ARTICLE

Pharmacogenetic and clinical predictors of voriconazole concentration in hematopoietic stem cell transplant recipients receiving CYP2C19-guided dosing

Jai N. Patel[1✉], Myra Robinson[2], Sarah A. Morris[1], Elizabeth Jandrisevits[1], Karine Eboli Lopes[1], Alicia Hamilton[3], Nury Steuerwald[3], Lawrence J. Druhan[4], Belinda Avalos[5], Edward Copelan[5], Nilanjan Ghosh[5] and Michael R. Grunwald[5]

CYP2C19-guided voriconazole dosing reduces pharmacokinetic variability, but many patients remain subtherapeutic. The aim of this study was to evaluate the effect of candidate genes and a novel *CYP2C* haplotype on voriconazole trough concentrations in patients receiving *CYP2C19*-guided dosing. This is a retrospective candidate gene study in allogeneic hematopoietic cell transplant (HCT) patients receiving *CYP2C19*-guided voriconazole dosing. Patients were genotyped for *ABCB1*, *ABCG2*, *CYP2C9*, *CYP3A4*, *CYP3A5*, and the *CYP2C* haplotype. Of 185 patients, 36% were subtherapeutic (of which 79% were normal or intermediate metabolizers). In all patients, *CYP2C19* ($p < 0.001$), age ($p = 0.018$), and letermovir use ($p = 0.001$) were associated with voriconazole concentrations. In the subset receiving 200 mg daily (non-RM/UMs), *CYP2C19* ($p = 0.004$) and *ABCG2* ($p = 0.015$) were associated with voriconazole concentrations; *CYP2C19* ($p = 0.028$) and letermovir use ($p = 0.001$) were associated with subtherapeutic status. CYP2C19 phenotype and letermovir use were significantly associated with subtherapeutic voriconazole concentrations and may be used to improve voriconazole precision dosing, while further research is needed to clarify the role of *ABCG2* in voriconazole dosing.

The Pharmacogenomics Journal (2023) 23:201–209; https://doi.org/10.1038/s41397-023-00320-z

INTRODUCTION

Voriconazole, a triazole antifungal agent, has broad-spectrum activity against yeasts and molds, including aspergillus [1]. The standard prophylaxis dose used in immunocompromised patients, such as those undergoing allogeneic hematopoietic cell transplantation (HCT), is 200 mg orally twice daily. Therapeutic drug monitoring is commonly employed based on the exposure-response relationship. A target trough concentration of 1.0–5.5 mg/l is typically used for prophylaxis and 2.0–5.5 mg/l for treatment of an invasive fungal infection (IFI). Subtherapeutic concentrations are associated with breakthrough IFIs, whereas supratherapeutic concentrations may be associated with increased adverse event risk [2].

Rates of subtherapeutic concentrations with standard prophylaxis dosing range from 40 to 62%, suggesting significant interpatient pharmacokinetic variability [3–5]. Voriconazole undergoes phase I metabolism via the hepatic enzyme cytochrome P450 2C19 (CYP2C19) [6]. Single nucleotide polymorphisms (SNPs) in the *CYP2C19* gene alter enzyme activity [7]. The *CYP2C19*17* allele results in enhanced enzyme activity and increased metabolism, whereas the **2* and **3* alleles result in loss-of-function and reduced metabolism. There is a significant association between CYP2C19 phenotype and voriconazole trough concentrations [8]. About 63% to 80% of rapid (RMs) or ultra-rapid metabolizers (UMs) (**1/*17* or **17/*17* genotypes, respectively) have subtherapeutic voriconazole concentrations with standard prophylaxis dosing, thus placing patients at risk of breakthrough IFIs [9–11]. Roughly one-third of White and Black patients are RMs or UMs [8]. Based on findings suggesting that RMs and UMs are at higher risk of drug failure and poor metabolizers (PMs) are at higher risk of adverse events, the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines recommend alternative antifungals in RMs, UMs, and PMs [8]. However, two prospective trials demonstrated that increasing the voriconazole prophylaxis dose from 200 to 300 mg in RMs and UMs significantly increased the likelihood of achieving target concentrations [12, 13], suggesting that dose modifications are also an option where voriconazole is the preferred antifungal agent.

We previously demonstrated that *CYP2C19*-guided voriconazole dosing significantly reduces the proportion of patients with subtherapeutic trough concentrations, particularly in RMs and UMs, and this is now standard of care at the Levine Cancer Institute [12]. However, 29% of patients had subtherapeutic concentrations at the initial steady-state level despite *CYP2C19*-guided dosing (0%, 26%, 50%, and 16% of PMs, intermediate metabolizers [IMs], normal metabolizers [NMs], and RMs/UMs, respectively) [12]. Although factors such as patient compliance,

[1]Department of Cancer Pharmacology and Pharmacogenomics, Levine Cancer Institute, Atrium Health, Charlotte, NC, USA. [2]Department of Biostatistics, Levine Cancer Institute, Atrium Health, Charlotte, NC, USA. [3]Molecular Biology and Genomics Core Laboratory, Levine Cancer Institute, Atrium Health, Charlotte, NC, USA. [4]Hematology/Oncology Translational Research Laboratory, Levine Cancer Institute, Atrium Health, Charlotte, NC, USA. [5]Department of Hematologic Malignancies and Blood Disorders, Levine Cancer Institute, Atrium Health, Charlotte, NC, USA. ✉email: Jai.Patel@atriumhealth.org

Received: 17 May 2023 Revised: 16 October 2023 Accepted: 26 October 2023
Published online: 4 November 2023

body composition, and drug interactions influence voriconazole pharmacokinetics, we hypothesize that other candidate genes may also play a role. Some studies suggest that SNPs in genes encoding for other CYP enzymes (CYP2C9, CYP3A4, CYP3A5) and drug transporters (ABCB1 and ABCG2) may influence voriconazole pharmacokinetics. More recently, a novel *CYP2C* haplotype, defined by rs2860840T and rs11188059G, was reported to be associated with increased metabolism of escitalopram and sertraline, two CYP2C19 substrates [14, 15].

