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ORIGINAL ARTICLE

Profiling of drug-metabolizing enzymes/transporters in CD33 + acute myeloid leukemia patients treated with Gemtuzumab-Ozogamicin and Fludarabine, Cytarabine and Idarubicin

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Genetic heterogeneity in drug-metabolizing enzyme/transporter (DMET) genes affects specific drug-related cancer phenotypes. To investigate the relationships between genetic variation and response to treatment in acute myeloid leukemia (AML), we genotyped 1931 variants on DMET genes in 94 CD33-positive AML patients enrolled in a phase III multicenter clinical trial combining Gemtuzumab-Ozogamicin (GO) with Fludarabine–Cytarabine–Idarubicin (FLAI) regimen, with the DMET Plus platform. Two *ADH1A* variants showed statistically significant differences (odds ratio (OR) = 5.68, $P = 0.0006$; OR = 5.35, $P = 0.0009$) in allele frequencies between patients in complete/partial remission and patients without response, two substitutions on *CYP2E1* (OR = 0.13, $P = 0.001$; OR = 0.09, $P = 0.003$) and one on *SLCO1B1* (OR = 4.68, $P = 0.002$) were found to differently influence liver toxicity, and two nucleotide changes on *SULTB1* and *SLC22A12* genes correlated with response to GO (OR = 0.24, $P = 0.0009$; OR = 2.75, $P = 0.0029$). Genetic variants were thus found for the first time to be potentially associated with differential response and toxicity in AML patients treated with a combination of GO–FLAI regimen.

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Keywords: AML; DMET; GO–FLAI; SNPs

INTRODUCTION

One of the most challenging goals of the present-day biomedical research is assessing which pharmacological treatment will be the most effective and, at the same time, with the fewest side effects for a specific patient. Such dare promises to throw medical sciences into the era of *personalized medicine*, in which the study of the individual's genotypic and phenotypic characteristics will drive the adoption of the most suitable drug in the attempt to increase the beneficial effects of pharmacotherapy while minimizing adverse reactions.[1] The rationale behind this approach is based on the evidences that in many cases a strong relationship exists between genetic variation and differential drug response.[2–5]

Pharmacogenetic studies have been traditionally focused on a limited set of candidate genes and/or pathways,[6–8] as well as on few variants, that are often too rare or highly population specific to lead to results with an appreciable clinical usefulness. On the contrary, in the very recent years, the advent of high-throughput genotyping platforms has favored a fine-scale assessment of the genetic variability hidden within cohorts of patients affected by the same disease, thus potentially highlighting specific gene classes responsible for the interindividual differences that may play a role in differential drug activation, metabolism, disposition and transport. Such functional classes mainly contain drug-metabolizing enzymes and transporters (DMET) genes whose interindividual variations, which alter their physiological expression or activity, have been demonstrated to influence the response to therapy.[1,7,9–11]

Acute myeloid leukemia (AML) is a heterogeneous group of clonal myeloid malignancies that predominantly affects middle-aged and elderly adults, and hence interindividual variability in the drug response of AML patients may be presumptively expected. All these malignancies are characterized by an arrest of maturation, along with uncontrollable proliferation, of hematopoietic progenitor cells. In general, the prognosis of patients with AML is currently based upon the presence or absence of cytogenetic abnormalities, leading to the identification of favorable, intermediate and unfavorable disease subgroups. Moreover, gene-expression profiles and specific genetic mutations (for example, in genes encoding *FMS-related tyrosine kinase 3* (*FLT3*), *nucleophosmin 1* (*NPM1*), *mixed lineage leukemia* (*MLL*) and *CCAAT/enhancer binding protein α* (*CEPBα*)) may identify good- and poor-risk normal cytogenetic AML subgroups.[12–17] Besides these specific differentiations, genetic variants in drug transporters and drug-metabolizing enzymes have been demonstrated to affect

