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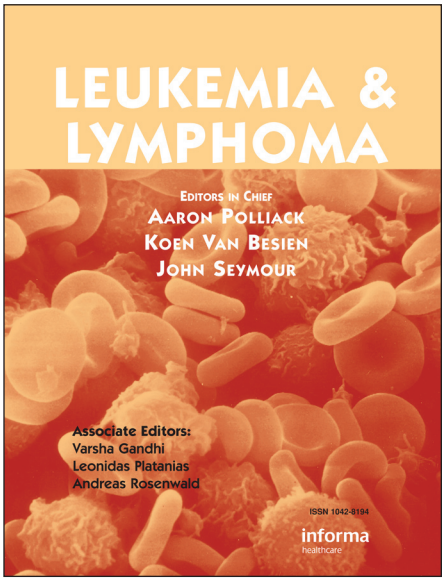

Pharmacogenetic Studies in Children with Acute Lymphoblastic Leukemia (ALL) in Argentina

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Abstract

The aim of this study was to evaluate the influence of the most common genetic variants in methylenetetrahydrofolate reductase (MTHFR), thiopurine methyltransferase (TPMT), and glutathione-S-transferases (GSTs) on the outcome of acute lymphoblastic leukemia (ALL) treatment in Argentinean children. Two hundred and eighty-six patients with ALL treated with two BFM-based protocols were analyzed. Ten genetic variants were studied. Toxicity was evaluated during the consolidation phase. Children who received 2 g/m²/day of methotrexate and carried at least one 677T allele in MTHFR showed an increased risk of developing severe leukopenia (p=0.004) and neutropenia (p=0.003). Intermediate-risk (IR) patients with a heterozygous TPMT genotype had a higher pEFS than those with a wild-type genotype. Genotyping of MTHFR polymorphisms might be useful to optimize consolidation therapy reducing the associated severe hematologic toxicity. Further studies are necessary to establish the usefulness of MTHFR and TPMT variants as additional markers to predict outcome in the IR group.

Pharmacogenetic Studies in Children with Acute Lymphoblastic Leukemia (ALL) in Argentina

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Short title: Pharmacogenetic Studies in Childhood ALL

Abstract

The aim of this study was to evaluate the influence of the most common genetic variants in methylenetetrahydrofolate reductase (*MTHFR*), thiopurine methyltransferase (*TPMT*), and glutathione-S-transferases (*GSTs)* on the outcome of acute lymphoblastic leukemia (ALL) treatment in Argentinean children. Two hundred and eighty-six patients with ALL treated with two BFM-based protocols were analyzed. Ten genetic variants were studied. Toxicity was evaluated during the consolidation phase. Children who received 2 g/m²/day of methotrexate and carried at least one *677T* allele in *MTHFR* showed an increased risk of developing severe leukopenia (p=0.004) and neutropenia (p=0.003). Intermediate-risk (IR) patients with a heterozygous *TPMT* genotype had a higher pEFS than those with a wild-type genotype. Genotyping of *MTHFR* polymorphisms might be useful to optimize consolidation therapy reducing the associated severe hematologic toxicity. Further studies are necessary to establish the usefulness of *MTHFR* and *TPMT* variants as additional markers to predict outcome in the IR group.

Keywords: ALL, *TPMT*, *MTHFR*, *GSTs*, pharmacogenetics, pediatrics

Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent malignant disease in childhood accounting for approximately 23% of all neoplastic diseases in patients younger than 15 years of age [1]. Over the last forty years, there has been a significant increase in the survival rate of patients with ALL and the probability of event-free survival is 70% to 80% in referral centers [2]. This important therapeutic progress has mainly been achieved through the optimization of existing treatments, the use of combined chemotherapy schedules, the incorporation of new drugs and more effective doses, the stratification of patients into risk groups, the identification of risk factors, the application of preemptive therapy to the central nervous system, and significant advances in supportive care [3].

With the advent of pharmacogenetics, the focus of attention was shifted to finding genetic variants in molecules that participate in the pharmacokinetic and pharmacodynamic processes of the drugs used in the treatment of acute leukemia. Genetic variants in the genes that codify for these molecules affect their normal function and, consequently, seem to modulate response to therapy [4,5].

Among these molecules, thiopurine methyltransferase (TPMT), best known for its role in the metabolism of the thiopurine drugs such as 6-mercaptopurine (6-MP), is an enzyme associated with ALL treatment, since it inactivates 6-MP by methylation [6,7]. Additionally, 5,10-methylenetetrahydrofolate reductase (MTHFR) is a key enzyme for intracellular folate homeostasis and metabolism and, as such, plays an important role in the methotrexate (MTX) pathway [8,9]. Both chemotherapeutic agents, 6-MP and MTX, are widely administered during the consolidation and continuation phases in the treatment of pediatric ALL. In addition, the glutathione transferase enzymes P1, M1, and T1 (GSTs) play a fundamental role in cellular detoxification and also influence individual response to treatment [10,11].

The allelic frequencies of genetic variants in these molecules have shown a marked variability among different populations resulting in a different response to the same drugs

and to different chemotherapy doses. Thus, in different populations response to treatment may differ, due not only to the characteristics of the disease and the doses of the drugs used, but also to the allelic frequencies of the molecules involved in the pharmacological processes [12].

In Argentina, there is limited information about the role these variants may have in the outcome of children with ALL. Since 2003, a pharmacogenetic study was designed and carried out at our center. The aim of the present study was to evaluate the influence of the most common genetic variants in the *MTHFR*, *TPMT*, *GSTP1*, *GSTT1*, and *GSTM1* genes on the outcome of ALL treatment (efficacy and toxicity) in Argentinean children affected by this disease.

