Pharmacogenetics of childhood acute lymphoblastic leukemia: an actor looking for a role

K. Schmiegelow

Department of Paediatrics and Adolescent Medicine, Juliane Marie Centre, The University Hospital Rigshospitalet, Denmark, and the Institute of Gynaecology, Obstetrics, and Paediatrics, The Medical Faculty, the University of Copenhagen, Copenhagen, Denmark

Correspondence: Kjeld Schmiegelow E-mail: kjeld.schmiegelow@rh.regionh.dk

Acknowledgments: This paper has received financial support from the Danish Childhood Cancer Foundation.

Hematology Education: the education program for the annual congress of the European Hematology Association

2013;7:293-302

A B S T R A C T

The 5-year overall survival of childhood acute lymphoblastic leukemia (ALL) is now 85-90% in the best contemporary protocols, but this reflects an overall intensification of therapy, and as many as one-third of all deaths in childhood ALL are caused by toxicities or secondary neoplasms. Many survivors are burdened by life-long sequelae that emphasize the need to develop more individualized treatment approaches. The treatment of ALL may include more than ten different anticancer agents that are used in different doses, combinations, and routes of administration for a total period of 2-3 years. In general, the pathways (and genes) affecting the pharmacokinetics and pharmacodynamics of these drugs are well known. As with all other drugs, the absorption, distribution, elimination and effect of the drugs varies widely between patients. Much of this diversity is genetically determined, reflecting the millions of genome variants between patients. Due to the complexity of the treatment, single pharmacogenetic variants will have little influence on cure rates or risk of toxicities. Instead, extensive panels of genetic variants need to be addressed. This review summarizes the advantages and challenges for implementing pharmacogenetic testing in the treatment strategies for childhood ALL.

Learning goals

At the conclusion of this activity, participants should be able to:
- understand and address challenges for implementation of pharmacogenetics in the treatment of childhood AL;
- describe characteristics of genomic variants that can be expected to have a significant impact on cure rates of childhood AL;
- describe strategies for identification of genomic variants that could potentially influence cure rates;
- describe the potential role of thiopurine methyltransferase variants for risk of relapse and risk of secondary cancer.

Introduction

Antileukemic therapy

The complex treatment of childhood acute lymphoblastic leukemia (ALL) includes an induction phase with 3-4 drugs to obtain clinical remission, followed by multi-agent consolidation and delayed intensification, central nervous system (CNS)-directed therapy and, finally, a maintenance phase lasting 2-3 years from the time of diagnosis. The induction and delayed intensification phases generally consist of a glucocorticosteroid, vincristine, an anthracycline and/or asparaginase. During the consolidation phase, other anticancer agents are introduced, such as the alkylating agent cyclophosphamide, and the antimetabolites methotrexate (MTX, a folate antagonist), thiopurines (purine analogs), and cytarabine (a pyrimidine analog). The backbone of maintenance therapy nearly always consists of MTX and a thiopurine with or without intermittent addition of other anticancer drugs.[1]

Through improved diagnostics, risk grouping and chemotherapy, the 5-year overall survival of childhood ALL has reached an impressive 85-90% in the best contemporary protocols.[2-18] However, this reflects an overall intensification of therapy, and as many as one-third of all deaths in childhood ALL are caused by toxicities (mostly infections) or secondary neoplasms (SMN).[19-23] This emphasizes the need to develop more individualized treatment approaches. Through drug dosing by body weight or body surface area, oncologists attempt to obtain the same treatment intensity for their patients. This is in vain since all anticancer agents vary several fold in critical pharmacokinetic parameters such as bioavailability, volume of distribution, peak concentration, clearance, and area under the plasma concentration-time curve.[24] This variation is fully compatible with clinicians' experience: some patients are cured, while others with the same leukemia subtype relapse; some patients tolerate chemotherapy well, while others are burdened by or even die from a variety of serious adverse events (SAE). Clinicians seem to be confronted with an impossible triangle: the disease, the treatment, and the host (Figure 1).

