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Association of ADME gene polymorphisms on toxicity to CDK4/6 inhibitors in patients with HR+ HER2- metastatic breast cancer

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ABSTRACT

A wide interindividual variability in therapeutic response to cyclin-dependent kinases 4 and 6 inhibitors (CDKis) palbociclib, ribociclib and abemaciclib, among patients with HR+/HER2- metastatic breast cancer has been reported. This study explored the impact of genetic polymorphisms in ADME genes (responsible for drug absorption, distribution, metabolism, and elimination) on CDKis safety profiles in 230 patients. Selected endpoints include grade 3/4 neutropenia at day 14 of the first treatment cycle, early dose-limiting toxicities (DLTs), and dose reductions within the initial three cycles. Our analysis revealed associations between these endpoints and polymorphisms in *CYP3A4*, *CYP3A5*, *ABCB1*, and *ABCG2* genes. Their impact on CDKis plasma concentrations (C_{trough}) was also examined. Specifically, *ABCB1* c.1236C>T and c.2677C>T polymorphisms correlated significantly with grade 3/4 neutropenia at day 14 (OR 3.94, 95% CI 1.32–11.75; p = 0.014 and OR 3.32, 95% CI 1.12-9.85; p = 0.030). Additionally, *ABCB1* c.3435C>T was associated with an elevated risk of early DLTs and dose reductions (OR 3.28, 95% CI 1.22–8.84, p = 0.019; OR 2.60, 95% CI 1.20–5.60, p = 0.015). Carriers of the *CYP3A4**22 allele also demonstrated in univariate a higher risk of early DLTs (OR 3.10, 95% CI 1.01–9.56, p = 0.049). Furthermore, individuals with the *ABCB1* 1236T–3435T–2677T(A) variant haplotype exhibited significant associations with grade 3/4 neutropenia at day 14 (OR 3.36, 95% CI 1.20–9.41; p = 0.021) and early DLTs in univariate (OR 3.08, 95% CI 1.19–7.95; p = 0.020). Homozygous carriers of the *ABCB1* T-T-T(A) haplotype tended to have a higher mean ribociclib C_{trough} (934.0 ng/mL vs. 752.0 ng/mL and 668.0 ng/mL). Regardless preliminary, these findings offer promising insights into the role of pharmacogenetic markers in CDKis safety profiles, potentially contributing to address the interindividual variability in CDKis responses.

1. Introduction

Palbociclib, ribociclib and abemaciclib are cyclin-dependent kinases 4 and 6 inhibitors (CDKis) whose use is well established in clinical practice for the treatment of advanced or metastatic hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2)-negative breast cancer in combination with aromatase inhibitor (letrozole/anastrozole/exemestane) or with the selective estrogen receptor degrader (SERD) fulvestrant [1–4].

Hematologic toxicities such as neutropenia, anaemia, and leukopenia are the most described side effects for CDKis. Grade ≥ 3 neutropenia occurs in approximately 60% of patients in the first treatment cycles of registration trials with a median onset of 15 days for both palbociclib and ribociclib, and a median duration of 7 and 12 days respectively [1,2]. Conversely, abemaciclib-treated patients exhibited a lower incidence of grade ≥ 3 neutropenia, occurring in only 25% of cases, with a median time to onset of 30 days and a median duration of 14 days. Gastrointestinal toxicities were more pronounced in

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abemaciclib-treated patients, with grade 3 diarrhea affecting approximately 14% of them within 6–8 days of treatment initiation and lasting 5–8 days [3].

To manage these toxicities, approximately 35–45% of patients required dose reductions due to any grade side effects, and about 10% of patients discontinued CDKis [1–3]. Such treatment adjustments, along with treatment suspensions or schedule changes are the recommended strategies for CDKis' toxicities management. However, such adjustments can potentially compromise treatment adherence by leading to inadequate dose intensity, which is critical for treatment success, especially in the case of CDKis taken over a long period of time [5]. Identifying predictive biomarkers of toxicities that lead to treatment adjustments (dose-limiting toxicities, DLTs) can play a pivotal role in ensuring adequate treatment adherence and improve patient's quality of life.

Some intrinsic factors have been shown to be closely associated with higher susceptibility to hematologic toxic events in CDKis' treatment. Asian ethnicity, a low baseline absolute neutrophil count (ANC) or low blood cell count have been linked to an increased risk of grade $\geq$ 3 neutropenia in patients treated with palbociclib and ribociclib [6–10] or abemaciclib [11]. Age greater than 70 years was also identified as a potential risk factor for a higher risk of grade $\geq$ 3 diarrhea during treatment with abemaciclib [11]. Additionally, higher plasmatic CDKis exposure has been associated with a greater toxicity risk. In particular, analysis of data from MONARCH 2 suggested that a higher abemaciclib exposure was associated with a higher risk of neutropenia [12]. Greater reductions in ANC and platelet levels were related with higher palbociclib area-under-the-curve values [13], and a correlation between higher palbociclib exposure and higher degree of neutropenia was found by a semi-mechanistic pharmacokinetic-pharmacodynamic model [14]. The relationship between ribociclib exposure and QTc prolongation was also characterized, and patients with a mean steady-state C_{max} of 2237 ng/mL presented a mean QTc prolongation of 22.87 ms [15].