Patients and Methods

Patient Population

The study included 286 patients with ALL older than 1 year of age diagnosed and treated at Hospital de Pediatría "Prof. Dr. Juan P. Garrahan" in Buenos Aires, Argentina. Patients were treated with Berlin-Frankfurt-Munich (BFM)-based protocols 1-LLA-96 (n= 67) [13] and 12-ALLIC-2002 (n=219) [14] based on the time of diagnosis. In the 1-ALL-96 protocol, patients from all risk groups received MTX at 5 g/m^2/day (4 cycles) during the consolidation phase (Phase M) of treatment [13].In the 12-ALLIC-2002 protocol, patients from the Standard and Intermediate risk groups received MTX at 2 g/m^2/day (4 cycles) and 6-mercaptopurine at 25 mg/m^2/day during phase M [14]. High-risk patients received MTX at 5 g/m^2 and high-risk block drugs during this phase. In both protocols, all risk-group patients received MTX at 25 mg/m^2 weekly, adjusted according to blood cell counts, during the continuation phase [13,14].

Patient samples were obtained at diagnosis after written informed consent from the parents or caregivers. The protocol for this study was approved by the local Ethics Committee at Hospital de Pediatría "Prof. Dr. Juan P. Garrahan".

Molecular Analysis

DNA was isolated from leukocytes of peripheral whole blood of each patient using conventional techniques [15].

A total of 10 variants in five genes (8 single nucleotide polymorphisms (SNPs) and two insertion/deletion) were assessed. TPMT*3A (c.615 G>A (rs1800460) and c.874 A>G (rs1142345), TPMT*3B (c.615 G>A), TPMT*3C (c.393 G>C) (rs1142345) genetic variants for the *TPMT* gene, c.665C>T (C677T, rs1801133) and c.1286A>C (A1298C, rs1801131) for *MTHFR,* and c.313A>G (p.Ile105Val) (rs1695) and c.341C>T (p.Ala114Val) (rs1138272) for the *GSTP1* gene were identified by PCR-RFLP[16-19] For the TPMT*2 (c.393 G>C) (rs1800462) variant, an allele-specific PCR was performed with minor modifications [16]. For the detection of the presence or absence of *GSTM1* or *GSTT1*, a multiplex PCR was carried out [20]. In the samples with presence of *GSTT1*, an additional gap PCR allowed the identification of three genotypes: wild type/wild type (wt/wt), wild type/deleted (wt/del), and deleted /deleted (del/del) [21]. For quality control purposes, for each batch of PCR-amplified samples, one sample containing a known genotype and one negative control were analyzed. In addition, duplicate analyses of around 20% of the samples, randomly selected, were performed, resulting in 100% agreement.

Toxicity Evaluation

Toxicity was evaluated in the consolidation phase (Phase M). Hematologic toxicity (anemia, leukopenia, granulocytopenia, thrombocytopenia), cutaneous and mucosal toxicity (stomatitis, diarrhea, skin changes), presence of infections and fever, renal toxicity (creatinine), liver toxicity (GOT/GPT ratio), cardiac toxicity (arrhythmias, heart function), and central and peripheral neurological toxicity were evaluated. Toxicity was graded following the World Health Organization (WHO) criteria (grades 0= null, 1 and 2= mild, 3 and 4= severe). Toxicity data were recorded on individual forms, once for each cycle, seven days after MTX administration, evaluating up to 4 cycles per patient. Toxicity evaluation was carried out in 161 patients. In total, 628 cycles were analyzed.

For statistical analysis, toxicity was defined as a dichotomous variable: presence of severe toxicity (grades 3 or 4, according to the WHO criteria) versus absence of severe toxicity (WHO grades: 0, 1, 2). Toxicity was evaluated by independent clinicians in a blinded fashion as to the patients' genotypes.

Survival Analysis

Survival analysis was performed in 202 patients accrued in the 12-ALLIC-02 protocol. Seventeen patients were excluded because of the following reasons: Down syndrome (9 patients); previous treatment (6 patients); change in lineage (1 patient), and chronic renal failure when ALL was diagnosed (1 patient). Event-free survival (EFS) was defined as the time from diagnosis to the first outcome event (relapse, second malignant neoplasms, or death in complete remission) or to the last follow-up date; induction failure and death during induction were considered events at time zero.

Statistical Analysis

MTHFR, TPMT, GSTP1, and GSTT1 genetic variants were tested for the Hardy–Weinberg equilibrium using the Chi-squared test. The association between toxicity and genotypes for TPMT and MTHFR was assessed with contingency tables and the Fisher's exact test. Stratified analyses were performed according to the MTX dose. Odds ratios and associated 95% confidence intervals (95% CIs) were reported as estimates of the association. Every variant was evaluated for association with every end point.

The total number of statistical tests performed was not quantified, and formal protection for multiple comparisons was not attempted. However, as a general protection against multiple comparisons, a two-sided significance level of 0.01 was used to show statistical significance for any finding. In those cases where a significant association was found using contingency tables (neutropenia and leukopenia), the exact Kruskal-Wallis test for contingency tables with an ordinal discrete variable (number of events of severe toxicity)

was performed. This analysis also takes into account that some patients repeated the toxicity episode while others only showed the adverse event once during the 4 cycles analyzed.

The probability of event free survival (pEFS) was estimated by Kaplan-Meier analysis [22]. Differences on survival curves were assessed by the log-rank test [23]. Cox proportional hazard analysis was used to model the risk of event given the different genotypes with the clinically relevant covariates: age, risk group, and bone marrow at day 15. The three clinically relevant covariates were chosen by stepwise Cox regression, backward elimination. The analyses were performed using the SPSS statistical package (version 15).

