Pathology & Oncology Research (2020) 26:743–752
https://doi.org/10.1007/s12253-019-00613-4

ORIGINAL ARTICLE

Association of *SLC28A3* Gene Expression and *CYP2B6*6* Allele with the Response to Fludarabine Plus Cyclophosphamide in Chronic Lymphocytic Leukemia Patients

Vojin Vukovic[1] · Teodora Karan-Djurasevic[2] · Darko Antic[1,3] · Natasa Tosic[2] · Tatjana Kostic[2] · Irena Marjanovic[2] · Marija Dencic-Fekete[4] · Vladislava Djurasinovic[1,3] · Sonja Pavlovic[2] · Biljana Mihaljevic[1,3]

Received: 30 November 2018 / Accepted: 8 February 2019 / Published online: 18 February 2019

Abstract

Fludarabine plus cyclophosphamide (FC) chemotherapy is the basis of treatment protocols used in management of chronic lymphocytic leukemia (CLL). In some patients, response to therapy may be affected by aberrant function of genes involved in pharmacokinetics and pharmacodynamics of the drugs. The aim of this research was to assess the impact of pharmacogenetic variability, namely expression of *SLC28A3* gene and the presence of *CYP2B6*6* variant allele, on the FC treatment efficacy. Forty-four CLL patients with functional *TP53* gene at the time of FC initiation were enrolled in this study. *CYP2B6* genotyping was performed by polymerase chain reaction and direct sequencing. *SLC28A3* expression was measured by quantitative reverse-transcriptase polymerase chain reaction. Significantly higher pretreatment levels of *SLC28A3* mRNA were detected in patients who failed to respond to FC in comparison to patients who achieved complete and partial response ($p = 0.01$). *SLC28A3* high-expressing cases were almost ten times more likely not to respond to FC than low-expressing cases (OR = 9.8; $p = 0.046$). However, association of *SLC28A3* expression with progression-free survival (PFS) and overall survival (OS) was not observed. *CYP2B6*6* allele, detected in 24 patients (54.6%), exerted no association with the attainment of response to FC, as well as with PFS and OS. The results of this study demonstrate that *SLC28A3* expression is a significant predictor of FC efficacy in CLL patients with intact *TP53*. Elevated *SLC28A3* mRNA levels are associated with inferior short-term response to FC, suggesting that, if validated on larger cohorts, *SLC28A3* expression may become a biomarker useful for pretreatment stratification of patients.

Keywords Chronic lymphocytic leukemia · Fludarabine · Cyclophosphamide · *SLC28A3* expression · *CYP2B6*6* allele · Response to therapy

Introduction

Chronic lymphocytic leukemia (CLL) is an incurable clonal disorder of mature B lymphocytes with well-defined morphological, immunophenotypic and molecular features [1]. Clinical course of CLL widely varies between decades-lasting stable lymphocytosis and progressive, treatment-resistant disease, ending lethally within a short period of time. One of the cornerstones of CLL treatment in contemporary era represents a combination of purine analog fludarabine and alkylating agent cyclophosphamide (FC) [2–4]. Addition of anti-CD20 monoclonal antibody rituximab to FC (FCR) improved overall response (OR) and complete response (CR), and prolonged both progression-free survival (PFS) and overall survival (OS). Therefore, FCR was established as the first-

Vojin Vukovic and Teodora Karan-Djurasevic equally contributed to this work.

✉ Vojin Vukovic
vojinvukovic@yahoo.com

[1] Clinic for Hematology, Clinical Center of Serbia, Belgrade, Serbia

[2] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

[3] School of Medicine, University of Belgrade, Belgrade, Serbia

[4] Institute of Pathology, School of Medicine, University of Belgrade, Belgrade, Serbia

line treatment choice for fit younger CLL patients [5, 6]. Although the therapeutic landscape has significantly expanded with new targeted molecules, fludarabine and cyclophosphamide have kept their place in CLL treatment within FCR combination [7].

However, a significant proportion of patients (up to 25% of treatment-naive and over 40% of previously treated patients) do not respond well, or relapse early after administration of fludarabine-containing regimens [8, 9]. Monoallelic or biallelic loss of functional *TP53,* due to deletion of 17p13 and/or *TP53* mutations, is the most powerful predictive and prognostic adverse factor pointing to patients with poor or short-lasting response to fludarabine-based chemo(immuno)therapy, as well as shorter OS [10–13]. In the pretreatment phase, only 5 to 8% of patients harbour *TP53* aberrations, but this percentage increases in every subsequent treatment line to 44% in fludarabine-refractory patients [14–16]. On the other hand, molecular and genetic profiling of CLL does not enable precise identification of patients with intact *TP53* who would not respond well to fludarabine-based treatment.

Mechanisms of anti-CLL drugs action are ascertained, but the impact of their pharmacodynamics and pharmacokinetics on treatment outcome is fairly underresearched [17, 18]. Host pharmacogenetics can potentially influence efficacy of fludarabine and cyclophosphamide by affecting disposition and/or physiological effect of the drugs.

In order to exhibit its cytotoxic effect, fludarabine accumulates in CLL cells and, phosphorylated to active 5′ triphosphate form, competitively inhibits synthesis and repairment of DNA and RNA transcription [17]. Accumulation of fludarabine and other purine analogues is mediated by human equilibrative (hENTs) and human concentrative (hCNTs) nucleoside transporters, encoded by solute carrier 29 (*SLC29*) and solute carrier 28 (*SLC28*) gene families, respectively. Out of 7 identified NTs (hENT1–4 and hCNT1–3), 4 have been confirmed to play a role in fludarabine uptake in CLL cells: hENT1, hENT2, hCNT2 and hCNT3 [19, 20]. Since abnormal expression and/or NT activity could lead to insufficient uptake of fludarabine, investigations have been conducted in order to determine whether this could influence treatment outcome in CLL. In a comprehensive research by Mackey et al, expression of several genes whose products are involved in fludarabine intracellular accumulation and metabolism was correlated with outcome of CLL patients treated with fludarabine monotherapy. High expression of *SLC28A3* gene, coding for hCNT3, emerged as the only independent factor, predicting shorter time to disease progression and lower rate of CR [21].

