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CONSENSUS ARTICLE

Pharmacogenetics and induction/consolidation therapy toxicities in acute lymphoblastic leukemia patients treated with AIEOP-BFM ALL 2000 protocol

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Drug-related toxicities represent an important clinical concern in chemotherapy, genetic variants could help tailoring treatment to patient. A pharmacogenetic multicentric study was performed on 508 pediatric acute lymphoblastic leukemia patients treated with AIEOP-BFM 2000 protocol: 28 variants were genotyped by VeraCode and Taqman technologies, deletions of *GST-M1* and *GST-T1* by multiplex PCR. Toxicities were derived from a central database: 251 patients (49.4%) experienced at least one gastrointestinal (GI) or hepatic (HEP) or neurological (NEU) grade III/IV episode during the remission induction phase: GI occurred in 63 patients (12.4%); HEP in 204 (40.2%) and NEU in 44 (8.7%). Logistic regression model adjusted for sex, risk and treatment phase revealed that *ITPA* rs1127354 homozygous mutated patients showed an increased risk of severe GI and NEU. *ABCC1* rs246240 and *ADORA2A* rs2236624 homozygous mutated genotypes were associated to NEU and HEP, respectively. These three variants could be putative predictive markers for chemotherapy-related toxicities in AIEOP-BFM protocols.

INTRODUCTION

Pediatric acute lymphoblastic leukemia (ALL) chemotherapy represents one of the greatest medical successes of the last decades. In the context of the AIEOP-BFM (Associazione Italiana Ematologia Oncologia Pediatrica/Berlin-Frankfurt-Münster), ALL 2000 protocol patients at intermediate risk reached a 5 years event-free survival of almost 80%,[1,2] in contrast to the 61% of the early 80s (AIEOP ALL 82).[3] However, the majority of patients treated in the 2000 Study experienced episodes of severe chemotherapy-related toxicities during the 2-years period of treatment. Nowadays, toxicities represent one of the major challenges faced by pediatric oncologists in treating ALL since severe adverse reactions could lead to therapy discontinuation with major effects on outcome and thus affect patient quality of life even afterward in long-term survivors.[4] ALL therapy comprises glucocorticoids, purine analogs, antimetabolites, alkylating agents, antimitotic drugs and anthracyclines, combined together at different dosages and timing. Severe adverse reactions are expected consequences of the prolonged, intense and simultaneous administration of these drugs; their pharmacological effects on target tissues (that is, bone marrow depression) may in fact be associated with damage on healthy cells too, and for therapies with multiple drugs it may be very difficult to identify the precise causative chemotherapeutic agent. Each of the cellular components directly or indirectly involved in the pharmacokinetics and pharmacodynamics of the drugs used as well as each of the effectors modulated by their action, could potentially be a carrier of a genetic mutation able to influence drug resistance or hypersensitivity. The genetic background of the patient could have therefore an important role to define the risk of toxic effects. Although numerous gene variants have been investigated for their association with toxicity induced by chemotherapy, few are those actually incorporated into clinical practice.[5,6] In the context of the ongoing AIEOP-BFM ALL 2009 protocol, only rs1124345, rs1800460 and rs1800462 variants in the thiopurine-methyltransferase (*TPMT*) gene affecting enzyme activity are used to individualize doses of 6-mercaptopurine (6-MP) in order to avoid the hematological toxicity in homozygous mutated patients.

In the study here reported, 28 single-nucleotide polymorphisms (SNPs) and 2 gene deletions potentially involved in efficacy and adverse effects of antileukemic drugs were evaluated in 508 AIEOP patients treated in the AIEOP-BFM ALL 2000 Study, and assessed for their impact on grade III/IV gastrointestinal (GI), hepatic (HEP) and neurological (NEU) toxicities during the remission induction and consolidation phase (protocol IA+IB).