Notably, palbociclib, ribociclib and abemaciclib share a common metabolic pathway mediated by cytochrome P450 isoenzyme (CYP)3 A. *In vitro* and *in vivo* studies have indicated that CDKis are substrate of the efflux transporter P-glycoprotein (P-gp), an adenosine triphosphate-binding cassette of subfamily B, member 1, encoded by the *ABCB1* gene, and of breast cancer resistance protein (BCRP), encoded by the *ABCG2* gene [16].

Several factors are thought to influence the variability of treatment efficacy and toxicity, including organ function, comedications, hormonal status, body weight, age, comorbidities, etc. Among them, single nucleotide polymorphisms (SNPs) are attracting huge interest to address the interindividual variability in drug response, whether in terms of efficacy or safety, since approximately 90–95% of people are carriers of at least one genetic variant that is likely to affect drug response[17]. In particular, SNPs in genes involved in the pharmacokinetic profile (absorption, distribution, metabolism, and excretion, ADME) could be predictive factors for the occurrence of toxicity, e.g. polymorphisms of the CYP450 family or drug transporters [18]. For other oral target therapies such as imatinib [19], gefitinib [20], nilotinib [21], or dasatinib [22] evidence about gene-drug interactions are available, while on CDKis there are preliminary data [7,23].

This study aims to explore a prospective cohort of 230 patients from a single institution to determine whether carriers of SNPs in ADME genes exhibit distinct CDKis' safety profiles and if this is reflected in differences in plasmatic CDKis exposure, measured by C_{trough}.

2. Methods

2.1. Study design

Patients diagnosed with HR-positive, HER2-negative metastatic breast cancer who initiated a CDKi treatment, either in combination with endocrine therapy or fulvestrant, were prospectively enrolled in the CRO–Aviano Integrated Pharmacological Counseling Program between 2020 and 2023 [24]. The study received approval from the internal ethics committee of CRO Aviano (CRO-2022–14) and was conducted in compliance with the principles outlined in the Helsinki Declaration. All participating patients provided written informed consent.

Data on baseline patient characteristics such as age, treatment setting, drug dose, menopausal status, body mass index (BMI), ANC, and adverse drug reactions (ADRs) were collected for the first three cycles of therapy (84 days). Data were prospectively collected from electronic medical records following some eligibility criteria: women over 18 treated with a CDKi for at least three months, first or second-line treatment, clinical and pharmacogenetic data available.

ADRs and hematologic laboratory data were recorded at each visit that coincided with the first day of the therapeutic cycle for each cycle between the first prescription and the cutoff date of the last observation. In addition, data were also collected at the interim visits corresponding to day 14 of the first two cycles of treatment. ADRs were classified by grade (G) according to the Common Terminology Criteria for Adverse Events (NTC-CTCAE Version 5.0) (November 2017).

Trough concentration at steady state (C_{trough}) was also collected in a subgroup of patients.

2.2. Outcome events

Some baseline patients' characteristics and the presence or absence of ADME gene polymorphisms were associated with safety outcomes: G3/4 neutropenia at day 14 of first cycle of treatment, DLTs within first cycle (28 days) defined as "early DLTs" and dose reductions.

DLTs were defined as occurring of one of the following: hematologic toxicities CTCAE G$\geq$ 4 (and G3 febrile neutropenia); non-hematologic toxicities CTCAE G$\geq$ 3; any toxicity on day 1 of the cycle that persists despite treatment interruption (14 $\pm$ 2 days of interruption) or any toxicity that requires treatment discontinuation.

Dose reduction in the first three months of treatment were only considered and were defined as reducing the dose of palbociclib from 125 mg to 100 mg or 75 mg; from 600 mg to 400 mg or 200 mg for ribociclib and from 300 mg to 200 mg or 100 mg for abemaciclib ($\geq$20% dose reduction).

Of the 230 patients, 195 were eligible for evaluation of G3/4 neutropenia at day 14 of first cycle of treatment; 203 for early DLTs and 222 were eligible for analysis of dose reductions. Exclusions were related to absence of data, early discontinued treatment during the observation period for reasons unrelated to toxicity or progression, and patients who received an off-label reduction.

2.3. Pharmacogenetic analysis

Pharmacogenetic analyses, after DNA extraction from blood samples, were performed by SNPline PCR Genotyping System platform using Kompetitive allele–specific assays (LGC Genomics, Hoddesdon, UK) following the manufacturer's instructions. According to CPIC guidelines and literature, polymorphisms in genes involved in the uptake, distribution, metabolism, and elimination of CDKis were analyzed: *CYP3A4* (*22, rs35599367); *CYP3A5* (*3, rs776746); *ABCB1* (c.1236C>T, rs1128503; c.3435C>T, rs1045642; c.2677G>T/A, rs2032582); and *ABCG2* (c.421C>A, rs2231142). Pyrosequencing technology from PyroMark Q48 (Qiagen, Hilden; Germany), was used for the analysis of *ABCB1* c.2677G>T/A to achieve triallelic discrimination. Positive and negative control samples were included in each analysis.

