Effect of Cytochrome P450 2C19 Genotyping and Therapeutic Drug Monitoring on Efficacy and Toxicity of Voriconazole Therapy

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science
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Abstract

Invasive fungal infections are a significant cause of morbidity and mortality in immunocompromised patients and voriconazole is often prescribed because of its potent activity. Treatment success of voriconazole therapy is highly dependent on maintaining therapeutic concentrations which is challenging due to the high inter- and intra-patient variability in drug exposure. The complex kinetics of voriconazole renders current manufacturers’ dosing guidelines ineffective. The variability in drug exposure observed in clinical settings has been linked to genetic polymorphisms in the cytochrome P450 2C19 gene, which encodes for the enzyme primarily responsible for voriconazole metabolism.

Therapeutic drug monitoring has been found to increase efficacy of voriconazole treatment. However, there is still inconsistency and delay in getting patients to reach target voriconazole concentrations. The aim of this thesis is to examine if implementation of cytochrome P450 2C19 real-time genotyping as part of voriconazole’s existing dosing algorithm can improve clinical outcomes.
Acknowledgments

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Chapter I: Introduction

1.1 Invasive Aspergillosis

1.1.1 Overview

The Aspergillus genus is a diverse group of moulds, first described in the 18\textsuperscript{th} century and named after the aspergillum because of a physical resemblance \cite{1}. By 1926, there were 69 known \textit{Aspergillus} species \cite{2}. There are over 200 known species today, of which only a few are pathogenic to humans \cite{1}. The most commonly isolated invasive moulds in clinical settings are \textit{Aspergillus fumigatus}, \textit{Aspergillus flavus}, and \textit{Aspergillus niger}, \textit{Aspergillus terreus}, and \textit{Aspergillus nidulans} \cite{1}. Colonization by \textit{Aspergillus} spp. can lead to invasive aspergillosis, tracheobronchitis, aspergilloma and chronic necrotizing aspergillosis.

Invasive aspergillosis (IA) is an invasive fungal infection (IFI) which left untreated, is a significant cause of morbidity and mortality. The incidence of IFIs has seen a steady rise over the last three decades, with fungi being increasingly recognized as significant pathogens in severely ill patients. This increase can be attributed to escalating use of broad-spectrum antibiotics, antineoplastic and immunosuppressive agents, expansion of transplantation medicine, improved survival of neonatal and other intensive care units, and the AIDS epidemic \cite{3}. Due to the development of newer antifungals such as voriconazole and caspofungin, IA associated mortality has decreased since the turn of the century \cite{4} although incidence of IFIs have seen a slight increase in solid organ transplant (SOT) patients \cite{5}.

1.1.2 Diagnosis

IA is asymptomatic in up to one-third of patients. The most immunocompromised patients are also the least likely to have symptoms and whose disease progression is relatively faster compared to immunocompetent patients who typically present with benign symptoms that progress slowly \cite{1}.

Early initiation of therapy for IA is essential for successful outcomes, however diagnosis is difficult due to the lack of characteristic clinical signs and symptoms and early microbiological proof of IA is rare \cite{6}. Open lung biopsy is the gold standard for diagnosis, although false negatives can still occur \cite{1}. Diagnosis is based on computed tomography (CT) scanning and non-
culture-based techniques such as galactomannan (aspergillus antigen) or fungal DNA detection in bronchoalveolar lavage (BAL) or blood samples. Early clinical symptoms of invasive pulmonary aspergillosis can include dry cough and fever which are not unequivocal indications of aspergillus establishment. Overall, a large number of presumed IA cases are unproven due to nonspecific clinical symptoms and unclear results from imaging diagnostic tests. A broad international consensus was established to define IFIs, providing better clarity and uniformity regarding the definitions of possible, probable and proven IA. These definitions were published in 2002, and then revised in 2008, with the intent for usage in the context of clinical research only.

1.1.2.1 Proven Invasive Aspergillosis

Proof of IA requires a positive culture result from a sample obtained from a body site that is normally sterile with clinical or radiologic abnormality consistent with infection. Alternatively, histopathological or cytopathological samples showing hyphae from needle aspirate or biopsy sample with evidence of associated tissue damage are needed to confirm IA.

1.1.2.2 Probable Invasive Aspergillosis

Most diagnoses of IA in immunocompromised patients do not fit the criteria necessary for a proven IA diagnosis as the samples required for histopathological, cytological evidence or a positive culture are obtained through invasive procedures. A diagnosis of probable aspergillosis requires fulfillment of criteria from host, microbiological and clinical criteria.

1.1.2.3 Possible Invasive Aspergillosis

The criteria for diagnosis of possible IA is similar to the criteria for diagnosis of probable IA, where appropriate host factors with supporting clinical evidence consistent with IA is needed, but with the exception that mycological evidence may be absent.

1.1.2.4 Antigen Detection

There are several antigens that have been researched for use in detecting aspergillus species, the most popular being the galactomannan antigen followed by the 1-3-β-D-glucan antigen which has been developed more recently. Other antigens have been evaluated but have not been proven to be successful in clinical settings. Galactomannan and 1-3-β-D-glucan are both found
in the cell wall of *Aspergillus* cells and *in vitro* studies have found them to be secreted during the growth phase \(^{11}\). Only the galactomannan antigen will be discussed here given that is the only antigen used for *Aspergillus* spp. detection at Toronto General Hospital.

Galactomannan is a family of galactofuranose (galf)-antigens that is detected by the commercial sandwich ELISA Platelia *Aspergillus* by Bio-Rad \(^{12}\). Studies have shown that galactomannan is somewhat unreliable as a sole test for IA. One study found the test to have an excellent sensitivity and specificity of 90% and 98%, respectively \(^{13}\). Others have found that sensitivity is as low as 20% in those receiving antifungal therapy \(^{14}\). The reason for this variability has not been determined and suggested explanations include antigen dissipation from the site of infection, variable amounts of galactomannan being released by different *Aspergillus* species, as well as variable effectiveness depending on the type of patient population \(^{6,15}\). There is evidence that the test performs particularly well among those with hematological malignancies and hematopoietic stem cell transplant (HSCT) recipients \(^{13}\). The breakpoint designating a positive versus negative result with the galactomannan antigen test continues to be an area of debate. Early evaluations of the test suggested cut-off values of 1.0 or 1.5 \(^{11}\). At Toronto General Hospital, the cut-off to positivity value used is ≥0.5 which is now the revised manufacturer’s recommended cut-off value \(^{12}\).