MATERIALS AND METHODS

Study design and population

Thirteen medium–large AIEOP Centers participated in this study (Supplementary Table 1), for a total of 785 Philadelphia-negative ALL patients treated with the AIEOP-BFM ALL 2000 trial

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(ClinicalTrials.gov identifier: NCT00613457). DNA was available for 518 children: 3 left the protocol during phase IA for clinical decision, 7 were excluded because affected by Down syndrome thus more prone to develop toxicities (Figure 1). Remaining 508 patients (mainly European Caucasians, median age at diagnosis 5 years (first–third quartile: 3–9); 270 (53.1%) males) were stratified into standard-, medium- and high-risk groups (Table 1). Remission induction phase IA+IB protocol is schematized in Figure 2. Parents or legal guardians informed consent was obtained before patient enrollment, protocol was approved by the ethics committee of each participating institution.

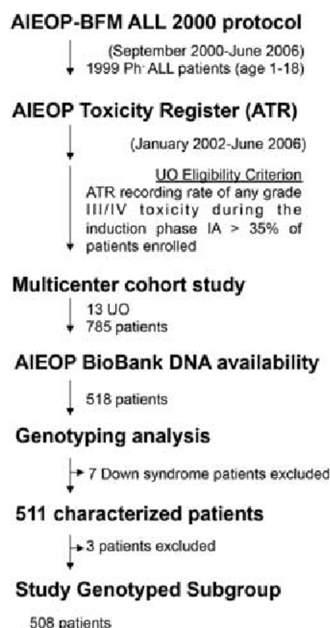

Figure 1. Flowchart for the selection of patients that resulted in the final Study Genotyped Subgroup. Inclusion criteria for both the Hemato-Oncology Units of the AIEOP network and patients were used. ATR, central AIEOP toxicity register; UO, hemato-oncology units.

DNA samples and genotyping

DNAs were kindly provided by SSD Clinical and Experimental Haematology Bio Bank, Department of Paediatrics, University of Padua, Italy (AIEOP Bio Bank). First-choice material was DNA collected from bone marrow at first diagnosis, however, depending on availability, also DNA at relapse was included. A literature search was performed by using public available electronic databases (www.pharmgkb.org; www.ncbi.nlm.nih.gov/pubmed), looking for sequence variants in genes involved in the response to drugs such as glucocorticoids, thiopurines, methotrexate (MTX), anthracyclines used during the induction remission therapy, as well as in genes encoding for phase I and II enzymes, efflux transporters and apoptotic proteins. The final panel included variants characterized at the molecular level and/or with known clinical impact in terms of efficacy and toxicity on childhood ALL and on other diseases outcome.

Genotyping analyses were performed blindly to patients' outcome: 25 SNPs were simultaneously genotyped using the VeraCode technology (Illumina, San Diego, CA, USA) according to the manufacturer's protocol; the three *TPMT* SNPs (rs1142345, rs1800460 and rs1800462) were genotyped using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA). Patients carrying two variant alleles are referred to as 'mutated' in the text. *GST-M1* and *GST-T1* gene deletions were assessed by multiplex PCR as described previously, with a genotyping method that stratifies individuals into those carrying at least one copy of the gene (normal genotype, Norm) and those missing the gene completely (zero copies, Null genotype).[7]

Toxicities

Toxicity episodes were graded according to the National Cancer Institute-Common Terminology Criteria (version 2.0, Supplementary Table S2) and derived from a central AIEOP database where they are recorded since 2002.

Statistical analysis

Among the 28 SNPs explored, 6 were not further considered as they did not satisfy quality control standard (deviation from Hardy–Weinberg equilibrium and genotyping success rate >85%). For *TPMT*, subjects were dichotomized as 'wild type' and 'heterozygous', the latter being those carrying at least one variant