2.4. TDM analysis

A whole blood sample was performed in eligible patients after 24 h from their last palbociclib or ribociclib intake and 12 h from abemaciclib last intake, which allowed the evaluation of C_{trough} with respectively coefficient of variation (CV, %). Plasma was collected by centrifugation

and stored at − 80 °C until analysis. Patients' samples were analyzed by a developed LC-MS/MS methods that have been reported previously [25,26]. At the time of sampling, information was also retrieved on adherence to treatment: doses not taken due to forgetfulness or following a medical advice, in order to understand whether the patients had taken the drug continuously for the days necessary to reach steady state.

2.5. Statistical analysis

All the statistical analyses were performed using STATA software and the results are reported as number and percentages and as Odds Ratio (OR) with relative 95% Confidence Interval (CI).

The associations between clinical characteristics and the different endpoints considered were evaluated using two-sided Fisher's exact test. Further, all the associations between the polymorphisms and haplotype and risk of experimented neutropenia G3/4 at day 14 of first cycle, early DLTs and dose reductions, were examined through univariate logistic regression analyses. For each single polymorphism, dominant, recessive, and additive genetic models were analyzed by combining heterozygous with homozygous genotypes. The best-fitting genetic model was selected according to the Wald chi-squared test. Additionally, multivariate logistic regression analyses were performed for significant covariates.

The median C_{trough} of three CDKis in patients carrying different genotypes were compared by Kruskal-Wallis test.

All results were considered statistically significant with p value < 0.05.

3. Results

3.1. Patients' characteristics

A total cohort of 230 patients treated with CDK4/6 inhibitors at National Cancer Institute, IRCCS, Aviano between 2020 and 2023 were included in the analysis. Demographics, baseline clinical patients' characteristics and the outcome events are presented in Table 1. All patients were self-reported Caucasians. Among ADRs were recorded as early DLTs (previously defined) the following toxicities: G4 neutropenia, G3 neutropenia which persists despite treatment interruption (14 ± 2 days), G4 thrombocytopenia, G4 leucopenia, G3 cardiovascular toxicity, G3 hypertransaminasemia, and G2 diarrhea which persists despite treatment interruption (14 ± 2 days).

3.2. Clinical characteristics and outcome events

In Table 2 are reported the association between clinical characteristics and the different endpoints studied. The occurrence of G3/4 neutropenia at day 14 of the first treatment cycle was found significantly higher in patients with low baseline ANC (i.e., ANC $< 3.6\times10^3/mm^3$ versus ANC $\geq 3.6\times10^3/mm^3$; $p < 0.001$). Early DLTs occurrence was also found to be significantly associated with low baseline ANC ($p = 0.017$). Regarding dose reduction, the only statistically difference emerged with age. Patients with aged older than the median value (61 years) underwent more dose reductions than patients aged younger the median value ($p = 0.011$).

3.3. ADME gene polymorphisms and outcome events

All the 230 patients were successfully genotyped. Twenty-one out of 230 patients (9.1%) were carriers of at least one *CY3A4**22 variant allele whereas 2/230 (0.9%) were *CYP3A5**1/*1, 20/230 (8.7%) were *CYP3A5**1/*3 and 208/230 (90.4%) *CYP3A5**3/*3. Regarding *ABCB1* SNPs, 41 of total 230 (17.8%) patients were carriers of *ABCB1* c.1236TT alleles (homozygous variant) while 117/230 (50.9%) were heterozygous (c.1236CT). The same frequencies were found for the carriers of the *ABCB1* c.2677G>T/A triallelic polymorphism: 42/230 (18.3%) patients were carriers of c.2677TT/TA (homozygous variant) and 116/230 (50.4%) were heterozygous (c.2677GT/GA). *ABCB1* c.1236C>T and *ABCB1* c.2677G>T/A were in linkage-disequilibrium. For *ABCB1* c.3435C>T SNP, 54/205 (23.5%) patients were homozygous carriers of variant allele (c.3435TT) and 107/230 (46.5%) were heterozygous (c.3435CT) indeed for *ABCG2* c.421C>A there were only 56/230 (24.3%) patients who are carriers of one variant allele (c.421CA, heterozygous). No significant deviation from Hardy-Weinberg equilibrium was found for all the SNPs analyzed.

Statistical analyses were performed to investigate the association between the presence of SNPs in ADME genes relevant for CDKis and toxicity outcome events (Table 3). The analyses concerning the presence of polymorphisms in genes encoding enzymes involved in CDKis metabolism show a significant association for carriers of *CYP3A4**22 allele with the risk of early DLTs (OR 3.10, 95%CI 1.01–9.56; $p = 0.049$).

Univariate analysis showed a significant association between polymorphisms in gene encoding for P-gp, as *ABCB1* c.1236C>T SNP and the risk of G3/4 neutropenia at day 14 of first cycle of CDKis treatment (OR 3.11, 95%CI 1.23–7.91; $p = 0.017$). Homozygous carriers of *ABCB1* c.1236C>T variant allele had higher occurrence of early DLTs (OR 2.30,

Table 1
A descriptive of patients' characteristics and outcomes events.