The leukemic motor (karyotype, gene expression profile, methylation pattern),[25-28] the degree of disease dissemination (white blood cell count (WBC), mediastinal mass, central nervous system or testicular leukemia),[29] and leukemic

chemosensitivity (*in vitro* drug resistance, post-induction minimal residual disease (MRD))[30-32] have revealed significant correlations between such leukemia characteristics and cure rates. But the leukemia is not a self-sufficient entity. It grows within, depends on, thrives on, and eventually may kill its host; all this is strongly dependent on, precisely, the host. As no two patients have leukemias with the exact same genetic aberrations, neither are any two patients identical with respect to the gene sequences that affect drug absorption, metabolism, excretion, cellular transport, targets and target pathways, i.e. drug-response phenotypes, including occurrence of toxicities.[33,34]

Genome variants

Each week hundreds of healthy and sick individuals have their genome sequenced and cancer patients have their aberrant tumor genome sequenced. But although we are flooded by genomic data, we are still far from understanding the impact of genomic variations on the phenotype of cancer patients. Ninety percent of the genomic variations consist of single nucleotide polymorphisms (SNP), *i.e.* single base differences in the DNA sequence occurring in at least 1% of the population or on average at every 100-300 base sites. Public databases such as the Single Nucleotide Polymorphism Database (dbSNP) (http://www.ncbi.nlm.nih.gov/snp?db=snp&otool=umnbmlib) offer information on these SNPs, including their unique reference identifier (rsID) (*e.g.* rs1045642 for *MDR1* 3435C>T), their genomic location, whether they are haplotype-tagged, and whether they are synonymous (confer no amino acid change) or non-synonymous (changes the amino acid). In addition to these estimated 15 million SNPs, other genetic variations include insertions, deletions, a variable number of tandem repeats (VNTR) of 2-60 bases, and copy number variation (CNV) in sizes ranging from 1 kilobase to several megabases. In addition, individual phenotypes may be affected by DNA methylation and histone modifications, of which at least the former is heritable and can remain stable through cell divisions. Since the completion of the Human Genome Project in 2001, several public databases have offered information on these genomic variations and their functional impact, *e.g.* hosted by the National Center for Biotechnology Information (NCBI), the International HapMap Project, the 1000 Genomes Project, and the Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB).

Childhood ALL: a model disease

There are multiple reasons for childhood ALL being a model cancer for understanding both the clinical potential and the practical challenges of pharmacogenetics[35] (Tables 1 and 2). First, ALL is the most common cancer in child-

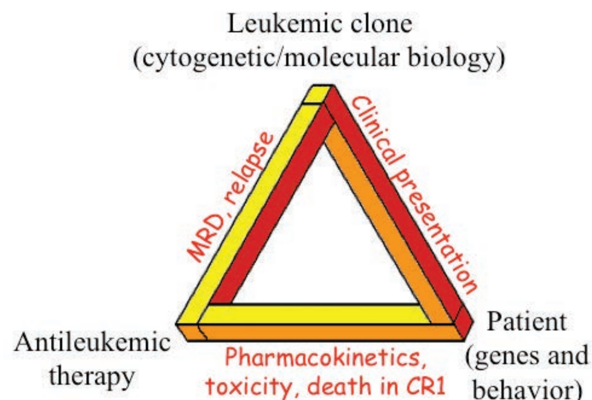

Figure 1. The impossible triangle.

Table 1. Advantages of using childhood ALL as a model for exploring the potential of pharmacogenetics.

Issue	Comment
1. Incidence	Most common cancer in childhood with an annual incidence in Europe and the US of approximately 3.5 cases per 100,000 children 0-14.9 years of age.
2. Subtype classification	The cytogenetic diversity and, *e.g.* gene expression profiles of childhood ALL is well described.
3. Collaborative trials	Patients are treated within large collaborative groups.
4. High chemosensitivity	Childhood ALL generally have high *in vivo* chemosensitivity, that is easily testable.
5. Precise MRD monitoring	Early treatment response can be determined precisely through quantification of minimal residual disease.
6. Toxicities	Serious adverse events are common, well described and potentially avoidable.
7. Huge variation in drug disposition	Huge variation in the pharmacokinetics of anticancer drugs
8. Narrow therapeutic index	The difference between the dose that causes effect and the dose that causes toxicity is small.
9. Individualized therapy by TDM ís complex or impossible	Individualized therapy by drug level measurements has had little influence on cure rates.
10. Pre-clinical testing	Leukemic clones can be cultured for testing of *in vitro* chemosensitivity and impact of pharmacogenetic polymorphisms, and data then correlated to clinical phenotypes.