Activation of cyclophosphamide, the other member of FC chemotherapy, requires its 4-hydroxylation which is catalyzed by several members of cytochrome P450 enzyme superfamily, including CYP2B6 isoform [18, 22]. *CYP2B6* gene is known to have more than 100 single nucleotide polymorphisms (SNPs), and more than 38 alleles (https://www.pharmvar.org/gene/CYP2B6). The most common variant allele is *CYP2B6*6*, occurring in 10% to over 60% of individuals in different populations [23]. *CYP2B6*6* is defined by the presence of 2 SNPs, c.516G > T in exon 4 (Q172H; rs3745274) and c.785A > G in exon 5 (K262R; rs2279343). Multiple studies have confirmed that CYP2B6*6 is associated with decreased in vitro and in vivo expression, in comparison to wild-type CYP2B6*1. The c.516G > T variant has been shown to affect pre-mRNA splicing, leading to 50–75% decrease in CYP2B6 protein level [24–27]. In vitro and in vivo studies have also indicated that Q172H and K262R amino acid substitutions alter CYP2B6 catalytic activity in a drug-specific manner, and that **6* allele is associated with a higher rate of 4-hydroxylation of cyclophosphamide [28–31]. Hence, a dilemma exists whether lower expression of CYP2B6*6 or its higher specific enzyme activity is more important for the final cytotoxic effect of cyclophosphamide. Johnson et al showed that CLL patients treated with FC exhibited inferior response if they expressed at least one **6* allele, marking *CYP2B6*6* as an independent predictive factor for treatment outcome [32].

The literature lacks a comprehensive analysis of the impact of host pharmacogenetics on standard treatment efficacy in CLL. In this study, we focused on a cohort of CLL patients without *TP53* abnormalities, who received FC at the time when rituximab was not accessible at our institution, with the aim of analysing the effect of *SLC28A3* expression and *CYP2B6*6* allele on treatment response. Since FC-based protocols are significantly toxic, it is of great importance to narrow the spectrum of patients who are eligible for this kind of treatment, in order to avoid unnecessary complications in the absence of therapeutic response. We tried to contribute to the achievement of this goal from the perspective of pharmacogenetics.

Materials and Methods

Patients and Samples

This study enrolled a total of 55 CLL patients from the Clinic for Hematology, Clinical Center of Serbia (Belgrade, Serbia), who were treated with fludarabine plus cyclophosphamide (FC). The patients were diagnosed with CLL according to the criteria of the National Cancer Institute-sponsored Working Group (NCI-WG) guidelines for CLL and update of these guidelines by the International Workshop on Chronic Lymphocytic Leukemia (IwCLL). The patients were characterized by typical immunophenotype of monoclonal B-cells with CLL score ≥ 4, less than 55% of prolymphocyte-like cells in peripheral blood smear, and > 5000×10^9/l of lymphocytes (Ly) in peripheral blood, with the exception of 2

patients who exhibited the pattern of small lymphocytic lymphoma [1, 33]. All the samples analysed in this study were collected prior to initiation of FC therapy. The research was conducted with the approval of the medical ethics committee of our institution, and in accordance with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study.

Evaluation of Response to FC

Response to treatment was evaluated according to the guidelines of NCI-WG and IwCLL, defining treatment outcomes as: complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) [1, 33]. Complete response (CR) was characterized by the absence of significant (>1.5 cm) lymphadenopathy and organomegaly, complete blood count reconstitution (>1500 of neutrophils per μL, <4000 of lymphocytes (Ly) per μL, hemoglobin (Hb) level > 110 g/L, and > 100,000 of platelets (PLT) per μL), <30% of bone marrow Ly without B-lymphoid nodules, and the absence of constitutional symptoms. Partial response (PR) was documented in patients who exhibited ≥50% decrement of pretreatment lymph nodes, spleen, and liver size if they were significantly enlarged initially, neutrophil count >1500/μL or increase of ≥50% over baseline, Ly count <4000/μL or decrease ≤50% of pretreatment count, Hb >110 g/L, PLT count >100,000/μL or ≥ 50% increment comparing to initial levels, as well as bone marrow infiltration ≥30% or presence of B-lymphoid nodules. Progressive disease (PD) was defined by at least one of the following: increase of lymph node, spleen or liver size for ≥50% over pretreatment diameters, ≥50% increase of Ly count (with absolute Ly count >5000/μL), decrease of Hb >2 g/L and/or decrease of PLT count ≥50% due to the bone marrow failure. Stable disease (SD) was considered as a response less than PR or progression without criteria for PD [1]. Patients who exhibited SD or PD following FC treatment were considered non-responders, as opposed to responders who achieved at least PR.

The duration of response was defined by progression-free survival (PFS) – the time from the initiation of FC to PD or death. Overall survival (OS) was measured from the time of diagnosis to the time of death from any cause or last follow-up. Time to first treatment (TTFT) was defined as time from diagnosis to the first therapy line [1].

Analytical Methods

For the purpose of *CYP2B6* genotyping, genomic DNA was isolated from peripheral blood samples using QIAamp DNA Blood Mini Kit (Qiagen). Detection of c.516G > T and c.785A > G was performed by amplification of exons 4 and 5 by polymerase chain reaction (PCR) using high-specificity primers designed by Lang et al, which disable coamplification of homologous pseudogene *CYP2B7* (CYP2B6-4F: 5'-GGTCTGCCCATCTATAAAC-3′; CYP2B6-4R: 5'-CTGATTCTTCACATGTCTGCG-3′; CYP2B6-5F: 5'-GACAGAAGGATGAGGGAGGAA-3′; CYP2B6-5R: 5'-CTCCCTCTGTCTTTCATTCTGT-3′) [27]. The obtained PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and directly sequenced with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in 3130 Genetic Analyzer (Applied Biosystems).

The expression of *SLC28A3* gene was analysed by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) using TaqMan chemistry. Mononuclear cells were isolated from peripheral blood samples by Ficoll-Paque Plus (GE Healthcare) density-gradient centrifugation and total cellular RNA was extracted using TRI reagent (Ambion). RNA was reverse-transcribed using RevertAid M-MuLV Reverse Transcriptase (Thermo Scientific) and random hexamer primers. qRT-PCR reactions were carried out using TaqMan Gene Expression Assay (Applied Biosystems) for *SLC28A3* (Hs00910439_m1) and TaqMan Universal Master Mix II (Applied Biosystems) in 7500 Real Time PCR system (Applied Biosystems). All samples were run in duplicates. Relative expression of *SLC28A3* mRNA was quantified by comparative ddCt method, using glyceraldehyde phosphate dehydrogenase (*GAPDH*) as endogenous control gene and HL-60 cell line as the calibrator.

Common cytogenetic abnormalities associated with CLL (deletions of 13q14, 17p13,11q22-q23 and trisomy 12q) were detected by fluorescence in-situ hybridization (FISH) on interphase nuclei obtained from peripheral blood, using a panel of locus-specific probes (Vysis/Abbott Laboratories). The *TP53* mutational status was determined by PCR amplification of coding exons 4–10 and flanking intronic regions followed by direct sequencing, as recommended in Pospisilova et al [34]. The analysis and the interpretation of the obtained results were performed using GLASS software (http://bat.infspire.org/genomepd/glass) and locus-specific IARC database (http://p53.iarc.fr/TP53GeneVariations.aspx). The percentage of CLL cells and CD38 expression were determined by flow cytometry [35, 36]. Immunoglobulin heavy variable gene (*IGHV*) mutational status was analysed as recommended in Ghia et al [37].