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each individual's response to antileukemia treatments, and thus the likelihood of experiencing an adverse drug reaction. The activity and high expression of the drug-transporter MDR1/P-glycoprotein, which is an ATP-dependent drug efflux pump, has been indeed shown to be associated with resistant disease,[18] whereas protein expression of *ABCG2* has been related to disease-free survival.[19] According to this, drug-metabolizing enzymes' genetic variants in genes involved in the metabolism of cytarabine (Ara-C), such as the deoxycytidine kinase and the cytidine deaminase, have been also demonstrated to impair the effectiveness and toxicity of Ara-C treatment. AML patients with higher deoxycytidine kinase expression demonstrated longer event-free survival than patients with low deoxycytidine kinase expression. Moreover, the 35708C > T 3′-untranslated region single-nucleotide polymorphism (SNP) has been linked to lower deoxycytidine kinase mRNA levels and lower Ara-CTP levels[20,21] and higher cytidine deaminase levels have been associated with disease recurrence and lower cytidine deaminase levels with longer duration of remission.[22]

These studies demonstrate that genetic characterization of AML patients is particularly crucial for selecting appropriate treatments. Nevertheless, how genetic variants in DMET genes interact to produce specific drug-related phenotypes in AML has not yet been investigated. In order to fill this gap, the DMET Plus platform was used to genotype 1931 variants on 225 DMET genes in a cohort of 94 CD33-positive Italian AML patients younger than 65 years, in search for unknown potential associations between genetic variability and clinical outcomes of drug treatment. All patients were homogeneously treated according to a clinical trial combining low dose of Gemtuzumab-Ozogamicin (GO), a chemotherapy agent composed of a humanized anti-CD33 antibody linked to the cytotoxic antitumor antibiotic Calicheamicin,[23,24] with Fludarabine, Cytarabine, and Idarubicin (FLAI) regimen as induction chemotherapy.

Table 1. Demographic, cytogenetic and molecular characteristics of the AML patients ($n = 94$) analyzed by the DMET platform

Variable	*N*
Age (median, range)	51 Years (19–65)
Sex	
M/F	45/49
De novo/sec	
De novo	76 (80.9%)
Secondary	18 (19.1%)
Risk	
High	62 (66%)
Standard	32 (34%)
Karyotype	
Normal	50 (53.2%)
FLT3	
Wild type	63 (67%)
ITD	8 (8.5%)
D835	16 (17%)
ITD+D835	3 (3.2%)
NA	5 (5.3%)
NPM1	
Pos	31 (33.0%)
Neg	58 (61.7%)
NA	5 (5.3%)

Abbreviations: AML, acute myeloid leukemia; DMET, drug-metabolizing enzyme/transporter; F, female; *FLT3*, *FMS-related tyrosine kinase 3*; M, male; NA, not available; Neg, negative; *NPM1*, *nucleophosmin 1*; Pos, positive; sec, secondary.

MATERIALS AND METHODS

We analyzed 94 CD33-positive AML patients, younger than 65 years, previously untreated and enrolled in a phase III multicenter clinical trial combining low dose of GO with FLAI regimen (Fludarabine, Cytarabine, and Idarubicin) as Induction chemotherapy (EudraCT: 2007-005248-26; ClinicalTrials.gov NCT00909168).

The induction regimen (GO–FLAI) included Fludarabine (25 mg sqm^{-1}) and Ara-C (2 g sqm^{-1}) on days 1–5, Idarubicin (10 mg sqm^{-1}) on days 1, 3 and 5 and GO (3 mg sqm^{-1}) on day 6. Consolidation included Idarubicin plus intermediate-dose Ara-C (AC). In the case of no response to GO–FLAI or in the case of AML patients who were waiting for allogeneic bone marrow transplantation, the patient could receive one course of high-dose AC. Intensification was based on allogeneic or autologous bone marrow transplantation.

Cytogenetic, multidrug-resistance phenotype, *FLT3* and *NPM1* mutation status analyses were performed at diagnosis in all patients. The main characteristics of the examined patients are shown in Table 1.

Genotype identification of 1931 SNPs and 5 copy number variations was performed using the DMET Plus GeneChip (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's instructions.

A full and detailed description of methods is provided in the Supplementary Methods.