The primary objective of this study was to evaluate the effect of these candidate genes and a novel *CYP2C* haplotype on voriconazole steady state trough concentrations in patients receiving *CYP2C19*-guided voriconazole prophylaxis.

METHODS

Study design and patient population

The original *CYP2C19*-guided voriconazole dosing study was a prospective observational study [12] in adults with hematologic malignancies undergoing allogeneic HCT and included patients with acute myeloid leukemia, myelodysplastic syndrome, chronic myeloid leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma, myeloproliferative neoplasms, chronic lymphocytic leukemia, and aplastic anemia. The study was reviewed and approved by Advarra Institutional Review Board (IRB). Upon inpatient admission for HCT, two buccal swabs were obtained and transferred to an internal Clinical Laboratory Improvement Amendment (CLIA)-certified molecular biology and genomics core laboratory for DNA extraction and *CYP2C19* genotyping (details under "Genotyping methods"). Data collected under this protocol included patient demographics, *CYP2C19* genotype, voriconazole dose, voriconazole steady-state trough concentrations, and concomitant medications.

A separate IRB approved protocol allowed additional specimen collection (buccal swabs) on the day of HCT admission from patients who provided informed consent for specimen banking. This protocol also allowed for clinical data abstraction from the electronic medical record (EMR), including patient demographics, disease characteristics, transplant data, medications, and laboratory values. DNA banked under this protocol was used to genotype for SNPs in *ABCB1*, *ABCG2*, *CYP2C9*, *CYP3A4*, *CYP3A5*, and the *CYP2C* haplotype (details under "Genotyping methods").

Antifungal administration and therapeutic drug monitoring

CYP2C19 phenotype was used to assign starting voriconazole doses. PMs, IMs, and NMs initiated voriconazole prophylaxis at the standard starting dose of 200 mg orally twice daily, whereas all RMs/UMs initiated voriconazole at 300 mg orally twice daily. Micafungin 50 mg intravenous daily was started on day +1 post HCT. Once patients could tolerate oral medications, micafungin was switched to oral voriconazole within ~1 week after HCT. Voriconazole was administered up to at least day +100 post-transplant, or until voriconazole was discontinued based on tolerability or presence of breakthrough fungal infection. A steady-state trough concentration was obtained after at least 5 days of continuous dosing. Blood specimens were sent to Viracor Eurofins Laboratories (Lee's Summit, MO) for quantitative analysis. Results were reported within ~2–3 days. The target steady-state trough concentration was 1.0–5.5 mg/l. If the initial steady state concentration was <1.0 mg/l, the total daily dose was increased by 100–200 mg; if the level was >5.5 mg/l, the total daily dose was decreased by 100–200 mg. Additional trough levels were obtained if a dose was changed or if the patient experienced voriconazole-related toxicities, per provider discretion.

Genotyping methods

DNA was extracted from buccal swabs using GenElute Mammalian Genomic DNA miniprep kit (MilliporeSigma, St Louis, MO), as directed. DNA concentration and integrity were verified using Qubit High Sensitivity DNA assay kit (Thermo Fisher Scientific, Waltham, MA) and gel electrophoresis.

For *CYP2C19* genotyping, TaqMan Drug Metabolism Enzyme (DME) Genotyping Assays were used to detect the following SNPs in *CYP2C19*: *2 (c.681G > A, SNP ID rs4244285), *3 (c.636G > A, SNP ID rs4986893), and *17 (c.-806C > T, SNP ID rs12248560) alleles (Assay ID C_25986767_70 for *1, *2; Assay ID C_27861809_10 for *1, *3; Assay ID C_469857_10 for *1, *17; Applied Biosystems, Foster City, CA). The *1 allele was considered default if *2, *3, and/or *17 was not present. Fifteen nanograms of DNA was used as the template for polymerase chain reaction with a VIIA-7 Real-Time PCR System (Applied Biosystems). The template was amplified by 50 cycles of denaturation at 95 °C for 15 s, annealing of primers and probe along with extension at 60 °C for 90 s in triplicate reactions. Fluorescence data were acquired during the combined anneal/extension step. The raw data from the genotyping assays were analyzed with TaqMan Genotyper Software. Genotyping results were mapped to star allele nomenclature using TaqMan AlleleTyper Software together with translation tables developed from established guidelines as set forth by the CPIC. The turnaround time for genotyping was 48–72 h and results were available prior to the first dose of voriconazole. *CYP2C* genotyping was performed as described for *CYP2C19* with the exception that TaqMan DME Genotyping Assays were used to detect rs2860840C>T (Assay ID C__11201742_10) and rs11188059G>A (Assay ID C__31983321_10).