Table 1. Descriptive characteristics of the Study Genotyped Subgroup

		Grade III/IV GI, HEP and NEU toxicity in IA+IB							
		GI/HEP/NEU	P	*GI (%)*	P	*HEP(%)*	P	*NEU (%)*	P
Total	508	251 (49.4)		63 (12.4)		204 (40.2)		44 (8.7)	
Gender									
F	238	122 (51.3)	0.43	35 (14.7)	0.14	100 (42.0)	0.42	18 (7.6)	0.41
M	270	129 (47.8)		28 (10.4)		104 (38.5)		26 (9.6)	
Age (years)									
1–9	406	196 (48.3)	0.26	44 (10.8)	0.03	159 (39.2)	0.06	34 (8.4)	0.28
10–14	86	44 (51.2)		18 (20.9)		34 (39.5)		10 (11.6)	
15–17	16	11 (68.7)		1 (6.3)		11 (68.8)		0 (0)	
Risk									
SR	131	65 (49.6)	0.99	16 (12.2)	0.42	51 (38.9)	0.56	15 (11.5)	0.33
MR	293	145 (49.5)		33 (11.3)		123 (42.0)		21 (7.2)	
HR	84	41 (48.8)		14 (16.7)		30 (35.7)		8 (9.5)	

Abbreviations: F, female; GI, gastrointestinal; HEP, hepatic; HR, high risk; M, male; MR, medium risk; NEU, neurological toxicity; SR, standard risk. *P*= P-value (Chi-square).

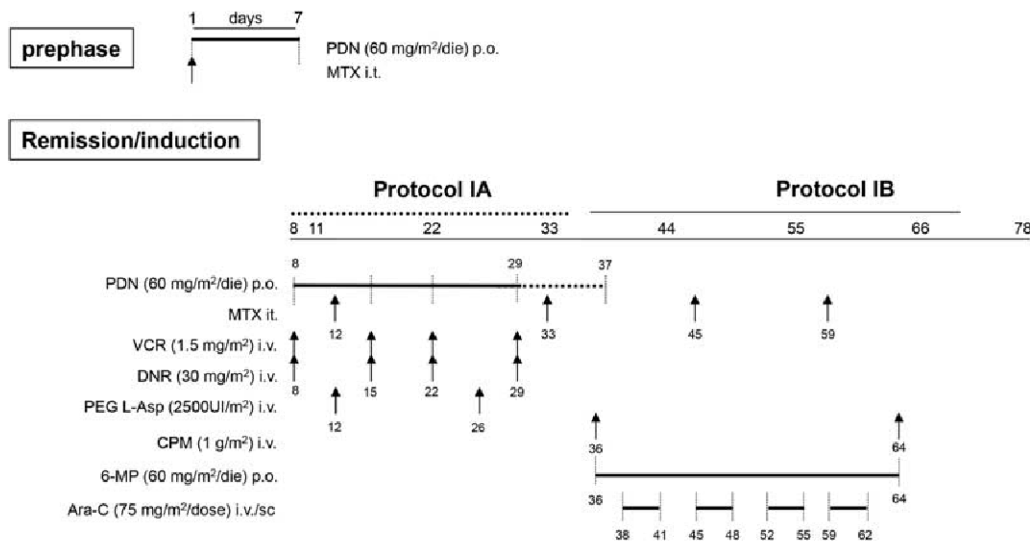

Figure 2. Induction phase in AIEOP-BFM ALL 2000 protocol (adapted from Stocco *et al.*[6]). On day 8, patients were randomized to steroid treatment with either PDN or DXM until day 28 (continuous line) with subsequent tapering doses (dashed line). MTX i.t.: 8, 10, 12 mg for <2, 2–3, $\geqslant 3$ years old children, respectively. Ara-C, cytarabine; CPM, cyclophosphamide; DNR, daunorubicin; DXM, dexamethasone; L-Asp, L-asparaginase; 6-MP, mercaptopurine; MTX, methotrexate; PDN, prednisone; VCR, vincristine.

allele in any of the three functional SNPs: indeed, *TPMT* rs1142345, rs1800460 and rs1800462 heterozygosis display similar functional effects on the enzyme. No patient in our cohort was *TPMT* mutated. The risk of grade III/IV severe treatment-related toxicity in remission induction was estimated within each genotype and within patients' characteristics (age class, sex and risk class). GI, HEP and NEU toxicities were separately considered: each was dichotomized as severe (grade III/IV) versus non-severe (grade I/II or absent), without discriminating among specific adverse episodes. Logistic regression model was used to assess the effect of genotypes (classified as wild type, heterozygote and mutated) on the onset of severe toxicity adjusting for gender, age and risk class. *P*-values <0.00048 were considered as significant to account for multiplicity by Bonferroni correction. Statistical analysis was performed using the software SAS version 9.2 (SAS Institute, NC, USA).