### 1.1.3 IA in Solid Organ Transplant Recipients

In SOT patients, diagnosis of invasive aspergillosis is usually made 2-4 weeks post-transplantation \(^{8}\). Risk factors for IA include prolonged neutropenia, neutrophil dysfunction, steroid therapy, and chemotherapy \(^{6}\). Lung and heart transplant recipients are at the highest risk of aspergillosis infections, which affects 14–18 % of patients \(^{16}\). Lung transplantations are at especially high risk because the respiratory tract is the primary entry point for aspergillosis moulds \(^{3}\) with incidence of IA in lung transplant recipients reported to be between 3-14% \(^{17}\). For other types of solid organ transplantations, approximately 7% of liver transplant recipients develop IA while renal transplant recipients pose the lowest risk at <1% \(^{17}\). Overall the 1-year cumulative mortality incidence for SOT patients with IA has been found to be 18-42% \(^{5,18}\).
1.1.3.1 Risk Factors

Several factors predispose individuals for invasive aspergillosis. General risk factors for most patient populations include prolonged qualitative or quantitative neutropenia (defined as a neutrophil count <100/µL and lasting for >10 days), hematological malignancy, AIDS, steroid therapy, cytotoxic drugs, and transplantation ³.

Risk factors associated with mortality in SOT patients include type of transplantation, hepatic insufficiency, malnutrition, proven IA, use of methyl prednisolone, CNS (central nervous system) IA, and disseminated IA ¹⁹. Cytomegalovirus infection, poor functioning of the allograft, and episodes of rejection have also been associated with IA ³.

1.1.4 Treatment Strategy

Early anti-fungal intervention has been associated with better clinical outcomes as well as diminished costs and resistance ²⁰,²¹. Fulfillment of criteria for proven IA rarely occurs in routine clinical practice and therefore empirical and pre-emptive approaches have been utilized in the absence of proven IA ⁶. Empirical approaches are typically based on persistent febrile neutropenia that is resistant to treatment with broad-spectrum antibiotics and targeted approaches are based on culture or histology ²². Administration of antifungals via the empirical approach has become more wide-spread because it has been shown to be more effective than changing empirical antibacterials ²³,²⁴. For pre-emptive therapy, patients that have a high risk of developing IA and show clinical signs and symptoms indicative of IA are given antifungal therapy ⁶. Pre-emptive approaches are usually based on biomarkers such as the aspergillus antigen galactomannan, radiographic signs such as the “halo” seen in CT scans and clinical symptoms such as cough, pleural pain, or paranasal sinus findings in high risk patients ²⁵.

The first-line choice for primary IA is voriconazole, which was found to be superior to amphotericin B ²⁶, leading to its approval by the FDA in 2002. Voriconazole exerts its effects by disrupting the fungal cell wall.

Other triazoles, lipid amphotericin B formulations and echinocandins may have a role in the treatment of IA in special situations ⁶. Combination therapy for synergistic activity with antifungals that disrupt the fungal cell wall synthesis (echinocandins) and those that disrupt ergosterol synthesis or function (triazoles and amphotericin B) may be useful for treatment of
refractory IA. Surgery is advocated only as a last resort if progression continues despite antifungal therapy or if life-threatening developments occur 22.

1.2 Voriconazole

Voriconazole, ((2R,3S)-2-(2,4-difluorophenyl)-3-(5-fluoro-4-pyrimidinyl)-1-(1H-1,2,4-triazol-1-yl)-2-butanol) is a synthetic second generation broad spectrum triazole anti-fungal. Voriconazole primarily exerts its actions through inhibition of fungal cytochrome (P450 or CYP)-dependent 14-α-sterol demethylase 27. In Canada, it is approved for treatment of invasive aspergillosis (IA), candidaemia in non-neutropenic patients, and the following candida infections: disseminated infections in skin, and infections in the abdomen, kidney, bladder wall, and wounds 28. Current guidelines recommend voriconazole as the first-line choice for primary IA 29. Voriconazole is currently being used at Toronto General Hospital for IA colonization with Aspergillus species and possible, probable or proven IA infections based on a composite of computed tomography positive (CT+), clinically defined and microbiologically positive findings.

1.2.1 Usage

Voriconazole can be administered intravenously (IV) as a lyophilized powder for solution or orally as tablets and powder for suspension. For adults, IV dosing is determined by patient weight where the loading dose consists of 6mg/kg once every 12 hours for the first day followed by 4mg/kg every 12 hours. Oral voriconazole is rapidly absorbed and has high bioavailability, estimated to be over 90% 30. Oral dosing is 200 mg every 12 hours for individuals over 401k according to the product monograph 31 however, the Infectious Disease Society of American (IDSA) recommends oral dosing be approximated to the IV dosage, rounded up to the nearest convenient pill size 9.

1.2.2 Safety

Commonly observed adverse events (AEs) associated with voriconazole include transient visual disturbances, visual and/or auditory hallucinations, hepatotoxicity that can be dose-limiting (caused by elevated serum bilirubin, alkaline phosphatase, and hepatic aminotransferase enzyme levels), skin rash, erythroderma, photosensitivity, perioral excoriations, nausea, vomiting, and diarrhea 31. Less common AEs include cardiovascular events such as prolonged QT intervals 32.
There is some evidence supporting a relationship between voriconazole plasma trough concentrations and voriconazole-related AEs, which is further discussed in Chapter 2.

1.2.3 Pharmacokinetics, Metabolism and Elimination

Voriconazole displays non-linear, saturable pharmacokinetics with greater than proportional increases in drug exposure. Voriconazole is extensively distributed into tissues in humans with a volume of distribution of 4.6 L/kg and shows good penetration into various tissues exceeding the MIC\textsubscript{90} values for \textit{Aspergillus} and \textit{Candida}. In healthy volunteers, the C\textsubscript{max} is attained in ≤1.7 hours after single or multiple oral doses. Voriconazole has an elimination half-life of 6.4-7.3 hours. Due to the long-half life, in the absence of loading doses, voriconazole target steady-state levels are achieved within 6 days while with loading doses, target levels can be achieved within the first 24 hours. Bioavailability of oral voriconazole is high at over 90%.

\textit{In vivo} studies have reported that voriconazole is extensively metabolized with less than 2% of the dose excreted exchanged. Voriconazole undergoes fluoropyrimidine N-oxidation by the cytochrome P450 enzymes \textit{CYP2C9}, \textit{CYP2C19}, and \textit{CYP3A4} to form voriconazole’s major metabolite, voriconazole N-oxide. \textit{CYP3A4} mediates the biotransformation of voriconazole into 4-hydroxyvoriconazole which is the next major metabolite. \textit{CYP2C19} has the highest affinity enzyme for voriconazole followed by \textit{CYP2C9} and \textit{CYP3A4}. The role of \textit{CYP2C9} and \textit{CYP3A4} in voriconazole metabolism is undetermined. Investigation with \textit{CYP2C9} inhibitors found no effect on voriconazole metabolism while \textit{CYP3A4} inhibitors affected voriconazole metabolism in those with deficient \textit{CYP2C19} enzymatic activity. This funding suggests \textit{CYP3A4} may have a more compensatory role in the absence of \textit{CYP2C19} activity.

