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REVIEW

Influence of ABCB1 polymorphisms upon the effectiveness of standard treatment for acute myeloid leukemia: A systematic review and meta-analysis of observational studies

JE Megías-Vericat[1,2], L Rojas[1,3], MJ Herrero[1,4], V Bosó[1,2], P Montesinos[5], F Moscardó[5], JL Poveda[2], MÁ Sanz[5] and SF Aliño[1,4,6]

The ABCB1 gene encodes for P-glycoprotein (P-gp), an efflux pump for a variety of xenobiotics. The role of ABCB1 polymorphisms in acute myeloid leukemia (AML) outcomes of standard chemotherapy (cytarabine plus anthracyclines) remains controversial. A systematic search was made of studies evaluating the association between ABCB1 polymorphisms 1236C>T, 2677G>T/A and 3435C>T and effectiveness variables. We found seven cohort studies (1241 patients) showing a significantly higher overall survival (OS) among carriers of the variant allele of 1236C>T at year 4 (odds ratio (OR): 1.47, 95% confidence interval (CI): 1.07–2.01), 2677G>T/A at years 4–5 (OR: 1.37, 95% CI: 1.01–1.86) and 3435C>T at years 3 (OR: 1.41, 95% CI: 1.03–1.94) and 4–5 (OR: 1.42, 95% CI: 1.05–1.91). In the subgroup analysis according to ethnicity, Caucasians carrying variant allele showed consistent results in OS. ABCB1 influence upon complete remission could not be demonstrated. Future studies based on larger populations and multiethnic groups should help clarify the effect of P-gp polymorphisms upon other outcomes.

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INTRODUCTION

Acute myeloid leukemia (AML) is primarily treated with cytarabine and anthracycline combinations with complete remission (CR) rates of 70–80%, although nearly 60% of these cases relapse because of the low efficacy of chemotherapy in eliminating minimal residual disease. Prognosis remains poor, with many patients dying because of intrinsic drug resistance of AML cells or relapse resulting from acquired resistance to initial therapy.[1,2]

The multidrug resistance 1 (MDR1) gene, also known as ABCB1 (ATP-binding cassette, subfamily B, member 1), encodes for P-glycoprotein (P-gp), which is a 170 kDa membrane glycoprotein responsible for the ATP-dependent cellular efflux of a variety of drugs (such as anthracyclines and epipodophyllotoxins, commonly used in AML therapy), xenobiotics and cellular metabolites across the plasma membrane, thus minimizing the exposure of potentially toxic compounds to the intracellular environment.[3–5] In addition to protection from xenobiotics, P-gp seems to play a role in carcinogenesis by regulating apoptosis and immune response.[6]

The human ABCB1 gene, which is mapped on chromosome 7q21.12, consists of 28 exons, and at least 50 synonymous single nucleotide polymorphisms (SNPs) have been reported.[7] The three most frequent ABCB1 gene polymorphisms in the Caucasian population are the synonymous SNPs 1236C>T (rs1128503) in exon 12 and 3435C>T (rs1045642) in exon 26, as well as the non-synonymous SNP 2677G>T/A (rs2032582) in exon 21, which results in the amino acid substitution Ser893Ala or Ser893Thr.

Overexpression and functional drug efflux of P-gp have been associated with poorer treatment outcome in AML, and numerous studies have confirmed that P-gp expression is an adverse prognostic factor for CR and survival in adult patients,[8–17] though in pediatric patients this effect remains unclear.[18,19] Genetic polymorphisms in ABC genes that affect the level of expression or function of these transporters could be associated with toxicity and survival in AML patients, because of decreased efflux from both somatic cells and AML cells. Different reports have indicated that the 3435C>T variant results in decreased levels of mRNA expression and P-gp activity.[20–23] However, this effect has been controversial in other studies, especially in Asian populations.[24–28] Similar results have been reported for 2677G>T/A.[29,30] A recent meta-analysis that investigated ABCB1 mRNA expression and response to therapy in AML associated mRNA overexpression to lower CR, especially in patients with anthracycline-based chemotherapy and Asian ethnicity—though with important heterogeneity in all the results. Nevertheless, the gene variants (3435C>T, 2677G>T/A and 1236C>T) were not found to be associated to CR.[31]

Considering the fact that the existing studies are inconclusive and meta-analyses published do not evaluate survival rates, we carried out a meta-analysis of all eligible observational studies to estimate the effect of the most studied ABCB1 polymorphisms

[1]Unidad de Farmacogenética, Instituto Investigación Sanitaria La Fe and Área del Medicamento Hospital Universitario y Politécnico La Fe. Av. Fernando Abril Martorell, Valencia, Spain; [2]Servicio de Farmacia, Área del Medicamento, Hospital Universitario y Politécnico La Fe. Av. Fernando Abril Martorell, Valencia, Spain; [3]Department of Internal Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Av. Libertador Bernardo O Higgins, Santiago, Chile; [4]Departamento Farmacología, Facultad de Medicina, Universidad de Valencia, Av. Blasco Ibáñez, Valencia, Spain; [5]Servicio de Hematología y Hemoterapia, Hospital Universitario y Politécnico La Fe. Av. Fernando Abril Martorell, Valencia, Spain and [6]Unidad de Farmacología Clínica, Área del Medicamento, Hospital Universitario y Politécnico La Fe. Av. Fernando Abril Martorell, Valencia, Spain. Correspondence: Dr MJ Herrero, Departamento Farmacologia, Hospital Universitario y Politecnico La Fe. Av, Fernando Abril Martorell, 106, Unidad de Farmacogenetica, Instituto Investigacion Sanitaria La Fe and Area del Medicamento, Valencia 46026, Spain.
E-mail: maria.jose.herrero@uv.es
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(1236C > T, 2677G > T/A or 3536C > T) upon AML treatment and to quantify the potential heterogeneity between studies. The results of this meta-analysis will contribute to clarify whether ABCB1 gene variants can affect overall survival (OS) and CR among treated AML patients.

MATERIALS AND METHODS

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[32]

Data sources and search strategy

We searched the MEDLINE, Cochrane Central Register, EMBASE, LILACS, ProQuest Medical Library, Web of Science and Database of Abstracts of Reviews of Effects (DARE) without date, language or status of publication restrictions (last search: 17 February 2014). In addition, we hand-searched the tables of contents of the main journals related to the fields of Hematology and Pharmacogenetics: Blood, Leukemia, British Journal of Haematology, Journal of Clinical Oncology, Lancet, Lancet Oncology, Pharmacogenetics and Genomics, Pharmacogenomics and Pharmacogenomics Journal. We also reviewed the available abstracts from the conferences of the American Society of Hematology, the European Hematology Association and the Spanish Society of Hematology and Hemotherapy. The reference lists of relevant reviews and studies were manually searched.

We used similar search terms to search all the registries and databases: acute myeloid leukemia, cytarabine or idarubicin or fludarabine, ABCB1 (or MDR1 or P-gp or ABCB1 gene or ABCB1 protein human), polymorphism (or single nucleotide or SNP or genetic polymorphisms or pharmacogenetics), effectiveness (or overall survival or disease free survival or survival or event free survival or relapse free survival or complete remission or treatment-related mortality or minimal residual disease) and toxicity (or drug toxicity or adverse drug effect or adverse drug reaction or drug induced toxicity).