RESULTS

The Study Genotyped Subgroup (median age at diagnosis 5 years (first-third quartiles: 3–9 years; 270 males) comprised 508 patients eligible to the AIEOP-BFM 2000 protocol (age: $\geqslant 1$ and <18 years, newly diagnosed with Philadelphia-negative ALL), enrolled after January 2002 in 13 Hemato-Oncological Units of the AIEOP network, for whom DNA and clinical data were available. Most of these patients were treated with the medium-risk protocol (293, 57.68%), whereas 131 (25.78%) and 84 (16.53%) of them were treated with the standard- and the high-risk protocols, respectively. Overall, 251 patients (49.4%) experienced at least one severe episode of grade III/IV GI, HEP and NEU toxicity in remission induction/consolidation phase, with similar rates among gender and risk class (Table 1). In detail, GI toxicity occurred in 63 patients (12.4%); whereas HEP toxicity occurred in 204 (40.2%) and NEU toxicity in 44 (8.7%). The incidence of grade III–IV GI toxicities was different among age classes ($P=0.03$), being higher for patients aged 10–14 years. Adolescents seemed more prone to develop severe hepatotoxicity episodes in comparison to their younger counterpart (15–17 years: 11 (68.8%) versus 1–14 years: 193 (39.2%).

Genotype distribution of allelic variants positive for quality control is shown in Table 2. Genotype effect on III/IV GI, HEP and NEU toxicity was evaluated by a logistic model adjusting for risk class, gender and age. Table 3 shows results of the three SNPs that emerged as significant for grade III/IV toxicity onset in the univariate analysis and remained significant in the multivariate analysis. The four patients carrying the mutated genotype of *ITPA* SNP rs1127354 showed a 13-fold increase in risk of developing severe NEU when compared with wild-type subjects (odds ratio (OR): 13.23, 95% confidence interval (CI): 1.74–100.65, $P=0.013$) and a 7.7-fold increase in risk of severe GI toxicity (OR: 7.73, 95% CI: 1.04–57.34, $P=0.046$). Other significant associations indicated the homozygous mutated genotype of *ADORA2A* SNP rs2236624 and of *ABCC1* SNP rs246240 as genetic traits associated to HEP (OR: 2.25, 95% CI: 1.03–4.88, $P=0.041$) and NEU (OR: 4.61, 95% CI: 1.12–19.02, $P=0.035$) toxicities, respectively. None of the above associations remained significant after adjustment for multiplicity correction.

Genotypes of *TPMT* and of the other genetic variants did not show any effect on the considered toxicities; results are reported in the Supplementary Tables 3–5.

DISCUSSION

Chemotherapy-induced toxicities are still an important clinical concern in ALL, as they might be responsible for therapy delays and drug dose reductions, therefore negatively affecting the final outcome. Some adverse drug reactions such as the hematological toxicities (myelosuppression, thrombocytopenia, anemia and neutropenia) could be considered extensions of the drug's desired pharmacological effects or their consequences (that is, infections), and were therefore not taken into account for this study.

Hepatotoxicity is one of the most common complications in pediatric ALL: 66.5% of children encountered grade II or higher liver toxicity at some point during therapy.[8] In our study, grade III/IV liver functional abnormalities were reported in 204 patients (40.2%), indicating a high occurrence of hepatotoxic episodes in the remission induction/consolidation phases. Adolescents showed a tendency in developing more grade III/IV hepatotoxic episodes and over 10 years children have higher grade III/IV GI toxicity: indeed, toxicities are a key issue in the use of high-intensity pediatric regimens in older patients as they experienced more side effects and are more at risk of toxic deaths.[9] The