1.3 Therapeutic Drug Monitoring for Voriconazole Therapy

Therapeutic drug monitoring (TDM) for voriconazole therapy involves monitoring voriconazole plasma trough concentrations when steady-state levels are reached and maintaining voriconazole plasma trough concentrations within a pre-determined target range by adjusting dosing in instances of supra- or subtherapeutic voriconazole trough levels. TDM is recommended for keeping voriconazole plasma trough concentrations within therapeutic ranges and is used at many centers during voriconazole therapy.
There is extensive literature finding that TDM increases the efficacy of voriconazole therapy while decreasing the incidence of discontinuation of voriconazole therapy due to voriconazole-related toxicity \(^{29}\). The literature has also established an association between voriconazole trough plasma concentrations and the efficacy and safety of voriconazole therapy \(^{38}\). A consensus therapeutic range for voriconazole plasma trough levels has not been established but a therapeutic range of 1.0 mg/L – 5.0 mg/L has been generally recommended \(^{39}\). A thorough review of the literature regarding TDM effect on the efficacy and safety of voriconazole therapy is given in Chapter 2.

Overall, studies have generally reported TDM to be helpful in achieving target therapeutic voriconazole levels \(^{40-42}\). However, TDM has the inherent problem of a delay in first-time dosing adjustments due to voriconazole’s half-life and the resulting time needed to reach steady-state concentrations. In clinical practice, TDM is typically not conducted until 4-7 days after starting voriconazole therapy \(^{43}\).

1.4 CYP2C19

1.4.1 Overview of CYP2C19

The *CYP2C19* gene possesses numerous allelic variants and single nucleotide polymorphisms (SNPs), of which 35 have been determined thus far \(^{44}\). The *CYP2C19* gene encodes for a 490 amino acid long protein and is located on chromosome 10 (10q24.1-q24.3) \(^{45}\).

*CYP2C19* genotypes are categorized as extensive metabolizer (EM), heterozygous extensive metabolizer (HEM), poor metabolizer (PM), and more recently, the ultra-rapid metabolizer (URM) phenotype. The EM phenotype has the *1/*1 genotype and is considered the wild-type (WT) phenotype. Presence of one of the non-functional alleles (*1/*2-*8) at the *CYP2C19* gene indicates a HEM phenotype, while the PM phenotype is reserved for carriers of two non-functional alleles (*2-*8/*2-*8). Presence of the *17* allele at a single site is enough to warrant categorization as a URM. It has been generally recognized that *CYP2C19* phenotypes can be predicted solely on the presence of the *2, *3, and *17* alleles. An overview of common *CYP2C19* SNPs and their respective alleles and enzymatic activity is provided in Table 1.1.
The product monograph states that HEMs and PMs have up to two and four-fold the voriconazole blood concentrations as EMs. Approximately 15-20% of Asian populations are expected to be PMs while only 3-5% of Caucasian and African populations are expected to be PMs. The literature has established that HEMs and PMs are more likely to develop toxicity at the standard 200 mg twice daily dosing while EMs may require higher than standard dosing to reach therapeutic concentrations.

1.4.2 CYP2C19 Variants and Impact on Function

1.4.2.1 Extensive Metabolizers

The CYP2C19*1 is the wild-type allele associated with normal enzymatic activity. Individuals with the *1/*1 are said to have the wild-type genetic status and are categorized as extensive metabolizers. The *1 allele is high in frequency in most major ethnic groups, approximately 60% in East Asians to approximately 87% in Middle Easterners except for Oceanians (approximately 24%).

1.4.2.2 Intermediate and Poor Metabolizers

The CYP2C19*2 and *3 variants are the most common non-functional alleles and have relatively high frequencies in the population. The CYP2C19*2 allele ranges from 12-15% in Caucasians to 29% in East Asians while Oceanians have the CYP2C19*2 allele at approximately 61%. The CYP2C19*3 allele ranges from approximately 0.50% in Caucasians to approximately 9% in East Asians while Oceanians are again the outlier, having the CYP2C19*3 allele frequency at approximately 15%. CYP2C19*2 is characterized by a 681G-->A substitution in exon 5 leading to a splicing defect while CYP2C19*3 has a G-->A point mutation in exon 4, leading to a premature stop codon. These single nucleotide polymorphisms (SNPs) lead to a defective protein product with no enzymatic activity. Carriers of one copy of a non-functional allele (*2-8) combined with one copy of the ultra-rapid metabolizer *17 allele present an interesting case for phenotypic categorization. Although adequate evidence regarding the CYP2C19 enzymatic activity of these loss-of-function and gain-of-functions genotypes is lacking, it seems the *17 may be unable to completely compensate for any non-functional alleles and at least in the case of a *2/*17 genotype, phenotypic categorization as an HEM is warranted.
The \( CYP2C19*4, *5, *6, *7, \) and \( *8 \) variants are associated with no enzymatic activity while the variants \( CYP2C19*9 - *35 \), excluding \( *17 \) have unknown enzymatic activity as of yet \(^{44}\). The \( CYP2C19*4 - *8 \) variants occur at very low frequencies, having approximately 0.35% at most (the \( CYP2C19*8 \) allele in Caucasians) to approximately 0.09% and undetermined frequencies in other major ethnic groups \(^{47}\).

1.4.2.3 Ultra-rapid Metabolizers

\( CYP2C19*17 \) is the only known genetic variant that is associated with increased enzymatic activity. The \( CYP2C19*17 \) allele has two SNPs in the 5’flanking region: \(-3402C\rightarrow T\) and \(-806C\rightarrow T\) substitutions leading to increased gene transcription \(^{49}\). Frequencies are highest in Caucasian populations at approximately 18-21% while East Asians have frequencies of approximately 2.7%. This promoter region polymorphism induces increased transcription of \( CYP2C19 \). Individuals who have this gain-of-function allele are considered ultra-rapid metabolizers (URMs).