TDM: therapeutic drug monitoring.

hood with an annual incidence in Europe and the US of approximately 3.5 cases per 100,000 children aged 0-14.9 years.[36] Second, the cytogenetic diversity and the gene expression profiles of childhood ALL is well described with respect to epidemiology, clinical characteristics, and biological profiles, which allows the clinical impact of pharmacogenetic polymorphisms to be explored within well-defined biological subsets.[25,37-39] Third, children with ALL are in general treated within collaborative national or multinational groups[2-18] with risk adapted and body size-based therapy, which allows large-scale exploration of whether interindividual variations in pharmacokinetics, treatment response, or toxicity can be explained by genetic polymorphisms.[33,40-42] Fourth, childhood ALL generally have a high *in vivo* chemosensitivity which increases the likelihood that variations in drug disposition significantly influence cure rates. Fifth, the early response to antileukemic therapy can be determined precisely through quantification of bone marrow minimal residual disease (MRD) using flowcytometry, chimeric gene transcripts, or clonal immune gene rearrangements.[32,43,44] This also allows us to explore the impact of pharmacogenetic variants during early phases of treatment, when patients receive a limited number of anticancer agents.[45,46] Sixth, serious adverse events (SAEs) are common. Not only will virtually all patients experience significant bone marrow and immunosuppression accompanied by a high risk of potentially life-threatening infections, but a number of other severe toxicities (*e.g.* osteonecrosis, pancreatitis, thrombosis, veno-occlusive disease) will occur in a small percentage of patients. These may be as burdensome as the leukemia itself, and their etiology can be questioned in genotype-phenotype association studies. Seventh, although drug dosing by body surface area facilitates comparison of the efficacy and toxicity of different protocols, it does not secure equal systemic drug exposure.[47,48] Eighth, the therapeutic index is very narrow for anticancer agents. Genetically determined variations in pharmacokinetics can thus affect the chances of cure or the risk of SAEs. Ninth, individualized therapy by drug level measurements has not consistently improved the cure rates,[49,50] and pharmacogenetic data can be added to drug concentration measurements to predict outcome. Finally, the malignant cells are readily available for *in vitro* chemosensitivity studies of the impact of the pharmacogenetic polymorphisms,[30,51,52] and clinical pharmacogenetic results can thus be explored through interference studies in leukemia cell lines. With all these clinical, technical and logistical advantages, and with the huge amount of cancer genome and host genome data pouring out worldwide from the laboratories of research institutions and collaborative childhood ALL groups, why then is pharmacogenetic information not routinely implemented in the treatment strategy of childhood ALL? It is not just because pediatric oncologists are conservative or skeptical, although psychological and cultural factors certainly may play a role. More important-

Table 2. Challenges for pharmacogenetically based individualization of ALL treatment.

Issue	Comment
1. Legitimized dose adjustment	*E.g.* dose reductions: risk of unacceptable toxicity (*e.g.* toxic death) outweighs the risk of relapse in higher risk patients or gives life-long sequelae in lower risk patients (*e.g.* osteonecrosis).
2. Convincing and defendable tailored therapy	PG-based dose adjustments must be defendable *statistically* (*i.e.* PG-outcome associations are supported by independent data sets), *biologically* (*i.e.* the PG-outcome associations are 'understood'), and *therapeutically* (*i.e.* effective alternatives to traditional treatment have been identified).
3. Prediction	Treatment adjustments by genetic polymorphism have predictable effects on efficacy/toxicity in individual patients.
4. Better strategy	Dose adjustments by pharmacogenetic data better/easier/cheaper than by toxicity and/or by drug concentration measurements.
5. No reverse effect	Reducing toxicity or increasing efficacy by PG-based drug dosing must not be upset by increased risk of 'reverse' events (*i.e.* less efficacy or more toxicity).
6. The target population for dose adjustments is well defined	Patient populations are not homogeneous, and PG-based drug dosing should be limited to the relevant leukemia subsets: a) the overall risk of a specific toxicity in the total population (e.g. AVN) should be significantly reduced (optimally >50%); b) interaction with leukemia subtype has been mapped.
7. Multiple genetic variants and long follow up	Multiple anticancer agents are given and their individual impact on cure rates is uncertain. Pharmacokinetics and pharmacodynamics of anticancer agents are complex and multiple genes are involved, and the effect of their genetic variants may be redundant or counteractive.
8. 'Conventional' therapy is not conventional	Clinicians already face the adverse effect of known or yet unidentified genetic variants and may on clinical grounds adjust therapy according to *e.g.* toxicity that can affect cure rates.
9. Clinical testing	PG-based treatment amendments must be tested in randomized trials of the specific and overall toxicities and relapse pattern.