RESULTS

Patient Characteristics

The genotype distribution of *MTHFR*, *TPMT* and *GSTs* variants in patients of protocols 12-ALLIC-2002 and 1-LLA-96 are summarized in Table I. Both protocols showed a similar genotype distribution and the frequencies of all variants were in Hardy–Weinberg equilibrium ($p>0.05$). The pattern of distribution was similar to other population reports, especially those of Caucasian origin [17,24-28]

Toxicity was evaluated in 161 patients. The distribution of patients in the different protocols, risk-group stratification, and administered treatment are summarized in Table II. The probability of Event-free survival data were assessed in 202 patients. For these patients, median age at diagnosis was 5.0 years (range, 1.1 to 17.2 years). The percentage of good responders to prednisone therapy was 92.1%. The median follow-up time was 56 months (range, 0–87 months). The main clinical characteristics of these 202 patients are summarized in Table III.

Genetic Variants and Toxicity Events in the Consolidation Phase

Severe leukopenia and severe granulocytopenia were the most frequent adverse events found in this cohort followed by severe hepatoxicity, anemia, thrombocytopenia, infections, and mucositis (Figure 1). In general, all these toxicities were detected in a higher rate in the group of patients receiving MTX at 5 g/m^2, and toxicity was more severe when high-risk blocks were administered to high-risk patients combining MTX at 5 g/m^2 with other chemotherapeutic agents.

In regard to the less common toxicities (presence of severe nausea, vomiting, diarrhea, or skin alterations), they were only detected in children who received MTX at 5 g/m^2. None of the 161 patients that were evaluated for toxicity presented mild or severe alterations of renal function, as measured by serum creatinine levels, or cardiotoxicity.

The influence of genetic variants on toxicity events was assessed in the three most frequent toxicities found in this cohort: hematological toxicity, hepatotoxicity, and mucositis.

Toxicity and MTHFR genetic variants

Children who received 2 g/m²/day of MTX and carried at least one 677T allele variant in *MTHFR* showed a significantly increased risk of developing severe leukopenia (p=0.004) during the consolidation phase (Figure 2A). Similar results were observed for *MTHFR* variants and severe neutropenia (Figure 2C). However, *MTHFR* polymorphisms did not seem to modulate MTX toxicity in patients who received 5 g/m²/day (Figure 2B and 2D).

Moreover, repeated episodes of toxicity in a single patient over a course of MTX are clinically less favorable. In our series, 13.3% of patients presented severe leukopenia in two of the four cycles evaluated. Only 2.4% of the patients presented severe leukopenia in all four cycles of MTX. About 25% of patients had one or two events of neutropenia (grade 3 or 4) and only 1.2% of patients experienced four episodes of severe neutropenia. Kruskall-Wallis analysis also showed that patients with the presence of at least one T

allele at position 677 showed a significantly higher frequency of severe neutropenia and leukopenia episodes when receiving 2 g/m^2/day of MTX ($p = 0.004$ for leukopenia and $p = 0.01$ for neutropenia).

The genetic variant C677T did not seem to modulate the incidence of severe anemia, severe thrombocytopenia, or the development of mucositis or severe hepatotoxicity. No significant association was found between the A1298C variant and the toxicities assessed.

Toxicity and TPMT Genetic Variants

The influence of *TPMT* variants on toxicity events was evaluated in 148 patients who received 6-MP (25 mg/m^2/day) during the consolidation phase. In the group of patients that carried variants in the *TPMT* gene (most of them heterozygous for *TPMT* variants) there was a higher proportion of severe hematologic toxicity, although the difference was not statistically significant (Figure 3). The presence of *TPMT* variants did not have any impact on the development of mucositis or hepatotoxicity.

Genetic Variants and Survival Analysis

The total number of events observed in 202 evaluable patients was 46 (22.8%). The most frequent adverse event was relapse (16.8%), mainly bone marrow relapse (55.8%), followed by testicular (8/46) and CNS relapse (2/46). A total of 3.9% of the children died during complete remission, 1.4% died during the induction phase, and 0.4% presented a second malignant disease. The pEFS for the evaluable patients according to risk groups was different: Standard Risk (86%), Intermediate Risk (73%) and High Risk (69%) ($p=0.1$).

No associations were found among the evaluated genetic variants and pEFS in the survival analysis, either in univariate or in multivariate analysis adjusted for the clinically relevant covariates (Figure 4).

DISCUSSION

The present study evaluated the influence of genetic variants of *MTHFR, TPMT* and *GSTs* genes on patients' response to ALL treatment, both in terms of toxicity and efficacy.

Regarding *MTHFR* genetic variants and drug-related toxicity, it was found that children who received 2 g/m²/day of methotrexate (MTX) and carried at least one 677T allele variant in *MTHFR* showed a significantly increased risk of developing severe leukopenia (leukocyte count less than $2.0x10^9$/L) and severe neutropenia (neutrophil count less than $1.0x10^9$/L) during the consolidation phase. Otherwise, the 677T allele variant did not seem to modulate the presence of severe adverse events in patients who received 5 g /m^2 of MTX. Probably the T677 variant in the *MTHFR* gene behaves as a low-penetrance allele and therefore, its effect may only be observed at lower doses (2 g/m^2/day). At a higher dose (5g/m^2/day), it is likely that other factors, such as the cumulative MTX dose, the patient's nutritional status and overall condition, liver function as well as other, until now unknown genetic factors, have a strong association with the presence of severe toxicity.