Statistical Analysis

Categorical data, presented as absolute numbers and frequencies, were tested using χ^2 test, Fisher's exact test and receiver operating characteristic (ROC) analysis. Continuous data, presented using medians and ranges, were analysed by Student's t test and Mann-Whitney rank-sum test. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the impact of clinical and genetic variables on response to FC therapy. Univariate analyses were conducted using Fisher's exact test, and multivariate analyses using logistic regression.

CYP2B6 genotype distributions were tested for conformity to Hardy-Weinberg equilibrium using χ^2 test. Haplotype phases and frequencies were estimated using Arlequin software (http://cmpg.unibe.ch/software/arlequin35/).

Comparison of TTFT, PFS, and OS between groups of patients was conducted using Kaplan-Meier method and log-rank test.

Statistical tests and creation of graphs were carried out using Sigma Stat 3.5 software (Systat Software Inc.). For all analyses, p values were 2-tailed and significance was defined as $p < 0.05$.

Results

Patient Demographics and Response to FC Treatment

Fifty-five CLL patients who received FC chemotherapy were initially involved in this study. In order to eliminate the possibility that loss or functional defect of *TP53* gene influenced response to treatment, the patients' samples were first retrospectively analysed for the presence of *TP53* mutations. Based on the obtained results and the results of FISH performed before the initiation of FC therapy, carriers of del17p13 and/or *TP53* mutations were excluded from further analysis. Ultimately, the study cohort consisted of 44 subjects, 33 men and 11 women. Baseline patients' characteristics at diagnosis, as well as at the time of FC initiation, are summarized in Table 1.

The majority of patients in our cohort (36 patients; 81.8%) received FC as first-line treatment, 4 patients (9.1%) as second-line, and 4 patients (9.1%) as third- and fourth-line. Median cumulative dose of administered fludarabine per patient was 450 mg/m^2 intravenously (range 100–450 mg/m^2), while median dose of cyclophosphamide per patient was 4500 mg/m^2 intravenously (range 900–4500 mg/m^2). Up to 6 cycles of FC were given (median 6, range 2–6). TTFT ranged from 1 to 71 months (median 6.5 months).

CR was observed in 18 patients (40.9%), PR in 18 patients (40.9%), SD in 5 patients (11.4%) and PD in 3 patients (6.8%). PFS (data available for 39 patients) ranged from 3 to 90 months (median 29 months); 4 patients were still in remission at the time of this research. OS (data available for 41 patients) ranged from 15 to 142 months (median 73 months).

Relationship between *CYP2B6*6* Allele and Clinical Outcome

The genotypes of the two *CYP2B6* SNPs were determined in all 44 subjects, and were found to be in conformance to Hardy-Weinberg equilibrium. The c.516G > T substitution was detected in 24 patients (54.6%), homozygous in 1 patient and heterozygous in 23 patients. The same result was obtained for c.785A > G substitution.

Assessment of *CYP2B6* allelotype was performed using Arlequin software. Since the investigated SNPs were found to be in complete linkage disequilibrium (D' = 1, $r^2 = 1$), only two haplotypes were deduced as the most probable: G_{516} A_{785} (interpreted as wild-type *CYP2B6*1* allele), and T_{516} G_{785} (interpreted as *CYP2B6*6* allele). The frequencies of haplotypic combinations **1/*1*, **1/*6* and **6/*6* were 45.4%, 52.3% and 2.3%, respectively.

In further analyses, 23 **1/*6* cases and 1 **6/*6* case were grouped together, as carriers of at least one *CYP2B6*6* variant allele.

The groups of **1/*1* and **6* carriers were first compared in order to establish whether they were different regarding clinical and biological features, namely age, gender, blood cell counts, Binet stage, β2-microglobulin and lactate dehydrogenase (LDH) levels, CD38 status, chromosomal abnormalities, and *IGHV* mutational status. No statistically significant differences were observed between these two groups of patients in terms of baseline characteristics, both at diagnosis and at the initiation of FC therapy. In addition, no significant difference in TTFT was detected.

Regarding efficacy of FC treatment, *CYP2B6*6* variant allele exerted no association with the achievement of response (CR and PR) vs no response (SD and PD). Furthermore, no association of *CYP2B6*6* with the number of treatment cycles administered, as well as with PFS and OS was observed.

Relationship between *SLC28A3* Expression and Clinical Outcome

The relative expression of *SLC28A3* was first dichotomised at the median value in order to compare the baseline characteristics of low-expressing and high-expressing cases. None of the clinical and biological parameters investigated (age, gender, blood cell counts, Binet stage, β2-microglobulin and LDH levels, CD38 status, chromosomal abnormalities, *IGHV* mutational status, TTFT) showed significant association with the expression level of *SLC28A3*, leading to conclusion that the groups of low-expressing and high-expressing cases were similar in terms of baseline characteristic, both at diagnosis and at the FC initiation. In addition, *SLC28A3* expression was not associated with *CYP2B6* allelotype.

Expression analysis of *SLC28A3* revealed significantly higher levels of *SLC28A3* mRNA in CLL patients who failed to respond to FC chemotherapy and experienced SD and PD following treatment (non-responders), in comparison to patients who achieved complete and partial response (responders) ($p = 0.01$) (Fig. 1). ROC analysis showed that *SLC28A3* expression prior to FC administration is a good discriminator of patients who will and patients who will not respond to treatment (Fig. 2). However, association of

Table 1 Clinical and biological characteristics of CLL patients

	at diagnosis	at FC initiation
no. of patients	44	44
Gender (male/female), no.	33/11	33/11
Age (years), median (range)	56.5 (38–75)	57.5 (39–76)
WBC [$\times 10^9$/l], ($n = 43/n = 37$)[a], median (range)	47.1 (7.1–570)	120 (7.1–570)
Ly [$\times 10^9$/l], ($n = 39/n = 34$)[a], median (range)	39.1 (2.7–558.6)	114.7 (2.7–558.6)
β2-microglobulin [mg/l] ($n = 32/ n = 25$)[a], median (range)	3.965 (0.2–11.5)	4.57 (0.2–11.5)
LDH [U/l], ($n = 38/n = 27$)[a], median (range)	382.5 (81–1042)	441 (81–1032)
Binet stage ($n = 43/n = 39$)[a], no.(%)		
A	12 (27.9%)	3 (7.7%)
B	22 (51.2%)	22 (56.4%)
C	9 (20.9%)	14 (35.9%)
CD38 status ($n = 36$), no.(%)		
positive (≥30%)	9 (25%)	–
negative (< 30%)	27 (75%)	–
Chromosomal aberrations ($n = 44$), no.(%)		
deletion 11q22-q23	–	9 (20.4%)
trisomy 12q	–	5 (11.4%)
deletion 13q14 (single)[b]	–	7 (15.9%)
no aberrations	–	23 (52.3%)
IGHV mutational status ($n = 43$), no.(%)		
mutated	7 (16.3%)	7 (16.3%)
unmutated	36 (83.7%)	36 (83.7%)