Patients' characteristics	Tot, n (%)
Number of enrolled patients	230
Age at enrollment	
Median years (IQR range)	61 (52 – 71)
Hormonal status	
Menopausal	180 (78.3)
Pre-menopausal	50 (21.7)
CDKis setting	
First line	192 (83.5)
Second line	38 (16.5)
BMI	
Median kg/m^2 (IQR range)	25.0 (21.8 – 28.4)
Drugs	
Palbociclib	136 (59.1)
Ribociclib	75 (32.6)
Abemaciclib	19 (8.3)
Anti-hormonal therapy	
Letrozole	141 (61.3)
Fulvestrant	85 (36.9)
Exemestane	2 (0.9)
Anastrozole	2 (0.9)
ANC baseline, *n*	205
$<3.6x10^3/mm^3$	85 (41.5)
$\geq3.6x10^3/mm^3$	120 (58.5)
Outcome events	Tot, n (%)
Neutropenia at day 14 of first cycle	
Patients eligible for evaluation of G3/4 neutropenia at day 14 of the first cycle	195
Patients who experienced a neutropenia G3/4 at day 14 of the first cycle	36 (18.5)
Early DLTs (within the first cycle/28 days)	
Patients eligible for evaluation of DLTs	203
Patients who experienced an early DLTs	24 (11.8)
Patients who experienced an early DLTs, and then underwent dose reduction within the first 3 cycles	16 (66.6)
Dose reduction (within the first 3 cycles/84 days)	
Patients eligible for evaluation of dose reduction	222
Patients who experienced a dose reduction	36 (16.2)
C_{trough}	
Palbociclib median value, ng/mL (IQR range)	62.5 (51.4 – 77.4)
Ribociclib median value, ng/mL (IQR range)	672.5 (493.5 – 985.8)
Abemaciclib median value, ng/mL (IQR range)	223.5 (170.1 – 281.0)

Abbreviations: IQR, interquartile range; BMI, body mass index; ANC, absolute neutrophil count; DLT, dose-limiting toxicities, C_{trough}, trough concentration at steady state.

Table 2
Association between patients' clinical characteristics and outcome events.

	NEUTROPENIA at DAY 14 of FIRST CYCLE (n = 195)		EARLY DOSE-LIMITING TOXICITIES (n = 203)		DOSE REDUCTION (n = 222)	
	Grade 3–4 (n = 36)*	Fisher's exact test *p* value	Day 1–28 (n = 24)*	Fisher's exact test *p* value	Day 1–84 (n = 36)*	Fisher's exact test *p* value
Age at the start of therapy						
< 61, n (%)	16 (44.4)	0.855	10 (41.7)	0.516	10 (27.8)	**0.011**
≥ 61, n (%)	20 (55.6)		14 (58.3)		26 (72.2)	
Menopausal status						
Menopausal, n (%)	30 (83.3)	0.651	20 (83.3)	0.607	30 (83.3)	0.513
Pre-menopausal, n (%)	6 (16.7)		4 (16.7)		6 (16.7)	
BMI (Kg/m^2)						
< 25 Kg/m^2, n (%)	18 (50.0)	0.716	13 (54.2)	0.523	22 (61.1)	0.101
≥ 25 Kg/m^2, n (%)	18 (50.0)		11 (45.8)		14 (39.9)	
Baseline ANC (x10^3/mm^3)	**(n = 30)**		**(n = 21)**		**(n = 30)**	
*≥ 3.6*x10^3/mm^3, *n (%)*	7 (23.3)	**< 0.001**	7 (33.3)	**0.017**	14 (46.7)	0.164
*< 3.6*x10^3/mm^3, *n (%)*	23 (76.7)		14 (66.7)		16 (53.3)	

*Only the number of patients who had the outcome event were reported
Abbreviations: BMI, body mass index; ANC, absolute neutrophil count.

Table 3
Association of ADME gene polymorphisms with outcome events.

