotherapy for acute lymphoblastic leukaemia. Lancet. 2000;356(9226):285–90.
72. Gidding CE, Meeuwsen-de Boer GJ, Koopmans P, Uges DR, Kamps WA, de Graaf SS. Vincristine pharmacokinetics after repetitive dosing in children. Cancer Chemother Pharmacol. 1999;44(3):203–9.
73. Groninger E, Meeuwsen-de Boer T, Koopmans P, et al. Vincristine pharmacokinetics and response to vincristine monotherapy in an up-front window study of the Dutch Childhood Leukaemia Study Group (DCLSG). Eur J Cancer. 2005;41(1):98–103.
74. Lonnerholm G, Frost BM, Abrahamsson J, et al. Vincristine pharmacokinetics is related to clinical outcome in children with standard risk acute lymphoblastic leukemia. Br J Haematol. 2008;142(4):616–21.
75. Shah NN, Merchant MS, Cole DE, et al. Vincristine sulfate liposomes injection (VSLI, Marqibo(R)): results from a phase i study in children, adolescents, and young adults with refractory solid tumors or leukemias. Pediatr Blood Cancer. 2016;63(6):997–1005.
76. Gidding CE, Kellie SJ, Kamps WA, de Graaf SS. Vincristine revisited. Crit Rev Oncol Hematol. 1999;29(3):267–87.
77. Diouf B, Crews KR, Lew G, et al. Association of an inherited genetic variant with vincristine-related peripheral neuropathy in children with acute lymphoblastic leukemia. JAMA. 2015;313(8):815–23.
78. Lavoie Smith EM, Li L, Hutchinson RJ, et al. Measuring vincristine-induced peripheral neuropathy in children with acute lymphoblastic leukemia. Cancer Nurs. 2013;36(5):E49–60.
79. Nishikawa T, Kawakami K, Kumamoto T, et al. Severe neurotoxicities in a case of Charcot-Marie-Tooth disease type 2 caused by vincristine for acute lymphoblastic leukemia. J Pediatr Hematol Oncol. 2008;30(7):519–21.
80. Egbelakin A, Ferguson MJ, MacGill EA, et al. Increased risk of vincristine neurotoxicity associated with low CYP3A5 expression genotype in children with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2011;56(3):361–7.
81. van Dalen EC, Raphael MF, Caron HN, Kremer LC. Treatment including anthracyclines versus treatment not including anthracyclines for childhood cancer. Cochrane Database Syst Rev. 2014;9:CD006647.
82. Kamps WA, Veerman AJ, van Wering ER, van Weerden JF, Slater R, van der Does-van den Berg A. Long-term follow-up of Dutch Childhood Leukemia Study Group (DCLSG) protocols for children with acute lymphoblastic leukemia, 1984–1991. Leukemia. 2000;14(12):2240–6.

83. Vora A, Goulden N, Wade R, et al. Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. Lancet Oncol. 2013;14(3):199–209.
84. Eksborg S. Anthracycline pharmacokinetics. Limited sampling model for plasma level monitoring with special reference to epirubicin (Farmorubicin). Acta Oncol. 1990;29(3):339–42.
85. Frost BM, Eksborg S, Bjork O, et al. Pharmacokinetics of doxorubicin in children with acute lymphoblastic leukemia: multi-institutional collaborative study. Med Pediatr Oncol. 2002; 38(5):329–37.
86. Bellott R, Auvrignon A, Leblanc T, et al. Pharmacokinetics of liposomal daunorubicin (DaunoXome) during a phase I–II study in children with relapsed acute lymphoblastic leukaemia. Cancer Chemother Pharmacol. 2001;47(1):15–21.
87. van Dalen EC, Michiels EM, Caron HN, Kremer LC. Different anthracycline derivates for reducing cardiotoxicity in cancer patients. Cochrane Database Syst Rev. 2010;5:CD005006.
88. Galettis P, Boutagy J, Ma DD. Daunorubicin pharmacokinetics and the correlation with P-glycoprotein and response in patients with acute leukaemia. Br J Cancer. 1994;70(2): 324–9.
89. Olson RD, Mushlin PS, Brenner DE, et al. Doxorubicin cardiotoxicity may be caused by its metabolite, doxorubicinol. Proc Natl Acad Sci U S A. 1988;85(10):3585–9.
90. Krajinovic M, Labuda D, Mathonnet G, et al. Polymorphisms in genes encoding drugs and xenobiotic metabolizing enzymes, DNA repair enzymes, and response to treatment of childhood acute lymphoblastic leukemia. Clin Cancer Res: Off J Am Assoc Cancer Res. 2002; 8(3):802–10.