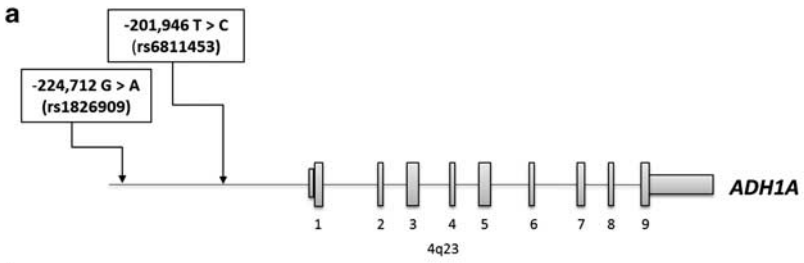
RESULTS

Genotyping quality results

Genotypes were evaluable for 91 of the 94 examined cases, as only samples with average call rates for the genotyped SNPs > 96% were retained after quality control filtering. The median call rate was 99.48% (range, 96.32–100%). Three samples were run in duplicate in order to assess genotyping accuracy and results with 'passed call rate' were compared across all the investigated nucleotide sites. When all SNPs were compared between the two duplicates of each sample, concordances of 98.91, 94.15 and 95.08% were found. However, the great majority of not-concordant results were due to the presence of No Call data (the confidence in assigning the data to the most likely genotype is poor, that is, genotypes with a score confidence value of < 0.1 are assigned as No Call for the locus of interest) in one of the two duplicates, and hence considering only SNPs showing a genotype call in both the duplicates raised the concordance values to 99.95, 99.95, and 99.89%, with a median repeatability of 99.93%. Moreover, three SNPs (rs2020863, rs2302948 and rs6811453) located in nonrelated genomic regions corresponding to *FMO2, SULT2B1* and *ADH1A* genes were analyzed by conventional Sanger sequencing in 13 patients previously analyzed with the DMET platform. Sequencing results confirmed DMET genotyping in all the 39 sequences. Association analyses were finally performed by exploiting information from the 938 variants that resulted nonmonomorphic in the examined AML cohort.

Association among SNPs and response to the induction cycle (GO–FLAI)

In an initial screening procedure, we investigated the association among SNPs and response to the induction cycle, which included a combination of FLAI and GO. Therefore, the genotyping profile of 80 AML patients in complete (85%) and partial (3%) remission was compared with that of 11 AML patients with no response (12%). According to both the allele and genotype association analyses, significant differences in the allele and genotype frequencies of two variants (rs6811453 and rs1826909), in high linkage disequilibrium (LD; $D' = 0.95$, $r^2 = 0.88$), in the alcohol



b

Gene and variant	Allele	Chr.	Position	Allele Freq. Responders	Allele Freq. Resistants	Fisher p value	OR
ADH1A -201946 T > C (rs6811453)	T	4	100414000	0.3	0.7	0.0006	5.68
ADH1A -224712 G > A (rs1826909)	A	4	100436766	0.3	0.7	0.0009	5.35

Figure 1. Localization of *ADH1A* variants associated with response to the induction cycle (Gemtuzumab-Ozogamicin with Fludarabine–Cytarabine–Idarubicin (GO–FLAI) (**a**); and their allele frequencies in the examined acute myeloid leukemia (AML) groups (**b**).

Table 2. Genotype distribution of variants potentially associated with the examined phenotypes

Variant	*Genotype and inheritance model*	*Resp/Ntox/Nf (%)*	*Res/Tox/Fr (%)*	*OR*	*CI*	*P-value*
rs6811453	C/C	48.1	0			
	C/T	45.6	60			
	T/T	6.3	40			
	Log-additive	88.8	11.2	8.08	2.31–28.29	0.0005
rs1826909	G/G	45.6	0			
	A/G	48.1	60			
	A/A	6.3	40			
	Log-additive	88.8	11.2	8.14	2.28–28.99	0.0009
rs2070673	T/T	60.3	92.6			
	A/T	33.3	7.4			
	A/A	6.3	0			
	Log-additive	70	30	0.14	0.03–0.6	0.0085
rs2515641	C/C	68.3	96.3			
	C/T	28.6	3.7			
	T/T	3.2	0			
	Log-additive	70	30	0.09	0.01–0.7	0.0059
rs4149056	T/T	87.3	59.3			
	C/T	12.7	33.3			
	C/C	0	7.4			
	Log-additive	70	30	4.55	1.68–12.33	0.0035
rs2302948	C/C	47.2	81.1			
	C/T	45.3	18.9			
	T/T	7.5	0			
	Log-additive	58.9	41.1	0.22	0.09–0.56	0.0023
rs11231825	C/C – C/T	94.3	73			
	T/T	5.7	27			
	Recessive	5.7	27	6.17	1.56–24.35	0.0044