A custom Ion AmpliSeq Pharmacogenetics Panel (Thermo Fisher Scientific, Waltham, MA) was used to batch genotype for SNPs in *ABCB1* (1236G > A, 2677C > T/A, 3435G > A), *ABCG2* (421G > T), *CYP2C9* (*2, *3, *4, *5, *6, *8, *9, *10, *11, *13, *15), *CYP3A4* (*1B, *22), and *CYP3A5* (*3, *6, *7). Sequencing libraries were prepared using an Ion Ampliseq Library Kit (Thermo Fisher Scientific) per manufacturer's instructions. Briefly, amplicons are ligated to ion-compatible adapters, followed by nick repair to complete the linkage between adapters and DNA inserts. The libraries are clonally amplified by emulsion PCR on ion sphere particles using the Ion OneTouch 200 Template Kit (Thermo Fisher Scientific) as directed. After amplification the template-positive ion sphere particles were enriched to maximize the number of sequencing reads produced using the Ion PGM Sequencing 200 Kit (Thermo Fisher Scientific) on an Ion PGM Sequencer (Thermo Fisher Scientific) and Ion 318 Chips (Thermo Fisher Scientific). For those samples in which library preparation, template preparation and chip loading were automated, an Ion Chef™ System (Thermo Fisher Scientific, Waltham, MA USA) was used together with Ion Ampliseq Kits for Chef DL8 (Thermo Fisher Scientific) and the Ion 510™ & Ion 520™ & Ion 530™ Kit—Chef (Thermo Fisher Scientific) as directed. The resultant enriched libraries were sequenced on an Ion GeneStudio™ S5 System (Thermo Fisher Scientific) using Ion 510™ Chips (Thermo Fisher Scientific). Ion Torrent Suite™ Software was used for base calling, preprocessing 3' trimming, quality control and assessment, and mapping. The Ion Pharmacogenomics Analysis Plugin was used to export data into AlleleTyper (Thermo Fisher Scientific) for mapping to star allele nomenclature together with translation tables. Cytochrome P450 star alleles were translated into metabolism phenotypes and transporter genotypes into function phenotypes based on CPIC guidelines [16].

Statistical analysis

The study sample size was based on convenience sampling. The primary endpoint of this study was initial voriconazole trough concentration, defined as both a continuous variable in mg/l and a binary variable (initial voriconazole trough concentration less than or greater than/equal to 1.0 mg/l). Additionally, voriconazole concentration was defined as a three-level categorical variable, with subtherapeutic (<1.0 mg/l), therapeutic (1.0–5.5 mg/l), and supratherapeutic (>5.5 mg/l) levels. Subgroup analyses were conducted on the subset of patients who received voriconazole 200 mg twice daily. Clinical and genetic characteristics were summarized with frequencies and proportions for categorical variables, while continuous variables were summarized with descriptive statistics. Fisher's exact tests were used to evaluate the associations between genetic variables and three-level categorized voriconazole level. Statistical significance was set at $\alpha \leq 0.05$.

General linear regression and logistic regression were utilized for modeling the continuous voriconazole concentration and the subtherapeutic status (dichotomized), respectively. The analysis was performed in all evaluable patients and in the subset receiving 200 mg twice daily given a substantial proportion of those that were subtherapeutic received this dose (particularly NMs). To meet the normality assumptions of general linear regression, voriconazole concentration was log-transformed. Univariable and multivariable modeling was conducted to evaluate the impact of clinical and genetic factors on voriconazole concentrations. Individual associations were evaluated with univariable models and independent predictors were identified with multivariable model selection, involving a combination of backward elimination and forward selection with entry/elimination criteria of 0.10. Statistical significance was set at $\alpha \leq 0.05$.

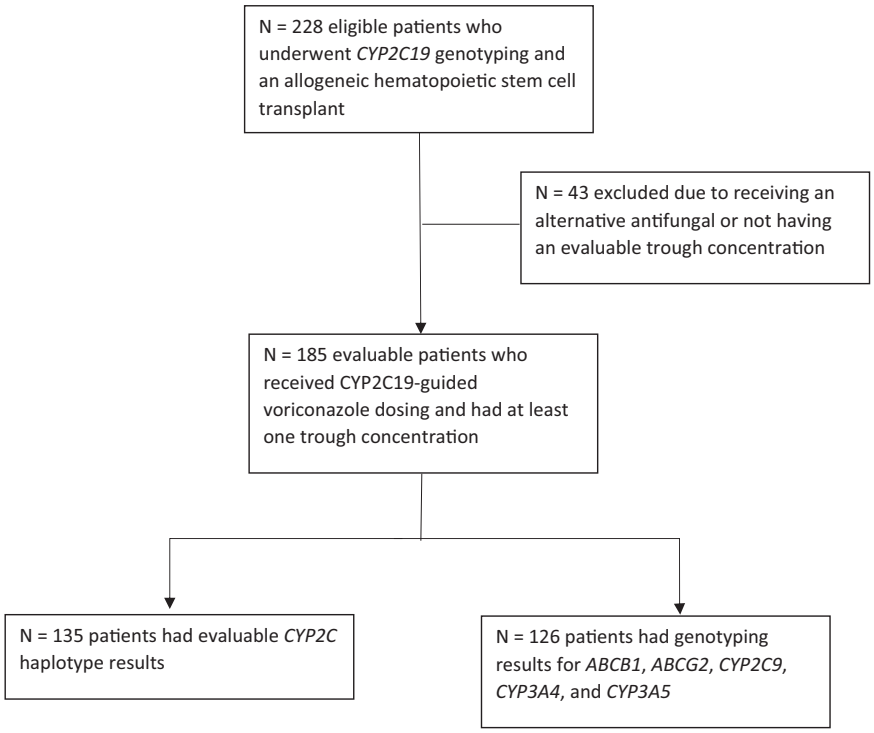

Fig. 1 Consort flow diagram. Of 228 eligible patients who underwent clinical CYP2C19 genotyping for voriconazole dosing post allogeneic hematopoietic stem cell transplantation, 185 evaluable patients who received voriconazole and had at least one trough concentration were included. Of these, 135 patients had evaluable results for CYP2C haplotype testing and 126 had results for the remaining candidate genes.