91. Lipshultz SE, Miller TL, Lipsitz SR, et al. Continuous versus bolus infusion of doxorubicin in children with ALL: long-term cardiac outcomes. Pediatrics. 2012;130(6):1003–11.
92. Aminkeng F, Bhavsar AP, Visscher H, et al. A coding variant in RARG confers susceptibility to anthracycline-induced cardiotoxicity in childhood cancer. Nat Genet. 2015;47(9): 1079–84.
93. Asselin BL, Whitin JC, Coppola DJ, Rupp IP, Sallan SE, Cohen HJ. Comparative pharmacokinetic studies of three asparaginase preparations. J Clin Oncol Off J Am Soc Clin Oncol. 1993;11(9):1780–6.
94. Albertsen BK, Schroder H, Ingerslev J, et al. Comparison of intramuscular therapy with Erwinia asparaginase and asparaginase Medac: pharmacokinetics, pharmacodynamics, formation of antibodies and influence on the coagulation system. Br J Haematol. 2001;115(4): 983–90.
95. Riccardi R, Holcenberg JS, Glaubiger DL, Wood JH, Poplack DG. L-asparaginase pharmacokinetics and asparagine levels in cerebrospinal fluid of rhesus monkeys and humans. Cancer Res. 1981;41(11 Pt 1):4554–8.
96. Appel IM, Pinheiro JP, den Boer ML, et al. Lack of asparagine depletion in the cerebrospinal fluid after one intravenous dose of PEG-asparaginase: a window study at initial diagnosis of childhood ALL. Leukemia. 2003;17(11):2254–6.
97. van der Sluis IM, Vrooman LM, Pieters R, et al. Consensus expert recommendations for identification and management of asparaginase hypersensitivity and silent inactivation. Haematologica. 2016;101(3):279–85.
98. Stams WA, den Boer ML, Holleman A, et al. Asparagine synthetase expression is linked with L-asparaginase resistance in TEL-AML1-negative but not TEL-AML1-positive pediatric acute lymphoblastic leukemia. Blood. 2005;105(11):4223–5.
99. Raja RA, Schmiegelow K, Frandsen TL. Asparaginase-associated pancreatitis in children. Br J Haematol. 2012;159(1):18–27.
100. Bhojwani D, Darbandi R, Pei D, et al. Severe hypertriglyceridaemia during therapy for childhood acute lymphoblastic leukaemia. Eur J Cancer. 2014;50(15):2685–94.
101. Tong WH, Pieters R, de Groot-Kruseman HA, et al. The toxicity of very prolonged courses of PEGasparaginase or Erwinia asparaginase in relation to asparaginase activity, with a special focus on dyslipidemia. Haematologica. 2014;99(11):1716–21.

102. Vrooman LM, Kirov II, Dreyer ZE, et al. Activity and toxicity of intravenous Erwinia asparaginase following allergy to E. coli-derived asparaginase in children and adolescents with acute lymphoblastic leukemia. Pediatr Blood Cancer. 2016;63(2):228–33.
103. Liu C, Yang W, Devidas M, et al. Clinical and genetic risk factors for acute pancreatitis in patients with acute lymphoblastic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2016;34(18):2133–40.
104. Fernandez CA, Smith C, Yang W, et al. Genome-wide analysis links NFATC2 with asparaginase hypersensitivity. Blood. 2015;126(1):69–75.
105. Yang L, Panetta JC, Cai X, et al. Asparaginase may influence dexamethasone pharmacokinetics in acute lymphoblastic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2008;26(12):1932–9.
106. Schmiegelow K, Attarbaschi A, Barzilai S, et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: a Delphi consensus. Lancet Oncol. 2016;17(6):e231–9.
107. Chen SH, Pei D, Yang W, et al. Genetic variations in GRIA1 on chromosome 5q33 related to asparaginase hypersensitivity. Clin Pharmacol Ther. 2010;88(2):191–6.
108. Fernandez CA, Smith C, Yang W, et al. HLA-DRB1*07:01 is associated with a higher risk of asparaginase allergies. Blood. 2014;124(8):1266–76.
109. Sur P, Fernandes DJ, Kute TE, Capizzi RL. L-asparaginase-induced modulation of methotrexate polyglutamylation in murine leukemia L5178Y. Cancer Res. 1987;47(5):1313–8.
110. Frandsen TL, Abrahamsson J, Lausen B, et al. Individualized toxicity-titrated 6-mercaptopurine increments during high-dose methotrexate consolidation treatment of lower risk childhood acute lymphoblastic leukaemia. A Nordic Society of Paediatric Haematology and Oncology (NOPHO) pilot study. Br J Haematol. 2011;155(2):244–7.