Study selection

We included studies that met the following inclusion criteria: (i) *in vivo* studies; (ii) studies involving AML patients subjected to standard induction therapy (cytarabine and idarubicin or other anthracyclines, plus fludarabine in case of relapse); (iii) studies reporting genotype frequencies of ABCB1 polymorphisms 1236C > T, 2677G > T/A or 3536C > T; (iv) studies evaluating the association between ABCB1 polymorphisms and the effectiveness or toxicity of chemotherapy. Studies involving patients with a diagnosis of AML French-American-British (FAB)—subtype M3 (promyelocytic leukemia) were excluded because of different treatment regimens involved.

Two authors (JM and LR) independently screened the titles and abstracts identified by the search, and conducted full text screening according to the study inclusion criteria. Disagreements were recorded and resolved by a third reviewer (MH).

Data extraction and quality assessment

Two authors extracted data in duplicate from the included studies using a pilot-tested data extraction sheet. The following information was collected: study design characteristics (methodological quality, publication status, language), baseline characteristics of the included patients (age, sex, ethnicity, AML status, FAB subtype, white blood cell count at diagnosis, cytogenetic risk and mutation status of FMS-related tyrosine kinase 3 internal tandem duplication (FLT3-ITD) and nucleophosmin (NPM1)), polymorphism frequencies of the main ABCB1 SNPs (1236C > T, 2677G > T/A, 3435C > T and the Hardy-Weinberg equilibrium), genotyping method and effectiveness or toxicity outcomes, including mean OS and/or CR or data to infer these values (for example, Kaplan–Meier plots or number of events) to calculate the odds ratio (OR) and 95% confidence interval (95%CI).

We contacted six authors to request missing outcome data, and obtained relevant additional information for one study. When a response was not received, we omitted the data (baseline characteristics) or inferred effectiveness data from the available information. When none of the necessary data were available, the study was excluded.

Methodological quality

Two reviewers (JM and LR) independently assessed the methodological quality of the included studies, without needing a third reviewer to resolve disagreements. The reviewers used the following criteria:

Selection:

1. Representativeness of exposed individuals in the community.
2. Selection from the same community.
3. Absence of clinical outcomes at the start of the study.
4. Standard method for measurement of effectiveness or toxicity outcomes.

Comparability:

1. Comparability of both groups on the basis of demographic characteristics: age, gender.
2. Control of confounding factors: ethnicity, different baseline pathologies, other therapies (transplantation, radiotherapy, different induction scheme).

Outcome:

1. Assessment of clinical outcome with bone marrow aspirates or biopsies.
2. Follow-up long enough for outcomes to occur (at least 24 months for effectiveness and 6 months for toxicity).
3. Adequacy of follow-up of cohorts.

The risk of bias of the included studies was defined by the number of criteria met: low risk of bias = all criteria were met; moderate risk of bias = one criterion was not met; and high risk of bias = two or more criteria were not met.

We evaluated agreement between the two reviewers by using weighted kappa statistics.

Analysis

We conducted the meta-analysis using the Mantel-Haenszel method for dichotomic outcomes with fixed effects model. We repeated the meta-analysis using the random effects model when heterogeneity was present. Studies with zero total events were not included in the analysis. The meta-analysis was performed using RevMan 5.1 software (The Cochrane Collaboration, Nordic Cochrane Center, Copenhagen, Denmark). The influence of the studied ABCB1 SNPs (1236C > T, 2677G > T/A, 3435C > T) was evaluated considering the genetic contrasts of dominant (CC vs CT/TT for 1236C > T and 3435C > T, GG vs GT/GA/TT/AA for 2677G > T/A) and recessive models (CC/CT vs TT for 1236C > T and 3435C > T, GG/GT/GA vs TT/AA for 2677G > T/A). The outcomes were reported as the odds ratios of OS and CR and their corresponding 95% CIs. This parameter was obtained by counting the number of events (death in OS and non-remission/relapse in CR) and the total number of patients observed among genotype groups (dominant or recessive model).

Heterogeneity was assessed using the chi-squared test and the I^2 statistic. Significant heterogeneity was defined by $P < 0.1$ or $I^2 > 50\%$.

We conducted a subgroup analysis for all the outcomes of the review based on the following predefined variables: age, sex, ethnicity, AML status, FAB subtype, white blood cell count at diagnosis, cytogenetic risk and mutation status. Interaction tests (chi-squared) were performed to evaluate the differences between subgroups.

Publication bias was assessed visually by evaluating the symmetry of the funnel plot of OR (log scale). This evaluation was complemented by Egger's test, with statistical significance defined by $P < 0.1$.

RESULTS

Our comprehensive search identified 963 citations from electronic databases and 17 from other sources. Of the 40 records selected for full text assessment, only 7 met the inclusion criteria (Figure 1).[26,27,33–37] All included studies were published in English. The agreement between reviewers for full text screening was close to 100% (kappa = 0.93).

In four studies, the mean percentage OS rate at some of the studied timepoints was inferred from the Kaplan–Meier plots.[26,33,36,37]

Study and patient characteristics

We included seven cohort studies (1241 patients).[26,27,33–37] The mean age of the included patients was 60.2 years, ranging from less than 1 to 86 years. Most of the patients were males (56.6%).

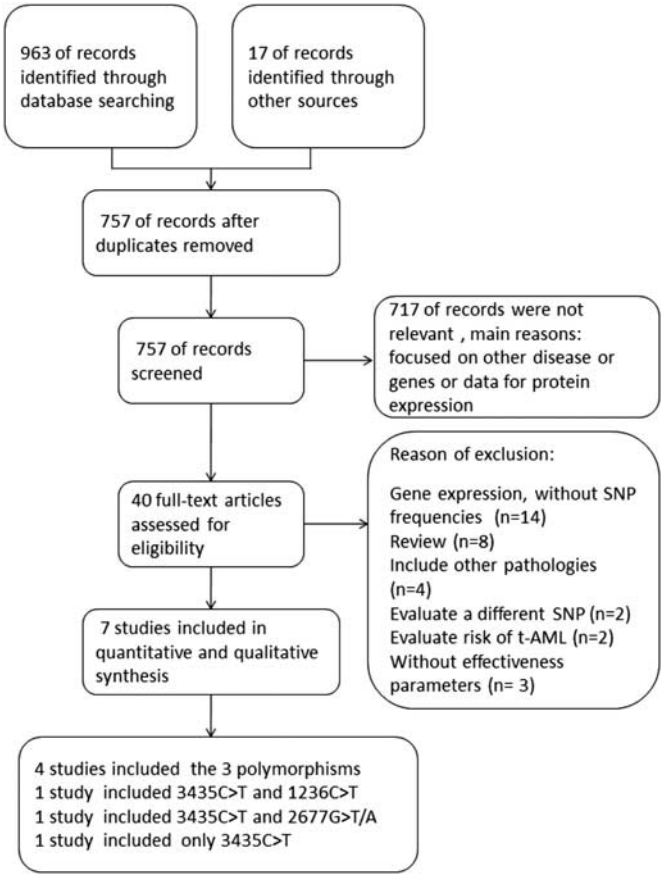

Figure 1. Summary of evidence search and selection.