		NEUTROPENIA at DAY 14 of FIRST CYCLE (n = 195)					Univariate logistic regression		Multivariate logistic regression[b]	
Gene	SNP		Grade 3-4 (n = 36)[a]			Model	Odds Ratio (95% CI)	*p* value	Odds Ratio (95% CI)	*p* value
			AA	*Aa*	*aa*					
CYP3A4	rs35599367	*22	33 (91.7)	3 (8.3)	0 (0.0)	Dominant	0.87 (0.24 – 3.19)	0.837		
CYP3A5	rs776746	*3	0 (0.0)	3 (8.33)	33 (91.7)	Additive	0.78 (0.23 – 2.66)	0.690		
ABCB1	rs1128503	c.1236C>T	6 (16.7)	24 (66.7)	6 (16.7)	Dominant	**3.11 (1.23 – 7.91)**	**0.017**	**3.94 (1.32 – 11.75)**	**0.014**
ABCB1	rs1045642	c.3435C>T	8 (22.2)	17 (47.2)	11 (30.6)	Dominant	2.01 (0.86 – 4.70)	0.107		
ABCB1	rs2032582	c.2677G>T/A	6 (16.7)	23 (63.9)	7 (19.4)	Dominant	**3.11 (1.23 – 7.91)**	**0.017**	**3.32 (1.12 – 9.85)**	**0.030**
ABCB1	haplotype	T-T-T(A)	8 (22.2)	22 (61.1)	6 (16.7)	Dominant	**2.62 (1.12 – 6.10)**	**0.026**	**3.36 (1.20 – 9.41)**	**0.021**
ABCG2	rs2231142	c.421C>A	24 (66.7)	12 (33.3)	0 (0.0)	Dominant	1.54 (0.70 – 3.6)	0.280		
		EARLY DOSE-LIMITING TOXICITIES (n = 203)					Univariate logistic regression		Multivariate logistic regression[b]	
Gene	SNP		Day 1-28 (n = 24)[a]			Model	Odds Ratio (95% CI)	*p* value	Odds Ratio (95% CI)	*p* value
			AA	*Aa*	*aa*					
CYP3A4	rs35599367	*22	19 (79.2)	5 (20.8)	0 (0.0)	Dominant	**3.10 (1.01 – 9.56)**	**0.049**	3.0 (0.81 – 11.11)	0.100
CYP3A5	rs776746	*3	0 (0.0)	2 (8.33)	22 (91.7)	Additive	0.88 (0.21 – 3.77)	0.862		
ABCB1	rs1128503	c.1236C>T	6 (25.0)	10 (41.7)	8 (33.3)	Recessive	2.30 (0.91 – 5.83)	0.080		
ABCB1	rs1045642	c.3435C>T	4 (16.7)	9 (37.5)	11 (45.8)	Recessive	**3.25 (1.35 – 7.83)**	**0.009**	**3.28 (1.22 – 8.84)**	**0.019**
ABCB1	rs2032582	c.2677G>T/A	4 (16.7)	12 (50.0)	8 (33.3)	Additive	**1.86 (1.00 – 3.46)**	**0.049**	2.24 (0.70 – 7.13)	0.174
ABCB1	haplotype	T-T-T(A)	6 (25.0)	10 (41.7)	8 (33.3)	Recessive	**3.08 (1.19 – 7.95)**	**0.020**	2.34 (0.80 – 6.84)	0.121
ABCG2	rs2231142	c.421C>A	19 (79.2)	5 (20.8)	0 (0.0)	Dominant	0.81 (0.29 – 2.29)	0.285		
		DOSE REDUCTION (n = 222)					Univariate logistic regression		Multivariate logistic regression[c]	
Gene	SNP		Day 1-84 (n = 36)[a]			Model	Odds Ratio (95% CI)	*p* value	Odds Ratio (95% CI)	*p* value
			AA	*Aa*	*aa*					
CYP3A4	rs35599367	*22	32 (88.9)	4 (11.1)	0 (0.0)	Dominant	1.43 (0.44 – 4.57)	0.552		
CYP3A5	rs776746	*3	0 (0.0)	4 (11.1)	32 (88.9)	Dominant	1.33 (0.42 – 4.23)	0.631		
ABCB1	rs1128503	c.1236C>T	11 (30.6)	15 (41.7)	10 (27.7)	Recessive	2.00 (0.87 – 4.58)	0.101		
ABCB1	rs1045642	c.3435C>T	10 (27.8)	11 (30.6)	15 (41.7)	Recessive	**2.88 (1.35 – 6.11)**	**0.006**	**2.60 (1.20 – 5.60)**	**0.015**
ABCB1	rs2032582	c.2677G>T/A	10 (27.8)	16 (44.4)	10 (27.8)	Recessive	1.92 (0.84 – 4.39)	0.120		
ABCB1	haplotype	T-T-T(A)	12 (33.3)	15 (41.7)	9 (25.0)	Recessive	2.25 (0.95 – 5.36)	0.067		
ABCG2	rs2231142	c.421C>A	25 (69.4)	11 (30.6)	0 (0.0)	Dominant	1.38 (0.63 – 3.02)	0.442		

Abbreviations: CI, Confidence Interval
[a] Only the number of patients who had the outcome event were reported
[b] By generalized linear model, adjusted by index drug and ANC baseline
[c] By generalized linear model, adjusted by index drug and age

95%CI 0.91–5.83; $p < .10$). A significant association was also reported between *ABCB1* c.3435C>T SNP and incidence of early DLTs (OR 3.25, 95%CI 1.35–7.83; $p = 0.009$) and dose reductions (OR 2.88, 95%CI 1.35–6.11; $p = 0.006$). Regarding the triallelic SNP, *ABCB1* c.2677G>T/A, a significant association was reported with the incidence of G3/4 neutropenia at day 14 of first cycle (OR 3.11, 95%CI 1.23–7.91; $p = 0.017$) and the incidence of early DLTs (OR 1.86, 95%CI 1.00–3.46; $p = 0.049$) while the significance was not reached with dose reductions.