<table>
<thead>
<tr>
<th>Allele</th>
<th>Nucleotide Change</th>
<th>Enzymatic Activity in vivo</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>( CYP2C19*1 )</td>
<td>None</td>
<td>Normal</td>
<td>50</td>
</tr>
<tr>
<td>( CYP2C19*2 )</td>
<td>19154G\rightarrow A</td>
<td>Absent</td>
<td>51</td>
</tr>
<tr>
<td>( CYP2C19*3 )</td>
<td>17948G\rightarrow A</td>
<td>Absent</td>
<td>52</td>
</tr>
<tr>
<td>( CYP2C19*17 )</td>
<td>--806C\rightarrow T</td>
<td>Increased</td>
<td>49,53,54</td>
</tr>
</tbody>
</table>

Table 1.1 Common alleles and their effect on enzymatic activity

1.4.3 Role of \( CYP2C19 \) in Voriconazole Metabolism

The current published literature is extensive in describing the effect of \( CYP2C19 \) genetic status on voriconazole pharmacokinetics, especially in healthy adult volunteers. Studies have found that \( CYP2C19 \) genotype plays an important role in determining voriconazole pharmacokinetics, and given the same dose, EMS, HEMs, and PMs have differing levels of exposure to
voriconazole. Overall, \textit{CYP2C19} genotype has been estimated to be responsible for approximately 30-50\% of the variation in voriconazole concentrations\textsuperscript{55}. A review and critical appraisal of the literature examining the effect \textit{CYP2C19} genetic status on voriconazole metabolism is provided in Chapter 2.

1.5 Statement of Problem

Non-linear, saturable pharmacokinetics in conjunction with polymorphisms in \textit{CYP2C19} partly explains the high interpatient variability in voriconazole concentrations. Subtherapeutic levels of voriconazole may result in loss of clinical efficacy leading to treatment failure while supratherapeutic drug levels may cause toxicity and treatment termination.

1.6 Rationale, Objectives and Hypotheses

It is not known if TDM along with \textit{CYP2C19} genotyping will improve treatment outcomes. Knowing and guiding voriconazole dosing based on a patient’s \textit{CYP2C19} genotype along with TDM may increase the likelihood of attaining trough concentrations within therapeutic range, and/or reduce the time to achieve target concentrations compared to TDM alone. As a result, this may provide better clinical efficacy and also decrease the incidence of voriconazole-related toxicity.

1.6.1 Primary Objectives

a) To determine if the proportion of patients who achieve therapeutic voriconazole trough concentrations at the initial steady-state trough level measurement (4-7 days post-start voriconazole therapy) through genotype-guided dosing in conjunction with TDM is greater than the proportion of patients who obtain therapeutic voriconazole trough concentrations via TDM alone.

Hypothesis: Of patients who receive genotype-guided dosing in conjunction with TDM, a greater proportion will reach target voriconazole trough concentrations at the initial trough level measurement compared to patients who receive solely TDM

b) To determine if genotype-guided dosing in conjunction with TDM will result in a fewer number of dose adjustments needed to achieve therapeutic voriconazole trough concentrations, compared to TDM alone.
Hypothesis: Patients who receive genotype-guided dosing in conjunction with TDM will require a fewer number of dosage adjustments to reach therapeutic voriconazole trough concentrations than patients who receive solely TDM.

c) To evaluate the genotype-guided dosages suggested for the genotyping plus TDM trial arm. Only HEMs and PMs will receive a genotype-guided dosage.

Hypothesis: It is expected that HEMs and PMs who receive genotype-guided dosing will be able to reach voriconazole target therapeutic levels at the initial trough level measurement in greater proportion than HEMs and PMs who receive solely TDM.

1.6.2 Secondary Objectives

a) To determine if genotype-guided dosing can increase treatment success (complete or partial response), decrease the incidence of voriconazole dose-related AEs, severity of toxicity, and incidence of termination of voriconazole therapy due to voriconazole dose-related AEs. AEs will be graded by criteria listed in the National Cancer Institute’s Common Terminology Criteria for Adverse Events (CTCAE) v4.0.

Hypothesis: Genotype-guided dosages will decrease the incidence of toxicity and decrease the incidence of termination of voriconazole therapy due to voriconazole dose-related AEs compared to the standard of care dosing.
2 Chapter II: Effect of therapeutic drug monitoring and cytochrome P450 2C19 genotyping on clinical outcomes of voriconazole: a systematic review

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2.1 Abstract

2.1.1 Background

Voriconazole is the primary therapy for the treatment of many serious fungal infections that are a significant cause of morbidity and mortality in critically ill patient populations. However, optimising dosage is complex as voriconazole displays high inter- and intra-patient variability in plasma concentrations. Various factors can cumulatively explain this variation, including voriconazole’s non-linear pharmacokinetics and patient specifics such as age, weight, disease state, polypharmacy, and genetic polymorphisms in the cytochrome P450 2C19 gene (CYP2C19). This review will critically examine the current knowledge on the clinical utility of therapeutic drug monitoring (TDM) in voriconazole therapy, the impact CYP2C19 genotype has on voriconazole plasma concentrations, and the role of prospective CYP2C19 genotyping in voriconazole therapy.

2.1.2 Methods

Two literature searches of bibliographic databases were conducted for original reports on: i) voriconazole therapeutic drug monitoring and clinical outcomes, and ii) voriconazole and CYP2C19 genetic polymorphisms. Reviewers independently extracted data and appraised the quality of included randomized controlled trials, cohort studies, and case series.

2.1.3 Results

The literature searches yielded 1539 unique references and a total of 51 studies were included. The use of therapeutic drug monitoring in voriconazole therapy is recommended due to
established voriconazole concentration and efficacy and toxicity relationships. Voriconazole plasma trough concentrations ≥1.0 mg/L were found to be associated with increased treatment success while plasma trough concentrations ≥5.5 mg/L and ≥4.0 mg/L were associated with increased incidences of neurotoxicity and hepatotoxicity, respectively. CYP2C19 polymorphisms were found to significantly affect voriconazole metabolism and concentrations but no relationship between CYP2C19 genotype and voriconazole efficacy and safety were found. CYP2C19 genotype-guided dosing in conjunction with TDM was reported to significantly reduce the time required to reach therapeutic voriconazole concentrations.

2.1.4 Conclusions

Voriconazole plasma concentrations and TDM guided therapy are predictors of treatment outcome but further research is needed to form a consensus target therapeutic range and dosage adjustment guidelines based on plasma levels. CYP2C19 polymorphisms are a predictor of voriconazole concentrations and metabolism but the clinical implications of CYP2C19 genotype have yet to be established. Large scale, high methodological quality trials are required to investigate the potential roles for prospective CYP2C19 genotyping and establish dosing recommendations for CYP2C19 guided voriconazole dosing.
2.2 Introduction

During the last 3-4 decades there has been an increase in the incidence of serious invasive fungal infections (IFIs). A major factor has been the increase in immunocompromised patient populations due to the AIDS epidemic, increased usage of immunosuppressant drugs and cancer chemotherapy 57.