Abbreviations: CI, 95% confidence interval; Fr, patients with fever reaction; Nf, patients without fever reaction; Ntox, patients without liver toxicity; OR, odds ratio; Res, GO-FLAI-resistant patients; Resp, GO-FLAI responders; Tox, patients with grade I/II liver toxicity.

dehydrogenase enzyme (*ADH1A*) were observed (Figure 1). In particular, the minor alleles of such SNPs were found to be significantly more represented in the AML-resistant subgroup, with a frequency of 70% (Figure 1), thus being considerably associated with resistance to the treatment. These results were further confirmed by association analyses performed at the genotype level. According to this, no ancestral homozygotes were observed in the group of resistant individuals that instead showed an outstanding higher percentage of derived homozygotes with respect to the responders' cohort, as well as a higher proportion of heterozygotes, with considerable odds ratios (ORs; Table 2). According to univariate analysis, these *ADH1A* variants were not associated with high-risk AML, *FLT3* and *NPM1* mutations, but strongly influenced response to the induction phase (Table 3), as well as their prognostic role was confirmed by multivariate analysis (Supplementary Table 1). Moreover, in our cohort, common genetic markers for risk assessment (*FLT3* and *NPM1* mutations) were not correlated with treatment response.

Association among SNPs and liver toxicity

As SNPs may also influence the presence/absence of liver toxicity as a consequence of chemotherapy drugs, we thereafter stratified genotypes according to this adverse response to treatment. Therefore, genotypes of 63 patients without liver toxicity (70%) were compared with those of 28 patients who experienced grade I/II liver toxicity (30%), finding out a significant difference in allele and genotype frequencies of a variant (rs2070673) occurring on a member of the cytochrome *P*450 family (*CYP2E1*). In such case, the ancestral allele was underrepresented in the group of AML patients showing liver toxicity with respect to that of individuals without adverse reaction to treatment (Figure 2). As a matter of fact, ancestral homozygotes were completely absent in the group characterized by liver toxicity, which also showed less heterozygotes and more derived homozygotes, confirming that the derived allele (T) is probably associated to toxicity, whereas the ancestral one may exert a protective action (Table 2). Another SNP (rs2515641), which is in moderate LD with the previously reported one ($D' = 0.86$, $r^2 = 0.41$), showed a pattern of statistically significant difference in allele and genotype frequencies of the two compared groups. Its derived allele was overrepresented in the sample of individuals without liver toxicity (Figure 2), as well as the derived homozygotes turned out to be completely absent in the group characterized by liver toxicity. Moreover, such cohort showed less heterozygotes and more ancestral homozygotes with respect to that of AML subjects without liver toxicity (Table 2).

On the contrary, the derived allele of the *SLCO1B1* rs4149056 turned out to be associated with adverse reaction to the used drugs (Table 2). In fact, it showed an increased frequency in the cohort of AML patients with liver toxicity (Figure 2), for which higher proportions of heterozygotes and derived homozygotes were also observed.

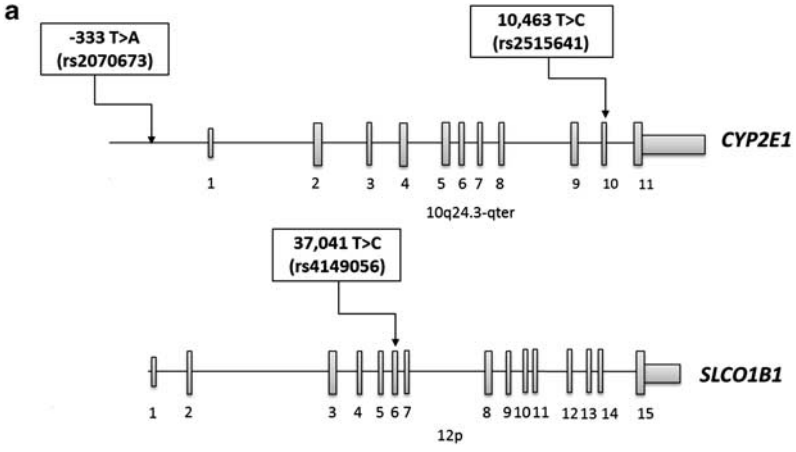
Table 3. Clinical and molecular variables related to *ADH1A* rs6811453 and rs1826909 in univariate analysis