111. Karran P, Attard N. Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer. Nat Rev Cancer. 2008;8(1):24–36.
112. Lowe ES, Kitchen BJ, Erdmann G, et al. Plasma pharmacokinetics and cerebrospinal fluid penetration of thioguanine in children with acute lymphoblastic leukemia: a collaborative Pediatric Oncology Branch, NCI, and Children's Cancer Group study. Cancer Chemother Pharmacol. 2001;47(3):199–205.
113. Vora A, Mitchell CD, Lennard L, et al. Toxicity and efficacy of 6-thioguanine versus 6-mercaptopurine in childhood lymphoblastic leukaemia: a randomised trial. Lancet. 2006;368(9544):1339–48.
114. Escherich G, Richards S, Stork LC, Vora AJ. Childhood acute lymphoblastic leukaemia collaborative G. Meta-analysis of randomised trials comparing thiopurines in childhood acute lymphoblastic leukaemia. Leukemia. 2011;25(6):953–9.
115. Lennard L, Richards S, Cartwright CS, et al. The thiopurine methyltransferase genetic polymorphism is associated with thioguanine-related veno-occlusive disease of the liver in children with acute lymphoblastic leukemia. Clin Pharmacol Ther. 2006;80(4):375–83.
116. Lafolie P, Hayder S, Bjork O, Peterson C. Intraindividual variation in 6-mercaptopurine pharmacokinetics during oral maintenance therapy of children with acute lymphoblastic leukaemia. Eur J Clin Pharmacol. 1991;40(6):599–601.
117. Balis FM, Holcenberg JS, Poplack DG, 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(10):3569–77.
118. 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(4):266–72.
119. Dervieux T, Brenner TL, Hon YY, et al. De novo purine synthesis inhibition and antileukemic effects of mercaptopurine alone or in combination with methotrexate in vivo. Blood. 2002;100(4):1240–7.
120. Hedeland RL, Hvidt K, Nersting J, et al. DNA incorporation of 6-thioguanine nucleotides during maintenance therapy of childhood acute lymphoblastic leukaemia and non-Hodgkin lymphoma. Cancer Chemother Pharmacol. 2010;66(3):485–91.

121. Ebbesen MS, Nersting J, Jacobsen JH, et al. Incorporation of 6-thioguanine nucleotides into DNA during maintenance therapy of childhood acute lymphoblastic leukemia-the influence of thiopurine methyltransferase genotypes. J Clin Pharmacol. 2013;53(6):670–4.
122. Nielsen SN, Grell K, Nersting J, Frandsen TL, Hjalgrim LL, Schmiegelow K. Measures of 6-mercaptopurine and methotrexate maintenance therapy intensity in childhood acute lymphoblastic leukemia. Cancer Chemother Pharmacol 2016; 78:983–94.
123. Relling MV, Gardner EE, Sandborn WJ, et al. Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. Clin Pharmacol Ther. 2013;93(4):324–5.
124. Schmiegelow K, Schroder H, Gustafsson G, et al. Risk of relapse in childhood acute lymphoblastic leukemia is related to RBC methotrexate and mercaptopurine metabolites during maintenance chemotherapy. Nordic Society for Pediatric Hematology and Oncology. J Clin Oncol Off J Am Soc Clin Oncol. 1995;13(2):345–51.
125. Bhatia S, Landier W, Hageman L, et al. Systemic exposure to thiopurines and risk of relapse in children with acute lymphoblastic leukemia: a Children's Oncology Group Study. JAMA Oncol. 2015;1(3):287–95.
126. Relling MV, Pui CH, Cheng C, Evans WE. Thiopurine methyltransferase in acute lymphoblastic leukemia. Blood. 2006;107(2):843–4.
127. Schmiegelow K, Forestier E, Kristinsson J, et al. Thiopurine methyltransferase activity is related to the risk of relapse of childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. Leukemia. 2009;23(3):557–64.
128. Lennard L, Cartwright CS, Wade R, Vora A. Thiopurine dose intensity and treatment outcome in childhood lymphoblastic leukaemia: the influence of thiopurine methyltransferase pharmacogenetics. Br J Haematol. 2015;169(2):228–40.
129. Appell ML, Berg J, Duley J, et al. Nomenclature for alleles of the thiopurine methyltransferase gene. Pharmacogenet Genomics. 2013;23(4):242–8.
130. McLeod HL, Siva C. The thiopurine S-methyltransferase gene locus – implications for clinical pharmacogenomics. Pharmacogenomics. 2002;3(1):89–98.
131. Stanulla M, Schaeffeler E, Flohr T, et al. Thiopurine methyltransferase (TPMT) genotype and early treatment response to mercaptopurine in childhood acute lymphoblastic leukemia. JAMA. 2005;293(12):1485–9.
132. Lennard L, Cartwright CS, Wade R, Vora A. Thiopurine methyltransferase and treatment outcome in the UK acute lymphoblastic leukaemia trial ALL2003. Br J Haematol. 2015;170(4):550–8.