RESULTS

Baseline characteristics

Of 228 eligible patients who underwent *CYP2C19* genotyping, 185 evaluable patients received *CYP2C19* genotype-guided voriconazole dosing and had at least one voriconazole trough concentration (Fig. 1). Reasons for being non-evaluable are reported in Fig. 1. Table 1 describes the demographics of the evaluable population. The median age was 60 (range 22–80), most were male (62%), White (80%), and had either leukemia or myelodysplastic syndrome (81%). Approximately 37% were CYP2C19 RMs or UMs, all of whom initiated voriconazole 300 mg twice daily. The distribution of genotypes/phenotypes are summarized in Supplementary Table S1. Of 185 evaluable patients, 135 had banked DNA and evaluable results for *CYP2C* genotype and 126 had evaluable results for the remaining genes.

Voriconazole concentration by CYP2C19 phenotypes

Voriconazole trough concentrations were subtherapeutic (<1 mg/l) in 36% of all patients ($N = 67$), supratherapeutic (>5 mg/l) in 3.2% ($N = 6$), and therapeutic in 60.5% ($N = 112$). The initial mean voriconazole trough concentrations in PMs, IMs, NMs, and RMs/UMs were 2.3, 1.5, 1.0, and 2.6 mg/l respectively. Of 68 RMs/UMs, 14 (20.6%) were subtherapeutic; of 69 NMs, 37 (53.6%) were subtherapeutic; and, of 44 IMs, 16 (36.4%) were subtherapeutic (no PMs were subtherapeutic). Overall, 53 of the 67 patients (79%) with subtherapeutic concentrations were NMs or IMs receiving the standard 200 mg twice daily dose. Using Fisher's exact tests, *CYP2C19* was the only gene significantly associated with subtherapeutic, therapeutic, or supratherapeutic voriconazole initial steady state trough concentration ($p < 0.001$). Supplementary Fig. S1 illustrates the percentage of patients with subtherapeutic, therapeutic, or supratherapeutic voriconazole initial steady state trough concentration stratified by each genotype or phenotype.

Table 1. Patient demographics.

	***N* = 185**	
	N	**%**
Age, years		
Median (range)	60	22–80
Sex		
Female	71	38.4%
Male	114	61.6%
Race		
Black	30	16.2%
White	147	79.5%
Other[a]	8	4.3%
BMI, kg/m^2		
Median (range)	27.8	18.4–54.8
Disease type		
Leukemia	112	60.5%
Lymphoma	25	13.5%
Myelodysplastic syndrome/Myelofibrosis	37	20.0%
Plasma cell disorder	3	1.6%
Other[b]	8	4.3%
Voriconazole starting dose		
200 mg twice daily	117	63.2%
300 mg twice daily	68	36.8%
Letermovir[c]	50	27.0%

[a]Other races include: Asian, Laotian, not indicated.
[b]Other disease types include: aplastic anemia, histiocytosis, plasmacytoid dendritic cell, severe aplastic anemia, myeloid sarcoma.
[c]Letermovir initiated before voriconazole.

Clinical and genetic predictors of voriconazole concentration

Multivariable regression was performed to identify clinical and genetic predictors of (1) mean voriconazole steady-state trough concentrations in the subset of patients who received 200 mg twice daily (i.e., all non-RM/UM patients), (2) mean voriconazole steady-state trough concentrations in all patients, (3) sub-therapeutic concentrations in the subset of patients who received 200 mg twice daily, and (4) sub-therapeutic concentrations in all patients.

Table 2 summarizes the general linear regression model results of the association between clinical and genetic factors with mean voriconazole trough concentrations in the subset of patients receiving 200 mg twice daily. In univariate analysis, CYP2C19 phenotype ($p = 0.004$), letermovir use ($p = 0.002$), and presence of the *CYP2C* TG haplotype ($p = 0.019$) were significantly associated with voriconazole trough concentrations. CYP2C19 NMs, those receiving letermovir, and those with the *CYP2C* TG haplotype had the lowest concentrations. In the multivariate model, CYP2C19 phenotype remained significantly associated with voriconazole concentrations ($p = 0.004$). ABCG2 phenotype was also associated with voriconazole concentrations ($p = 0.015$) with poor function (PF) or decreased function (DF) patients having higher voriconazole concentrations compared to normal function (NF) patients.

Table 3 summarizes the logistic regression model results of the association between clinical and genetic factors with odds of having subtherapeutic voriconazole concentrations (<1 mg/l) in the subset receiving 200 mg twice daily. In univariate analysis, CYP2C19 phenotype (NM vs. PM/IM) (OR 2.33, 95% CI 1.08–5.00, $p = 0.032$), letermovir use (yes vs. no) (OR 3.99, 95% CI 1.71–9.28, $p < 0.001$), and ABCG2 phenotype (PF/DF vs. NF) (OR 0.24, 95% CI 0.06–0.91, $p = 0.035$) were significantly associated with the odds of having subtherapeutic voriconazole concentrations. CYP2C19 phenotype (OR 2.50, 95% CI 1.11–5.56, $p = 0.028$) and letermovir use (OR 4.21, 95% CI 1.76–10.05, $p = 0.001$) were independent predictors in the multivariable model.

Supplementary Table S2 summarizes the linear regression model results of the association between clinical and genetic factors with mean voriconazole trough concentrations in all patients. While several factors (age, CYP2C18 rs2860840, CYP2C diplotype, CYP2C19 phenotype, CYP3A4 phenotype, disease, and letermovir use) were associated with voriconazole trough concentrations in the univariate analysis, only age ($p = 0.018$), CYP2C19 phenotype ($p < 0.001$), and letermovir use ($p = 0.001$) were retained in the multivariable model. Supplementary Table S3 summarizes the logistic regression model results of the association between clinical and genetic factors with odds of having subtherapeutic voriconazole concentrations (<1 mg/l) in all patients. Only CYP2C19 phenotype ($p < 0.001$) and letermovir use ($p < 0.001$) were retained in the multivariable model.