The predominant ethnic groups were Caucasian (70.8%) and Asian (22.6%). Regarding AML status, 92.3% constituted *de novo* AML and 7.7% secondary AML. A total of 88.4% of the patients were classified into FAB subtypes, with a predominant representation of M2 (40.9%) and M1 (21.0%). Cytogenetic risk was evaluated in 88.4% of the patients: 61% of them had normal cytogenetic characteristics, while 27.1% had unfavorable ones and 8.5% had favorable cytogenetic risk. The mutation status was only evaluated in three studies[27,33,35] for FLT-ITD (31% of the total patients, 29.7% with the mutation) and in one study for NPM1.[33]

Most studies showed all genotype distributions in accordance with the expectations of the Hardy–Weinberg equilibrium, except two studies.[36,37] The genotyping method used consisted of PCR-restriction fragment length polymorphism in four studies,[27,35–37] whereas different methods with previous PCR were used in the other three studies.[26,33,34] The baseline characteristics and clinical outcomes for each study regarding 1236C>T, 2677G>T/A, and 3435C>T genotypes are listed in Table 1.

Toxicity was only evaluated in two studies,[34,37] with no significant associations between the allelic variants and the maximum grade of such adverse events. Toxicity could not be evaluated in our meta-analysis because of insufficient information.

Subgroup analyses were conducted for OS and CR based on the ethnic origin of the patients. We did not analyze other predefined variables (age, sex, AML status, FAB subtype, white blood cell count at diagnosis, cytogenetic risk and mutation status), because the information in the studies was very limited, and such data were rarely associated to the genotype frequencies (only partially so in three studies[26,35,37]).

Risk of bias

Among the evaluated studies, two met all nine criteria and were consequently categorized as presenting a low risk of bias.[36,37] Three studies met eight criteria (intermediate risk of bias)[27,33,34] and two met seven criteria (high risk of bias)[26,35] (Table 2). The level of agreement between the reviewers in assessing the risk of bias was high (kappa = 0.83).

Meta-analysis

We analyzed OS of the different genotypes with dominant and recessive models, with fixed effects, and only recorded significant results with the dominant model (Table 3). The same happened with the Caucasian patient subgroup. We found no significant results for CR using both analytical models (Table 4). The random effects model was only used when the heterogeneity level was high.

OS for 1236C>T

Five studies analyzed OS for 1236C>T polymorphism (925 patients) (Table 1).[26,33,34,36,37] We evaluated OS at 4 years and calculated OR and 95% CI. We estimated OS at 4 years using the Kaplan–Meier plots in two studies that calculated OS at 5 years.[36,37] These two studies did not censure the time from transplantation in the survival analysis, in contrast to the other three studies.[26,33,34]

Evidence was observed suggesting that the presence of allele T is associated to increased OS. The dominant model obtained statistical significance (OR: 1.47, 95% CI: 1.07–2.01, I^2: 62%; Figure 2), though significant heterogeneity was detected using the fixed effect model. In a *post hoc* sensitivity analysis, we excluded the study of Scheiner *et al.*[36] because of the divergent results obtained, and the new pooled estimate was consistent with an increase in OS among patients with allele T (OR: 1.62, 95% CI: 1.16–2.25, I^2: 44%).

The analysis of bias in the publications yielded significant findings in the funnel plot, again because of the study of Scheiner

Table 1. Characteristics of the studies included in the meta-analysis for ABCB1 polymorphism

Study	n	*Age (range)*	*Sex: male/female (%)*	*Ethnia*	*HWE*	*Genotype frequencies (%) of 1236C>T, 2677G>T/A and 3435C>T*			*AML status (%)*		*WBC count 109 per liter (range)*	*FAB subtype*	*Cytogenetic risk (%)*				*Mutation status*	*Chemo-therapy scheme*	*Clinical outcome*
						HS	*HET*	*HM*	*De novo*	*Secondary*			*Fav*	*Normal*	*Unfav*	*NR*			
Green *et al.*[33]	100	63 (20–85)	52/48	Caucasian	Yes	34	44	22	100	0	NR	NR	0	100	0	0	Reported	Ara C +ANT or MIT +/or Others	OS at 4 years
						31	43/2/2	22											
						19	47	34											
Hampras *et al.*[34]	261	61,5 (20–85)	52/48	Caucasian (86%) Others (14%)	Yes	36.4	48.7	14.9	75	25	86% < 100.000 14% > 100.000	Reported	7	50	43	0	NR	Ara C +ANT	OS[a]
						39.8	45.6	14.6											
						30.3	44.4	25.3											
Hur *et al.*[35]	200	44 (NR)	63,5/36,5	Asian	Yes	NR	NR	NR	100	0	42 ± 60	Reported	17,5	60	20,5	2	Reported	Ara C +ANT	OS at 5 years and CR
						NR	NR	NR											
						35.5	46.5	18.0											
Illmer *et al.*[26]	405	53 (17–78)	NR	Caucasian	Yes	36.7	45.4	17.7	100	0	NR	Reported	5.4	66.2	24	4.4	NR	Ara C+ MIT+ETOP +AMSA	OS at 4 years and CR
						34.7	48.3	17.5											
						22.7	32.3	14.1											
Kim *et al.*[27]	81	39 (15–72)	54/46	Asian	Yes	NR	NR	NR	100	0	40.8 ± 6.3	Reported	23	62	15	0	Reported	Ara C+IDA	OS at 3 years and CR
						25.9	34.6/9.9/13.6	13.6/2.5											
						39.5	48.1	12.3											
Scheiner *et al.*[36]	109[b]	34 (< 1–86)	59.6/40.4	Others: White (69,7%) Non-white (30,3%)[c]	No	38.8	50.5	10.7	72.5	18.3	32.5 (0.22–660)	Reported	NR	NR	NR	NR	NR	Ara C+IDA	OS at 5 years
						NR	NR	NR											
						24.3	60.2	15.5											
Van der Holt *et al.*[37]	150[d]	67 (60–85)	57/43	Caucasian	Yes[e]	42	30	28	79	21	18.8 (0.8–389)	Reported	NR	NR	23	77	NR	Ara C +DAUNO	OS at 5 years and CR
						41	32	27											
						28	34	38											

Abbreviations: AML, acute myeloid leukemia; AMSA, amsacrine; ANT, anthracycline; CR, complete remission; DAUNO, daunorubicin; ETOP, etoposide; FAB, French-American-British; Fav, favorable; HET, heterozygous; HM, homozygous mutant; HS, homozygous wild-type; HWE, Hardy–Weinberg equilibrium; IDA, idarubicin; MIT, mitoxantrone; NR, not reported; OS, overall survival; Unfav, unfavorable; WBC, white blood cell. [a]Time is not defined in the original article.[34] According to their results, the OS data were imputed this OS to 3 and 4–5 years. [b]Allele frequency only reported in 103 patients and treatment outcomes only in 44 patients (AML M3 subtype, secondary AML and patients with comorbidities or poor performance status were excluded). [c]Ethnia: because of the marked heterogeneity of the Brazilian population, the patients were classified as whites or non-whites. We classified these patients in a different group named 'others'. [d]Allele frequency and treatment outcomes only reported in 115 patients for 1236C>T, 142 patients for 2677G>T/A and 130 patients for 3435C>T. [e]In accordance with HWE, except for 3435C>T genotypes.