133. Nielsen SN, Grell K, Nersting J, Abrahamson J, Lund B, Kanerva J, Jónsson OG, Vaitkeviciene G, Pruunsild K, Hjalgrim LL, Schmiegelow K. Population-based, prospective analysis of DNA thioguanine nucleotide levels during maintenance therapy of childhood acute lymphoblastic leukemia. Lancet Oncol (In press).
134. Yang JJ, Landier W, Yang W, et al. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2015;33(11):1235–42.
135. Moriyama T, Nishii R, Perez-Andreu V, et al. NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet. 2016;48(4):367–73.
136. 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(4):288–92.
137. Nygaard U, Toft N, Schmiegelow K. Methylated metabolites of 6-mercaptopurine are associated with hepatotoxicity. Clin Pharmacol Ther. 2004;75(4):274–81.
138. Rivard GE, Infante-Rivard C, Hoyoux C, Champagne J. Maintenance chemotherapy for childhood acute lymphoblastic leukaemia: better in the evening. Lancet. 1985;2(8467):1264–6.
139. Schmiegelow K, Glomstein A, Kristinsson J, Salmi T, Schroder H, Bjork O. Impact of morning versus evening schedule for oral methotrexate and 6-mercaptopurine on relapse risk for children with acute lymphoblastic leukemia. Nordic Society for Pediatric Hematology and Oncology (NOPHO). J Pediatr Hematol Oncol. 1997;19(2):102–9.

140. Koren G, Langevin AM, Olivieri N, Giesbrecht E, Zipursky A, Greenberg M. Diurnal variation in the pharmacokinetics and myelotoxicity of mercaptopurine in children with acute lymphocytic leukemia. Am J Dis Child. 1990;144(10):1135–7.
141. Koren G, Ferrazzini G, Sohl H, Robieux I, Johnson D, Giesbrecht E. Chronopharmacology of methotrexate pharmacokinetics in childhood leukemia. Chronobiol Int. 1992;9(6):434–8.
142. Clemmensen KK, Christensen RH, Shabaneh DN, et al. The circadian schedule for childhood acute lymphoblastic leukemia maintenance therapy does not influence event-free survival in the NOPHO ALL92 protocol. Pediatr Blood Cancer. 2014;61(4):653–8.
143. Schmiegelow K, Levinsen MF, Attarbaschi A, et al. Second malignant neoplasms after treatment of childhood acute lymphoblastic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2013;31(19):2469–76.
144. Relling MV, Rubnitz JE, Rivera GK, et al. High incidence of secondary brain tumours after radiotherapy and antimetabolites. Lancet. 1999;354(9172):34–9.
145. Schmiegelow K, Al-Modhwahi I, Andersen MK, et al. Methotrexate/6-mercaptopurine maintenance therapy influences the risk of a second malignant neoplasm after childhood acute lymphoblastic leukemia: results from the NOPHO ALL-92 study. Blood. 2009;113(24):6077–84.
146. Stanulla M, Schaeffeler E, Moricke A, et al. Thiopurine methyltransferase genetics is not a major risk factor for secondary malignant neoplasms after treatment of childhood acute lymphoblastic leukemia on Berlin-Frankfurt-Munster protocols. Blood. 2009;114(7):1314–8.
147. Mikkelsen TS, Thorn CF, Yang JJ, et al. PharmGKB summary: methotrexate pathway. Pharmacogenet Genomics. 2011;21(10):679–86.
148. Cronstein BN, Merrill JT. Mechanisms of the effects of methotrexate. Bull Rheum Dis. 1996;45(5):6–8.
149. Goldman ID, Matherly LH. The cellular pharmacology of methotrexate. Pharmacol Ther. 1985;28(1):77–102.
150. Chabner BA, Allegra CJ, Curt GA, et al. Polyglutamation of methotrexate. Is methotrexate a prodrug? J Clin Invest. 1985;76(3):907–12.
151. Schroder H, Clausen N, Ostergaard E, Pressler T. Pharmacokinetics of erythrocyte methotrexate in children with acute lymphoblastic leukemia during maintenance treatment. Cancer Chemother Pharmacol. 1986;16(2):190–3.
152. Schroder H. Methotrexate pharmacokinetics in age-fractionated erythrocytes. Cancer Chemother Pharmacol. 1986;18(3):203–7.
153. Schroder H. Methotrexate kinetics in myeloid bone marrow cells and peripheral neutrophils. Cancer Chemother Pharmacol. 1987;19(1):42–6.
154. Fotoohi AK, Albertioni F. Mechanisms of antifolate resistance and methotrexate efficacy in leukemia cells. Leuk Lymphoma. 2008;49(3):410–26.