Variable	*rs6811453 C/C rs1826909 G/G* N = 39	*rs6811453 C/T rs1826909 G/A* N = 43	*rs6811453 T/T rs1826909 A/A* N = 9	*P-value*
FLT3				
Mut	16 (41%)	10 (23%)	2 (22%)	0.25
Wild type	23 (59%)	31 (72%)	6 (67%)	
NA	—	2 (5%)	1 (11%)	
NPM1				
Mut	12 (31%)	13 (30%)	5 (56%)	0.37
Wild type	27 (69%)	25 (58%)	4 (44%)	
NA	—	5 (12%)	—	
Risk				
High	27 (69%)	25 (58%)	8 (89%)	0.18
Standard	12 (31%)	18 (42%)	1 (11%)	
Induction Res				
Resistant	—	7 (16%)	4 (44%)	0.0006
CR+PR	39 (100%)	36 (84%)	5 (56%)	

Abbreviations: CR, complete response; *FLT3*, *FMS-related tyrosine kinase 3*; Mut, mutated; NA, not available; *NPM1*, *nucleophosmin 1*; PR, partial response; Res, response.

Association among SNPs and infusion-related reactions to GO

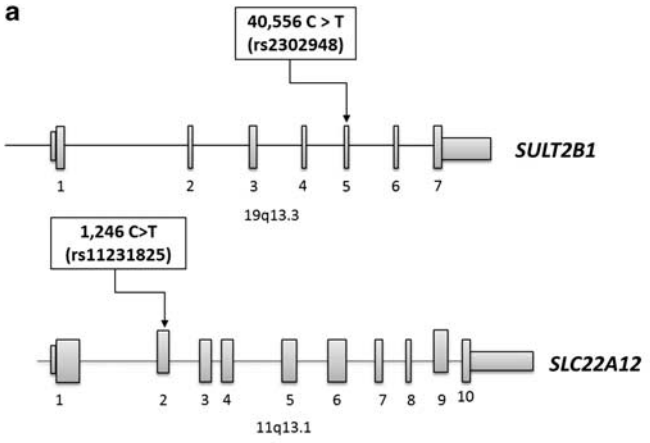
Finally, we performed an association analysis between genetic variants and infusion-related reactions to GO. In this analysis, genotypes of 37 patients (41%) who experienced fever were compared with that of 54 patients who did not (59%). The derived allele of rs2302948 located in the *sulfotransferase family cytosolic 2B member 1* (*SULTB1*) gene was found to be associated with a condition of no fever (Figure 3), seeming to act as a protective factor. The ancestral allele of another SNP (rs11231825) was significantly overrepresented in AML patients showing fever



b

Gene and variant	Allele	Chr.	Position	Allele Freq. No Toxicity	Allele Freq. Grade I/II Toxicity	Fisher p value	OR
CYP2E1 **-333 T >A (rs2070673)**	A	10	135190557	0.23	0.04	0.001	0.13
CYP2E1 **10463 T>C, F421F (rs2515641)**	T	10	135201352	0.17	0.02	0.003	0.09
SLCO1B1 **37041 T>C, V174A (rs4149056)**	C	12	21222816	0.06	0.24	0.002	4.68

Figure 2. Localization of *CYP2E1* and *SLCO1B1* variants associated with liver toxicity (**a**) and their allele frequencies in the examined acute myeloid leukemia (AML) groups (**b**).



b

Gene and variant	Allele	Chr	Position	Allele Freq. No Fever	Allele Freq. Fever	Fisher p value	OR
SULT2B1 40556 C > T, L213L (rs2302948)	T	19	53787877	0.30	0.09	0.0009	0.24
SLC22A12 1246 C>T, H142H (rs11231825)	T	11	64116850	0.22	0.43	0.0029	2.75

Figure 3. Localization of *SULT2B1* and *SLC22A12* variants associated with infusion-related reactions to Gemtuzumab-Ozogamicin (GO) (**a**) and their allele frequencies in the examined acute myeloid leukemia (AML) groups (**b**).

reaction (Figure 3). Both these results were confirmed by association analyses performed at the genotype level, with the rs2302948 heterozygotes and derived homozygotes being much more represented in the group of AML subjects without fever reaction, as well as the rs11231825 ancestral homozygotes being overrepresented in the opposite group (Table 2).