DISCUSSION

We previously demonstrated that *CYP2C19*-guided voriconazole dosing significantly reduces the proportion of patients with subtherapeutic trough concentrations in those undergoing allogeneic HCT—this is particularly true for CYP2C19 RMs and UMs [12]. However, a substantial proportion of patients, especially CYP2C19 NMs, are still subtherapeutic at the initial steady state level (<1 mg/l) and may require higher up-front doses. To our knowledge, this is the first study to investigate genetic and clinical predictors of voriconazole trough concentrations in patients receiving *CYP2C19*-guided dosing. Apart from CYP2C19 phenotype, we identified that letermovir use consistently resulted in lower voriconazole concentrations. We also identified potential associations with *ABCG2* and the *CYP2C* TG haplotype that warrant further study.

Substantial interpatient pharmacokinetic variability exists in voriconazole trough concentrations when using flat or weight-based dosing. Numerous prior reports demonstrate that voriconazole trough concentrations vary by CYP2C19 phenotypes [7, 8]. Original reports by Patel et al. [12] and Hicks et al. [13] demonstrated that *CYP2C19*-guided dosing in adults improves the attainment of target trough concentrations. Prior to completion of these studies, CPIC guidelines were also published summarizing the evidence supporting the association between CYP2C19 phenotype and voriconazole pharmacokinetics and response, and recommending that alternative antifungals be considered in CYP2C19 PMs and RM/UMs [8]. While increasing the voriconazole dose in CYP2C19 RM/UMs significantly increases the overall proportion of patients achieving target concentrations, a substantial number of non-RM/UM patients remain subtherapeutic; however, there are no prior studies investigating contributing factors in this subgroup.

Prior candidate gene studies have evaluated other potential SNPs associated with voriconazole concentrations in patients receiving standard dosing. A study of 177 Thai patients with IFIs receiving voriconazole treatment demonstrated no significant association between SNPs in *CYP3A4*, *ABCB1*, and *FMO3* with voriconazole concentrations [17]. Another study of 68 pediatric Chinese patients receiving voriconazole treatment similarly failed to demonstrate an association between *CYP3A4* and voriconazole concentrations [18]. In a larger study of 233 pediatric patients receiving voriconazole treatment, SNPs in *SLCO1B3*, *ABCG2*, and *ABCB1* were significantly associated with trough concentrations [19]. A study of 36 pediatric patients receiving voriconazole treatment demonstrated that *CYP2C19*, *CYP3A4*, *ABCC2*, and *ABCG2* were associated with voriconazole concentrations [20]. However, the minor allele frequency for *CYP3A4* was 5%, and the study sample size was small. A systematic review and meta-analysis including 203 patients and 754 voriconazole trough concentrations from six studies demonstrated that voriconazole trough concentrations were independently influenced by age, dose, C-reactive protein level, *CYP2C19* genotype, and *CYP3A4* genotype [21]. Prior studies suggesting that *CYP3A4* genotype is independently associated with voriconazole concentrations are small (low minor allele frequency of the *CYP3A4*22* allele) and include potential confounders such as proton pump inhibitor use [22–24]. Like some prior studies, we identified lack of an association between certain genes, including *CYP2C9*, *CYP3A4/5*, and *ABCB1*, with voriconazole concentrations but did identify a potential signal with *ABCG2*, suggesting that NF patients have lower trough concentrations compared to PF or DF patients. However, this finding may have little clinical significance given *ABCG2* was not associated with odds of having subtherapeutic concentrations. Larger studies are needed to validate the association between *ABCG2* and voriconazole concentrations and whether *ABCG2* genotype can inform dosing, in addition to *CYP2C19*.

To our knowledge, this is the first report to investigate the association between the novel *CYP2C* haplotype and voriconazole concentrations. Braten et al. identified three new haplotypes of the *CYP2C* locus (TG, TA, and CG). In a study of 875 previously genotyped escitalopram-treated patients, the *CYP2C* haplotype was significantly associated with ultrarapid metabolism of escitalopram, whereby the serum concentrations of escitalopram in homozygous *CYP2C:TG* and *CYP2C19*17* carriers were 25 and 17% lower compared with *CG* and *TA* carriers [14]. Subsequently, Braten et al. also demonstrated the same haplotype was significantly associated with sertraline exposure [15]. Given the similarities in hepatic metabolism between these selective serotonin reuptake inhibitors and voriconazole, we investigated the association of the same haplotypes on voriconazole trough concentrations. In the univariate analysis of patients receiving voriconazole 200 mg twice daily (non-RM/UM patients), *TG* carriers had significantly lower mean voriconazole trough concentrations compared to non-*TG* carriers (1.1 vs. 1.5; $p = 0.019$); however, this effect was not retained in the multivariable model when

Table 2. Association of clinical and genetic factors with mean voriconazole trough concentration (mg/l) in patients receiving 200 mg twice daily.