155. Panetta JC, Sparreboom A, Pui CH, Relling MV, Evans WE. Modeling mechanisms of in vivo variability in methotrexate accumulation and folate pathway inhibition in acute lymphoblastic leukemia cells. PLoS Comput Biol. 2010;6(12):e1001019.
156. Borsi JD, Wesenberg F, Stokland T, Moe PJ. How much is too much? Folinic acid rescue dose in children with acute lymphoblastic leukaemia. Eur J Cancer. 1991;27(8):1006–9.
157. Skarby TV, Anderson H, Heldrup J, et al. High leucovorin doses during high-dose methotrexate treatment may reduce the cure rate in childhood acute lymphoblastic leukemia. Leukemia. 2006;20(11):1955–62.
158. Bertino JR. “Rescue” techniques in cancer chemotherapy: use of leucovorin and other rescue agents after methotrexate treatment. Semin Oncol. 1977;4(2):203–16.
159. Wolfrom C, Hartmann R, Fengler R, Bruhmuller S, Ingwersen A, Henze G. Randomized comparison of 36-hour intermediate-dose versus 4-hour high-dose methotrexate infusions for remission induction in relapsed childhood acute lymphoblastic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 1993;11(5):827–33.
160. von Stackelberg A, Hartmann R, Buhrer C, et al. High-dose compared with intermediate-dose methotrexate in children with a first relapse of acute lymphoblastic leukemia. Blood. 2008;111(5):2573–80.
161. Borsi JD, Moe PJ. Systemic clearance of methotrexate in the prognosis of acute lymphoblastic leukemia in children. Cancer. 1987;60(12):3020–4.

162. Csordas K, Hegyi M, Eipel OT, Muller J, Erdelyi DJ, Kovacs GT. Comparison of pharmacokinetics and toxicity after high-dose methotrexate treatments in children with acute lymphoblastic leukemia. Anti-Cancer Drugs. 2013;24(2):189–97.
163. Yarlagadda SG, Perazella MA. Drug-induced crystal nephropathy: an update. Expert Opin Drug Saf. 2008;7(2):147–58.
164. Sand TE, Jacobsen S. Effect of urine pH and flow on renal clearance of methotrexate. Eur J Clin Pharmacol. 1981;19:453–6.
165. Garneau AP, Riopel J, Isenring P. Acute Methotrexate-Induced Crystal Nephropathy. The New England journal of medicine. 2015;373:2691–3.
166. Skarby T, Jonsson P, Hjorth L, et al. High-dose methotrexate: on the relationship of methotrexate elimination time vs renal function and serum methotrexate levels in 1164 courses in 264 Swedish children with acute lymphoblastic leukaemia (ALL). Cancer Chemother Pharmacol. 2003;51(4):311–20.
167. Rasmussen MM, Christensen RH, Gregers J, Heldrup J, Nersting J, Schmiegelow K. Can SLC19A1 80G>A polymorphisms predict risk of extremely delayed MTX excretion after high dose of methotrexate? J Pediatr Hematol Oncol. 2013;35(5):417–8.
168. Widemann BC, Schwartz S, Jayaprakash N, et al. Efficacy of glucarpidase (carboxypeptidase g2) in patients with acute kidney injury after high-dose methotrexate therapy. Pharmacotherapy. 2014;34(5):427–39.
169. Svahn T, Mellgren K, Harila-Saari A, Åsberg A, Kanerva J, Jónsson O, Vaitkeviciene G, Mikkelssen TS, Schmiegelow K, Heldrup J. Delayed elimination of high dose methotrexate and use of Carboxypeptidase G2 in pediatric patients during treatment for acute lymphoblastic leukemia. Ped Blood Cancer 2017.
170. Chessells JM, Leiper AD, Tiedemann K, Hardisty RM, Richards S. Oral methotrexate is as effective as intramuscular in maintenance therapy of acute lymphoblastic leukaemia. Arch Dis Child. 1987;62(2):172–6.
171. Whittle SL, Hughes RA. Folate supplementation and methotrexate treatment in rheumatoid arthritis: a review. Rheumatology (Oxford). 2004;43(3):267–71.
172. Schroder H, Clausen N, Ostergard E, Pressler T. Folic acid supplements in vitamin tablets: a determinant of hematological drug tolerance in maintenance therapy of childhood acute lymphoblastic leukemia. Pediatr Hematol Oncol. 1986;3(3):241–7.
173. Richards S, Pui CH, Gayon P. Childhood acute lymphoblastic leukemia collaborative G. Systematic review and meta-analysis of randomized trials of central nervous system directed therapy for childhood acute lymphoblastic leukemia. Pediatr Blood Cancer. 2013;60(2):185–95.