Table 2. Methodological quality of studies

Study	Selection				Comparability		Outcome		
	Representativeness of exposed individuals in the community	*Cohorts drawn from the same community*	*Standard diagnostic method to measure outcomes (OS, CR)*	*Demonstration that outcome was not present at start of study*	*Comparability of cohorts (age, gender)*	*Control of confounders (ethnicity, other pathologies, other therapies)*	*Assessment of outcome with biopsy*	*Was follow-up long enough for outcomes to occur (≥24 months)*	*Adequacy of follow-up of cohorts*
High quality									
Hur *et al.*[35]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Illmer *et al.*[26]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Moderate quality									
Green *et al.*[33]	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes
Hampras[34]	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Kim *et al.*[27]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes
Low quality									
Schéiner *et al.*[36]	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Van der Holt *et al.*[37]	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

Abbreviations: OS, overall survival; CR, complete response.

et al.,[36] which fell slightly outside the funnel, and in Egger's test ($P = 0.06$).

OS for 2677G > T/A

Five of the included studies analyzed OS regarding 2677G > T/A polymorphism (Table 1), with the inclusion of a total of 989 patients.[26,27,33,34,37] We evaluated OS at 3 or 4 years and calculated OR and 95% CI. In one study, OS at 4 years was estimated using the Kaplan–Meier plot of OS at 4 years.[37] This study was the only publication that did not censure the time from transplantation in the survival analysis. Our results demonstrated an effect of 2677G > T/A in OS with the dominant model (OR: 1.37, 95% CI: 1.01–1.86, I^2: 33%) (Figure 3). No asymmetry was observed in the funnel plot. The *P*-value for Egger's test was nonsignificant ($P = 0.13$).

OS for 3435C > T

Seven studies analyzed OS for 3435C > T polymorphism (Table 1), involving a total of 1221 patients.[26,27,33–37] OS was evaluated at 3 years and at 4 or 5 years. In turn, OS was estimated at 3 years using the Kaplan–Meier plots in four studies that calculated OS at 4 or 5 years,[26,33,36,37] but this was not necessary for OS at 4 or 5 years. Three studies did not censure the time from transplantation in the survival analysis.[35–37]

The pooled effect estimate suggested a higher survival at 3 years in T allele carriers (6 studies, 1021 patients[26,27,33,34,36,37]) that proved statistically significant in the dominant model (OR: 1.41, 95% CI: 1.03–1.94, I^2: 8%) (Figure 4). Likewise, we observed significantly greater survival associated to the variant in OS at 4 or 5 years (6 studies, 1140 patients[26,33–37]), using the dominant model (OR: 1.42, 95% CI: 1.05–1.91, I^2: 17%) (Figure 5).

No asymmetry was observed in the funnel plot for OS at 3 and 4–5 years. The *P*-value for Egger's test was nonsignificant.

CR for 1236C > T

Only two studies evaluated CR regarding 1236C > T polymorphism,[26,37] with a total of 520 patients, all of which were Caucasians. The pooled effect estimate showed no association between CR and 1236C > T polymorphism for either the dominant model (OR: 1.33, 95% CI: 0.93–1.90, I^2: 0%) (Figure 6) or the recessive model (OR: 1.12, 95% CI: 0.72–1.74, I^2: 52%).

CR for 2677G > T/A

Three studies measured CR for 2677G > T/A polymorphism,[26,27,37] with a total of 628 patients (two studies in Caucasians and one in an Asian population). The pooled estimate showed no association between CR and 2677G > T/A polymorphism for either the dominant model (OR: 1.05, 95% CI: 0.75–1.47, I^2: 74%) (Figure 7) or the recessive model (OR: 1.37, 95% CI: 0.91–2.07, I^2: 0%).

The analysis of publication bias yielded significant findings in the funnel plot (Kim *et al.*[27] fell slightly outside the funnel) and in Egger's test ($P = 0.11$).

CR for 3435C > T

Four studies evaluated CR for 3435C > T polymorphism,[26,27,35,37] with a total of 816 patients (two studies in Caucasians and two in Asian patients). We found no significant association between CR and this SNP for either the dominant model (OR: 1.03, 95% CI: 0.75–1.41, I^2: 63%) (Figure 8) or the recessive model (OR: 1.03, 95% CI: 0.73–1.44, I^2: 0%).

We found asymmetry in publication bias (*P*-value for Egger's test $P = 0.05$).

SUBGROUP ANALYSIS

OS: we found an interaction for ethnicity at 3 years for 3435C > T, at 4 years for 1236C > T and at 4–5 years for 2677G > T/A and

Table 3. Summary of ORs with 95% CIs for ordinary genetic contrasts of the association between the ABCB1 1236C>T, 2677G>T/A and 3435C>T polymorphisms and overall survival with fixed effects

Contrast	*Overall subgroup*	n	*OR (95% CI)*	I^2 *(%)*	P[a]
OS at 4 years with 1236C>T					
CC vs CT/TT	All studies	925	1.47 (1.07–2.01)	62%[b]	0.02
CC/CT vs TT	All studies	925	1.09 (0.74–1.60)	15%	0.66
OS at 3–4 years with 2677G>T/A					
GG vs GT/TT	All studies	989	1.37 (1.01–1.86)	33%	0.04
GG/GT vs TT	All studies	989	1.11 (0.78–1.60)	28%	0.55
OS at 3 years with 3435C>T					
CC vs CT/TT	All studies	1021	1.41 (1.03–1.94)	8%	0.03
CC/CT vs TT	All studies	1021	1.25 (0.92–1.71)	12%	0.16
OS at 4–5 years with 3435C>T					
CC vs CT/TT	All studies	1140	1.42 (1.05–1.91)	17%	0.02
CC/CT vs TT	All studies	1140	1.22 (0.91–1.65)	0%	0.18

Abbreviations: CI, confidence interval; OR, odds ratio; OS, overall survival. [a]*P* value of test of overall effect. [b]Significant heterogeneity using the fixed-effect model. We calculated with random effects (OR: 1.41, 95% CI: 0.75–2.65, I^2: 62%, $P=0.28$). In a *post-hoc* sensitivity analysis, we excluded the study of Scheiner *et al.*[35] in order to the divergent results, and we obtained an acceptable heterogeneity with fixed effects (OR: 1.62, 95% CI: 1.16–2.25, I^2: 44%, $P=0.004$).