Table 2. Variants distribution in Study Genotyped Subgroup

Gene	Gene category	SNP	HGVS alternate names	wt (%)	hz (%)	mut (%)	NA	Ref.
CYP3A5	[1] VCR	rs776746	NM_000777.4:c.219-237G > A,	427 (87.3)	57 (11.7)	5 (1.0)	19	25–27,15
NQO1	[1] DOX	rs1800566	NM_000903.2:c.559C > T (p.Pro187Ser)	296 (57.9)	141 (27.6)	27 (5.3)	54	29,28
GSTP1	[2]	rs1138272	NM_000852.3:c.341C > T(p.Ala114Val)	446 (90.7)	45 (9.1)	1 (0.2)	16	
NR3C1	[3], GC	rs41423247	NM_000176.2:c.1184+646C > G	239 (49.5)	203 (42.0)	41 (8.5)	25	30–32
NR3C1	[3], GC	rs6190	NM_000176.2:c.68G > A(p.Arg23Lys)	447 (97.7)	11 (2.3)	0 (0.0)	20	33
IL10	[3], GC	rs1800896	NM_000572.2:c.-1117A > G	162 (35.4)	217 (47.4)	79 (17.2)	50	34
SERPINE1	[3], GC	rs6092	NM_000602.4:c.43G > A(p.Ala15Thr)	367 (79.8)	85 (18.5)	8 (1.7)	48	35
TPMT	[3], TP	rs1142345	NM_000367.2:c.719A > G(p.Tyr240Cys)	441 (95.2)	22 (4.8)	0 (0.0)	45	
		rs1800460	NM_000367.2:c.460G > A(p.Ala154Thr)					
		rs1800462	NM_000367.2:c.238G > C (p.Ala80Pro)					
ITPA	[3], TP	rs1127354	NM_033453.3:c.94C > A (p.Pro32Thr)	428 (89.4)	47 (9.8)	4 (0.8)	29	36
GGH	[3], MTX	rs719235	NM_003878.2:c.-354G > T,	256 (57.0)	163 (36.3)	30 (6.7)	59	18,37,17
ADORA2A	[3], MTX	rs2236624	NM_000675.4:c.333-527T > C	304 (62.8)	150 (31.0)	30 (6.2)	24	11
ABCC1	[4]	rs35592	NM_004996.3:c.1219-176T > C	254 (52.7)	182 (37.8)	46 (9.5)	26	22
ABCC1	[4]	rs246240	NM_004996.3:c.616-7942A > G	362 (77.4)	95 (20.3)	11 (2.4)	40	22
ABCC1	[4]	rs3784864	NM_004996.3:c.616-1641G > A	70 (15.1)	217 (46.8)	177 (38.1)	44	22
ABCC1	[4]	rs11075291	NM_004996.3:c.49-3198G > A	113 (24.5)	222 (48.2)	126 (27.3)	47	22
ABCC2	[4]	rs17222723	NM_000392.4:c.3563T > A (p.Val1188Glu)	398 (82.4)	78 (16.1)	7 (1.5)	25	
BCL2L11	[5]	rs12613243	NM_001204106.1:c.125-10115T > C	405 (88.2)	51 (11.1)	3 (0.7)	49	38
NRP2	[6]	rs10932125	NM_003872.2:c.2426-4659C > G	115 (24.8)	232 (50.0)	117 (25.2)	44	39
ANKS1B	[6]	rs17028658	NM_001204081.1:c.691-2700 A > G	402 (84.1)	69 (14.4)	7 (1.5)	30	40
NEU3	[6]	rs7115499	NM_006656.5:c.143G > A (p.Arg48Gln)	395 (84.4)	69 (14.7)	4 (0.9)	40	
				Norm		Null		
GST-M1	[2]	Deletion	NA	217 (42.7)		236 (46.5)	55	7
GST-T1	[2]	Deletion	NA	352 (69.3)		79 (15.6)	77	7