DISCUSSION

Medicines that work wonders for some can be ineffective, or even toxic, to others. This different behavior can be attributed to several reasons, such as the person's age, weight and lifestyle, as well as specific drug interaction with other medicines, but it has also been already proved that the genetic component has an important influence on the success of individual treatment response.[1]

In the present study, we have exploited the potential of a recently introduced, exploratory, large-scale approach made possible by the current high-throughput genotyping technology and having unprecedented informativity and resolution. The DMET platform, covering $>90\%$ of the most biologically relevant drug absorption, distribution, metabolism and excretion markers,[1,25] was indeed used, obtaining genotype information about 1931 variants distributed along 225 genes. Moreover, unlike other SNP detection methods, which interrogate markers with an average minor allele frequency of $\sim 20\%$, the ADME Core markers being part of the DMET Plus Panel have allelic frequencies $<9\%$, although more common genetic variants are also included. Therefore, we succeeded in exhaustively investigating genetic structure and related pharmacogenetic profiles within a cohort of AML samples enrolled in a clinical trial combining low dose of GO with FLAI regimen as induction chemotherapy, by means of association analyses based on a high number of genetic markers and thus implying multiple comparisons. Nevertheless, according to the exploratory and hypothesis-generating purpose of this study, a conventional 1% threshold for statistical significance was set. This necessarily means that identified findings might contain false positives and thus the results of potential interest will need to be further evaluated in independent AML cohorts to definitively elucidate the involvement of related variants in the examined phenotypes.

Association among the surveyed SNPs and response to the induction cycle (complete or partial *vs.* resistant), which was perfectly the same for all patients included in the study, was first tested. According to both allele and genotype association analyses, significant differences in allele and genotype frequencies of two variants in high LD (rs6811453 and rs1826909) and located on the *ADH1A* were observed. The enzyme encoded by *ADH1A* metabolizes the conversion of ethanol to acetaldehyde, which is thereafter converted to acetate by aldehyde dehydrogenases, or which is accumulated within the cell, creating protein and DNA adducts. Literature data already support the evidence that SNPs of both alcohol and aldehyde dehydrogenase genes lead to the production of enzymes with altered kinetic properties.[26,27] In particular, the pathophysiological effects of these variants may be mediated by abnormal accumulation of acetaldehyde, which is subsequently converted to crotonaldehyde by polyamines in dividing cells and forms mutagenic 1,N2-propanodeoxyguanosine adducts.[28] Both the identified *ADH1A* genetic variants, whose minor alleles turned out to be associated with complete or partial resistance to the induction cycle in the present study, are located in the promoter region of the gene, ~ 200 kb upstream with respect to the first exon, and their detailed role in the metabolism of chemotherapy drugs has not yet been described. According to this, it may be plausible that these SNPs do not play a direct role in the metabolism of the combination of FLAI regimen and GO used in the induction phase, but that other still unidentified variants, which are not included in the DMET Plus Panel, located in such genomic region, and in strong LD with them, actually alter *ADH1A* expression and/or functionality, thus contributing to the failure of the treatment response by the carriers.

When genotypes were stratified according to liver toxicity, significant differences in allele and genotype frequencies of two *CYP2E1* variants and one *SLCO1B1* nucleotide substitution were found between the subgroup of AML patients experiencing grade I/II liver toxicity and that showing no adverse reaction.

The *CYP2E1* gene is a member of the cytochrome *P*450 gene family and is involved in the alcohol metabolism and, in