	Univariable results			Multivariable model[a]		
	LSMean	**StdErr**	***p* value**	**LSMean**	**StdErr**	***p* value**
CYP2C19 ($n = 117$)						
PM	2.3	0.5	0.004	3.0	0.5	0.004
IM	1.5	0.1		2.0	0.2	
NM	1.0	0.1		1.3	0.2	
Letermovir before voriconazole ($n = 117$)						
Yes	0.9	0.2	0.002			
No	1.4	0.1				
CYP2C9 ($n = 78$)						
PM/IM	1.2	0.2	0.335			
NM	1.5	0.1				
CYP3A5 ($n = 78$)						
PM	1.3	0.1	0.737			
IM/NM	1.6	0.2				
CYP3A4 ($n = 78$)						
PM/IM	1.2	0.6	0.892			
NM	1.4	0.1				
ABCG2 ($n = 78$)						
PF/DF	1.7	0.2	0.072	2.3	0.3	0.015
NF	1.3	0.1		1.9	0.2	
ABCB1 3435G > A ($n = 78$)						
PF	1.3	0.2	0.250			
DF	1.3	0.2				
NF	1.7	0.2				
ABCB1 2677C > A ($n = 78$)						
PF	1.3	0.3	0.392			
DF	1.2	0.2				
NF	1.7	0.2				
ABCB1 1236G > A ($n = 78$)						
PF	1.3	0.3	0.952			
DF	1.5	0.2				
NF	1.4	0.2				
CYP2C18 rs2860840 C > T ($n = 83$)						
C/C	1.5	0.2	0.111			
C/T	1.4	0.2				
T/T	1.1	0.2				
CYP2C18 rs11188059 G > A ($n = 83$)						
A/A	1.1	0.4	0.567	1.9	0.4	0.086
A/G	1.6	0.3		2.5	0.3	
G/G	1.3	0.1		1.9	0.2	
CYP2C18 Haplotype ($n = 83$)						
T/G	1.1	0.2	0.019			
Non-T/G	1.5	0.1				
Haplotype 1 ($n = 83$)						
CG	1.6	0.1	0.021			
TA	1.1	0.4				
TG	1.1	0.2				
Haplotype 2 ($n = 83$)						
CG	1.3	0.1	0.752			
TA	1.4	0.2				
TG	1.3	0.4				

Table 2. continued

	Univariable results			Multivariable model[a]		
	LSMean	**StdErr**	***p* value**	**LSMean**	**StdErr**	***p* value**
CYP2C diplotype ($n = 83$)						
CG/CG	1.5	0.2	0.076			
CG/TA	2.0	0.3				
CG/TG	1.1	0.2				
TA/TA	1.1	0.4				
TG/TA	0.8	0.4				
TG/TG	1.3	0.4				
Race						
White	1.2	0.1	0.388			
Non-White	1.5	0.2				
Gender						
Female	1.2	0.1	0.177			
Male	1.3	0.1				
Disease						
Leukemia	1.2	0.1	0.378			
Lymphoma	1.3	0.3				
MDS/MF	1.6	0.2				
Other	1.0	0.3				
Age, years						
Slope (change for 1 unit increase)	0.002	0.007	0.533			
BMI, kg/m^2						
Slope (change for 1 unit increase)	0.016	0.016	0.239			

LSMean least squares mean estimate, *StdErr* standard error of the least squares mean estimate, *PM* poor metabolizer, *IM* intermediate metabolizer, *NM* normal metabolizer, *PF* poor function, *DF* decreased function, *NF* normal function.
[a]Sample size for the final multivariable model was $N = 75$ due to the missingness observed in some variables.

accounting for other variables. Further, there was no association between the CYP2C haplotype and odds of having subtherapeutic concentrations. We also conducted an additional analysis limited to CYP2C19 NMs, but there was no association between the novel haplotype and mean voriconazole concentration or odds of subtherapeutic concentration (data not shown), which may be limited by the small sample size ($n = 45$ CYP2C19 NMs with haplotype information). Larger studies are warranted to determine the effect of the novel *CYP2C* haplotype on voriconazole concentrations and potential clinical utility when combined with *CYP2C19* genotype information.

In addition to genetic factors, we also evaluated the impact of clinical factors on voriconazole concentrations. Race, sex, disease, and BMI were not significantly associated with voriconazole concentrations; however, presence of letermovir resulted in about 4-fold higher probability of having subtherapeutic voriconazole concentrations when analyzed across all patients and in those limited to receiving 200 mg twice daily. Letermovir is a CYP2C19 inducer and the letermovir-voriconazole drug interaction is listed in the FDA package insert [25]. Two small studies in allogeneic hematopoietic cell transplant recipients also showed that letermovir significantly reduces voriconazole concentrations [26, 27]. A study of healthy subjects who received concomitant letermovir and voriconazole showed that voriconazole AUC and maximum serum concentration were reduced by 44% and 39%, respectively [28]. It is reasonable to consider starting voriconazole at higher doses (e.g., 300 mg twice daily) like in CYP2C19 RMs/UMs, followed by therapeutic drug monitoring; however, additional prospective studies are needed to confirm the safety and efficacy of this approach.

It is important to recognize the limitations of this study, including its retrospective nature, small sample sizes for subgroup analyses, and unknown clinical relevance of pharmacokinetic findings. Although a substantial number of patients underwent clinical *CYP2C19* genotyping, fewer patients had voriconazole trough levels and banked DNA for retrospective genotyping of candidate genes. Further, subgroup analyses were performed on those receiving 200 mg twice daily since one of the objectives was to identify candidate genes associated with mean voriconazole concentration and/or subtherapeutic levels in non-RM/UM patients. Other patient-related factors were not assessed such as compliance, however, most patients were still inpatient at the time of initial voriconazole steady state trough collection. Lastly, we did not include data on incidence of IFIs or voriconazole-related side effects. In our prior study [12], we reported fewer IFIs with *CYP2C19*-guided dosing compared to historical control data, but no such evaluation was performed in this study as the objective was to identify other clinical and genetic factors associated with voriconazole pharmacokinetics only.

In conclusion, while *CYP2C19* genotype-guided dosing improves the ability to achieve voriconazole target trough concentrations, many patients are still subtherapeutic. In the first study to evaluate clinical and genetic predictors of voriconazole concentration in patients already receiving *CYP2C19*-guided dosing, we identified that concomitant letermovir, *ABCG2*, and possibly the novel *CYP2C* haplotype may further modulate mean voriconazole trough concentrations. If validated in larger independent cohorts, these clinical and genetic variables can be used to identify the most appropriate up-front prophylactic dose (e.g., 200 mg vs. 300 mg twice daily), followed by therapeutic drug monitoring to further

Table 3. Association of clinical and genetic factors with odds of subtherapeutic voriconazole trough concentration in patients receiving 200 mg twice daily.