Abbreviations: [1], Phase I metabolism enzymes; [2], Phase II metabolism enzymes; [3], Drug cellular pathway-related targets and effectors; [4], Transporters; [5], Apoptosis-related genes; [6], Others; DOX, doxorubicin; GC, glucocorticoids; HGVS, human genome variation society: alternate variant names given as HGVS nomenclature (http://www.hgvs.org/mutnomen), where 'c.' stays for coding DNA-level with respect to the RefSeq database (listing both database accession and version number as 'NM number') and 'p.' for protein level; hz, heterozygous; MTX, methotrexate; mut, mutated; Ref. bibliographic references; SNP, single-nucleotide polymorphisms; TP, thiopurine; VCR, vincristine; wt, wild type.

metabolic and physiological profiles differ between children and adolescents: hormonal status could influence the pharmacokinetic and pharmacodynamic profiles of patients, and therefore the therapy response. As expected, severe toxicities were independent from risk group because patients underwent the same induction/consolidation chemotherapy (IA+IB) regardless of their risk category (Figure 2).

Identifying the precise agent causative of liver injury is difficult and not straightforward, as several of the antileukemic drugs employed in protocol IA+IB might be involved. Among variants considered in this study, only SNP rs2236624 (C > T) in the adenosine A2A receptor gene (*ADORA2A*) was significantly associated with hepatotoxicity: in multivariate analysis, the mutated genotype (TT) seemed to be a trait predisposing to grade III/IV toxicity episodes in comparison to subjects carrying at least one C allele. Observed genotype distribution was in accordance with literature data: the minor allele frequency (T, 21.8%) was consistent with that reported for HapMap populations of the Western and Northern European ancestry (23.5%). This SNP lies in the intron sequence of the *ADORA2A* gene, located on chromosome 22q11.23. The SNP functional role has yet to be clarified; however, it has an intermediate regulatory potential (http://genome.ucsc.edu). Being in linkage disequilibrium with 15 other currently known SNPs, rs2236624 could be only the nominal genetic variant associated to the investigated phenotype. PharmGKB includes *ADORA2A* variants as novel genetic candidates for MTX pharmacogenomics.[10] According to a study of Hider and co-workers on the association between *ADORA2A* polymorphisms and outcome of MTX-treated rheumatoid arthritis patients, carriage of at least one T allele in SNP rs2236624 increased the risk of all considered MTX adverse effects (including liver transaminase level > 2ULN, upper limit of normal), and particularly of GI complications.[11] In our patients, MTX was only intrathecally given (8–12 mg, depending on age). MTX undergoes saturable efflux transport across the brain–blood barrier, particularly via ABCG2 and likely via the organic anion transporter OAT3.[12] Literature data revealed that after 12 mg intrathecal MTX administration, the mean antifolate plasma concentration reached a peak of 0.2 μM.[13] Such concentration is in the range of that reported in pediatric patients with juvenile rheumatoid arthritis after MTX oral administration in doses of 6.4–11.2 mg m^{-2} per week (http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/ClinicalChemistryandClinicalToxicologyDevicesPanel/UCM348939.pdf).

ADORA2A receptors are expressed in the liver, on Kupffer cells and hepatocytes, where they modulate TNF-alpha production and hepatic glycogen metabolism, respectively, on stellate cells, where they modulate endothelin receptor function, as well as on venous and microvascular endothelium, where they stimulate angiogenesis. Ohta *et al.* demonstrated in different models of *in vivo* inflammatory liver injury and systemic inflammation that stimulation of these receptors by endogenously released adenosine resulted in tissue-protecting properties. In comparison to wild-type animals, ADORA2A-deficient mice showed enhanced sepsis and liver damage, demonstrated by an increase in serum alanine aminotransferase and liver tissue histology.[14] These observations suggest a regulatory role of ADORA2A in liver inflammatory conditions, and let us hypothesize that rs2236624 could be a genetic trait predisposing to hepatotoxic injury rather than a specific drug-related genetic marker. Replication of the observed clinical association in independent cohorts, and also in other hepatic diseases, as well as the characterization of rs2236624 (or SNP in linkage disequilibrium) effect on receptor function and mRNA stability are potential areas of further investigation.