AVN: avascular necrosis; PG: pharmacogenetic.

ly, there is an array of challenges that complicates the clinical implementation of pharmacogenetic data (Table 2).

First, upward or downward dose adjustments and, to an even greater extent, even elimination of specific drugs due to expected severe toxicities, need to be justified by an unacceptable balance between treatment efficacy and toxicity. Second, to implement pharmacogenetic data into first-line treatment strategies, such treatment adaptations must be defendable *statistically* (i.e. validated by independent data sets), *biologically* (clinicians as well as patients are likely to require that they 'understand' the genotype-phenotype associations), and *therapeutically* (effective treatment alternatives have been identified). Third, treatment adjustments guided by genetic profiles must have predictable effects on efficacy/toxicity in the individual patient. Thus, it is not sufficient to know that patients with a specific SNP profile are at increased risk of a specific toxicity (*e.g.* severe immunosuppression), clinicians will need to know the relevant degree of dose adjustments to compensate for the adverse genetic profile. Fourth, already today patients may receive modified therapy according to plasma concentrations of the maternal drug (*e.g.* MTX concentrations or asparaginase activity),[53] the intracellular levels of cytotoxic metabolites (*e.g.* 6-meracptopurine, 6MP, metabolites = thioguanine nucleotides),[50] or the target effect (*e.g.* post-induction MRD, or absolute neutrophil counts (ANC) during maintenance therapy).[31,42] In such cases, we need data that demonstrate that host genotyping will offer more effective / less toxic treatment guidelines compared to such traditional phenotyping. Fifth, the current childhood ALL drug combinations and doses have evolved through decades of empirical testing, including numerous randomized trials. Even though retrospective host genomic data may indicate that certain patients are at increased risk of relapse with standard dosing, there are virtually no published data that demonstrate that pharmacokinetic-based treatment intensification will not lead to more toxic deaths. Sixth, the target population needs to be precisely defined since the benefit of treatment adjustments may differ among the childhood ALL subsets, and very few of the published studies have been performed within well-defined ALL subsets.[38,45] In addition, most publications on genotype-phenotype associations report odds ratios of 2.0-3.0 at most. Figure 2A and B illustrates that even if all events could be avoided in a subpopulation with an odds ratio of 3.0, this may have little impact on the overall risk of the toxicity in question and, furthermore, will not be beneficial for most patients with the specific phenotype. Seventh, unless the end point is early MRD monitoring,[34,45,54,55] quantification of efficacy requires years of follow up, and a polymorphism that influences the efficacy of a drug given during the early treatment phases is likely to be modified by subsequent treatment with other agents. This may, in part, help explain the diverse results obtained in childhood ALL pharmacogenetic studies.[33,56,57] In addition, thousands of genetic polymorphisms may affect the most commonly used antileukemic agents, which hampers the interpretation of their individual significance, and increases the risk of type I errors. And it certainly does not help that SNPs are sometimes reported differently with respect to their genomic position.[33] Also, due to the low frequency of many of the SNPs, the statistical power of most published studies has been very low. Thus, even with a 1:1 distribution of two alleles, more than 500 patients will be needed to show a 10% reduction in cure for one of the SNPs, if the overall cure rate is 75%. Finally, many polymorphisms are linked in haplotypes, which complicates interpretation of their individual clinical impact and also the understanding on how a specific gene variant affects phenotype. A polymorphism that reduces clearance of one drug, *e.g.* glucocorticosteroids, may induce increased transcription of CYP3A enzymes, which subsequently may increase the clearance of other anticancer agents.[58] Eighth, the relationship between a given polymorphism and relapse rate may easily be misinterpreted, since some polymorphisms increase the risk of, for example, myelo- or hepatotoxicity, which subsequently may lead clinicians to decrease the dose intensity for such patients and thus potentially increase their risk of relapse.[42,59,60] Ninth, since multiple rare toxicities and relapse sites are to be questioned in trials of individualized therapy, addressing only one of these events may have little impact on the overall risk of SAEs or on cure rates. Alternatively, randomized trials can compare conventional ALL treatment with complex genetic profiling and multiple treatment amendments to explore to what extent this tailored therapy approach influences the *overall* burden of therapy and *overall* cure rates, i.e. a proof of principle strategy.