Table 4. Summary of ORs with 95% CIs for ordinary genetic contrasts of the association between the ABCB1 1236C>T, 2677G>T/A and 3435C>T polymorphisms and complete remission with fixed effects

Contrast	*Overall subgroup*	n	*OR (95% CI)*	I^2 *(%)*	P[a]
CR with 1236C>T					
CC vs CT/TT	All studies	520	1.33 (0.93–1.90)	0%	0.12
CC/CT vs TT	All studies	520	1.12 (0.72–1.74)	52%[b]	0.61
CR with 2677G>T/A					
GG vs GT/TT	All studies	628	1.05 (0.75–1.47)	74%[c]	0.78
GG/GT vs TT	All studies	628	1.37 (0.91–2.07)	0%	0.13
CR with 3435C>T					
CC vs CT/TT	All studies	816	1.03 (0.75–1.41)	63%[d]	0.87
CC/CT vs TT	All studies	816	1.03 (0.73–1.44)	0%	0.89

Abbreviations: CI, confidence interval; CR, complete remission; OR, odds ratio. [a]*P* value of test of overall effect. [b]Significant heterogeneity using the fixed-effect model. We calculated with random effects (OR: 1.04, 95% CI: 0.52–2.06, I^2: 52%, $P=0.92$). [c]Significant heterogeneity using the fixed-effect model. We calculated with random effects (OR: 0.78, 95% CI: 0.33–1.84, I^2: 74%, $P=0.57$). [d]Significant heterogeneity using the fixed-effect model. We calculated with random effects (OR: 0.86, 95% CI: 0.47–1.56, I^2: 63%, $P=0.62$).

3435C>T. Caucasian participants carrying the T allele showed significantly greater OS (3 years 3435C>T, OR: 1.44, 95% CI: 1.02–2.04, I^2: 43%; 4 years 1236C>T: 1.62, 95% CI: 1.16–2.25, I^2: 44%; 4–5 years 2677G>T/A: 1.39 95% CI: 1.01–1.91, I^2: 49%; 4–5 years 3435C>T: 1.41, 95% CI: 1.03–2.10, I^2: 43%, respectively).

We found no significant correlations regarding CR.

Sensitivity analyses

No significant differences were found after excluding outlier studies in the OS analysis of 1236C>T[36] and in the CR analysis of 2677G>T/A and 3435C>T,[27] thus indicating robustness and reliability of the results. No significant discrepancies were found when estimates of the pooled effects were obtained using fixed or random effect models.

DISCUSSION

The findings of this meta-analysis suggest that ABCB1 polymorphisms in AML probably influence the effectiveness of standard chemotherapy, specifically in relation to OS where the variants might have a positive effect because of lesser P-gp expression. The pooled effect estimate indicated that the variant allele of all three studied ABCB1 SNPs significantly increased OS using the dominant model. This effect was more evident in Caucasian patients at 3 years for 3435C>T, at 4 years for 1236C>T and at 4–5 years for 2677G>T/A and 3435C>T. We found significant heterogeneity in our analysis for 1236C>T, which was attributable to a single study.[36] This was the only publication mixing adults and children, and was carried out in a Brazilian population, characterized by different variant frequencies compared with Caucasians, and with high ethnic diversity. When this study was excluded in the *post hoc* sensitivity analysis, the heterogeneity disappeared and the new pooled estimate was consistent with an increased risk of OS among allele T carriers.

We observed no significant effect in relation to CR, maybe because the number of studies was smaller than in reference to OS. A previous meta-analysis likewise recorded no significant association between CR and these three polymorphisms using the generalized OR, a genetic model-free approach and the genetic models (recessive and dominant).[31] The studies included were the same, and the results obtained were similar to those of our own meta-analysis. Our data showed a tendency towards greater CR when variant allele in 1236C>T and 2677G>T/A is present. Previous studies showed the overexpression of P-gp to be associated to lower OS[11–16,26] and CR.[9–14,16,17,37] The three variants of ABCB1 SNPs investigated in the present study result in a decrease in ABCB1 expression,[20–23] and theoretically should increase OS and CR, in agreement with our results and those of another study.[33] However, two studies reported a decreased expression of P-gp with wild-type genotypes,[26,27] and better outcomes in one of them.[27]

Toxicity was one of the outcomes which we planned to evaluate in this meta-analysis, but the limited published data precluded such evaluation. We found only two studies that analyzed associations between toxicity and ABCB1 polymorphisms,[34,37] and no significant results were obtained.

The subgroup analysis according to ethnic origin yielded different results in Caucasian and Asian populations referred to some outcomes—particularly CR. Caucasian and Asian populations have different genotype frequencies (Hapmap project), but we also detected a different effect of these SNPs upon AML outcomes. We detected lower CR with variant allele in Asian populations on studying genotypes 2677G>T/AA and 3435C>T. Similar results were obtained in relation to OS at 3 years for 3435C>T using the recessive model. However, we did not observe great changes in OS on considering 2677G>T/A. The reason might be that different genetic backgrounds and differences in the environment existed among different ethnicities and individuals. The literature reports different P-gp expression in these populations in 3435C>T polymorphism, with a higher expression of mRNA in duodenal enterocytes of healthy Japanese subjects with the TT genotype,[25,28] and the opposite effect in Caucasians.[20–23] Some authors postulate that ethnic differences between Caucasians and Asians can be explained by differential ABCB1 expression including transcriptional initiation and RNA maturation, or by a possible relationship between 3435C>T and different haplotypes, depending on whether the subjects are Caucasian or Asian.[25,27,28] These differences in ABCB1 expression

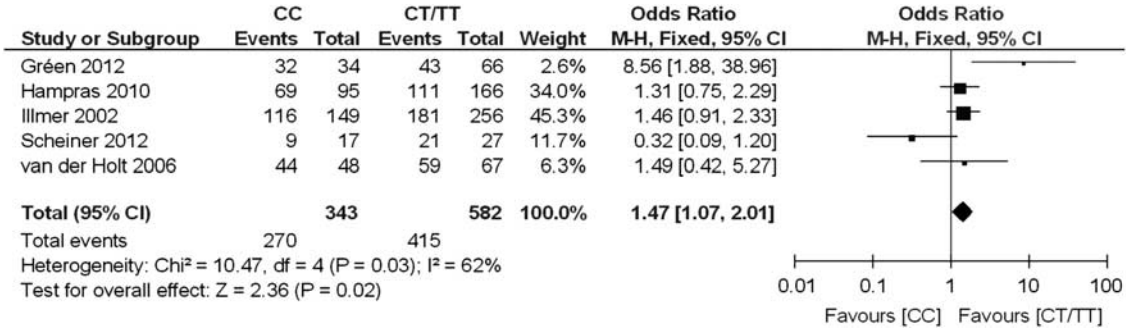

Figure 2. Overall survival at 4 years for 1236C>T (dominant model CC vs CT/TT).

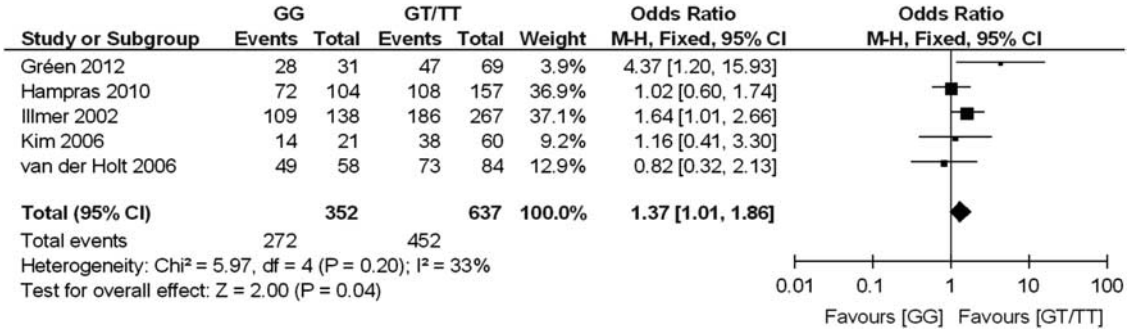

Figure 3. Overall survival at 3–4 years for 2677G>T/A (dominant model GG vs GT/TT).