Table 3. Multivariate analysis for the association of genotypes and the onset of severe treatment-related toxicities during the remission induction phase (IA+IB)

		Grade III/IV Tox		Logistic model				
				Univariate		Multivariate		
SNP (gene)	TOT	Yes (%)	No	OR (95%CI)	P-value	OR (95%CI)	P-value[a]	P-value[b]
	GI							
	508	63 (12.4)	445					
rs1127354 (ITPA)								
wt	428	54 (12.6)	374	1		1		
hz	47	4 (8.5)	43	0.64 (0.22;1.87)	0.418	0.71 (0.24;2.07)	0.527	1
mut	4	2 (50)	2	6.93 (0.96;50.2)	0.056	7.73 (1.04;57.34)	0.046	1
NA	29	3	26					
	HEP							
	508	204 (40.2)	304					
rs2236624 (ADORA2A)								
wt	304	119 (39.1)	185	1		1		
hz	150	54 (36)	96	0.87 (0.58;1.31)	0.517	0.87 (0.58;1.31)	0.496	1
mut	30	18 (60)	12	2.33 (1.08;5.02)	0.030	2.25 (1.03;4.88)	0.041	1
NA	24	13	11					
	NEU							
	508	44 (8.7)	464					
rs1127354 (ITPA)								
wt	428	34 (7.9)	394	1		1		
hz	47	3 (6.4)	44	0.79 (0.23;2.68)	0.705	0.77 (0.23;2.63)	0.673	1
mut	4	2 (50)	2	11.59 (1.58;84.86)	0.016	13.23 (1.74;100.65)	0.013	1
NA	29	5	24					
rs246240 (ABCC1)								
wt	362	28 (7.7)	334	1		1		
hz	95	8 (8.4)	87	1.10 (0.48;2.49)	0.825	1.10 (0.48;2.52)	0.816	1
mut	11	3 (27.3)	8	4.47 (1.12;17.81)	0.034	4.61 (1.12;19.02)	0.035	1
NA	40	5	35					

Abbreviations: CI, confidence interval; GI, gastrointestinal; HEP, hepatic; hz, heterozygous; mut, mutated; NA, not applicable; NEU, neurological toxicity; OR, odds ratio; SNP, single-nucleotide polymorphism; TOT, total; Tox, toxicity; wt, wild type. Only associations that emerged as significant for grade III/IV toxicity onset in both univariate and multivariate analysis are shown. [a]Adjusted for risk class, gender and age. [b]Adjusted for multiplicity.

GI toxicity (severe nausea/vomiting, diarrhea and stomatitis) occurred with an incidence of 12.4% and NEU with an incidence of 8.7%, almost double than those reported in the remission induction phase of St Jude Children's Research Hospital protocol (SJCRH) Total XIIIB (6.6% and 3.3%, respectively), likely because of differences in the therapeutic schemes and phase duration.[15] In multivariable analysis, the four homozygous mutated individuals for rs1127354 SNP had higher risk of developing severe GI and NEU complications. This SNP lies in exon 2 of the inosine triphosphate pyrophosphatase (*ITPA*) gene, located on chromosome 20p13; frequency of the minor A allele (10%) in our population was in accordance with the literature data for Caucasians (7%). The 94C>A transversion results in a proline-to-threonine substitution at position 32 of the ITPA protein, leading to the complete depletion of enzymatic activity in homozygous mutated individuals and a 75% reduction in heterozygous subjects.[16] The ITPA enzyme has an important role in the adenosine metabolism pathway, balancing the purine nucleotide pools in the cell. By hydrolyzing the non-canonical inosine triphosphate and xanthosine triphosphate (ITP/dITP/XTP), ITPA avoids the accumulation of these unusual (d)NTPs and catalyzes their reduction in the corresponding monophosphate forms, important intermediates for canonical (d)ATP and (d)GTP synthesis. The pharmacological action of 6-MP (daily administered during the IB protocol) and/or of MTX (intrathecally given during the induction/consolidation phase) could be influenced by *ITPA* genotypes. To authors' knowledge, no previous report indicates rs1127354 as a pharmacogenetic marker for chemotherapy-induced GI or NEU toxicity. In the context of the SJCRH Total XIIIB protocol, Stocco *et al.* found that ALL patients with a variant *ITPA* allele had a higher probability of developing severe febrile neutropenia during continuation therapy when doses of 6-MP had been adjusted for *TPMT* genotype.[17] De Beaumais *et al.* found that wild-type *TPMT*/variant *ITPA* ALL patients treated with the European Organization for Research and Treatment of Cancer-58951 protocol had higher 6-methylmercaptopurine metabolites in erythrocytes during maintenance therapy in comparison to wild-type *TPMT*/wild-type *ITPA* and that 6-methylmercaptopurine threshold above 5,000 pmol/8×10^8 red blood cells was associated with an increased risk of hepatotoxicity.[18] In Asian populations, Kim and co-workers did not found a difference in the cumulative incidence of grade III/IV febrile neutropenia according to *ITPA* genotypes in Korean ALL pediatric patients, whereas Malaysian patients with *ITPA* 94 A allele seemed more likely to develop fever and liver toxicity in therapeutic protocols non-individualized for *TPMT*.[19,20]