MTX is an antimetabolite that alters folate metabolism by inhibiting dihydrofolate reductase and thymidylate synthase, thereby preventing the synthesis of DNA precursors and thus inhibiting cell proliferation [29]. The presence of the MTHFR enzyme of a lower activity due to the C677T variant contributes to an increase of the imbalance of intracellular folate levels generated by MTX; additionally, it is associated with an increase in homocysteine, which can induce apoptosis [30]. Probably, as a consequence of these effects, more hematologic toxicity events were observed in our series. The contribution of this variant to an increased sensitivity to MTX was detected in a pilot study *ex vivo* using cryopreserved lymphoblasts from children with ALL, which had MTX-related toxicity [8]. This observation, however, could not be confirmed in another study performed *ex vivo* in a larger population of children with ALL, probably because the experiments performed used

high levels of folate, which possibly masked the changes in the folate distribution induced by the polymorphism [31].

The findings of the current study related to *MTHFR* variants are in concordance with those reported by D´ Angelo *et al* 2001 [32] and Ongaro *et al* 2009 [33]. In these studies, in agreement with our findings, the patients with TT or CT genotypes for the C677T variant had a higher risk of hematologic toxicity. Moreover, Liu *et al* 2011 [34], who also evaluated toxicity during the consolidation phase, did not find any association between the C677T variant and the presence of toxicity when 5 g/m^2 of MTX was administered. In line with the finding that increased toxicity was associated with the *MTHFR* genetic variant shown by the clinical studies, Celtikci *et al* [30] also showed in animal studies that the presence of reduced MTHFR activity, either by deficiency of one or both alleles, increased myelosuppression and apoptosis induced by MTX. On the other hand, an American and a French-Canadian pharmacogenetic studies did not find any association between the C677T variant and the presence of severe toxicity [35,36]. The controversial results shown by various reports may be due to several reasons: small population size, the different clinical setting and the treatment schedule, demographic characteristics of patients, the phase of treatment evaluated, etc, [35,36].

Regarding the A1298C variant in *MTHFR*, it does not appear to influence the presence of adverse events when it was analyzed independently, as described in the majority of the studies evaluating the role of this polymorphism [32].

The TPMT pathway is the main mechanism of intracellular inactivation of 6-MP in hematopoietic tissues [37]. The presence of polymorphisms in *TPMT* determines low enzymatic activity, lower metabolic inactivation, and higher systemic exposure to thioguanine nucleotides, predisposing patients to severe hematologic toxicity [25,38,39]. In a study including patients from the protocol Total XII from St Jude Children´s Hospital, the authors concluded that patients with a heterozygous genotype for *TPMT* need a 35%-

50% decrease in doses of 6- MP. [25] .Moreover, TPMT deficient patients require approximately a 10-fold dose reduction of the drug to prevent hematopoietic toxicity[40]. This dose adjustment based on *TPMT* genotype did not affect event-free survival of patients [41]. In our study, among children with hematologic toxicity grade 3 or 4, a higher proportion of heterozygous for *TPMT* variants was found, although it was not statistically significant. This finding was expected since during the consolidation phase the dose administered of 6-MP was three times lower than the dose considered (75 mg/m^2/day) in the St Jude Hospital´s study [25,40]. The absence of an association between the *TPMT* heterozygous genotype and toxicity was also described by members of the BFM group who used 6-MP at doses not higher than 60/m^2/day [24].

In reference to the association between the studied genetic variants and treatment efficacy, our analysis showed that the evaluable patients of the three risk groups presented a different pEFS. The most frequent event observed was bone marrow relapse. In the evaluated patients, the variants in *MTHFR*, *TPMT* and *GSTs* did not significantly affect the pEFS in univariate analysis, not even when the COX model, which included the previously defined covariables (age at the moment of diagnosis and bone marrow on day 15), was built.

Although survival analysis did not show a significant association with any of the genetic variants, the survival curves by *MTHFR* or *TPMT* genotype showed an interesting distribution. The intermediate-risk patients TT homozygous for the C677T variant had a higher pEFS than the patients with the CC or CT genotype. In line with this finding, de Deus [42] reported that Brazilian pediatric patients with ALL with the 677TT genotype showed a better overall survival than patients with the 677CC or 677CT genotypes for the *MTHFR* gene. Recently, the BFM group using a candidate gene approach reported that patients in the standard and intermediate risk groups who carried the AA genotype for the

A1298C variant in *MTHFR*, presented with a better pEFS [43]. Interestingly, the *677T* and *1298A* alleles have been found to be in strong linkage disequilibrium [44].

Regarding the *TPMT* genetic variants, it was observed that intermediate-risk group patients carrying a wild type genotype for *TPMT* presented a lower (not significant) pEFS than heterozygous patients. The presence of variants in *TPMT* determines a lower methylation grade of 6-MP and higher intracellular levels of thioguanine nucleotides, responsible for the antileukemic effect of this antimetabolite. This would explain a better treatment outcome in patients with genetic variants in *TPMT*. Stanulla *et al* 2005 [24] reported that patients heterozygous for allelic variants of *TPMT* had a significantly lower rate of minimal residual disease positivity compared to patients with homozygous wild-type alleles on day 78. This finding suggested that the 6-MP dose in patients with the wild type genotype would not be enough.