conjunction with *ADH*, it contributes to increased acetaldehyde production. It also catalyzes the biotransformation of numerous xenobiotics of pharmaceutical and toxicological interest and it takes part in the biotransformation of endogenous compounds, such as ketone bodies, glycerol and different fatty acids, and generates reactive oxygen species that can damage cellular and mitochondrial components including mitochondrial DNA and cytochrome *c* oxidase.[29] Recently, immortalized B cells homozygous for the derived rs2070673 allele (T), which we have found to be overrepresented in the group of AML patients showing liver toxicity with respect to that of individuals without adverse reaction to treatment, have been demonstrated to have increased *CYP2E1* expression and enzymatic activity. This pattern was further associated to increased comet rate and percentage of DNA tails in such cells, and thus to high levels of DNA damage.[30] It is intriguing to note that in our AML subgroup characterized by liver toxicity, 92.6% of subjects were homozygotes for the derived rs2070673 allele and no homozygotes for the ancestral allele were present. According to these evidences, the presence of the rs2070673 variant allele may also lead to higher levels of *CYP2E1* transcription and enzymatic activity in AML cells, thus explaining the mechanism that potentially underlies its association to liver toxicity in response to the adopted chemotherapy drugs.

On the contrary, frequency patterns observed for *CYP2E1* rs2515641 suggest that its derived allele (T), being more represented in the sample of individuals without liver toxicity, may exert a protective action, whereas the more diffused ancestral allele can be somehow involved in increased susceptibility to such adverse reaction. However, as the ancestral allele of this SNP is in partial LD with the rs2070673 derived allele, we cannot rule out the hypothesis that its association signal simply reflects that of the more biologically plausible rs2070673 one.

The *SLCO1B1* rs4149056-derived allele also turned out to be overepresented in the cohort of AML patients with liver toxicity, thus resulting associated with adverse reaction to the used drugs. The *SLCO1B1* gene encodes a solute carrier organic anion transporter family member 1B1, a liver-specific cell membrane influx transporter that mediates sodium-independent uptake of many endogenous compounds, playing an important role in the clearance of bile acids and organic anions from the liver, and being also involved in the removal of drug compounds from the blood into the hepatocytes. Different polymorphisms in this gene have been already associated with impaired transporter function. In particular, the nonsynonymous rs4149056, located on exon 6, has been already related to statin metabolism and has resulted in a strong association with an increased risk of statin-induced myopathy.[31] Therefore, the significantly higher proportions of rs4149056-derived homozygotes and heterozygotes in the cohort of AML patients with liver toxicity with respect to the group showing no adverse reaction again support a crucial role of *SLCO1B1* in drug metabolism and define rs4149056 as a potential useful marker to predict or prevent reaction to therapy.

Finally, possible relationships between DMET genetic variants and infusion-related reactions to GO were also investigated, finding two different SNPs being differentially associated with the presence (rs11231825 ancestral allele) or absence (rs2302948-derived allele) of a fever reaction after GO infusion. This latter polymorphism is located on the *SULT2B1* gene encoding for a cytoplasmic protein involved in renal excretion of different compounds, such as hormones, neurotransmitters and drugs, through sulfonation and subsequent water solubility increase. Although there is no evidence for rs2302948 to directly affect *SULT2B1* catalytic activity, alterations in renal function due to the presence of its ancestral allele, or more plausibly to the action of an unidentified SNP-derived allele in LD with it, have the potential to cause an inflammatory status that occurs with fever. A similar mechanism can also be invoked for rs11231825 that was located on the *SLC22A12* gene encoding for the solute carrier family 22 organic anion/cation transporter member 12, a urate transporter and urate-anion exchanger that regulates the level of blood urate. Several *SLC22A12* variants have been already associated with altered reabsorption of uric acid by kidneys, contributing to hyperuricemia or hypouricemia[32,33] and thus being responsible for abnormal renal function.

In conclusion, we performed an exploratory, fine-scale assessment of the genetic structure of a cohort of AML patients, and succeeded in relating SNPs located on multidrug enzymes and transporter genes with the efficacy and toxicity of a combination of GO with FLAI regimen. According to this approach, we identified for the first time a pharmacogenetic panel made up of one gene (*ADH1A*) potentially associated with AML clinical outcome and four genes (*CYP2E1*, *SLCO1B1*, *SULT2B1*, and *SLC22A12*) plausibly associated with treatment toxicity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Author contributions: GM and II: project conception; II, MS, AL: manuscript writing; II, AL, SF, EA and AA: DMET array analysis; MS: statistical analyses; II, MS and GM: data interpretation; AL and AF: Sanger sequencing validation analysis; AC, CP, AM, ET, MCA, MM, FC, DR, DD, FG, MG and FP: clinical data and biological sample collection; GI MAR and MB: final approve.

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