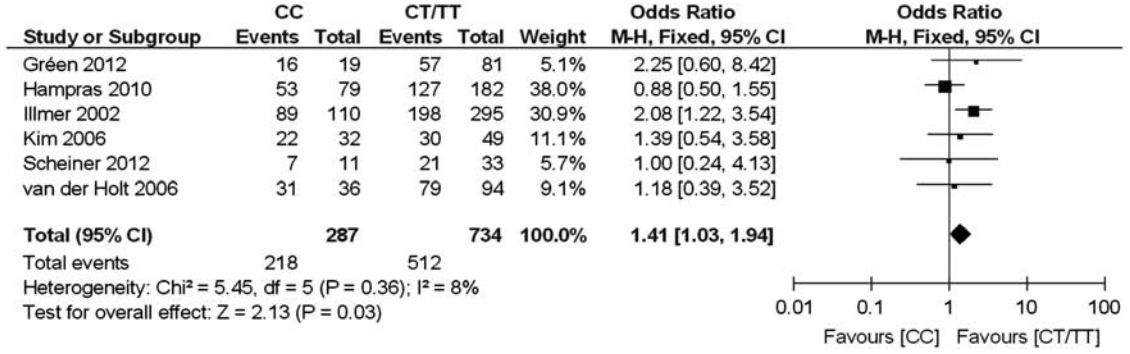

Figure 4. Overall survival at 3 years for 3435C>T (dominant model CC vs CT/TT).

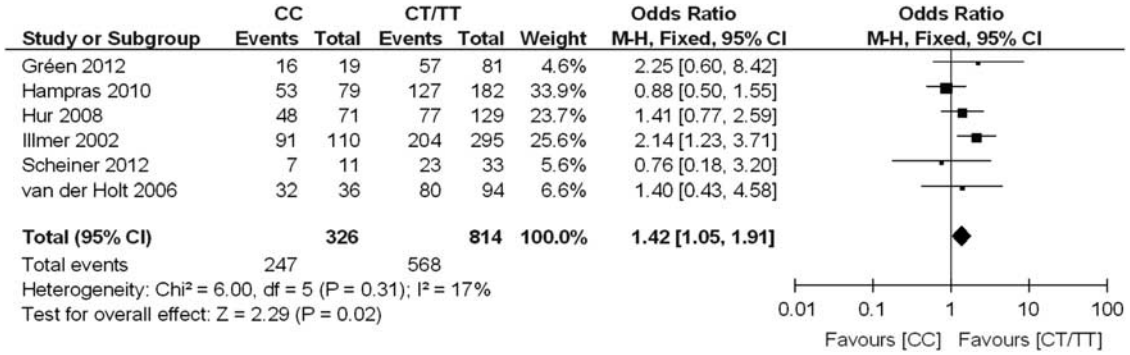

Figure 5. Overall survival at 4–5 years for 3435C>T (dominant model CC vs CT/TT).

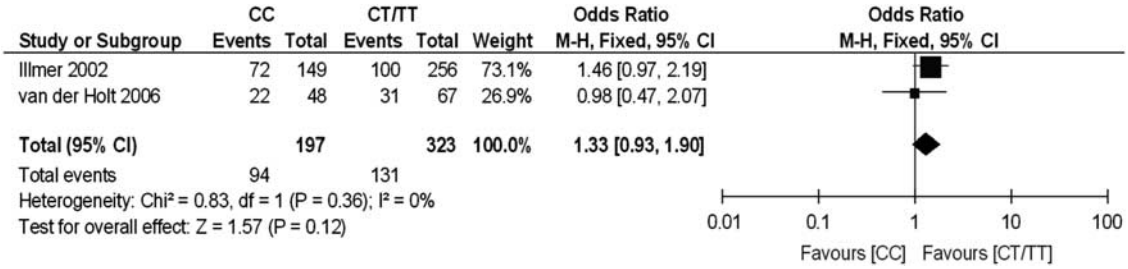

Figure 6. Complete remission for 1236C > T (dominant model CC vs CT/TT).

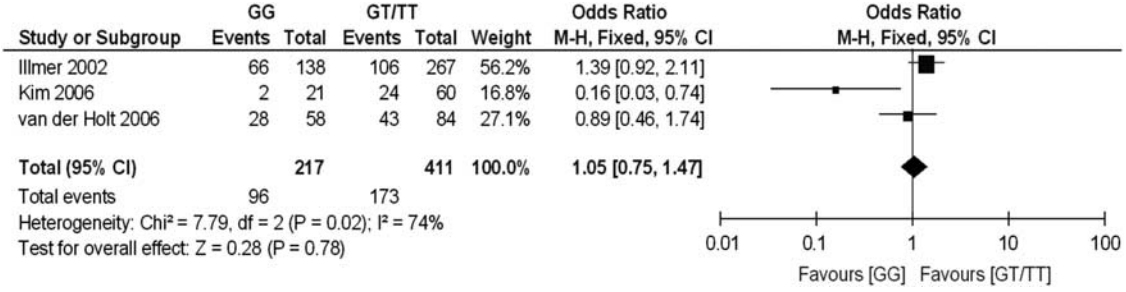

Figure 7. Complete remission for 2677G > T/A (dominant model GG vs GT/TT).

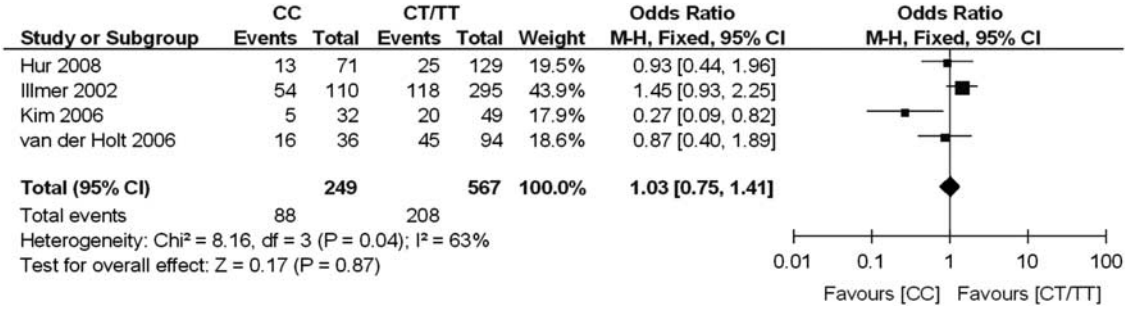

Figure 8. Complete remission for 3435C > T (dominant model CC vs CT/TT).

between healthy Caucasian[29] and Asian[30] volunteers were also observed regarding G2677. The effect of 1236C > T polymorphism in Asian populations was not reported in the included studies.