174. Kager L, Cheok M, Yang W, et al. Folate pathway gene expression differs in subtypes of acute lymphoblastic leukemia and influences methotrexate pharmacodynamics. J Clin Invest. 2005;115(1):110–7.
175. Ramsey LB, Panetta JC, Smith C, et al. Genome-wide study of methotrexate clearance replicates SLCO1B1. Blood. 2013;121(6):898–904.
176. Radtke S, Zolk O, Renner B, et al. Germline genetic variations in methotrexate candidate genes are associated with pharmacokinetics, toxicity, and outcome in childhood acute lymphoblastic leukemia. Blood. 2013;121(26):5145–53.
177. Baslund B, Gregers J, Nielsen CH. Reduced folate carrier polymorphism determines methotrexate uptake by B cells and CD4+ T cells. Rheumatology (Oxford). 2008;47(4):451–3.
178. Gregers J, Christensen IJ, Dalhoff K, et al. The association of reduced folate carrier 80G>A polymorphism to outcome in childhood acute lymphoblastic leukemia interacts with chromosome 21 copy number. Blood. 2010;115(23):4671–7.
179. Campbell JM, Bateman E, Stephenson MD, Bowen JM, Keefe DM, Peters MD. Methotrexate-induced toxicity pharmacogenetics: an umbrella review of systematic reviews and meta-analyses. Cancer Chemother Pharmacol. 2016;78(1):27–39.
180. Aplenc R, Thompson J, Han P, et al. Methylenetetrahydrofolate reductase polymorphisms and therapy response in pediatric acute lymphoblastic leukemia. Cancer Res. 2005;65(6):2482–7.

181. Schroder H, Agger KE, Rosthoj S, Carlsen NT, Schmiegelow K. Antibacterial prophylaxis with trimethoprim-sulfamethoxazole during induction treatment for acute lymphoblastic leukemia. Dan Med Bull. 2001;48(4):275–7.
182. Poulsen A, Demeny AK, Bang Plum C, Gjerum Nielsen K, Schmiegelow K. Pneumocystis carinii pneumonia during maintenance treatment of childhood acute lymphoblastic leukemia. Med Pediatr Oncol. 2001;37(1):20–3.
183. Beach BJ, Woods WG, Howell SB. Influence of co-trimoxazole on methotrexate pharmacokinetics in children with acute lymphoblastic leukemia. Am J Pediatr Hematol/Oncol. 1981;3(2):115–9.
184. Watts CS, Sciasci JN, Pauley JL, et al. Prophylactic trimethoprim-sulfamethoxazole does not affect pharmacokinetics or pharmacodynamics of methotrexate. J Pediatr Hematol Oncol. 2016;38:449.
185. Levinsen M, Shabaneh D, Bohnstedt C, et al. Pneumocystis jiroveci pneumonia prophylaxis during maintenance therapy influences methotrexate/6-mercaptopurine dosing but not event-free survival for childhood acute lymphoblastic leukemia. Eur J Haematol. 2012; 88(1):78–86.
186. Schmiegelow K, Bretton-Meyer U. 6-mercaptopurine dosage and pharmacokinetics influence the degree of bone marrow toxicity following high-dose methotrexate in children with acute lymphoblastic leukemia. Leukemia. 2001;15(1):74–9.
187. van Kooten Niekerk PB, Schmiegelow K, Schroeder H. Influence of methylene tetrahydrofolate reductase polymorphisms and coadministration of antimetabolites on toxicity after high dose methotrexate. Eur J Haematol. 2008;81(5):391–8.
188. Balis FM, Holcenberg JS, Zimm S, et al. The effect of methotrexate on the bioavailability of oral 6-mercaptopurine. Clin Pharmacol Ther. 1987;41(4):384–7.
189. Innocenti F, Danesi R, Di Paolo A, et al. Clinical and experimental pharmacokinetic interaction between 6-mercaptopurine and methotrexate. Cancer Chemother Pharmacol. 1996;37(5): 409–14.
190. Nygaard U, Schmiegelow K. Dose reduction of coadministered 6-mercaptopurine decreases myelotoxicity following high-dose methotrexate in childhood leukemia. Leukemia. 2003;17(7):1344–8.
191. Jordheim LP, Dumontet C. Review of recent studies on resistance to cytotoxic deoxynucleoside analogues. Biochim Biophys Acta. 2007;1776(2):138–59.
192. Veerman AJ, Hogeman PH, van Zantwijk CH, Bezemer PD. Prognostic value of 5'nucleotidase in acute lymphoblastic leukemia with the common-ALL phenotype. Leuk Res. 1985; 9(10):1227–9.