The results obtained in the present study offer an important contribution to a better understanding of the treatment response of a group of children with ALL diagnosed and treated at Hospital de Pediatría “Prof. Dr. Juan P. Garrahan”. Furthermore, they represent the initial steps toward applied pharmacogenetic studies in a group of patients treated homogenously in the field of pediatric oncology in Argentina. Genotyping of *MTHFR* polymorphisms might be useful to optimize consolidation therapy, reducing the associated severe hematologic toxicity. On the basis of the data obtained in this study, the potential pharmacogenetic role of the variants evaluated as genetic determinants of the efficacy and toxicity of ALL therapy remains quite uncertain and any definitive conclusion should be drawn with caution. It would be necessary to study these polymorphisms in a larger population of patients under similar treatment conditions in order to validate the results presented in our series. The technological advances for the identification of other genetic variants and a better comprehension of the application of statistical tools will lead to the development of new models that would optimize patient characterization and thereby

contribute to improving their quality of life by means of tailored drug and dose selection to be used in each particular case.

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FIGURE LEGENDS

Figure 1. Distribution of at least one Severe Toxicity Event in Patients with ALL during the Consolidation Phase. Severe Anemia: hemoglobin <8 g/dL; Severe Leukopenia: WBC < $2.0x10^9$/L; Neutropenia Grade 3 (G3): 0.5-0.9 $x10^9$/L, and grade 4 (G4): < 0.5 $x10^9$/L; Severe Thrombocytopenia < 50 $x10^9$/L, following WHO criteria.

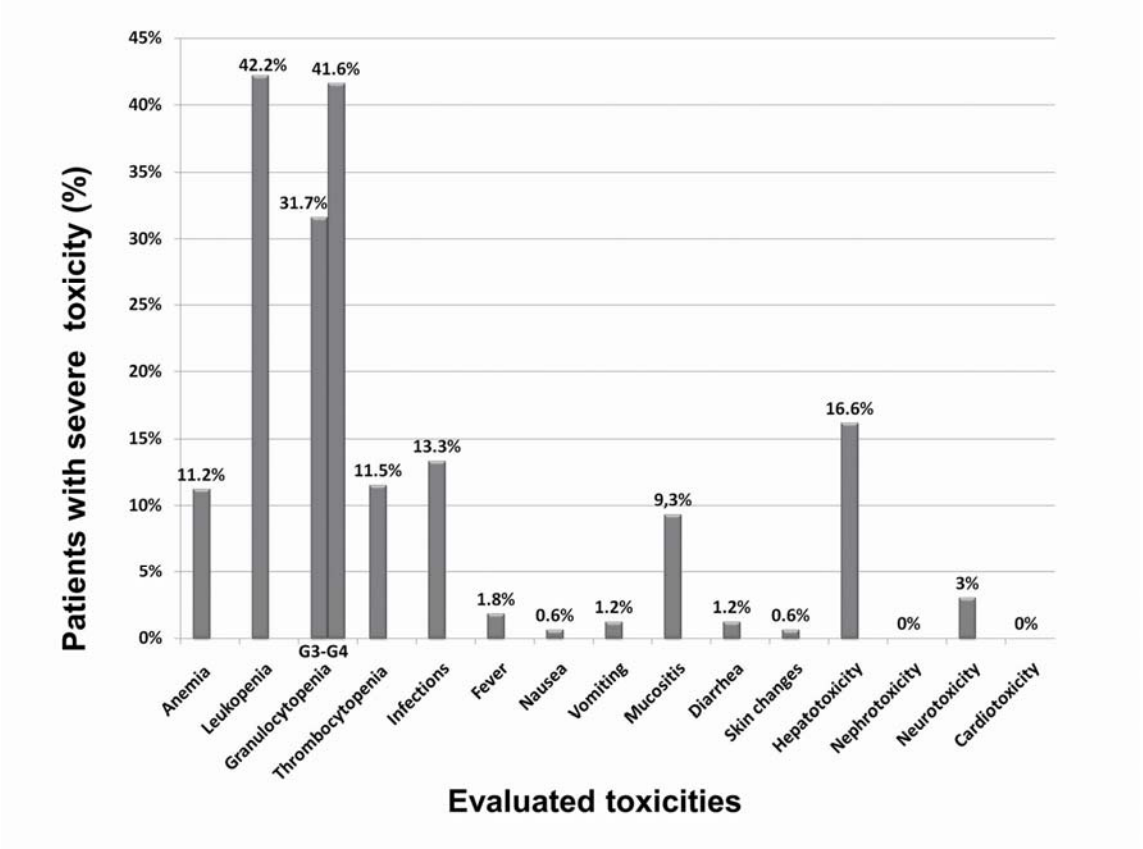


Figure 2. Distribution of Severe Leukopenia and Neutropenia (Events according to C677T Variant in *MTHFR.* A and C: Patients who received 2 g/m^2/day of MTX. **B and D**: Patients who received 5 g/m^2/day of MTX. Severe leukopenia: leukocyte count less than 2.0x10^9/L. Severe neutropenia: neutrophil count less than 1.0x10^9/L.