TPMT variant genotypes have been associated to the GI toxicity during consolidation therapy in patients treated according to the SJCRH Total XIIIB protocol (75 mg m^{-2} of 6-MP daily given).[21] This predictive role was not confirmed in the AIEOP-BFM ALL 2000 validation cohort likely because of the lower dosage of 6-MP used during consolidation (25 mg m^{-2} per day). However, the present study reveals that *TPMT* genotypes are not predictive of GI toxicity even during the induction phase, when higher doses of 6-MP are used (60 mg m^{-2} per day). It seems therefore that in the AIEOP-BFM ALL protocols the *TMPT* predictive genetic effect is limited to the hematological toxicity.

The SNP rs246240 is an intronic variant of the efflux transport gene *ABCC1* (ATP-binding cassette, subfamily C, member 1). ABCC1 transports a broad range of antineoplastic agents, including MTX, anthracyclines, vinca-alkaloids as well as numerous glucuronidated, glutathionized and sulphated derivates. This SNP has been associated to MTX-induced adverse events in adult patients affected by psoriasis with wild-type subjects showing a 2.2-fold increased risk in developing HEP and specially GI toxicity.[22] Our multivariate analysis did not confirm such clinical associations, showing a significant correlation between the mutated genotype and the occurrence of severe neurotoxic episodes. Although the molecular function of the variant on ABCC1 protein and activity is unknown, we can hypothesize that the mutated genotype leads to an increased pump activity. At the polarized blood–brain barrier ABCC1 is expressed on the abluminal (basal) surface of the capillary endothelium, hence an increased pump activity could result in increased drug levels in the brain.[23] Indeed, biodistribution studies in mice showed that ABCC1 expression and functionality differs between the brain and the liver/testis, promoting the accumulation of known ABCC1 substrates compounds in the brain and resulting in drug removal in peripheral tissues.[24]

In conclusion, this pharmacogenetic retrospective study on ALL pediatric patients identified three polymorphisms as putative predictive genetic traits for grade III–IV GI, HEP and NEU toxicity developed during the induction phase of the AIEOP-BFM ALL 2000 protocol. Of particular interest is the *ITPA* rs1127354, whose role on 6-MP pharmacokinetics could be validated in the current AIEOP-BFM ALL 2009 protocol that maintains almost the same induction therapeutic scheme of the previous one. If significant, the *ITPA* rs1127354 could have a direct fallout in optimizing future AIEOP protocols. All proposed genetic variants could be investigated in the future for their effects on other grade III–IV toxicities developed during the induction phase as well as during other phases of the treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

RF was the principal investigator and contributed to study design, genetic analysis, data interpretation and paper writing; PR is responsible for the study design and statistical analysis; NB recruited patients and collected the clinical data; FF and VC discussed results and revised the manuscript; AB, AC, CM, MZ, RP, FP, GB, MCP and FL recruited patients and collected the clinical data; PD contributed for genetic analysis; MGV and GD discussed results and revised the manuscript; MR contributed to study design, co-ordinated the clinical part, discussed results and revised the manuscript.

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