2.2.1 Voriconazole

Voriconazole is a synthetic second generation broad spectrum triazole antifungal that has been shown to be active in vitro and in clinical settings against Aspergillus, Cryptococcus, Candida, Fusarium and Scedosporium apiospermum species 31. Voriconazole is approved by the FDA for the treatment of invasive aspergillosis, candidaemia in neutropenic patients, disseminated Candida infections, esophageal candidiasis and for serious infections due to Scedosporium apiospermum and Fusarium spp. including Fusarium solani, in patients intolerant of, or refractory to, other therapy 31. Voriconazole primarily exerts its actions through inhibition of fungal cytochrome (P450 or CYP)-dependent 14-α-sterol demethylase by metabolized voriconazole 27.

2.2.2 Metabolism

Voriconazole can be administered intravenously (IV) as a lyophilized powder for solution, orally as tablets, or as a powder for suspension 31. Bioavailability of oral voriconazole is high at over 90% 30. Voriconazole is extensively metabolized by the cytochrome P450 system, having less than 2% excreted unchanged 35. Biotransformation of voriconazole into its main metabolite voriconazole-N-oxide involves several human hepatic cytochrome P450 enzymes of which voriconazole has the highest affinity for CYP2C19, followed by CYP2C9, and then CYP3A4 31. CYP2C19 is the primary enzyme responsible for voriconazole’s metabolism into its main metabolite voriconazole-N-oxide (~72% of plasma metabolites) and there is also evidence that CYP2C19 may be involved with the formation of voriconazole’s other metabolites including hydroxyl-voriconazole and dihydroxy-voriconazole 30. Genetic polymorphisms in CYP2C19 have been estimated to be responsible for approximately 30-50% of the variation in voriconazole concentrations 35. Mutations in the CYP3A4 gene can affect its enzymatic activity as well, but
these variants are relatively rare and their effect on voriconazole pharmacokinetics are not well established\textsuperscript{58,59}.

### 2.2.3 Trough concentrations

Treatment outcomes with voriconazole have been shown to be associated with voriconazole plasma concentrations; several reviews and meta-analyses have established that treatment failure and toxicity are associated with sub- and supra-therapeutic voriconazole concentrations\textsuperscript{39,38,60}. Some common side effects of voriconazole include transient visual disturbances, visual and/or auditory hallucinations, hepatotoxicity and skin rash\textsuperscript{61,62}.

Optimising dosage is complex as voriconazole displays high variability in inter- and intra-patient plasma concentrations\textsuperscript{63}. Non-linear pharmacokinetics, as well as patient age, weight, comorbidities, concomitant medications, and genetic make-up all contribute to the high inter- and intra-patient variability observed.

Therapeutic drug monitoring (TDM) allows for dosage adjustments to be implemented in response to patient supra- or sub-therapeutic plasma levels. Voriconazole dose individualization based on TDM is recommended for most treatment settings by several guidelines\textsuperscript{29,39,64–67}.

However, in clinical settings, voriconazole trough levels are typically obtained and tested 4-7 days after the start of voriconazole therapy when steady state is reached\textsuperscript{68,69} and dosage adjustments based on those results made at least 24-48 hours after drawing the level\textsuperscript{41,42,70}. Considering that early diagnosis and initiation of accurate anti-fungal therapy is important to treatment outcomes of patients with IFIs\textsuperscript{20,21} there is an inherent problem where voriconazole dosage optimization is not initiated until potentially ≥5-7 days after the initiation of voriconazole.

### 2.2.4 Genetic Polymorphisms in \textit{CYP2C19}

\textit{CYP2C19} is the enzyme encoded for by the cytochrome P450 \textit{CYP2C19} gene and mediates the metabolism of numerous commercially available medications. The metabolism of several classes of drugs and medications have been shown to be affected by genetic mutations in the \textit{CYP2C19} gene, including proton pump inhibitors, antidepressants, benzodiazepines, clopidogrel, and voriconazole\textsuperscript{47}. 
The \textit{CYP2C19} gene is highly polymorphic and possesses numerous allelic variants of which 35 have been determined thus far\textsuperscript{44}. Several alleles have been shown to be prevalent in highly variable frequencies in different ethnicities and linked to increased or decreased protein expression, or functional ability of the CYP2C19 isoenzyme\textsuperscript{47}.

The \textit{*1} allele is the wild-type variant and displays normal enzymatic activity. Homozygotes for the wild-type allele (having the genotype *1/*1) are categorized as having the extensive metabolizer (EM) phenotype. The frequency of the \textit{*1} allele is high in most major ethnic groups: 60\%-69\% in Asians, Africans, and Caucasian\textsuperscript{47}.

\textit{CYP2C19*2} is the most common of the \textit{CYP2C19} loss-of-function alleles (\textit{CYP2C19*2 to*8}), and ranges from 12-15\% in Caucasians, and 29-35\% in Asians\textsuperscript{47}. This is followed by the \textit{CYP2C19*3} allele, which ranges from <~0.5\% in Caucasians, and ~9\% in East Asians. The \textit{CYP2C19*4 to*8} variants occur at much lower frequencies\textsuperscript{47}. The presence of a single non-functional allele denotes a heterozygous extensive metabolizer (HEM) phenotype, while the poor metabolizer (PM) phenotype is defined by the presence of two non-functional alleles.

\textit{CYP2C19*17} is the only known allele that is associated with increased enzymatic activity which is attributed to increased \textit{CYP2C19} gene transcription\textsuperscript{49}. Carriers of the \textit{*17} allele that are homozygous (*17/*17) or heterozygous with the WT allele (*1/*17) have the ultra-rapid metabolizer (URM) phenotype. Frequencies are highest in Caucasian populations at ~18-21\% while East Asians have frequencies of ~2.7\%\textsuperscript{47}.

\textbf{2.2.5 Pharmacogenetics}

Pharmacogenetics is the study of genetic differences in drug metabolic pathways, which can determine individual responses to therapy. Since therapeutic voriconazole concentrations are associated with better clinical outcomes, and voriconazole metabolism is mediated significantly by \textit{CYP2C19}, there may be benefit to incorporating patients’ \textit{CYP2C19} metabolic status into the existing dosing algorithms. In particular, utilizing prospective \textit{CYP2C19} genotyping to guide initial voriconazole dosing before TDM is conducted may lead to better outcomes. This review will critically examine the current knowledge of voriconazole plasma concentrations and efficacy/toxicity relationships, the impact \textit{CYP2C19} genotype has on voriconazole plasma
concentrations, and the role of \textit{CYP2C19} genotype on the clinical outcomes of voriconazole therapy.

\subsection*{2.3 Methods}

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines \cite{71} and recommendations of the Cochrane Handbook for Systematic Reviews \cite{72} were used as framework for this review.

\subsubsection*{2.3.1 Search Strategy and Data Sources}

Two literature searches were conducted with the help of a librarian, one constructed around the search terms for voriconazole and therapeutic drug monitoring and the other around the search terms voriconazole and genetics. The search terms were adapted for each database as necessary. The full list of search terms used can be found in the Appendix A.