How then can we identify the genomic variants that have the strongest impact on the efficacy and/or toxicity of specific anticancer agents? Such variants should either: i) markedly influence the disposition or target of drugs that are critical for cure and widely used (*e.g.* glucocorticosteroids, asparaginase, antimetabolites); or ii) alter the activity of metabolizing enzymes or transporters that address several important antileukemic agents; or iii) have a combined effect on a complex pathway affected by widely used antileukemic agents (thiopurine therapy or DNA repair), where the cumulative effect of many SNPs are significantly

A N= 1000 pts	N	Risk	OR	N w/ AVN	Fraction AVN
PG-group A	900	5%	1.0	45	75%
PG-group B	100	15%	3.0	15	25%

B N= 1000 pts	N	Risk	OR	N w/ AVN	Fraction AVN
PG-group A	900	5%	1.0	45	45%
PG-group B	100	55%	11.0	55	55%

Figure 2. (A) Risk of AVN in PG-defined subsets. Even if a change in therapy completely eliminates the risk of AVN for group B who have a 3-fold increased risk of AVN, the overall *occurrence* of AVN among all patients (group A+B) would be reduced by only 25%, and 6 of 7 group B patients will not benefit from the intervention. (B) Risk of AVN in PG-defined subsets. If a change in therapy eliminated the risk for group B, the overall occurrence of AVN would be reduced by 55%; 55% of the patients may benefit from the intervention. AVN: avascular bone necrosis; PG: pharmacogenetic; OR: odds ratio.

associated with outcome even though the effect of each individual SNP is small. The lengthy and complex multi-drug approach to childhood ALL with an interplay of thousands of genetic variants makes it likely that only very few pharmacogenetic variants will have a significant independent influence. Instead, thousands of variants should be questioned in parallel by genome-wide association studies,[34,61,62] targeted sequencing,[63] or whole genome sequencing,[64] although the latter is still too costly to be widely applied. Subsequently, extensive bioinformatic data mining and complex pathway analysis is required.

Drug disposition

Drug metabolizing enzymes are divided into phase I enzymes which metabolize the functional part of the drug leading to activation or inactivation, and phase II enzymes which conjugate drugs with endogenous substances making them more water-soluble and suitable for excretion. These detoxifying pathways and drug efflux systems are very polymorphic and often share the same anticancer agent as a substrate, and polymorphisms in such genes are thus likely to influence treatment response.[58,65,66]

Cytochrome P450 enzymes

The cytochrome P450 (CYP) phase I enzymes, and particularly the CYP3A subfamily, are involved in the activation (*e.g.* cyclophosphamide and epipodophyllotoxins) or inactivation (*e.g.* glucocorticosteroids and vinca alkaloids) of many anticancer agents. Furthermore, the glucocorticosteroids induce CYP3A enzymes, which may influence the clearance of the glucocorticosteroids themselves, but also of other anticancer agents such as vincristine.[58,67,68] Most of the *CYP* genes are highly polymorphic, and although the clinical consequences remain uncertain, several studies have indicated that these variants may influence the risk of relapse in childhood ALL.[33,69-71]