Abbreviations: *WBC* white blood cells, *Ly* lymphocytes, *LDH* lactate dehydrogenase, *IGHV* immunoglobulin heavy variable gene

[a] number of patients for whom the values of the given parameter at diagnosis/at FC initiation were available

[b] deletion 13q14 occurring as sole chromosomal abnormality detected by FISH

SLC28A3 mRNA levels with the number of FC cycles administered, PFS and OS was not observed in our cohort.

At the univariate level, the only statistically significant predictors of response to FC were found to be the line of therapy and *SLC28A3* expression (Table 2). Patients in 2nd and later-line treatment, as well as *SLC28A3* high-expressing patients, were more likely to fail to respond to FC when compared to patients in 1st line treatment and *SLC28A3* low-expressing patients (OR = 8; 95% CI = 1.42–45.23; $p = 0.026$ for treatment line and OR = 9.8; 95% CI = 1.09–88.23; $p = 0.046$ for *SLC28A3*). In multivariate analysis controlling for age, Binet stage and deletion 11q22-q23, only *SLC28A3* expression exerted a trend towards an independent association with the response to FC, but without reaching statistical significance (OR = 8.77; 95% CI = 0.76–101.49; $p = 0.082$) (Table 2).

Discussion

Extreme clinical heterogeneity of CLL has inevitably imposed the need of personalised approach to the management of patients. Molecular and cellular markers used in clinical practice (cytogenetic abnormalities, *IGHV* mutational status, CD38 expression) exert high prognostic value, but their predictive significance, in the context of currently available treatment protocols, is still under evaluation [1, 38, 39]. At present, p53 deficiency (due to del17p13 and/or *TP53* mutations) is the only predictive marker that has been validated and translated into treatment decision-making [10, 40].

Besides the biomarkers that reflect the pathobiology of the malignant clone itself, germline and somatic mutations, as well as aberrant expression of pharmacogene(s) can severely impact the efficacy of the chosen therapy. Although there is growing evidence that patient-to-patient variability in the treatment outcome may stem, at least in part, from the variability in the host pharmacogenetics, the data for CLL are still scarce and incomplete.

In this study, we investigated two pharmacogenetic markers, *CYP2B6*6* allele and *SLC28A3* expression, that have been previously implicated in CLL. Our aim was to evaluate their association with the response to therapy in patients treated with fludarabine plus cyclophosphamide combination. Since p53 status is the main determinant of FC efficacy, only patients with functional *TP53* at the time of FC initiation were included into the study cohort.

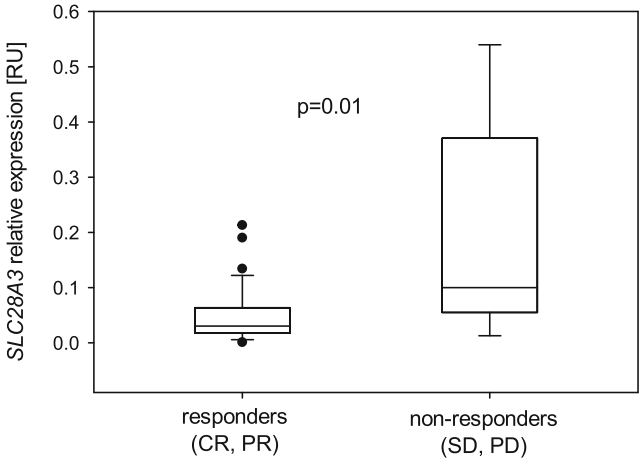

Fig. 1 Relative expression of *SLC28A3* mRNA in mononuclear cells of responders vs non-responders to FC chemotherapy. qRT-PCR analysis showed a significantly higher expression of *SLC28A3* in CLL patients who experienced stable disease and progressive disease following FC treatment (non-responders) in comparison to patients who achieved partial and complete response (responders) ($p = 0.01$; Mann-Whitney Rank Sum Test). Abbreviations: SD = stable disease; PD = progressive disease; CR = complete response; PR = partial response; RU = relative units

*CYP2B6*6* allele was detected in 54.6% of our CLL patients (52.3% heterozygotes and 2.3% homozygotes), which is frequency higher than expected in Caucasian populations [23, 27]. This result may be a consequence of the relatively small size of our cohort but, on the other hand, high incidence of *CYP2B6*6* allele may imply its link with elevated susceptibility to CLL. *CYP2B6* SNPs and specific haplotypes, associated with reduced activity, have already been suggested as risk factors predisposing to the development of certain solid tumors and hematological malignancies [41–45]. We did not further explore this issue, since it was beyond the scope of this study.

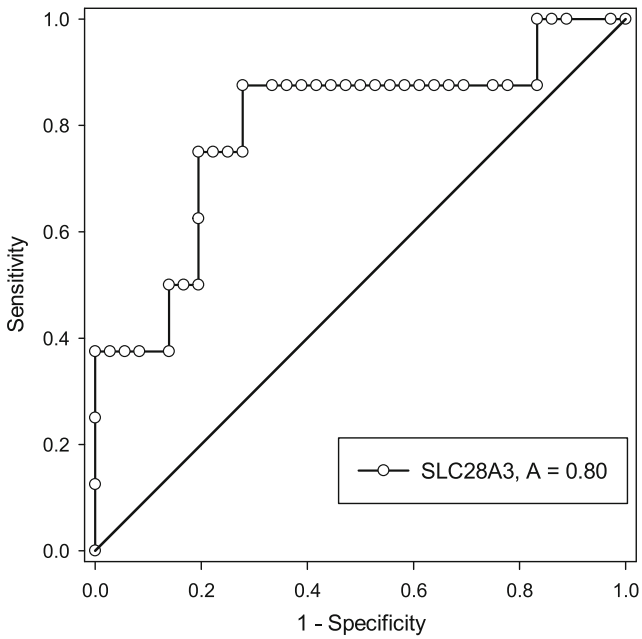

Fig. 2 ROC analysis of *SLC28A3* expression in responders vs non-responders to FC chemotherapy. High level of *SLC28A3* mRNA expression was found to be a good predictor of failure to achieve complete response and partial response to FC treatment (A = 0.80; 95% CI = 0.6031–0.9872; $p = 0.009697$; sensitivity = 0.8750; specificity = 0.7222). Abbreviations: ROC = receiver operating characteristic; A = area under the ROC curve; CI = confidence interval