Another factor that could explain the variability of the results of the different studies is the difference in drugs used in relation to induction therapy. One of the main chemotherapy agents used in all of the studies was cytarabine, which is not a P-gp substrate. On the other hand, anthracyclines are a well-known substrate for P-gp, which exhibits a greater efflux effect with classical anthracyclines such as doxorubicin or daunorubicin than with others such as idarubicin. In this regard, the latter drug is a highly lipophilic substance and seems to be a weak substrate for P-gp—though this point is controversial.[38–40] The two studies that used cytarabine with idarubicin as induction therapy obtained greater heterogeneity in the results.[27,36]

Heterogeneity is a potential problem when interpreting the results of all meta-analyses. In our case, significant heterogeneity was detected in one of our statistically significant results (OS analysis of 1236C > T with the dominant model), attributable to a single study[36] that combined non-Caucasian children and adults. When this study was excluded in the *post hoc* sensitivity analysis, the heterogeneity decreased. The other three cases corresponded to CR analysis, in which we had a small number of studies with divergent results in Caucasian and Asian patients. This explains the changes in the results on recalculating OR using the random effects model in genotypes G2627T/A and 3435C > T (with similar results in 1236C > T). Such heterogeneity decreased on analyzing only the Caucasian subgroup of patients in all of the outcomes.

Some limitations of this meta-analysis should be addressed. Firstly, AML is a complex disease with a multifactorial etiology, and the role of drug efflux pumps in relation to AML outcomes is not fully understood. Secondly, our results are based on non-adjusted estimates, whereas a more precise analysis should be conducted if more detailed individual data were available, such as OS at the same timepoints or censuring the time from transplantation in the survival analysis in all the studies. Thirdly, we found that the genotype distributions were not in agreement with Hardy–Weinberg equilibrium in two studies.[35,36] On the other hand, regarding the subgroup analysis according to ethnicity, the Caucasian race was the only group with enough patients to allow an evaluation of effectiveness, in contrast to the other groups. Lastly, subgroup analyses referred to other variables (age, sex, AML status, FAB

subtype and so on) were not performed in our work because of insufficient available data in the primary studies. The inclusion of studies in adult and pediatric patients could influence the results obtained, nevertheless, we found no significant differences after exclusion of the only one study that evaluated pediatric patients.[36]

The strength of our meta-analysis lies in the comprehensive literature search strategies, using inclusive criteria and appropriate statistical analyses of the main AML outcomes. ABCB1 has been previously meta-analyzed in other contexts such as cancer risk,[41] leukemia risk[42,43] or mRNA expression and CR,[31] but not in relation to AML survival. Our results confirm that ABCB1 polymorphisms are not associated to CR, as in the previous meta-analysis,[31] and for the first time, OS is associated to these gene variants. Finally, we tested the robustness of our results using several sensitivity analyses.

In conclusion, this meta-analysis found a significant association between the three ABCB1 polymorphisms evaluated and OS, especially among Caucasian populations. The polymorphisms showed no association to CR. Future studies based on larger populations and involving different age and ethnic groups should help clarify the effect these SNPs upon AML outcomes. Moreover, further studies addressing gene-gene and gene-environment interactions may help us to understand the role of ABCB1 polymorphisms in relation to the effectiveness of chemotherapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Löwenberg B. Acute myeloid leukemia: the challenge of capturing disease variety. *Hematology Am Soc Hematol Educ Program* 2008: 1–11.
2 Huntly B, Gilliland G. Pathobiology of acute myeloid leukemia. In: Hoffman (ed.) *Hematology. Basic principles and practice*. Churchill Livingstone: Philadelphia, 2005: pp 1057–1069.
3 Bodor M, Kelly EJ, Ho RJ. Characterization of the human MDR1 gene. *AAPS J* 2005; **7**: E1–E5.
4 Allikmets R, Gerrard B, Hutchinson A, Dean M. Characterization of the human ABC superfamily: isolation and mapping of 21 new genes using the expressed sequence tags database. *Hum Mol Genet* 1996; **5**: 1649–1655.
5 Yamada O, Ozaki K, Furukawa T, Machida M, Wang YH, Motoji T *et al.* Activation of STAT5 confers imatinib resistance on leukemic cells through the transcription of TERT and MDR1. *Cell Signal* 2011; **23**: 1119–1127.
6 Johnstone RW, Ruefli AA, Smyth MJ. Multiple physiological functions for multidrug transporter P-glycoprotein? *Trends Biochem Sci* 2000; **25**: 1–6.
7 Breier A, Barancik M, Sulova Z, Uhrik B. P-glycoprotein—implications of metabolism of neoplastic cells and cancer therapy. *Curr Cancer Drug Targets* 2005; **5**: 457–468.
8 Samdani A, Vijapurkar U, Grimm MA, Spier CS, Grogan TM, Glinsmann-Gibson BJ *et al.* Cytogenetics and P-glycoprotein (PGP) are independent predictors of treatment outcome in acute myeloid leukemia (AML). *Leukemia Res* 1996; **20**: 175–180.
9 Leith CP, Kopecky KJ, Chen IM, Eijdems L, Slovak ML, McConnell TS *et al.* Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/Pglycoprotein, MRP1, and LRP in acute myeloid leukemia: a Southwest Oncology Group Study. *Blood* 1999; **94**: 1086–1099.
10 Leith CP, Kopecky KJ, Godwin J, McConnell T, Slovak ML, Chen IM *et al.* Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. *Blood* 1997; **89**: 3323–3329.
11 Benderra Z, Faussat AM, Sayada L, Perrot JY, Chaoui D, Marie JP *et al.* Breast cancer resistance protein and P-glycoprotein in 149 adult acute myeloid leukemias. *Clin Cancer Res* 2004; **10**: 7896–7902.
12 Wuchter C, Leonid K, Ruppert V, Schrappe M, Buchner T, Schoch C *et al.* Clinical significance of P-glycoprotein expression and function for response to induction chemotherapy, relapse rate and overall survival in acute leukemia. *Haematologica* 2000; **85**: 711–721.
13 Schaich M, Soucek S, Thiede C, Ehninger G, Illmer T. MDR1 and MRP1 gene expression are independent predictors for treatment outcome in adult acute myeloid leukaemia. *Br J Haematol* 2005; **128**: 324–332.
14 Damiani D, Tiribelli M, Calistri E, Geromin A, Chiarvesio A, Michelutti A *et al.* The prognostic value of P-glycoprotein (ABCB) and breast cancer resistance protein (ABCG2) in adults with de novo acute myeloid leukemia with normal karyotype. *Haematologica* 2006; **91**: 825–828.
15 Huh HJ, Park CJ, Jang S, Seo EJ, Chi HS, Lee JH *et al.* Prognostic significance of multidrug resistance gene 1 (MDR1), multidrug resistance-related protein (MRP) and lung resistance protein (LRP) mRNA expression in acute leukemia. *J Korean Med Sci* 2006; **21**: 253–258.
16 Michieli M, Damiani D, Ermacora A, Masolini P, Raspadori D, Visani G *et al.* P-glycoprotein, lung resistance-related protein and multidrug resistance associated protein in de novo acute non-lymphocytic leukaemias biological and clinical implications. *Br J Haematol* 1999; **104**: 328–335.
17 Shi P, Zha J, Guo X, Chen F, Fan Z, Huang F *et al.* Idarubicin is superior to daunorubicin in remission induction of de novo acute myeloid leukemia patients with high MDR1 expression. *Pharmacogenomics* 2013; **14**: 17–23.
18 Sievers EL, Smith FO, Woods WG, Lee JW, Bleyer WA, Willman CL *et al.* Cell surface expression of the multidrug resistance Pglycoprotein (P-170) as detected by monoclonal antibody MRK-16 in pediatric acute myeloid leukemia fails to define a poor prognostic group: a report from the Childrens Cancer Group. *Leukemia* 1995; **9**: 2042–2048.
19 Steinbach D, Furchtbar S, Sell W, Lengemann J, Hermann J, Zintl F *et al.* Contrary to adult patients, expression of the multidrug resistance gene (MDR1) fails to define a poor prognostic group in childhood AML. *Leukemia* 2003; **17**: 470–471.
20 Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W. Multidrug resistance polypeptide 1 (MDR1, ABCB1) variant 3435C>T affects mRNA stability. *Pharmacogenet Genomics* 2005; **15**: 693–704.
21 Hoffmeyer S, Burk O, von Richter O, Arnold HP, Brockmoller J, Johne A *et al.* Functional polymorphisms of the human multidrug- resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci USA* 2000; **97**: 3473–3478.
22 Hitzl M, Drescher S, van der Kuip H, Schäffeler E, Fischer J, Schwab M *et al.* The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56+ natural killer cells. *Pharmacogenetics* 2001; **11**: 293–298.
23 Seedhouse CH, Grundy M, White P, Li Y, Fisher J, Yakunina D *et al.* Sequential influences of leukemia-specific and genetic factors on p-glycoprotein expression in blasts from 817 patients entered into the National Cancer Research Network acute myeloid leukemia 14 and 15 trials. *Clin Cancer Res* 2007; **13**: 7059–7066.
24 Jamroziak K, Balcerczak E, Cebula B, Janus A, Mirowski M, Robak T. No influence of 3435C>T ABCB1 (MDR1) gene polymorphism on risk of adult acute myeloid leukemia and P-glycoprotein expression in blast cells. *Ther Drug Monit* 2006; **28**: 707–711.
25 Nakamura T, Sakaeda T, Horinouchi M, Tamura T, Aoyama N, Shirakawa T *et al.* Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther* 2002; **71**: 297–303.
26 Illmer T, Schuler US, Thiede C, Schwarz UI, Kim RB, Gotthard S *et al.* MDR1 gene polymorphisms affect therapy outcome in acute myeloid leukemia patients. *Cancer Res* 2002; **62**: 4955–4962.
27 Kim DH, Park JY, Sohn SK, Lee NY, Baek JH, Jeon SB *et al.* Multidrug resistance-1 gene polymorphisms associated with treatment outcomes in de novo acute myeloid leukemia. *Int J Cancer* 2006; **118**: 2195–2201.
28 Sakaeda T, Nakamura T, Horinouchi M, Kakumoto M, Ohmoto N, Sakai T *et al.* MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. *Pharm Res* 2001; **18**: 1400–1404.
29 Lamba J, Strom S, Venkataramanan R, Thummel KE, Lin YS, Liu W *et al.* MDR1 genotype is associated with hepatic cytochrome P4503A4 basal and induction phenotype. *Clin Pharmacol Ther* 2006; **79**: 325–338.
30 Kurata Y, Ieiri I, Kimura M, Morita T, Irie S, Urae A *et al.* Role of human MDR1 gene polymorphism in bioavailability and interactionof digoxin, a substrate of P-glycoprotein. *Clin Pharmacol Ther* 2002; **72**: 209–219.
31 Doxani C, Voulgarelis M, Zintzaras E. MDR1 mRNA expression and MDR1 gene variants as predictors of response to chemotherapy in patients with acute myeloid leukaemia: a meta-analysis. *Biomarkers* 2013; **18**: 425–435.