193. Estlin EJ, Ronghe M, Burke GA, Yule SM. The clinical and cellular pharmacology of vincristine, corticosteroids, L-asparaginase, anthracyclines and cyclophosphamide in relation to childhood acute lymphoblastic leukaemia. Br J Haematol. 2000;110(4):780–90.
194. Ratain MJ, Mick R, Schilsky RL, Vogelzang NJ, Berezin F. Pharmacologically based dosing of etoposide: a means of safely increasing dose intensity. J Clin Oncol Off J Am Soc Clin Oncol. 1991;9(8):1480–6.
195. Stewart CF, Arbuck SG, Fleming RA, Evans WE. Relation of systemic exposure to unbound etoposide and hematologic toxicity. Clin Pharmacol Ther. 1991;50(4):385–93.
196. Evans WE, Rodman JH, Relling MV, et al. Differences in teniposide disposition and pharmacodynamics in patients with newly diagnosed and relapsed acute lymphocytic leukemia. J Pharmacol Exp Ther. 1992;260(1):71–7.
197. Rodman JH, Abromowitch M, Sinkule JA, Hayes FA, Rivera GK, Evans WE. Clinical pharmacodynamics of continuous infusion teniposide: systemic exposure as a determinant of response in a phase I trial. J Clin Oncol Off J Am Soc Clin Oncol. 1987;5(7):1007–14.
198. Clark PI, Slevin ML. The clinical pharmacology of etoposide and teniposide. Clin Pharmacokinet. 1987;12(4):223–52.
199. Relling MV, Mahmoud HH, Pui CH, et al. Etoposide achieves potentially cytotoxic concentrations in CSF of children with acute lymphoblastic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 1996;14(2):399–404.

200. Levinsen M, Marquart HV, Groth-Pedersen L, et al. Leukemic blasts are present at low levels in spinal fluid in one third of childhood acute lymphoblastic leukemia cases. Pediatr Blood Cancer. 2016;63:1935.
201. Taskinen M, Oskarsson T, Levinsen M, et al. The effect of central nervous system involvement and irradiation in childhood ALL: lessons from the NOPHO ALL-92 and ALL-2000 protocols. Br J Haematol. 2016. doi:10.1002/pbc.26191.
202. Pui CH, Howard SC. Current management and challenges of malignant disease in the CNS in paediatric leukaemia. Lancet Oncol. 2008;9(3):257–68.
203. Kose F, Abali H, Sezer A, Mertsoylu H, Disel U, Ozyilkan O. Little dose, huge toxicity: profound hematological toxicity of intrathecal methotrexate. Leuk Lymphoma. 2009;50(2): 282–3.
204. Peyrl A, Sauermann R, Traunmueller F, et al. Pharmacokinetics and safety of intrathecal liposomal cytarabine in children aged <3 years. Clin Pharmacokinet. 2009;48(4):265–71.
205. Levinsen M, Harila-Saari A, Grell K, et al. Efficacy and toxicity of intrathecal liposomal cytarabine in first-line therapy of childhood acute lymphoblastic leukemia. J Pediat Hematol Onc. 2016;38(8):602–609.
206. Bohnstedt C, Levinsen M, Rosthoj S, et al. Physicians compliance during maintenance therapy in children with down syndrome and acute lymphoblastic leukemia. Leukemia. 2013; 27(4):866–70.
207. Thompson PA, Murry DJ, Rosner GL, et al. Methotrexate pharmacokinetics in infants with acute lymphoblastic leukemia. Cancer Chemother Pharmacol. 2007;59(6):847–53.
208. Lonnerholm G, Valsecchi MG, De Lorenzo P, et al. Pharmacokinetics of high-dose methotrexate in infants treated for acute lymphoblastic leukemia. Pediatr Blood Cancer. 2009;52(5):596–601.
209. Lucchesi M, Guidi M, Fonte C, et al. Pharmacokinetics of high-dose methotrexate in infants aged less than 12 months treated for aggressive brain tumors. Cancer Chemother Pharmacol. 2016;77(4):857–64.
210. Lonnerholm G, Frost BM, Soderhall S, de Graaf SS. Vincristine pharmacokinetics in children with down syndrome. Pediatr Blood Cancer. 2009;52(1):123–5.
211. Hempel G, Relling MV, de Rossi G, et al. Pharmacokinetics of daunorubicin and daunorubicinol in infants with leukemia treated in the interfant 99 protocol. Pediatr Blood Cancer. 2010;54(3):355–60.
212. van der Sluis I, Moricke A, Escherich G, et al. Pediatric acute lymphoblastic leukemia: efficacy and safety of recombinant E. coli-asparaginase in infants (less than one year of age) with acute lymphoblastic leukemia. Haematologica. 2013;98(11):1697–701.
213. Pui CH, Pei D, Campana D, et al. Improved prognosis for older adolescents with acute lymphoblastic leukemia. J Clin Oncol Off J Am Soc Clin Oncol. 2011;29(4):386–91.