The importance of *CYP2B6* pharmacogenetics for the treatment outcome has not been extensively studied in CLL. The only research that investigated the value of *CYP2B6*6* allele as a predictive biomarker was conducted by Johnson et al, involving patients recruited within LRF CLL4 trial [3, 32]. The authors showed that *CYP2B6*6* allele was an independent determinant of inferior response to FC; the carriers of at least one **6* allele were less likely to achieve complete response to FC and had fewer adverse events related to the toxicity of FC in comparison to **1/*1* carriers, indicating decreased efficacy of cyclophosphamide activation in these patients. In addition, the authors observed a trend, although non-significant, towards shorter PFS in **6* carriers. Similarly to the study of Johnson et al, in our study baseline characteristics of patients and treatment exposure were not significantly different between **1/*1* and **6* carriers. However, we could not corroborate other findings of the aforementioned research. In our cohort, no association was detected between *CYP2B6*6* allelotype and the response to FC treatment, as well as with PFS and OS. The possible cause of this discrepancy may be the substantial difference in cohort sizes between our study and Johnson et al publication, since our two cohorts were comparable in terms of patients' gender and dosing regimen, both of which have previously been reported to affect pharmacokinetics of cyclophosphamide [46–50].

We have also investigated the effect of *SLC28A3* gene expression, whose protein product hCNT3 is involved in cellular uptake of fludarabine. Reduced intracellular accumulation is one of the mechanisms of resistance to fludarabine, and it has been previously documented that *SLC28A3* exerts strong association with the treatment outcome [17, 21]. CLL patients expressing high levels of *SLC28A3* mRNA have been shown to be less likely to achieve complete response, and had shorter PFS following fludarabine monotherapy than patients with low levels of *SLC28A3* mRNA [21]. In addition, quantification of hCNT3 protein in pre-treatment tissue samples also revealed an independent association of high hCNT3 levels with shorter PFS and a trend towards lower response rates in patients treated with fludarabine [51].

The results of our study show that *SLC28A3* expression affects, in a similar manner, the response to FC combination. The level of *SLC28A3* mRNA, measured in samples obtained prior to FC initiation, was found to be a significant predictor of response to treatment, since in our cohort *SLC28A3* high-expressing patients were almost 10 times more likely not to

Table 2 Analysis of variables associated with nonattainment of response to FC treatment

Variable *	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
Age (>57.5 vs ≤57.5)	0.54	0.11–2.59	0.698	0.96	0.83–1.09	0.505
Gender (M vs F)	1.0	0.17–5.87	1			
Binet stage (B + C vs A)	0.32	0.02–4.26	0.403	1.16	0.07–20.08	0.920
CD38 status (positive vs negative)	0.7	0.12–4.18	1			
Deletion 11q22-q23 (carriers vs non-carriers)	1.38	0.23–8.36	0.659	1.34	0.45–3.96	0.597
IGHV mutational status (unmutated vs mutated)	1.2	0.12–11.87	1			
Line of treatment (≥2nd vs 1st)	8.0	1.42–45.23	0.026	5.17	0.43–62.64	0.197
CYP2B6 (**6* vs **1/*1*)	3.0	0.53–16.9	0.259			
SLC28A3 expression (high vs low)	9.8	1.09–88.23	0.046	8.77	0.76–101.49	0.082

*the second category in brackets was considered as reference

respond to FC than low-expressing patients (OR = 9.8; p = 0.046). At univariate level, *SLC28A3* mRNA expression and line of FC treatment were the only variables associated with therapeutic response. In multivariate analysis incorporating age, Binet stage, deletion 11q22-q23 and line of treatment, only high *SLC28A3* expression showed a trend towards nonattainment of response to FC, but without reaching statistical significance (OR = 8.77; p = 0.082). It should be noted that we did not observe association of *SLC28A3* expression with any of the clinical parameters and molecular/cellular prognostic factors, so that the groups of low- and high-expressing cases were comparable regarding baseline characteristics. However, in contrast to previous studies, we did not detect association between *SLC28A3* expression and the duration of PFS and OS. The fact that the correlation between *SLC28A3* expression and the response to FC did not translate into PFS and OS differences suggests that the effect of *SLC28A3* is short-term and limited to the period of drug administration.

The seemingly paradoxical relationship between high levels of hCNT3 protein with the fludarabine resistance instead of increased effect of the drug was, to some extent, clarified by the findings that hCNT3 is not localised in the plasma membrane of CLL cells but in cytosol and intracellular membranes, and that there is no detectable hCNT3-related nucleoside transport [20, 21]. Taking that into account, Mackey et al proposed several mechanisms to explain the association of hCNT3 expression with the response to fludarabine: 1) high hCNT3 expression is merely a marker of CLL subtype intrinsically insensitive to fludarabine, 2) up-regulation of hCNT3 correlates with other genes involved in fludarabine resistance and 3) localisation in membranes of organelles may modulate fludarabine toxicity [21]. Interestingly, it has been demonstrated that all-*trans*-retinoic acid (ATRA), used in the treatment of acute promyelocytic leukemia, induces translocation of pre-existing hCNT3 protein to the plasma membrane via transforming growth factor β1-dependent mechanism in MEC1 cell line and primary CLL cells, leading to increased hCNT3-mediated fludarabine transport and sensitivity [52, 53]. Thus, including ATRA into nucleoside-based chemotherapy is a potential solution for overcoming fludarabine resistance by enhancing the CLL cells' capacity to uptake the drug.

Conclusions

To the best of our knowledge, this study is the first that deals with pharmacogenetic markers that are responsible for both fludarabine and cyclophosphamide action within a cohort of patients with functional *TP53* treated with FC. While our results did not demonstrate the link between *CYP2B6*6* allele and the response to FC chemotherapy, the level of *SLC28A3* expression was shown to be a significant predictor of the treatment efficacy in CLL patients. Further studies on larger cohorts are needed to validate its predictive power and applicability for more precise risk stratification of candidates for fludarabine-based treatment in CLL.