Glutathione S-transferases

The phase II enzymes glutathione *S*-transferases (GSTs) include *GSTP1* 313A>G which alters substrate affinity, *GSTP1* A114V which changes the catalytic activities, and the *GSTM1* and *GSTT1* null alleles both of which, in the case of homozygozity, lead to absence of activity. Since the GST enzymes metabolize a number of anticancer agents, including glucocorticosteroids, vincristine, anthracyclines, methotrexate, cyclophosphamide, and epipodophyllotoxins, polymorphisms in these genes are likely to influence the prognosis in childhood ALL, and at least a few, but not all, studies have shown that poor metabolizers have a decreased risk of relapse.[72-76]

Drug transporters

Many cancer cells have a multi-drug resistance (MDR) phenotype.[77] The classic form of MDR is caused by increased activity of transmembrane protein-mediated efflux of anticancer drugs. Most of the multidrug efflux proteins belong to the superfamily of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP), and breast cancer resistance protein (BCRP). The lung resistance protein (LRP) is not an ABC transporter, but is also part of the MDR scenario.[77] The genes encoding these transporters are highly polymorphic, and their substrates include many anticancer agents, including vincristine, anthracyclines, methotrexate, thiopurines, and epipodophyllotoxins.[33] Yet, these polymorphisms do not seem to have a significant influence on relapse rate or toxicity in childhood ALL (reviewed by Borst *et al.*[33]).

Pharmacogenetics of childhood ALL illustrated by 6-mercaptopurine and methotrexate

The thiopurines 6-mercaptopurine (6MP) and 6-thioguanine (6TG) are among the most important drugs in the treatment of ALL.[78,79] The bioavailability of oral 6MP is highly variable.[79] A major fraction is broken down in first pass metabolism to the inactive thiouric acid. The remainder is methylated by thiopurine methyltransferase (*TPMT*) or enzymatically converted into 6TG and then to 6-thioguanine nucleotides (6TGN). 6TGN are the most important cytotoxic metabolites of thiopurines as they are incorporated into DNA (DNA-6TGN) causing DNA-damage and cell death.[80] Red blood cell levels of free 6TGN (Ery-6TGN) have been related to the risk of relapse,[50,81,82] but dosing 6MP according to Ery-6TGN does not improve cure rates[50] because Ery-6TGN levels are inadequate surrogates for events in the nucleated target cells, where the end point metabolites are DNA-6TGN.[83,84] Methylated 6MP metabolites enhance DNA-6TGN incorporation, due to inhibition of purine *de novo* synthesis[83,85-88] and is, furthermore, associated with hepatotoxicity.[89] This may explain why patients with low methylated 6MP metabolite levels in red blood cells (Ery-MeMP), *e.g.* in *TPMT* deficient patients, may tolerate Ery-6TGN levels ten times higher than *TPMT* wild-type patients.[90] Similarly, lack of methylated 6MP metabolites may explain why replacing 6MP with 6TG, as tested by the US CCG, the German COALL and the British UKALL groups, failed to improve ALL cure rates, even though children receiving 6TG had 6-fold higher Ery-6TGN levels.[85,91]

MTX inhibits folate-dependent processes, such as nucleotide *de novo* synthesis, and this affects cell proliferation and survival.[92,93] MTX is transported by Reduced Folate Carrier (*RFC1*) into target cells[94] and is then conjugated with up to seven glutamates (MTXpg).[95,96] MTX molecules with longer polyglutamate tails are retained longer intracellularly and have higher affinities for the target enzymes.[97,98]

Since patients differ widely in 6MP and MTX disposition, all international study groups recommend dose adjustments by the degree of myelotoxicity.[99] However,

since the WBC varies between healthy individuals,[100,101] the on-treatment WBC is a weak surrogate for the treatment intensity.