	Univariable results			Multivariable model		
	OR	**95% CI**	***p* value**	**OR**	**95% CI**	***p* value**
CYP2C19 ($n = 117$)						
NM vs. PM/IM	2.33	1.08–5.00	0.032	2.50	1.11–5.56	0.028
Letermovir ($n = 117$)						
Y vs. N	3.99	1.71–9.28	<0.001	4.21	1.76–10.05	0.001
CYP2C9 ($n = 78$)						
PM/IM vs. NM	1.60	0.62–4.17	0.336			
CYP3A5 ($n = 78$)						
PM vs. IM/NM	1.36	0.44–3.60	0.670			
CYP3A4 ($n = 78$)						
PM/IM vs. NM	3.55	0.31–41.03	0.310			
ABCG2 ($n = 78$)						
PF/DF vs. NF	0.24	0.06–0.91	0.035			
ABCB1 3435G > A ($n = 78$)						
PF vs. NF	0.67	0.16–2.74	0.358			
DF vs. NF	1.62	0.56–4.69				
ABCB1 2677C > A ($n = 78$)						
PF vs. NF	1.11	0.27–4.67	0.571			
DF vs. NF	1.69	0.61–4.70				
ABCB1 1236G > A ($n = 78$)						
PF vs. NF	1.14	0.26–4.95	0.342			
DF vs. NF	2.10	0.72–6.15				
CYP2C18 rs2860840 C > T ($n = 83$)						
C/C vs. T/T	0.54	0.17–1.73	0.536			
C/T vs. T/T	0.54	0.16–1.83				
CYP2C18 rs11188059 G > A ($n = 83$)						
A/A vs. G/G	0.71	0.12–4.18	0.920			
A/G vs. G/G	1.07	0.33–3.44				
CYP2C18 Haplotype ($n = 83$)						
T/G vs. Non T/G	2.02	0.81–4.99	0.130			
Haplotype 1 ($n = 83$)						
CG vs. TG	0.50	0.20–1.27	0.317			
TA vs. TG	0.47	0.08–2.95				
Haplotype 2 ($n = 83$)						
CG vs. TG	0.68	0.13–3.65	0.897			
TA vs. TG	0.67	0.11–4.17				
CYP2C Diplotype ($n = 83$)						
CG/CG vs. TG/TG	0.61	0.11–3.44	0.506			
CG/TA vs. TG/TG	0.29	0.03–2.69				
CG/TG vs. TG/TG	0.82	0.13–5.08				
TA/TA vs. TG/TG	0.50	0.05–5.15				
TG/TA vs. TG/TG	4.00	0.27–60.32				
Race						
Non-White vs. White	1.15	0.47–2.78	0.760			
Gender						
Female vs. Male	1.11	0.53–2.33	0.788			
Disease						
Leukemia vs. Other	0.79	0.18–3.38	0.899			
Lymphoma vs. Other	1.14	0.21–6.37				
MDS/MF vs. Other	0.73	0.14–3.82				

Table 3. continued

	Univariable results			Multivariable model		
	OR	**95% CI**	***p* value**	**OR**	**95% CI**	***p* value**
Age, years						
1 year increase	1.00	0.97–1.03	0.832			
BMI, kg/m^2						
1 unit increase	0.96	0.90–1.02	0.193			

OR odds ratio, *CI* confidence interval, *PM* poor metabolizer, *IM* intermediate metabolizer, *NM* normal metabolizer, *PF* poor function, *DF* decreased function, *NF* normal function.