We also examined the *ABCB1* haplotype (c.1236C>T, c.3435C>T, and c.2677G>T/A). Thirty-four patients out of 230 (14.8%) were homozygous carriers of variant haplotype (1236T–3435T–3677T(A)), 114/230 (49.6%) were heterozygous carriers and 84/230 (36.5%) were noncarriers. A significant association was reported with the carriers of variant haplotype and neutropenia at day 14 of first cycle (OR 2.62, 95%

CI 1.12–6.10; $p = 0.026$) and early DLTs (OR 3.08, 95%CI 1.19–7.95; $p = 0.020$). Also, homozygous carriers of variant haplotype had higher incidence of dose reductions (OR 2.25, 95%CI 0.95 – 5.36; $p < .10$).

A multivariate analysis was performed. Regarding neutropenia at day 14, the three statistically significant associations found in univariate analysis remained consistent also after adjustment. The association of *ABCB1* c.3435C>T with early DLTs and dose reductions remained essentially unchanged after adjusting for potentially confounding factors (Table 2).

3.4. ADME gene polymorphisms and $C_{through}$

A total of 122 samples were collected for the C_{trough} evaluation, more precisely 49 samples for palbociclib, 44 samples for ribociclib and 29 for abemaciclib. Each sample was performed at standard dose for all 3 CDKis and at steady state. The C_{trough} median value for palbociclib was 62.5 ng/mL (%CV 33.6), for ribociclib was 672.5 ng/mL (%CV 47.2) and for abemaciclib was 223.5 ng/mL (%CV 45.5), as reported in Table 1. We evaluated whether the exposure to CDKis was different in the carriers of the *ABCB1* haplotype and of the *CYP3A4**22 polymorphism (Figs. 1 and 2).

No differences in median plasma palbociclib concentrations were observed between the groups of patients carrying the *ABCB1* T-T-T(A) haplotype in homozygosity or heterozygosity and the noncarriers (58.0 ng/mL [%CV 25.6], 65.7 ng/mL [%CV 34.2] and 63.3 ng/mL [% CV 36.2] respectively; $p = 0.9432$). As well as between the carriers of the *CYP3A4**22 variant allele and noncarriers (65.70 ng/mL [%CV 10.9] and 62.5 ng/mL [%CV 34.6] respectively; $p = 0.9844$). For ribociclib, the median plasma concentration is higher in carriers of the homozygous *ABCB1* T-T-T(A) variant haplotype than in heterozygous carriers and noncarriers (934.0 ng/mL [%CV 43.4], 752.0 ng/mL [%CV 61.4] and 668.0 ng/mL [%CV 38.2] respectively; $p = 0.5724$). The same was observed between the carriers of the *CYP3A4**22 variant allele and noncarriers (718.0 ng/mL [%CV 49.7] and 672.5 ng/mL [%CV 47.5] respectively; $p = 0.7057$). Finally, for abemaciclib, small differences in median plasma concentrations were observed among the three groups of *ABCB1* haplotype (276.0 ng/mL, 203.5 ng/mL [%CV 39.0] and 238.6 ng/mL [%CV 51.0] respectively; $p = 0.4090$), although there was only one patient in the homozygous carrier group. As well as between the group of carriers of *CYP3A4**22 variant allele and noncarriers there were small differences. The median value of the patients who were carriers of the *CYP3A4**22 variant allele was 209.3 ng/mL (%CV 9.6), whereas the patients who were noncarriers of the mutation had a median plasma concentration of 264.7 ng/mL (%CV 41.6) ($p = 0.4409$).

4. Discussion

Interindividual variability in therapeutic outcomes is one of the main problems in anticancer drugs treatment that has never been fully addressed. This is especially true for orally administered anticancer drugs, including CDKis, where the route of administration further affects oral bioavailability. In addition, patients receiving oral target therapies are likely to be treated for a longer period of time compared with cytotoxic chemotherapies. Therefore, although less severe, toxicities with target agents are often symptomatic and persistent and still require dose reductions, treatment suspensions or schedule modifications for their management. DLTs may therefore compromise treatment adherence and consequently, maximum clinical benefit [5,27]. A recent real-word study of 396 patients treated with CDKis highlighted that neutropenia and diarrhea were the most common side effects. Of the patients who experienced neutropenia, 36% required dose reductions and 10% permanently discontinued treatment; while patients who experienced diarrhea, 27% required dose reductions and 21% required permanently discontinued treatment [28].

In our study involving 230 HR+/HER2- metastatic breast cancer patients, we investigated pharmacogenetic polymorphisms in ADME genes to identify potential markers for stratifying patients at risk of experiencing CDKis' associated toxicities, specifically G3/4 neutropenia, early DLTs, and dose reductions. We also examined their impact on plasmatic CDKis' exposure.