32 Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP *et al.* The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009; **339**: b2700.
33 Gréen H, Falk IJ, Lotfi K, Paul E, Hermansson M, Rosenquist R *et al.* Association of ABCB1 polymorphisms with survival and in vitro cytotoxicty in de novo acute myeloid leukemia with normal karyotype. *Pharmacogenomics J* 2012; **12**: 111–118.
34 Hampras SS, Sucheston L, Weiss J, Baer MR, Zirpoli G, Singh PK *et al.* Genetic polymorphisms of ATP-binding cassette (ABC) proteins, overall survival and drug toxicity in patients with Acute Myeloid Leukemia. *Int J Mol Epidemiol Genet* 2010; **1**: 201–207.
35 Hur EH, Lee JH, Lee MJ, Choi SJ, Lee JH, Kang MJ *et al.* C3435T polymorphism of the MDR1 gene is not associated with P-glycoprotein function of leukemic blasts and clinical outcome in patients with acute myeloid leukemia. *Leuk. Res* 2008; **32**: 1601–1604.
36 Scheiner MA, da Cunha Vasconcelos F, da Matta RR, Dal Bello Figueira R Jr, Maia RC. ABCB1 genetic variation and P-glycoprotein expression/activity in a cohort of Brazilian acute myeloid leukemia patients. *J Cancer Res Clin Oncol* 2012; **138**: 959–969.
37 van der Holt B, Van den Heuvel-Eibrink MM, Van Schaik RH, van der Heiden IP, Wiemer EA, Vossebeld PJ *et al.* ABCB1 gene polymorphisms are not associated with treatment outcome in elderly acute myeloid leukemia patients. *Clin Pharmacol Ther* 2006; **80**: 427–439.
38 Berman E, McBride M. Comparative cellular pharmacology of daunorubicin and idarubicin in human multidrug-resistant leukemia cells. *Blood* 1992; **79**: 3267–3273.
39 Tsimberidou AM, Paterakis G, Androutsos G, Anagnostopoulos N, Galanopoulos A, Kalmantis T *et al.* Evaluation of the clinical relevance of the expression and function of P-glycoprotein, multidrug resistance protein and lung resistance protein in patients with primary acute myelogenous leukemia. *Leuk Res* 2002; **26**: 143–154.
40 Lacayo NJ, Duran GE, Sikic BI. Modulation of resistance to idarubicin by the cyclosporin PSC 833 (valspodar) in multidrug-resistant cells. *J Exp Ther Oncol* 2003; **3**: 127–135.
41 Sheng X, Zhang L, Tong N, Luo D, Wang M, Xu M *et al.* MDR1 C3435T polymorphism and cancer risk: a meta-analysis based on 39 case-control studies. *Mol Biol Rep* 2012; **39**: 7237–7249.
42 Zhang BB, Xuan C, Deng KF, Wu N, Lun LM. Association between the MDR1 gene variant C3435T and risk of leukaemia: a meta-analysis. *Eur J Cancer Care (Engl)* 2013; **22**: 617–625.
43 Yan Y, Liang H, Xie L, He Y, Li M, Li R *et al.* Association of MDR1 G2677T polymorphism and leukemia risk: evidence from a meta-analysis. *Tumour Biol* 2014; **35**: 2191–2197.