Acknowledgements This work was supported by grant No. III 41004, Ministry of Education, Science and Technological Development, Republic of Serbia.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, Hillmen P, Keating MJ, Montserrat E, Rai KR, Kipps TJ International Workshop on Chronic Lymphocytic L (2008) Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the international workshop on chronic lymphocytic leukemia updating the National Cancer Institute-working group 1996 guidelines. Blood 111(12):5446–5456. https://doi.org/10.1182/blood-2007-06-093906
2. Grever MR, Lucas DM, Dewald GW, Neuberg DS, Reed JC, Kitada S, Flinn IW, Tallman MS, Appelbaum FR, Larson RA, Paietta E, Jelinek DF, Gribben JG, Byrd JC (2007) Comprehensive assessment of genetic and molecular features predicting outcome in patients with chronic lymphocytic leukemia: results from the US intergroup phase III trial E2997. J Clin Oncol 25(7):799–804. https://doi.org/10.1200/JCO.2006.08.3089
3. Catovsky D, Richards S, Matutes E, Oscier D, Dyer M, Bezares RF, Pettitt AR, Hamblin T, Milligan DW, Child JA, Hamilton MS, Dearden CE, Smith AG, Bosanquet AG, Davis Z, Brito-Babapulle V, Else M, Wade R, Hillmen P, Group UKNCRIHOCS, Group NCLLW (2007) Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 trial): a randomised controlled trial. Lancet 370(9583):230–239. https://doi.org/10.1016/S0140-6736(07)61125-8
4. Eichhorst BF, Busch R, Hopfinger G, Pasold R, Hensel M, Steinbrecher C, Siehl S, Jager U, Bergmann M, Stilgenbauer S, Schweighofer C, Wendtner CM, Dohner H, Brittinger G, Emmerich B, Hallek M, German CLLSG (2006) Fludarabine plus cyclophosphamide versus fludarabine alone in first-line therapy of younger patients with chronic lymphocytic leukemia. Blood 107(3):885–891. https://doi.org/10.1182/blood-2005-06-2395
5. Hallek M, Fischer K, Fingerle-Rowson G, Fink AM, Busch R, Mayer J, Hensel M, Hopfinger G, Hess G, von Grunhagen U, Bergmann M, Catalano J, Zinzani PL, Caligaris-Cappio F, Seymour JF, Berrebi A, Jager U, Cazin B, Trneny M, Westermann A, Wendtner CM, Eichhorst BF, Staib P, Buhler A, Winkler D, Zenz T, Bottcher S, Ritgen M, Mendila M, Kneba M, Dohner H, Stilgenbauer S, International Group of I, German Chronic Lymphocytic Leukaemia Study G (2010) Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet 376(9747):1164–1174. https://doi.org/10.1016/S0140-6736(10)61381-5
6. Ghielmini M, Vitolo U, Kimby E, Montoto S, Walewski J, Pfreundschuh M, Federico M, Hoskin P, McNamara C, Caligaris-Cappio F, Stilgenbauer S, Marcus R, Trneny M, Dreger P, Montserrat E, Dreyling M, Panel Members of the 1st ECCoML (2013) ESMO guidelines consensus conference on malignant lymphoma 2011 part 1: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL) and chronic lymphocytic leukemia (CLL). Ann Oncol 24(3):561–576. https://doi.org/10.1093/annonc/mds517
7. Committee EG (2017) Appendix 4: chronic lymphocytic leukaemia: eUpdate published online 27 June 2017 (www.esmo.org/Guidelines/Haematological-Malignancies). Ann Oncol 28 (suppl_4):iv149-iv152. https://doi.org/10.1093/annonc/mdx242
8. Flinn IW, Neuberg DS, Grever MR, Dewald GW, Bennett JM, Paietta EM, Hussein MA, Appelbaum FR, Larson RA, Moore DF Jr, Tallman MS (2007) Phase III trial of fludarabine plus cyclophosphamide compared with fludarabine for patients with previously untreated chronic lymphocytic leukemia: US intergroup trial E2997. J Clin Oncol 25(7):793–798. https://doi.org/10.1200/JCO.2006.08.0762
9. Robak T, Dmoszynska A, Solal-Celigny P, Warzocha K, Loscertales J, Catalano J, Afanasiev BV, Larratt L, Geisler CH, Montillo M, Zyuzgin I, Ganly PS, Dartigeas C, Rosta A, Maurer J, Mendila M, Saville MW, Valente N, Wenger MK, Moiseev SI (2010) Rituximab plus fludarabine and cyclophosphamide prolongs progression-free survival compared with fludarabine and cyclophosphamide alone in previously treated chronic lymphocytic leukemia. J Clin Oncol 28(10):1756–1765. https://doi.org/10.1200/JCO.2009.26.4556
10. Edelmann J, Gribben JG (2017) Managing patients with TP53-deficient chronic lymphocytic leukemia. J Oncol Pract 13(6):371–377. https://doi.org/10.1200/JOP.2017.023291
11. Zenz T, Eichhorst B, Busch R, Denzel T, Habe S, Winkler D, Buhler A, Edelmann J, Bergmann M, Hopfinger G, Hensel M, Hallek M, Dohner H, Stilgenbauer S (2010) TP53 mutation and survival in chronic lymphocytic leukemia. J Clin Oncol 28(29):4473–4479. https://doi.org/10.1200/JCO.2009.27.8762
12. Rossi D, Cerri M, Deambrogi C, Sozzi E, Cresta S, Rasi S, De Paoli L, Spina V, Gattei V, Capello D, Forconi F, Lauria F, Gaidano G (2009) The prognostic value of TP53 mutations in chronic lymphocytic leukemia is independent of Del17p13: implications for overall survival and chemorefractoriness. Clin Cancer Res 15(3):995–1004. https://doi.org/10.1158/1078-0432.CCR-08-1630
13. Malcikova J, Smardova J, Rocnova L, Tichy B, Kuglik P, Vranova V, Cejkova S, Svitakova M, Skuhrova Francova H, Brychtova Y, Doubek M, Brejcha M, Klabusay M, Mayer J, Pospisilova S, Trbusek M (2009) Monoallelic and biallelic inactivation of TP53 gene in chronic lymphocytic leukemia: selection, impact on survival, and response to DNA damage. Blood 114(26):5307–5314. https://doi.org/10.1182/blood-2009-07-234708
14. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, Lichter P (2000) Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med 343(26):1910–1916. https://doi.org/10.1056/NEJM200012283432602
15. Zenz T, Habe S, Denzel T, Mohr J, Winkler D, Buhler A, Sarno A, Groner S, Mertens D, Busch R, Hallek M, Dohner H, Stilgenbauer S (2009) Detailed analysis of p53 pathway defects in fludarabine-refractory chronic lymphocytic leukemia (CLL): dissecting the contribution of 17p deletion, TP53 mutation, p53-p21 dysfunction, and miR34a in a prospective clinical trial. Blood 114(13):2589–2597. https://doi.org/10.1182/blood-2009-05-224071
16. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, Kluth S, Bozic I, Lawrence M, Bottcher S, Carter SL, Cibulskis K, Mertens D, Sougnez CL, Rosenberg M, Hess JM, Edelmann J, Kless S, Kneba M, Ritgen M, Fink A, Fischer K, Gabriel S, Lander ES, Nowak MA, Dohner H, Hallek M, Neuberg D, Getz G, Stilgenbauer S, Wu CJ (2015) Mutations driving CLL and their evolution in progression and relapse. Nature 526(7574):525–530. https://doi.org/10.1038/nature15395
17. Galmarini CM, Mackey JR, Dumontet C (2001) Nucleoside analogues: mechanisms of drug resistance and reversal strategies. Leukemia 15(6):875–890
18. Emadi A, Jones RJ, Brodsky RA (2009) Cyclophosphamide and cancer: golden anniversary. Nat Rev Clin Oncol 6(11):638–647. https://doi.org/10.1038/nrclinonc.2009.146
19. Young JD (2016) The SLC28 (CNT) and SLC29 (ENT) nucleoside transporter families: a 30-year collaborative odyssey. Biochem Soc Trans 44(3):869–876. https://doi.org/10.1042/BST20160038
20. Molina-Arcas M, Bellosillo B, Casado FJ, Montserrat E, Gil J, Colomer D, Pastor-Anglada M (2003) Fludarabine uptake mechanisms in B-cell chronic lymphocytic leukemia. Blood 101(6):2328–2334. https://doi.org/10.1182/blood-2002-07-2236
21. Mackey JR, Galmarini CM, Graham KA, Joy AA, Delmer A, Dabbagh L, Glubrecht D, Jewell LD, Lai R, Lang T, Hanson J, Young JD, Merle-Beral H, Binet JL, Cass CE, Dumontet C