refine dosing. While further studies are needed to confirm the effects of *ABCG2* and *CYP2C* haplotype on voriconazole dosing, there is sufficient data to recommend using CYP2C19 phenotype and presence of concomitant letermovir to guide initial dosing for voriconazole prophylaxis post-transplantation. Additional studies are needed in those receiving treatment dosing. Given the relationship between voriconazole trough concentrations and clinical efficacy, it is imperative that personalized approaches to dosing are used to improve drug efficacy, especially in high-risk immunocompromised patients.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Herbrecht R. Voriconazole: therapeutic review of a new azole antifungal. Expert Rev Anti Infect Ther. 2004;2:485–97.
2. Smith J, Safdar N, Knasinski V, Simmons W, Bhavnani SM, Ambrose PG, et al. Voriconazole therapeutic drug monitoring. Antimicrob Agents Chemother. 2006;50:1570–2.
3. Marks DI, Pagliuca A, Kibbler CC, Glasmacher A, Heussel CP, Kantecki M, et al. Voriconazole versus itraconazole for antifungal prophylaxis following allogeneic haematopoietic stem-cell transplantation. Br J Haematol. 2011;155:318–27.
4. Trifilio S, Ortiz R, Pennick G, Verma A, Pi J, Stosor V, et al. Voriconazole therapeutic drug monitoring in allogeneic hematopoietic stem cell transplant recipients. Bone Marrow Transplant. 2005;35:509–13.
5. Trifilio S, Pennick G, Pi J, Zook J, Golf M, Kaniecki K, et al. Monitoring plasma voriconazole levels may be necessary to avoid subtherapeutic levels in hematopoietic stem cell transplant recipients. Cancer. 2007;109:1532–5.
6. Theuretzbacher U, Ihle F, Derendorf H. Pharmacokinetic/pharmacodynamic profile of voriconazole. Clin Pharmacokinet. 2006;45:649–63.
7. Owusu Obeng A, Egelund EF, Alsultan A, Peloquin CA, Johnson JA. CYP2C19 polymorphisms and therapeutic drug monitoring of voriconazole: are we ready for clinical implementation of pharmacogenomics? Pharmacotherapy. 2014;34:703–18.
8. Moriyama B, Obeng AO, Barbarino J, Penzak SR, Henning SA, Scott SA, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP2C19 and voriconazole therapy. Clin Pharm Ther. 2017;102:45–51.
9. Hassan A, Burhenne J, Riedel KD, Weiss J, Mikus G, Haefeli WE, et al. Modulators of very low voriconazole concentrations in routine therapeutic drug monitoring. Ther Drug Monit. 2011;33:86–93.
10. Hamadeh IS, Klinker KP, Borgert SJ, Richards AI, Li W, Mangal N, et al. Impact of the CYP2C19 genotype on voriconazole exposure in adults with invasive fungal infections. Pharmacogenet Genomics. 2017;27:190–6.
11. Trubiano JA, Crowe A, Worth LJ, Thursky KA, Slavin MA. Putting CYP2C19 genotyping to the test: utility of pharmacogenomic evaluation in a voriconazole-treated haematology cohort. J Antimicrob Chemother. 2015;70:1161–5.
12. Patel JN, Hamadeh IS, Robinson M, Shahid Z, Symanowski J, Steuerwald N, et al. Evaluation of CYP2C19 genotype-guided voriconazole prophylaxis after allogeneic hematopoietic cell transplant. Clin Pharm Ther. 2020;107:571–9.
13. Hicks JK, Quilitz RE, Komrokji RS, Kubal TE, Lancet JE, Pasikhova Y, et al. Prospective CYP2C19-guided voriconazole prophylaxis in patients with neutropenic acute myeloid leukemia reduces the incidence of subtherapeutic antifungal plasma concentrations. Clin Pharm Ther. 2020;107:563–70.
14. Braten LS, Haslemo T, Jukic MM, Ivanov M, Ingelman-Sundberg M, Molden E, et al. A novel CYP2C-haplotype associated with ultrarapid metabolism of escitalopram. Clin Pharm Ther. 2021;110:786–93.
15. Braten LS, Ingelman-Sundberg M, Jukic MM, Molden E, Kringen MK. Impact of the novel CYP2C:TG haplotype and CYP2B6 variants on sertraline exposure in a large patient population. Clin Transl Sci. 2022;15:2135–45.
16. Caudle KE, Dunnenberger HM, Freimuth RR, Peterson JF, Burlison JD, Whirl-Carrillo M, et al. Standardizing terms for clinical pharmacogenetic test results: consensus terms from the Clinical Pharmacogenetics Implementation Consortium (CPIC). Genet Med. 2017;19:215–23.
17. Chuwongwattana S, Jantararoungtong T, Prommas S, Medhasi S, Puangpetch A, Sukasem C. Impact of CYP2C19, CYP3A4, ABCB1, and FMO3 genotypes on plasma voriconazole in Thai patients with invasive fungal infections. Pharm Res Perspect. 2020;8:e00665.
18. Fan X, Zhang H, Wen Z, Zheng X, Yang Y, Yang J. Effects of CYP2C19, CYP2C9 and CYP3A4 gene polymorphisms on plasma voriconazole levels in Chinese pediatric patients. Pharmacogenet Genomics. 2022;32:152–8.
19. Allegra S, Fatiguso G, Francia S, Pirro E, Carcieri C, Cusato J, et al. Pharmacogenetic of voriconazole antifungal agent in pediatric patients. Pharmacogenomics. 2018;19:913–25.
20. Tilen R, Paioni P, Goetschi AN, Goers R, Seibert I, Muller D, et al. Pharmacogenetic analysis of voriconazole treatment in children. Pharmaceutics. 2022;14:1289–302.
21. Bolcato L, Khouri C, Veringa A, Alffenaar JWC, Yamada T, Naito T, et al. Combined impact of inflammation and pharmacogenomic variants on voriconazole trough concentrations: a meta-analysis of individual data. J Clin Med. 2021;10:2089.
22. Walsh TJ, Moriyama B, Penzak SR, Klein TE, Caudle KE. Response to "Impact of CYP3A4 genotype on voriconazole exposure: new insights into the contribution of CYP3A4*22 to metabolism of voriconazole". Clin Pharm Ther. 2018;103:187.
23. Duflot T, Schrapp A, Bellien J, Lamoureux F. Impact of CYP3A4 genotype on voriconazole exposure. Clin Pharm Ther. 2018;103:185–6.
24. He HR, Sun JY, Ren XD, Wang TT, Zhai YJ, Chen SY, et al. Effects of CYP3A4 polymorphisms on the plasma concentration of voriconazole. Eur J Clin Microbiol Infect Dis. 2015;34:811–9.
25. United States Food and Drug Administration. Prevymis prescribing information. 2023. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/209939orig1s000,209940orig1s000lbl.pdf.
26. Nakashima T, Inamoto Y, Fukushi Y, Doke Y, Hashimoto H, Fukuda T, et al. Drug interaction between letermovir and voriconazole after allogeneic hematopoietic cell transplantation. Int J Hematol. 2021;113:872–6.
27. Hikasa S, Shimabukuro S, Osugi Y, Ikegame K, Kaida K, Fukunaga K, et al. Decrease in voriconazole concentration-to-dose ratio after letermovir initiation: a retrospective, observational study. Bone Marrow Transplant. 2021;56:949–51.
28. Duong A, Sweet A, Jain R, Hill JA, Pergam SA, Boeckh M, et al. Clinically significant drug interaction: letermovir and voriconazole. J Antimicrob Chemother. 2020;75:775–7.

AUTHOR CONTRIBUTIONS

JNP, MR, SAM, and EJ wrote the manuscript; JNP, MR, and SAM designed the research; all authors performed the research; JNP, MR, SAM, and EJ analyzed the data; all authors reviewed and approved the manuscript.

COMPETING INTERESTS

JNP serves as a paid consultant for VieCure and Clarified Precision Medicine.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41397-023-00320-z.

Correspondence and requests for materials should be addressed to Jai N. Patel.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.