EMBASE, MEDLINE/PubMed, Scopus, and the Cochrane Central Register of Controlled Trials were searched from their inception through February 16, 2016. To locate grey literature, ResearchGate (www.researchgate.net) and Google Scholar (www.googlescholar.com) were searched. In addition, the references cited by eligible original articles and other published reviews were manually searched.

\subsubsection*{2.3.2 Eligibility Criteria}

Randomized controlled trials (RCTs), prospective and retrospective observational cohort studies, and case series (with sample size \( \geq 10 \)) were eligible for inclusion. Only studies in English and having complete datasets were eligible. Non-primary literature (reviews, meta-analyses, editorials and commentaries) were ineligible for inclusion but their findings are discussed.

All eligible studies from the two separate literature searchers were categorized according to the aim of their research question. Eligible references from the search surrounding the terms ‘voriconazole’ and ‘drug monitoring’ were categorized as studies examining Voriconazole TDM and Efficacy or Safety Relationship. Eligible studies from the second search surrounding the search terms ‘voriconazole’ and ‘genetics’ were categorized into one of 3 study categories depending on the study objective: 1) CYP2C19 Polymorphisms and Voriconazole
Pharmacokinetic Relationship, 2) CYP2C19 Polymorphisms and their Efficacy and Toxicity Relationship, or 3) CYP2C19 Genotype Guided Dosing and Voriconazole Efficacy and Safety.

There were specific inclusion and exclusion criteria for each type of study. For studies looking at voriconazole TDM and efficacy or safety, trials that did not measure efficacy or safety outcomes in relationship to patient voriconazole trough levels were excluded. For studies looking at CYP2C19 polymorphisms and voriconazole pharmacokinetics, studies needed to report the pharmacokinetic parameters that correlated to CYP2C19 phenotypes. For studies examining the relationship between CYP2C19 genetic status and clinical outcomes, trials needed to report efficacy or safety outcomes associated with CYP2C19 polymorphisms. For studies looking at CYP2C19 genotype-guided dosing and voriconazole efficacy and safety, trials needed to measure the effect of CYP2C19 genotyping on clinical efficacy or toxicity.

2.3.3 Initial Screening & Full Text Review

The title and abstract screening process was independently conducted by two reviewers (J.L. and M.B.). Citations were eligible for full text review if at least one reviewer voted for their inclusion using the predefined inclusion criteria. Full-text manuscripts of potentially eligible articles were read by 3 reviewers (J.L, M.B., and B.H.). Disagreements regarding article eligibility were resolved through discussion and if consensus could not be reached, articles were included if there was majority (2 of 3 reviewers) in agreement.

2.3.4 Data Extraction

Three reviewers (J.L., A.V., and A.A.) independently extracted the data on patients (eg. age, sex, and ethnicity), methods (study type), interventions (eg. TDM, dosage adjustments, genotyping), and outcomes (efficacy, toxicity, trough concentrations) using an a priori developed data extraction forms (see Appendix B). Any discrepancies were discussed and resolved by consensus or majority.

2.3.5 Quality Assessments

Risk of bias (ROB) was assessed by 3 reviewers (J.L., A.V., and A.A) independently using a priori developed quality assessment forms (see Appendix C). RCTs were assessed using the Cochrane ROB tool which assesses ROB on 7 domains: random sequence generation,
allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias. Reviewers assessed each section as having high, low, or unclear ROB. Cohort studies were appraised using the Newcastle-Ottawa Scale (NOS)\textsuperscript{74}. The NOS for cohort studies assigns a maximum of 9 points for ROB over 3 domains: 1) selection of study groups (four points), 2) ascertainment of exposure (three points), and 3) ascertainment of outcomes (3 points). Case series were appraised using the critical appraisal checklist for descriptive studies in the Joanna Briggs Institute Reviewer’s Manual which uses a series questions to assess the validity of the study methodology\textsuperscript{75}. Case series were deemed to have a low risk of bias if no more than 2 questions scored “unclear” or “no”. If studies scored “unclear” or “no” in no more than 4 sections, the risk of bias was deemed to be “medium”. If studies scored “unclear” or “no” in more than 4 sections, the risk of bias was deemed to be “high”.

2.4 Results

The literature searches yielded 1796 potentially relevant references. After the removal of duplicates and initial screening, there were 135 potentially eligible references requiring full text review. There were a total of 51 studies that were included after 84 references were excluded. Their quality assessments can be found in Appendix D. The study selection process is outlined in Figure 2.1.
2.4.1 Voriconazole TDM and Efficacy or Toxicity Relationship

Our literature search found 30 studies meeting our eligibility criteria that examined the effect of TDM on either voriconazole treatment efficacy or toxicity 40–42,76–102 (see Table 2.1 for study characteristics). Two randomized controlled trials were found, one of which was prematurely discontinued due to problems with study enrollment and the remaining citations (28) were retrospective or prospective cohort and case series studies 42,99.

2.4.1.1 Efficacy Relationship

Fifteen studies investigating the effect of TDM on voriconazole therapy established statistically significant relationships between voriconazole concentration and efficacy and recommended usage of TDM 40–42,76–78,81,86–89,95,98,99,101,102.
In a South Korean single-center randomized controlled trial investigating the effect of TDM on voriconazole efficacy and safety, 110 patients given voriconazole for targeted or empiric therapy were 1:1 randomized to either a TDM intervention arm (n=55) or control arm (n=53) \(^{42}\). All patients received standard weight-based dosing of 6mg/kg loading and 4mg/kg maintenance dosage. For those in the intervention arm, voriconazole dosage adjustments were made based on trough levels drawn at steady state. Treatment success was significantly higher in the TDM arm than the control arm (81% vs 59%; p=0.04). There was no relationship between TDM and the incidence of all voriconazole-related adverse events, but patients in the TDM arm had significantly lower rates of AE-related discontinuation of voriconazole therapy compared to those in the control group. The study reported that when faced with intolerable voriconazole-related adverse events, clinicians in the non-TDM arm had to discontinue voriconazole, whereas in the TDM arm, if adverse events and supra-therapeutic voriconazole concentrations coincided, clinicians could make dose adjustments that were predicted to mitigate toxicity.