(2005) Quantitative analysis of nucleoside transporter and metabolism gene expression in chronic lymphocytic leukemia (CLL): identification of fludarabine-sensitive and -insensitive populations. Blood 105(2):767–774. https://doi.org/10.1182/blood-2004-03-1046

22. Roy P, Yu LJ, Crespi CL, Waxman DJ (1999) Development of a substrate-activity based approach to identify the major human liver P-450 catalysts of cyclophosphamide and ifosfamide activation based on cDNA-expressed activities and liver microsomal P-450 profiles. Drug Metab Dispos 27(6):655–666
23. Zanger UM, Klein K (2013) Pharmacogenetics of cytochrome P450 2B6 (CYP2B6): advances on polymorphisms, mechanisms, and clinical relevance. Front Genet 4:24. https://doi.org/10.3389/fgene.2013.00024
24. Desta Z, Saussele T, Ward B, Blievernicht J, Li L, Klein K, Flockhart DA, Zanger UM (2007) Impact of CYP2B6 polymorphism on hepatic efavirenz metabolism in vitro. Pharmacogenomics 8(6):547–558. https://doi.org/10.2217/14622416.8.6.547
25. Hofmann MH, Blievernicht JK, Klein K, Saussele T, Schaeffeler E, Schwab M, Zanger UM (2008) Aberrant splicing caused by single nucleotide polymorphism c.516G>T [Q172H], a marker of CYP2B6*6, is responsible for decreased expression and activity of CYP2B6 in liver. J Pharmacol Exp Ther 325(1):284–292. https://doi.org/10.1124/jpet.107.133306
26. Tsuchiya K, Gatanaga H, Tachikawa N, Teruya K, Kikuchi Y, Yoshino M, Kuwahara T, Shirasaka T, Kimura S, Oka S (2004) Homozygous CYP2B6 *6 (Q172H and K262R) correlates with high plasma efavirenz concentrations in HIV-1 patients treated with standard efavirenz-containing regimens. Biochem Biophys Res Commun 319(4):1322–1326. https://doi.org/10.1016/j.bbrc.2004.05.116
27. Lang T, Klein K, Fischer J, Nussler AK, Neuhaus P, Hofmann U, Eichelbaum M, Schwab M, Zanger UM (2001) Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. Pharmacogenetics 11(5):399–415
28. Zhang H, Sridar C, Kenaan C, Amunugama H, Ballou DP, Hollenberg PF (2011) Polymorphic variants of cytochrome P450 2B6 (CYP2B6.4-CYP2B6.9) exhibit altered rates of metabolism for bupropion and efavirenz: a charge-reversal mutation in the K139E variant (CYP2B6.8) impairs formation of a functional cytochrome p450-reductase complex. J Pharmacol Exp Ther 338(3):803–809. https://doi.org/10.1124/jpet.111.183111
29. Ariyoshi N, Ohara M, Kaneko M, Afuso S, Kumamoto T, Nakamura H, Ishii I, Ishikawa T, Kitada M (2011) Q172H replacement overcomes effects on the metabolism of cyclophosphamide and efavirenz caused by CYP2B6 variant with Arg262. Drug Metab Dispos 39(11):2045–2048. https://doi.org/10.1124/dmd.111.039586
30. Xu C, Ogburn ET, Guo Y, Desta Z (2012) Effects of the CYP2B6*6 allele on catalytic properties and inhibition of CYP2B6 in vitro: implication for the mechanism of reduced efavirenz metabolism and other CYP2B6 substrates in vivo. Drug Metab Dispos 40(4):717–725. https://doi.org/10.1124/dmd.111.042416
31. Xie HJ, Yasar U, Lundgren S, Griskevicius L, Terelius Y, Hassan M, Rane A (2003) Role of polymorphic human CYP2B6 in cyclophosphamide bioactivation. Pharmacogenomics J 3(1):53–61. https://doi.org/10.1038/sj.tpj.6500157
32. Johnson GG, Lin K, Cox TF, Oates M, Sibson DR, Eccles R, Lloyd B, Gardiner LJ, Carr DF, Pirmohamed M, Strefford JC, Oscier DG, Gonzalez de Castro D, Else M, Catovsky D, Pettitt AR (2013) CYP2B6*6 is an independent determinant of inferior response to fludarabine plus cyclophosphamide in chronic lymphocytic leukemia. Blood 122(26):4253–4258. https://doi.org/10.1182/blood-2013-07-516666
33. Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O'Brien S, Rai KR (1996) National Cancer Institute-sponsored working group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. Blood 87(12):4990–4997
34. Pospisilova S, Gonzalez D, Malcikova J, Trbusek M, Rossi D, Kater AP, Cymbalista F, Eichhorst B, Hallek M, Dohner H, Hillmen P, van Oers M, Gribben J, Ghia P, Montserrat E, Stilgenbauer S, Zenz T, European Research Initiative on CLL (2012) ERIC recommendations on TP53 mutation analysis in chronic lymphocytic leukemia. Leukemia 26(7):1458–1461. https://doi.org/10.1038/leu.2012.25
35. Wood BL, Arroz M, Barnett D, DiGiuseppe J, Greig B, Kussick SJ, Oldaker T, Shenkin M, Stone E, Wallace P (2007) 2006 Bethesda international consensus recommendations on the immunophenotypic analysis of hematolymphoid neoplasia by flow cytometry: optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. Cytometry B Clin Cytom 72(Suppl 1):S14–S22. https://doi.org/10.1002/cyto.b.20363
36. Davis BH, Holden JT, Bene MC, Borowitz MJ, Braylan RC, Cornfield D, Gorczyca W, Lee R, Maiese R, Orfao A, Wells D, Wood BL, Stetler-Stevenson M (2007) 2006 Bethesda international consensus recommendations on the flow cytometric immunophenotypic analysis of hematolymphoid neoplasia: medical indications. Cytometry B Clin Cytom 72 Suppl 1:S5–13. https://doi.org/10.1002/cyto.b.20365
37. Ghia P, Stamatopoulos K, Belessi C, Moreno C, Stilgenbauer S, Stevenson F, Davi F, Rosenquist R, European Research Initiative on CLL (2007) ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia. Leukemia 21(1):1–3. https://doi.org/10.1038/sj.leu.2404457
38. Rosenquist R, Cortese D, Bhoi S, Mansouri L, Gunnarsson R (2013) Prognostic markers and their clinical applicability in chronic lymphocytic leukemia: where do we stand? Leuk Lymphoma 54(11):2351–2364. https://doi.org/10.3109/10428194.2013.783913
39. Stilgenbauer S (2015) Prognostic markers and standard management of chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program 2015:368–377. https://doi.org/10.1182/asheducation-2015.1.368
40. Montserrat E, Dreger P (2016) Treatment of chronic lymphocytic leukemia with del(17p)/TP53 mutation: allogeneic hematopoietic stem cell transplantation or BCR-signaling inhibitors? Clin Lymphoma Myeloma Leuk 16(Suppl):S74–S81. https://doi.org/10.1016/j.clml.2016.02.013
41. Justenhoven C, Pentimalli D, Rabstein S, Harth V, Lotz A, Pesch B, Bruning T, Dork T, Schurmann P, Bogdanova N, Park-Simon TW, Couch FJ, Olson JE, Fasching PA, Beckmann MW, Haberle L, Ekici A, Hall P, Czene K, Liu J, Li J, Baisch C, Hamann U, Ko YD, Brauch H (2014) CYP2B6*6 is associated with increased breast cancer risk. Int J Cancer 134(2):426–430. https://doi.org/10.1002/ijc.28356
42. Berkoz M, Yalin S (2009) Association of CYP2B6 G15631T polymorphism with acute leukemia susceptibility. Leuk Res 33(7):919–923. https://doi.org/10.1016/j.leukres.2008.11.014
43. Yuan ZH, Liu Q, Zhang Y, Liu HX, Zhao J, Zhu P (2011) CYP2B6 gene single nucleotide polymorphisms and leukemia susceptibility. Ann Hematol 90(3):293–299. https://doi.org/10.1007/s00277-010-1085-z
44. Daraki A, Zachaki S, Koromila T, Diamantopoulou P, Pantelias GE, Sambani C, Aleporou V, Kollia P, Manola KN (2014) The G(5)(1)(6)T CYP2B6 germline polymorphism affects the risk of acute myeloid leukemia and is associated with specific chromosomal abnormalities. PLoS One 9(2):e88879. https://doi.org/10.1371/journal.pone.0088879
45. Daraki A, Kakosaiou K, Zachaki S, Sambani C, Aleporou-Marinou V, Kollia P, Manola KN (2016) Polymorphisms and haplotypes of