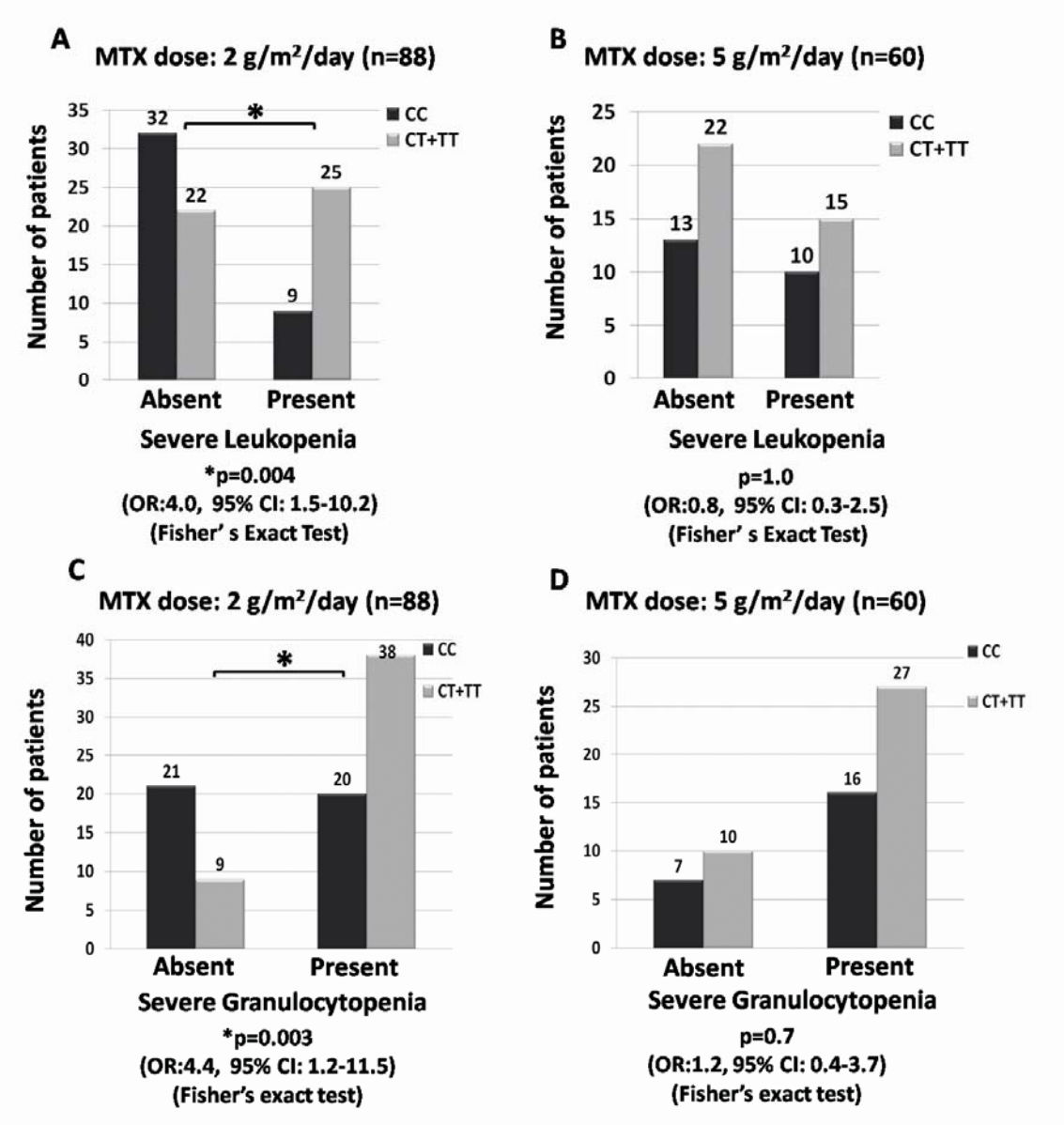


Figure 3. Distribution of Hematologic Toxicity Events according to *TPMT* Genotypes in 148 patients. **TPMTHH:** TPMT*3A, *3B, *3C or *2 variants in heterozygous (n=23) or homozygous (n=1) state for *TPMT*. **TPMTWT**: *absence of* the analyzed genetic variants (n=124). **Severe leukopenia**: leukocyte count less than $2.0x10^9$/L; **severe neutropenia**: neutrophil count less than $1.0x10^9$/L; **severe anemia**: hemoglobin <8 g/dL; **severe thrombocytopenia**: < 50 $x10^9$/L, following WHO criteria.