The other randomized controlled trial was a prematurely discontinued multi-center trial investigating the effect of TDM on the incidence of AEs and treatment failures. Twenty-nine adult patients were prescribed voriconazole for possible, probable, or proven IA. Patients were randomized using a 1:1 allocation ratio to either the clinician-driven dosing control arm (n=15) or TDM dosing (n=14) intervention arm \(^{99}\). Patients in both arms were given intravenous or oral voriconazole at 4mg/kg every 12 hours. TDM trial arm participants had trough levels measured at steady-state, 5 (±3) days within starting voriconazole, and dosage adjustments were subsequently made based on trough level results. TDM was repeated every two weeks and whenever a dosage adjustment was made. In the control arm, voriconazole dosing was made based on clinician judgement. Trough levels were drawn for control arm participants as well but results were blinded to clinicians. The trial found that significantly more participants in the TDM arm than the control arm had positive outcomes of antifungal therapy (85.7% vs 46.7%; p=0.05). No significant relationships were found between TDM and the incidence of voriconazole toxicity. The study reported that relative to the TDM arm, control arm participants frequently had out-of-range trough levels. At the end of therapy, mean voriconazole trough levels of patients in the control arm were lower than those in the TDM arm (1.3 ± 1.7 mcg/ml vs. 4.6 ± 2.4 mcg/ml; p=0.008)
Other observational and descriptive studies also found voriconazole concentration and treatment outcome relationships and reported that TDM was an essential tool for clinicians in making appropriate dosage adjustments. In an observational study with 52 patients, authors found a lack of response in patients with voriconazole trough levels ≤1 mg/L vs. ≥1 mg/L; 46% vs. 12% (p=0.02). This association of efficacy with voriconazole trough concentrations ≥1.0mg/L was similarly reported in several other studies. A retrospective study of 34 Japanese patients with hematological malignancies found voriconazole trough levels ≥2 mg/L were associated with voriconazole efficacy (100% of patients with troughs ≥2mg/L responded vs. 33% of patients with troughs <2mg/L responded). Association of efficacy with voriconazole trough concentrations ≥2.0mg/L was also reported in other studies.

In all, a clear relationship between voriconazole trough concentrations and treatment success has been established and voriconazole TDM is recommended. However, the suggested minimum trough concentrations for efficacy have ranged from 0.25-2.2mg/L. The lower threshold in the targeted therapeutic range for both randomized controlled trials was 1.0 mg/L. This limit comes from earlier in vitro data that established 0.5-1 mcg/ml as the minimum inhibitory concentration (MIC) for voriconazole efficacy against relevant pathogenic fungi. Based on the number and methodological quality of the studies reporting an efficacy relationship with trough concentrations ≥1.0mg/L, this review agrees that the lower threshold of ≥1.0mg/L should be used as the cut-off for efficacy.

2.4.1.2 Toxicity Relationship

Eleven studies reported statistically significant associations between voriconazole trough concentrations and voriconazole-related adverse effects. The most frequent types of toxicities reported were hepatotoxicity, neurotoxicity, and cutaneous toxicity while reports of cardiotoxicity and metabolic disturbances were rarer. Neurotoxicities included visual disturbances, encephalopathy, photophobia, and hallucinations. Hepatotoxicity was most frequently defined as liver enzymes elevated above grade 2 according the criteria of NCI CTCAE or >3-5 times upper limit of normal (ULN). Two studies defined hepatotoxicity as liver enzymes elevated ≥ grade 1 according to CTCAE criteria.

The relationship between neurological toxicity and higher voriconazole concentrations has been reported by several studies. One retrospective study reported that of 16 patients
with voriconazole trough concentrations over >5.5 mg/L, 5 patients had encephalopathy whereas encephalopathy was not present in those with trough levels ≤5.5 mg/L (p=0.02 \textsuperscript{78}). Another retrospective study found that trough levels in patients reporting neurological adverse events were significantly higher compared to patients who reported no neurological toxicity (6.5 mg/L vs 1.6 mg/L, p <0.01) \textsuperscript{40}.

For the incidence of hepatotoxicity, few studies found statistically significant associations to voriconazole trough concentrations. A prospective study found that patients with higher median voriconazole levels was associated with having elevated levels of bilirubin (3.5 mcg/ml vs 2.4 mcg/ml, p =0.03) \textsuperscript{96}. Another recent study found that hepatotoxicity was more frequently observed in patients whose trough concentrations were >5 mcg/ml compared to patients with trough concentrations ≤5 mcg/ml (90% vs 31.6%, p=0.01). Trough levels in patients with and without hepatotoxicity were 5.55±2.73 mcg/mL and 2.36± 1.67 mcg/mL respectively (p<0.01).

Voriconazole-induced hepatotoxicity should also be investigated further as there is no consensus on a relationship between voriconazole trough concentrations and hepatotoxicity. It is plausible that trough concentrations (C\textsubscript{min}) may not be the ideal metric to predict hepatotoxicity and one study suggested that sustained (over two trough level measurements) voriconazole supra-therapeutic concentrations are associated with hepatotoxicity \textsuperscript{91}. Interestingly, a greater proportion of studies conducted in Asian populations have been able to report a relationship between voriconazole trough concentrations and hepatotoxicity. However, these findings have not been observed in studies conducted in predominantly Caucasian populations. It is plausible that this discrepancy is influenced by genetic polymorphisms in CYP2C19, given the higher proportion of HEMs and PMs in Asian populations and the higher proportion of EMs and URMs in Caucasian populations.

Taking these findings together, it is conceivable that only some proportion of the population can be exposed to the prolonged supra-therapeutic concentrations that induce hepatotoxicity. More specifically, URMs and possibly EMs may be less capable of reaching or surpassing for prolonged periods to cause hepatotoxicity. Indeed, genetic association studies have found that in single oral dose settings, PMs can have nearly two-fold the peak drug concentration (C\textsubscript{max}) and half-life (t\textsubscript{1/2}), six-fold the total exposure over time (AUC\textsubscript{0-∞}) and one-
sixth the total body clearance (CL/F) as URMs. In multiple oral dose scenarios, PMs can have 2.25 times the C\text{max} as EMs and over 3 times the AUC\text{0-\infty}.

Overall, a relationship between voriconazole trough concentrations and adverse events can be established with the existing evidence, and TDM is recommended clinically to reduce the incidence of adverse events. In particular, TDM has been shown to be valuable in decreasing the frequency of discontinuations due to voriconazole-related adverse events. There is no established higher threshold for target therapeutic voriconazole ranges. This review found that trough levels >5.5 mg/L were associated with neurotoxicity and trough levels >4.0 mg/L were associated with hepatotoxicity.

2.4.1.3 Pediatrics

A retrospective cohort study from Korea that compared treatment outcomes of children receiving TDM (n=31) with those that did not receive TDM (n=30) found that rates of treatment failure in the control group were higher than those in the TDM group (78.6% vs 40%, p=0.038). Rates of voriconazole discontinuation due to drug-related adverse events however, was more frequent in the TDM group than in the control group (26% vs 92.3%, p=0.001). An efficacy and concentration relationship was also established where patients whose voriconazole levels were above 1.0 mg/L for over 50% of their treatment duration had greater rates of treatment success (71.4% vs 9.1%, p=0.013). Finally, the study reported that in order to reach target therapeutic range (1-5.5 mg/L), 8.3 mg/kg was the mean dose required for children <12 years of age and 6.9 mg/kg was the mean dose required for children ≥12 years of age.