the CYP2B6 detoxification gene in the predisposition of acute myeloid leukemia (AML) and induction of its cytogenetic abnormalities. Cancer Genet 209(11):525–533. https://doi.org/10.1016/j.cancergen.2016.10.004

46. Gandhi M, Aweeka F, Greenblatt RM, Blaschke TF (2004) Sex differences in pharmacokinetics and pharmacodynamics. Annu Rev Pharmacol Toxicol 44:499–523. https://doi.org/10.1146/annurev.pharmtox.44.101802.121453
47. Lamba V, Lamba J, Yasuda K, Strom S, Davila J, Hancock ML, Fackenthal JD, Rogan PK, Ring B, Wrighton SA, Schuetz EG (2003) Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J Pharmacol Exp Ther 307(3):906–922. https://doi.org/10.1124/jpet.103.054866
48. Ren S, Kalhorn TF, McDonald GB, Anasetti C, Appelbaum FR, Slattery JT (1998) Pharmacokinetics of cyclophosphamide and its metabolites in bone marrow transplantation patients. Clin Pharmacol Ther 64(3):289–301. https://doi.org/10.1016/S0009-9236(98)90178-3
49. Chang TK, Yu L, Maurel P, Waxman DJ (1997) Enhanced cyclophosphamide and ifosfamide activation in primary human hepatocyte cultures: response to cytochrome P-450 inducers and autoinduction by oxazaphosphorines. Cancer Res 57(10):1946–1954
50. McCune JS, Salinger DH, Vicini P, Oglesby C, Blough DK, Park JR (2009) Population pharmacokinetics of cyclophosphamide and metabolites in children with neuroblastoma: a report from the Children's oncology group. J Clin Pharmacol 49(1):88–102. https://doi.org/10.1177/0091270008325928
51. Tsang RY, Santos C, Ghosh S, Dabbagh L, King K, Young J, Cass CE, Mackey JR, Lai R (2008) Immunohistochemistry for human concentrative nucleoside transporter 3 protein predicts fludarabine sensitivity in chronic lymphocytic leukemia. Mod Pathol 21(11):1387–1393. https://doi.org/10.1038/modpathol.2008.110
52. Fernandez-Calotti P, Pastor-Anglada M (2010) All-trans-retinoic acid promotes trafficking of human concentrative nucleoside transporter-3 (hCNT3) to the plasma membrane by a TGF-beta1-mediated mechanism. J Biol Chem 285(18):13589–13598. https://doi.org/10.1074/jbc.M109.055673
53. Fernandez-Calotti PX, Lopez-Guerra M, Colomer D, Pastor-Anglada M (2012) Enhancement of fludarabine sensitivity by all-trans-retinoic acid in chronic lymphocytic leukemia cells. Haematologica 97(6):943–951. https://doi.org/10.3324/haematol.2011.051557