214. Hough R, Rowntree C, Goulden N, et al. Efficacy and toxicity of a paediatric protocol in teenagers and young adults with Philadelphia chromosome negative acute lymphoblastic leukaemia: results from UKALL 2003. Br J Haematol. 2016;172(3):439–51.
215. Knoester PD, Underberg WJ, Beijnen JH. Clinical pharmacokinetics and pharmacodynamics of anticancer agents in pediatric patients (review). Anticancer Res. 1993;13(5C): 1795–808.
216. Frost BM, Lonnerholm G, Koopmans P, et al. Vincristine in childhood leukaemia: no pharmacokinetic rationale for dose reduction in adolescents. Acta Paediatr. 2003;92(5):551–7.
217. Silverman LB, Supko JG, Stevenson KE, et al. Intravenous PEG-asparaginase during remission induction in children and adolescents with newly diagnosed acute lymphoblastic leukemia. Blood. 2010;115(7):1351–3.
218. Peyrl A, Sauermann R, Chocholous M, et al. Pharmacokinetics and toxicity of intrathecal liposomal cytarabine in children and adolescents following age-adapted dosing. Clin Pharmacokinet. 2014;53(2):165–73.
219. Donelli MG, Zucchetti M, Robatto A, et al. Pharmacokinetics of HD-MTX in infants, children, and adolescents with non-B acute lymphoblastic leukemia. Med Pediatr Oncol. 1995;24(3):154–9.

220. Toft N, Birgens H, Abrahamsson J, et al. Risk group assignment differs for children and adults 1–45 yr with acute lymphoblastic leukemia treated by the NOPHO ALL-2008 protocol. Eur J Haematol. 2013;90(5):404–12.
221. Frandsen TL, Heyman M, Abrahamsson J, et al. Complying with the European Clinical Trials directive while surviving the administrative pressure – an alternative approach to toxicity registration in a cancer trial. Eur J Cancer. 2014;50(2):251–9.
222. Toft N, Birgens H, Abrahamsson J, et al. Toxicity profile and treatment delays in NOPHO ALL2008-comparing adults and children with Philadelphia chromosome-negative acute lymphoblastic leukemia. Eur J Haematol. 2016;96(2):160–9.
223. Schmiegelow K, Heyman M, Gustafsson G, et al. The degree of myelosuppression during maintenance therapy of adolescents with B-lineage intermediate risk acute lymphoblastic leukemia predicts risk of relapse. Leukemia. 2010;24(4):715–20.
224. Hijiya N, Panetta JC, Zhou Y, et al. Body mass index does not influence pharmacokinetics or outcome of treatment in children with acute lymphoblastic leukemia. Blood. 2006;108(13):3997–4002.
225. Orgel E, Genkinger JM, Aggarwal D, Sung L, Nieder M, Ladas EJ. Association of body mass index and survival in pediatric leukemia: a meta-analysis. Am J Clin Nutr. 2016;103(3):808–17.
226. Kendrick JG, Carr RR, Ensom MH. Pediatric obesity: pharmacokinetics and implications for drug dosing. Clin Ther. 2015;37(9):1897–923.
227. Griggs JJ, Mangu PB, Anderson H, et al. Appropriate chemotherapy dosing for obese adult patients with cancer: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol Off J Am Soc Clin Oncol. 2012;30(13):1553–61.
228. Schmiegelow K. Treatment-related toxicities in children with acute lymphoblastic leukaemia predisposition syndromes. Eur J Med Genet. 2016; doi:10.1016/j.ejmg.2016.02.006.
229. Lee P, Bhansali R, Izraeli S, Hijiya N, Crispino JD. The biology, pathogenesis and clinical aspects of acute lymphoblastic leukemia in children with down syndrome. Leukemia. 2016;30:1816.
230. Hefti E, Blanco JG. Pharmacokinetics of chemotherapeutic drugs in pediatric patients with down syndrome and leukemia. J Pediatr Hematol Oncol. 2016;38(4):283–7.
231. Buitenkamp TD, Mathot RA, de Haas V, Pieters R, Zwaan CM. Methotrexate-induced side effects are not due to differences in pharmacokinetics in children with down syndrome and acute lymphoblastic leukemia. Haematologica. 2010;95(7):1106–13.
232. Bienemann K, Burkhardt B, Modlich S, et al. Promising therapy results for lymphoid malignancies in children with chromosomal breakage syndromes (Ataxia teleangiectasia or Nijmegen-breakage syndrome): a retrospective survey. Br J Haematol. 2011;155(4):468–76.
233. Holmfeldt L, Wei L, Diaz-Flores E, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet. 2013;45(3):242–52.