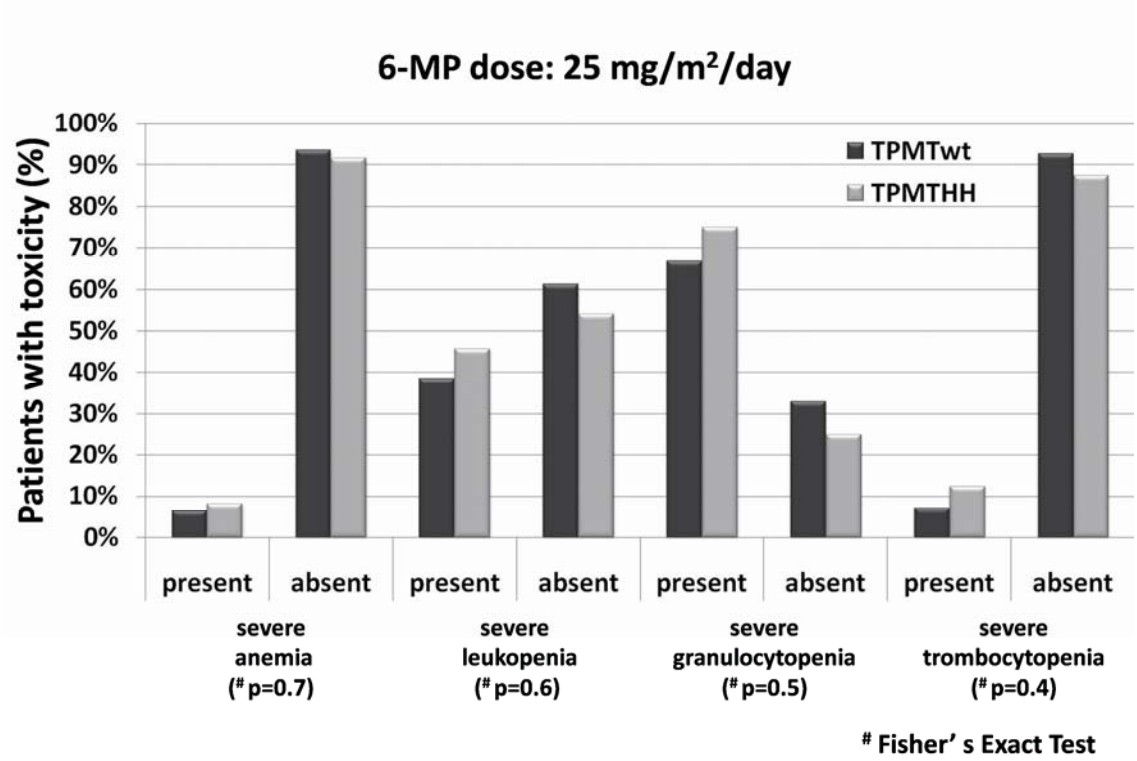
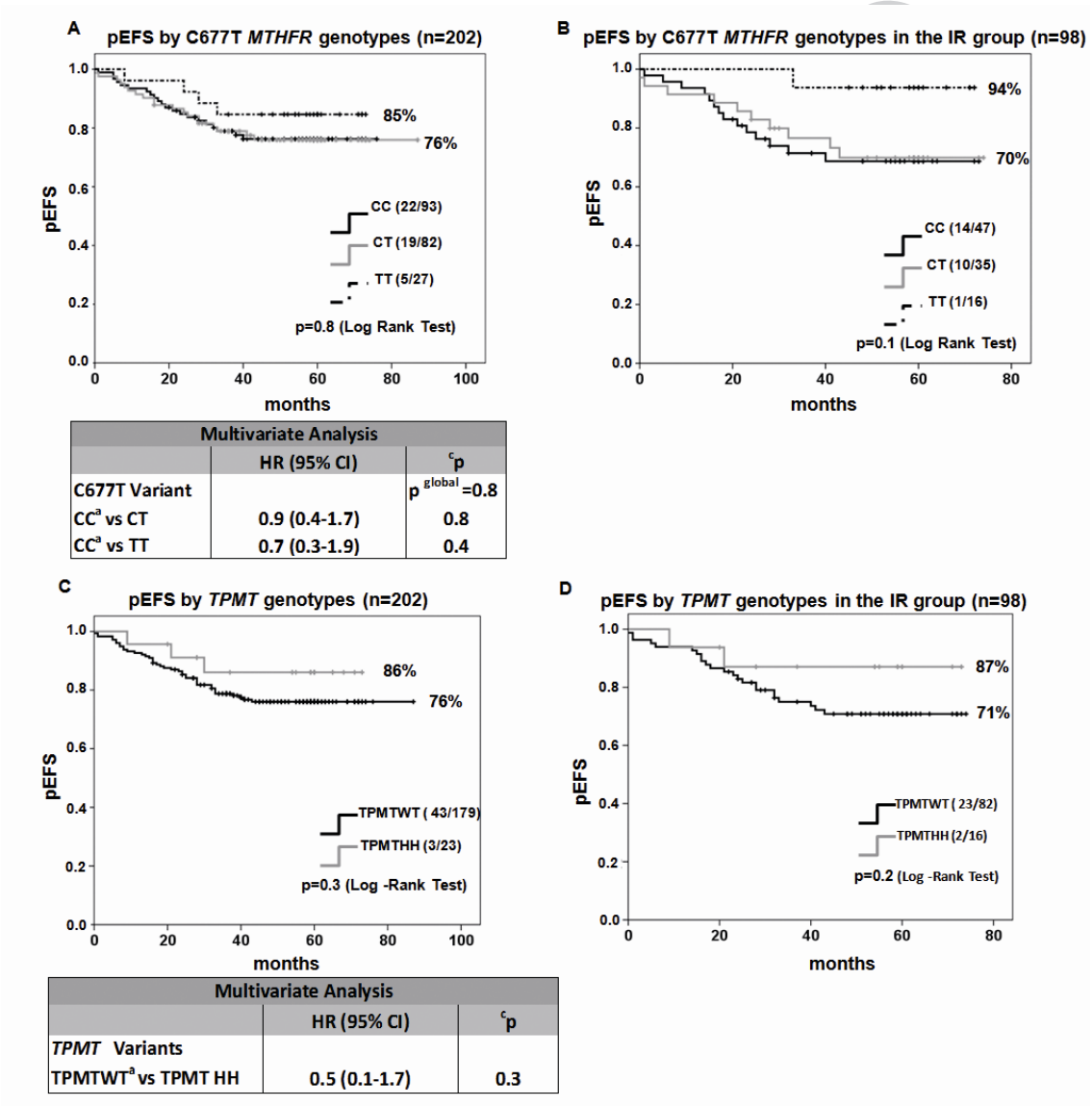


Figure 4. Genetic Variants and Survival Analysis. Survival curves according to MTHFR (A) or TPMT (C) genotypes for all evaluated patients. Survival curves according to MTHFR (B) or TPMT (D) genotypes for Intermediate-Risk (IR) patients. TPMTHH: TPMT*3A, *3B, *3C or *2 variants in heterozygous or homozygous state for TPMT. TPMTWT: absence of the analyzed genetic variants. pEFS: probability of event free survival. aReference category. cAdjusted by risk, age and bone marrow at day15.