To date pharmacogenetic predictive biomarkers of CDKis toxicities have not been identified, with only preliminary results available. Iwata et al. in 2021 identified *ABCB1* c.1236C>T polymorphism as potential independent risk factors for grade 3–4 neutropenia in non-Asian patients at day 15 of first treatment cycle with palbociclib ($p < .10$) [7]. While, Maeda et al. in 2022 found that patients who were carriers of the *ABCB1* c.2677G>T/A polymorphism had higher tendency of abemaciclib withdrawal or dose reductions within first 4 treatment weeks ($p < .10$)

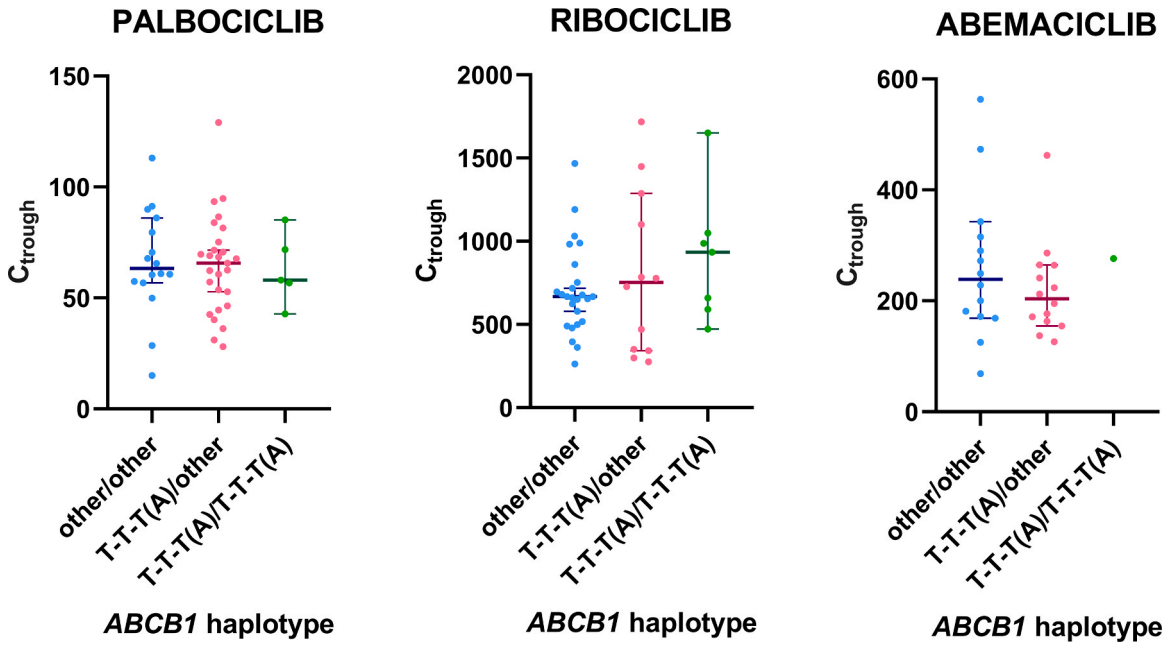

Fig. 1. Association between *ABCB1* variant haplotype (1236T–3435T–2677 T/A) and C_{trough} of each CDKi. Box plots depict the median (horizontal bar) and 95% Confidence Interval (CI). The dots represent individual concentration values.

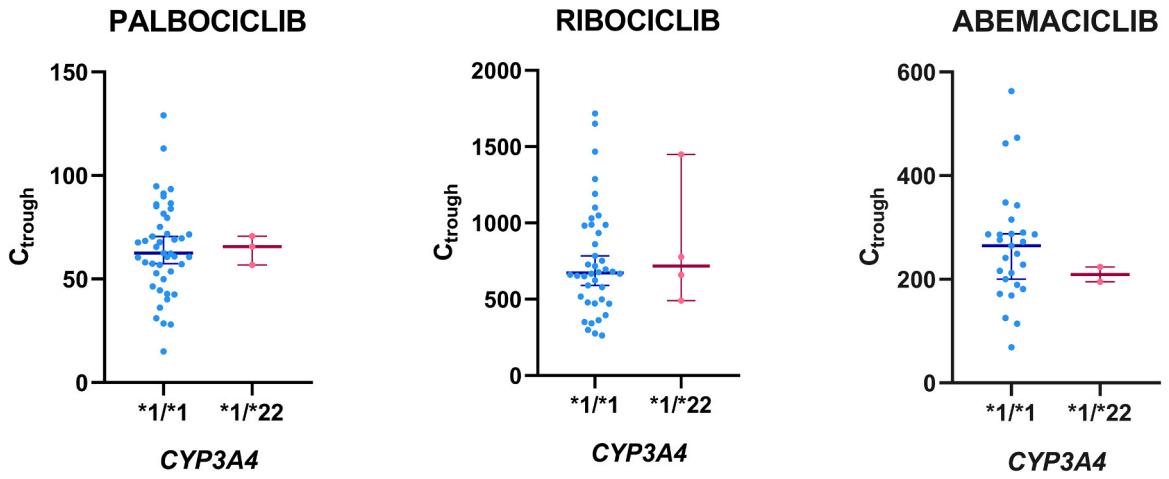

Fig. 2. Association between *CYP3A4**22 polymorphism and C_{trough} of each CDKi. Box plots depict the median (horizontal bar) and 95% Confidence Interval (CI). The dots represent individual concentration values.

[23].

Our findings suggest a significant association for *ABCB1* c.1236C>T, c.2677G>T/A with G3/4 neutropenia at day 14 of first cycle and for *ABCB1* c.3435C>T, c.2677G>T/A, *CYP3A4**1/*22 with early DLTs whereas only *ABCB1* c.3435C>T was significantly associated with dose reductions.