Pharmacogenetic variants may significantly influence the response to 6MP therapy[102] with low activity TPMT variants being studied most extensively.[103] The intermediate low-activity *TPMT* heterozygous patients have high intracellular 6TGN levels, reduced tolerance to 6MP,[103-105] a higher cure rate,[42] but also a higher risk of second cancers,[20,106] although not all groups have confirmed this, possibly due to different 6MP dosing strategies.[46] In the most extreme situation, TPMT deficient patients (homozygous for low-activity alleles) may develop life-threatening myelosuppression at standard 6MP doses.[90,107] Unfortunately, dose increments of 6MP in *TPMT* wild-type patients to obtain higher intracellular 6TGN levels and improved chance of cure, will not mirror the situation in TPMT low-activity patients, since the extra 6MP is shunted to methylated metabolites causing more liver toxicity.[89,108] and in some patients even increased risk of relapse.[50] Interestingly, the superior cure rates for patients with TPMT low-activity does not seem to be dependent on the degree of myelosuppression during maintenance therapy.[42] Still, there are no studies to demonstrate that 6MP dose reductions for patients heterozygous for *TPMT* low activity alleles to reduce their risk of secondary cancer will not lead to an increased risk of relapse.

A large number of studies have shown that the clinical variation in response to MTX reflects polymorphisms in genes involved in MTX and folate disposition (reviewed in[33,56,109]). However, many of the studies have been small, most only address one or a few of the genetic polymorphisms involved in the disposition of MTX, and subsequent larger studies or meta-analyses have not confirmed previous results.[57]

The reduced folate carrier *RFC1* 80G>A is the most investigated polymorphism in the *RCF1* gene (=*SCL19A1*). The *RFC1* gene is located on chromosome 21, which probably explains the high MTX sensitivity in children with high hyperdiploidy (which nearly always includes trisomy 21[27]) and in Down syndrome.[110,111] The A allele results in higher MTX plasma concentrations in AA homozygous patients[38,112] and has, furthermore, been more associated with gene dose-related higher cure rates in patient cohorts treated on protocols with high cumulative doses of MTX.[38] Other alleles related to higher plasma MTX concentrations include variants of the *ABC C-family* (=*MRP2*).[62,113]

Several functional polymorphisms have been found in the gene encoding folyl-polyglutamyl synthetase (*FPGS*),[114] but pharmacogenetic studies in ALL are lacking, and in rheumatoid arthritis, SNPs in the *FPGS* gene seem not to affect MTX efficacy or toxicity.[115] In contrast, low activity SNPs in γ-glutamyl hydrolase, such as *GGH* 452C>T, may increase intracellular MTX_{PG} and MTX cytotoxicity on leukaemic cells.[116] In addition, the *GGH* –401C>T genotype has been associated with decreased MTX_{PG} levels in patients with rheumatoid arthritis, indicating increased GGH activity,[117] but the clinical significance of these SNPs has not been mapped in childhood ALL. Numerous studies have been performed on several other genes related to folate metabolism, including *thymidylate synthetase* and its triple repeat (3R) polymorphism in the enhancer region of the gene,[73,118-122] *methylene-tetrahydrofolate reductase* (an important enzyme in the folate-homocysteine cycle)[123] where two SNPs in the gene encoding MTHFR have been extensively studied (*i.e. MTHFR* 677C>T and *MTHFR* 1298A>C) but with limited association with MTX effects,[57] and m*ethionine synthetase* and *methionine synthase reductase* that both play a role in the homocysteine-methionine pathway.[124,125] Finally, *methylene-tetrahydrofolate dehydrogenase* plays a role in purine *de novo* synthesis, and has been associated with risk of relapse, but with no association to toxicity.[126]

Conclusion and perspectives

The low-activity alleles of *TPMT* so far represent the only example of implementation of pharmacogenetically-based drug dosing in ALL protocols, and then only in a few treatment centers.[99,127] This shows that it has been difficult, other than *TPMT* polymorphisms, to establish clear associations between polymorphisms and treatment response. Ideally, clinical pharmacogenetic studies should be performed in a protocol- and ALL cytogenetic subtype-specific manner and should address both cure rates and pattern of toxicities. The genetic screening of patients needs to explore hundreds of SNPs to give a combined gene-dosage effect (*e.g.* individual SNP risk profile) rather than just question one or a few variants.[63] Until whole genome sequencing can be offered at sufficiently low costs to allow its application to all patients on a protocol, targeted *SNP profiling* will require extensive preparatory work in order to identify the genes and variants most relevant to include in an extensive targeted genotyping approach. Subsequently, a customized genotyping platform for childhood ALL needs to be designed to fully explore pharmacogenetics relating to efficacy and toxicity to allow individually tailored therapy.

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