Multivariate Analysis		
	HR (95% CI)	[c]p
C677T Variant		p^{global}=0.8
CC[a] vs CT	0.9 (0.4-1.7)	0.8
CC[a] vs TT	0.7 (0.3-1.9)	0.4

Multivariate Analysis		
	HR (95% CI)	[c]p
TPMT Variants		
TPMTWT[a] vs TPMT HH	0.5 (0.1-1.7)	0.3

TABLE LEGENDS

Table I. Genotype Distribution for MTHFR, TPMT and GSTs Genetic Variants in patients with ALL

Genetic Variants	12-ALLIC-2002 Protocol		1-LLA-96 Protocol	
MTHFR 677C > T (c.665C>T)				
CC	102	46.6%	26	38.8%
CT	88	40.2%	30	44.8%
TT	29	13.2%	11	14.3%
Total	219		67	
		*p=0.5		
MTHFR 1298 A > C (c.1286A>C)				
AA	137	62.6%	34	50.7%
AC	72	32.9%	31	46.3%
CC	10	4.6%	2	3.0%
Total	219		67	
		*p=0.1		
TPMT				
wild-type	196	89.5%	58	86.6%
Hetero for TPMT variants	22	10.0%	9	13.4%
Homo TPMT*3A	1	0.5%	0	
Total	219		67	
		*p=0.6		
GSTP1 A313 >G; ILE105VAL				
ILE/ILE	84	38.4%	26	38.8%
ILE/VAL	93	42.5%	30	44.8%
VAL/VAL	42	19.2%	11	16.4%
Total	219		67	
		*p=0.9		
GSTP1 C341 >T; ALA114VAL				
ALA/ALA	204	93.2%	64	95.5%
ALA/VAL	15	6.8%	3	4.5%
VAL/VAL	-	-	-	
Total	219		67	
		*p=0.6		
GSTM1 deletion				
nodel	102	46.6%	30	44.8%
del/del	117	53.4%	37	55.2%
Total	219		67	
		*p=0.8		
GSTT1 deletion				
nodel/nodel	99	45.2%	33	49.3%
nodel/del	91	41.6%	25	32.5%
del/del	29	13.2%	9	11.7%
Total	219		67	
		*p=0.8		

**1-ALL-96 vs 12-ALLIC2002; Fisher´s exact Test. TPMT variants: Hetero TPMT*3A, Hetero TPMT*3B,Hetero TPMT*3C and Hetero TPMT*2 . TPMT wild type: absence of the analyzed genetic variants. del: deletion; nodel: non deletion.*

Table II. Patient Distribution by Protocol, Risk Group and Treatment Scheme

Scheme received during Consolidation Phase	n	Treatment Protocol	SR	IR	HR
2 g MTX*	88	12-ALLIC 2002	26	57	-
		1-LLA 96	1	4[b]	-
5 g MTX*	60	12-ALLIC 2002	-	1[a]	-
		1-LLA 96	29	30	-
5 g MTX+ other drugs[#]	13	12-ALLIC 2002	-	-	10
		1-LLA 96	-	-	3

** patients also received 25mg/m^2/day of 6-MP y intratecal MTX. [a]Patient with T-ALL who received 5g/m^2/day of MTX. [b] Reduced dosis due to toxicity. SR: Standard Risk Group, IR: Intermediate Risk Group, HR: High Risk Group.[#]This group of patients was not considered for statistical analysis due to its small sample size.*

Table III. Clinical and Laboratory Data of the Patients under Protocol 12-ALLIC-2002 (n=202)			
Characteristic		*number of patients*	*%*
Sex			
	male	*107*	*53.0*
	female	*95*	*47.0*
Age at diagnosis (years old)			
	1< age < 6	*113*	*55.9*
	6 ≤ age < 10	*44*	*21.8*
	age ≥10	*45*	*22.3*
Initial WBC (x 10^9/L)			
	WBC <20/L	*130*	*64.4*
	20/L ≤ WBC <100/L	*48*	*23.8*
	WBC ≥100/L	*24*	*11.9*
Prednisone response			
	good#	*186*	*92.1*
	poor#	*16*	*7.9*
Immunophenotype			
	B	*180*	*89.1*
	T	*22*	*10.9*
DNA index			
	< 1	*2*	*1.1*
	1≤ index < 1.16	*138*	*73.0*
	≥1.16	*49*	*25.9*
	unkown	*13*	*-*
ETV6-RUNX1 (TEL-AML)			
	positive	*28*	*15.3*
	negative	*155*	*84.7*
	not evaluable	*19*	*-*
TCF3-PBX1 (E2A-PBX1)			
	positive	*10*	*5.5*
	negative	*173*	*94.5*
	not evaluable	*19*	*-*
BCR-ABL1(BCR-ABL)			
	positive	*3*	*1.6*
	negative	*180*	*98.4*
	not evaluable	*19*	*-*
MLL-AFF1 (MLL-AF4)			
	positive	*2*	*1.1*
	negative	*181*	*98.9*
	not evaluable	*19*	*-*
Day 15 Bone Marrow (BM) Response			
	M1 (< 5% of blasts in BM)	*151*	*74.8*
	M2 (5-25 of blasts in BM)	*34*	*16.8*
	M3 (>25% of blasts in BM)	*16*	*7.9*
	*ND**	*1*	*-*
Risk Group			
	Standart (S)	*75*	*37.1*
	Intermediate (I)	*98*	*48.5*
	High (H)	*29*	*14.4*
Events			
	absent	*156*	*77.2*
	present	*46*	*22.8*

#Good: <1000 leukemic blood blasts/µL on treatment day 8; poor: >1000/µL. *ND: no determinate. WBC white blood cells.