Multivariate regression analysis confirmed the association found with G3/4 neutropenia at day 14 and dose reductions, with *ABCB1* c.3435C>T remaining significant for early DLTs. Consistent ORs were maintained for the other SNPs. Importantly, the observed effects of polymorphisms on safety outcomes were independent from the CDKi used by the patient.

Furthermore, when examining the cumulative effect of all *ABCB1* SNPs (c.1236C>T, c.3435C>T, and c.2677G>T/A) as a haplotype, heterozygous carriers of variant haplotype (1236T–3435T–2677T(A)) had a higher risk of neutropenia at day 14 ($p = 0.026$), while homozygous carriers of the T-T-T(A) haplotype faced an increased risk of incidence of early DLTs ($p = 0.020$) and dose reductions ($p < .10$).

We hypothesized that reduced P-gp function, associated with *ABCB1* T-T-T(A)/T-T-T(A) haplotype [29–31], might lead to higher plasma drug concentrations. A reduced P-gp function could be associated with a lower extrusion back to the intestinal lumen of CDKis, higher plasmatic exposure to the drug and, potentially, higher incidence of toxicities. To verify this hypothesis, we investigated whether homozygous carriers of the T-T-T(A) haplotype also had an increased C_{trough} for each CDKi used. Our findings indicated that homozygous carriers of the T-T-T(A) haplotype tended to have higher median C_{trough} of ribociclib. To date, the data highlighting CDKis as P-gp substrates came from *in vitro* studies only for palbociclib [32] and abemaciclib [33,34], while for ribociclib there is also evidence in genetically modified mouse models [35]. Notably, only ribociclib has been proven to be a substrate of intestinal P-gp [36].

Even if *CYP3A4**22 has been previously associated with decreased CYP3A4 enzyme activity, in our study a lower exposure to abemaciclib was highlighted for carriers of the *CYP3A4**22 variant allele, regardless the $C_{through}$ values were retrieved only from two patients.

The study's primary strength rests in being among the first to comprehensively examine various ADME gene variants in the context of CDKis, representing a significant step in addressing the intricate issues of interindividual variability in their therapeutic responses. By investigating the genetic associations with safety outcomes, such as G3/4 neutropenia at day 14, early DLTs, and dose reductions, the research not only delivers predictive insights, but it represents an important starting point toward personalized CDKis treatment. Notably, the study's multivariate analysis, incorporating correction for the specific CDKi used, ensures that the clinical impact observed for the variant alleles remains robust irrespective of the inhibitor employed.

Certainly, the size of the study cohort is a major limitation. In addition, the observed large confidence intervals resulting from the association with polymorphisms and the plasma exposure are probably due to sample size and the stratification performed for the outcome's evaluation. Moreover, a larger number of polymorphisms including also rarer variants, should be also investigated along with evaluation of drug-drug-gene interactions and association with adherence endpoints.

Finally, this study confirms the data reported in the literature identifying a low count of ANC baseline as a strong intrinsic risk factor for the development of G3/4 neutropenia on day 14 of the first treatment cycle ($p < 0.001$) [6–10]. In our cohort, baseline ANC appears as well to be associated with the development of early DLTs ($p = 0.017$), as hematologic toxicities were the most represented DLTs in the first treatment cycle.

In conclusion, we have identified a group of patients who are at higher risk for developing DLTs from CDKis and are carriers of pharmacogenetic variants in ADME genes (*ABCB1* and *CYP3A4*). Particularly for ribociclib, *ABCB1* polymorphisms and haplotype may be associated with variability in exposure.

If confirmed in *ad hoc* studies, the pre-therapeutic screening for clinically relevant genotypes in ADME genes could be valuable for addressing interindividual variability, enhancing decision-making in the management of CDKis safety profiles. Combining genetic insights with clinical aspects also provides the potential to refine risk stratification for toxicity, which, while less severe, can still impact treatment adherence and the potential benefits derived from it, offering a streamlined pathway for improving the overall quality of life for patients undergoing CDKi-based treatment.

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CRediT authorship contribution statement

EP: Investigation, Visualization, Methodology, Data curation, Writing – original draft, Formal analysis, Validation. **RR:** Conceptualization, Methodology, Investigation, Writing – review & editing, Formal analysis, Validation, Funding acquisition, Supervision. **GT:** Writing – review & editing, Supervision. **MM:** Visualization, Software. **EC:** Resources. LG, MB, SC, BM and FP Patient's Provision. **RR, EP, BP, SG,**

EDM and **MO**: Formal analysis, Validation.

Declaration of Competing Interest

LG reports consulting or advisory role: Lilly, Novartis. FP reports honoraria: Roche, MSD, AstraZeneca, Novartis, Lilly, Pfizer, Pierre Fabre, and Daiichi Sankyo; consulting or advisory role: Roche, Amgen, Lilly, Novartis, Pfizer, and Eisai; research funding: Eisai, AstraZeneca, and Roche; travel, accommodations, and expenses: Roche and Celgene. MB reports consulting or advisory role: GSK, MSD, Roche, EISAI; research funding: Astrazeneca; data monitoring committee: Novartis. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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