A retrospective study of 11 children from ages (0.8-14.8) found that 3 of the 4 patients that had therapeutic failure also had voriconazole trough levels <2 mg/L. Two patients that experienced voriconazole-related adverse events had trough levels of 12.14 mg/L and 8.52 mg/L respectively. The study found that dosing regimens varied greatly from 6 mg/kg/day to 26.1 mg/kg/day. In another pediatric study of 30 immunocompromised children with probable or proven IFIs, supratherapeutic trough concentrations above 5.5 mg/L were related to an increased frequency of neurological and cutaneous adverse events. The relationship between voriconazole concentrations and hepatotoxicity in pediatric populations is less well-defined and several pediatric observational studies found no relationship between voriconazole trough level and hepatotoxicity. Those between the age of two and twelve have been reported to
require 7 mg/kg Q12 dosing and neonates and toddlers require even higher mg/kg voriconazole dosing\textsuperscript{89,92}. These findings show pediatric dosing to be inversely correlated with age and weight.

Overall, voriconazole concentrations are highly variable in pediatric populations and it has been reported that extreme dosages of voriconazole have been required to reach target therapeutic levels with limited toxicity (>30 mg/kg/day)\textsuperscript{84,92}. The usage of TDM in voriconazole therapy has been reported to reduce discontinuation rates due to drug-related toxicity, and this finding has also been corroborated in adult populations\textsuperscript{42}. This review agrees that TDM is recommended as a tool of paramount importance for clinicians to monitor voriconazole trough levels in pediatric populations\textsuperscript{29}.

2.4.2 CYP2C19 Polymorphisms and Voriconazole Pharmacokinetic Relationships

We found 11 trials meeting our eligibility criteria that examined the effect of \textit{CYP2C19} genotype on voriconazole pharmacokinetics alone\textsuperscript{30,111,112,114–121}. Their main characteristics can be found in Table 2.2.

An early study in healthy Japanese volunteers given oral voriconazole 200 mg or 300 mg BID over 10 days found that PMs (n=2) had a 3.8-5.8 fold higher total AUC\textsubscript{0–\infty} (area under the curve) than EMs (n=6)\textsuperscript{114}. Several other studies on healthy volunteers reported similar findings in both single and multiple dose settings where when given oral or intravenous voriconazole, PMs had consistently higher AUCs than EMs by 2-3 fold\textsuperscript{30,111,112,115}.

In a study where 20 healthy Chinese volunteers were given single dose oral voriconazole, those with the *2/*2 genotype had approximately 4.8 times the AUC\textsubscript{0-24} and 6.6 times the AUC\textsubscript{0-\infty} of those with the *1/*17 genotype\textsuperscript{111}. Interestingly, another study of 35 predominantly Caucasian, healthy participants reported PMs had approximately 3.5 times the AUC\textsubscript{0-\infty} as URM, which is only approximately half (3.5/6.6) the difference noted in the previous study\textsuperscript{115}. A factor that may partially explain this discrepancy is in the study with predominantly Caucasian participants, 2 of the 10 URM had genotypes of *2/*17. A combination of one copy of a non-functional allele with one copy of the *17 allele presents an interesting case for phenotypic categorization. Although there is limited evidence regarding the CYP2C19 enzymatic activity of
the *2/*17 genotype it seems the *17 is unable to completely compensate for the loss of function allele and in the case of a *2/*17, phenotypic categorization as an HEM is warranted 48.

A study on adult patients treated for probable or proven IFIs, evaluated the effect of CYP2C19 polymorphisms on voriconazole clearance and dose requirement 121. The study found WT patients required significantly lower mean voriconazole doses to achieve therapeutic levels compared to *17 heterozygotes and homozygotes (2.57±0.25 mg/kg for *1/*1 vs 3.94±0.39 mg/kg for *1/*17; p<0.001 and 6.75±0.54 mg/kg for *17/*17; p<0.0001). Carriers of the *17 allele (n= 17) had significantly lower voriconazole trough concentrations than *1 homozygotes (n= 11) (p<0.001) Similarly, *1 homozygotes (n= 11) had lower concentrations than *2 homozygotes (n= 1) (p= 0.00636). Unfortunately, two individuals heterozygous for *2 and *17 were excluded from the analyses for unknown reasons.

Overall, there are significant differences in the exposure, clearance, and voriconazole trough levels between CYP2C19 genotypes for healthy volunteers and patients given voriconazole for possible, probable or proven IFIs. There is also evidence that the voriconazole dosage required for patients to achieve therapeutic levels is mediated by CYP2C19 genotype.

2.4.3 CYP2C19 Polymorphisms and their Efficacy and Toxicity Relationship

We found 10 trials examining the association between CYP2C19 genotype and the clinical outcome of voriconazole therapy 61,102,105,122–128 (see Table 2.3).

2.4.3.1 Effect of CYP2C19 Genotype on Efficacy

Only one prospective and one retrospective study evaluated the association between CYP2C19 genotype and efficacy of voriconazole therapy, with both studies finding no relationship 102,128. The prospective study found that of the 32 patients (31%) who experienced treatment failure, there was no significant relationship between CYP2C19 genotype and efficacy in terms of treatment response, all-cause or IA-attributed mortality 128. The retrospective trial found no relationship between CYP2C19 genotype and treatment success 102. However, the study found that there was a relationship between voriconazole trough concentrations and CYP2C19 polymorphisms where EMs (1.98±0.17 mg/L) and HEMs (2.36±1.59 mg/L) each had
significantly lower trough levels than PMs (3.67±2.54 mg/L). In addition, there was a significant
difference between mean voriconazole trough levels in those with a successful response (n=118; 
Cₘᵢₙ= 2.37 mg/L) with those with a lack of response (n=26; Cₘᵢₙ=1.16 mg/L) (p<0.05) ¹⁰².

2.4.3.2 Effect of CYP2C19 Genotype on Toxicity

Ten studies evaluating the association between CYP2C19 genotype and toxicity of voriconazole 
therapy were located. In a retrospective study examining the relationship between CYP2C19 
genotype and toxicity, comparisons were made between patients with the WT genotype (n= 63) 
which they defined as those lacking *2 and *3, against those having at least one copy of the two 
non-functional alleles (n= 23) ¹²⁷. The study found that CYP2C19 genotype was not a predictor 
of hepatotoxicity or any elevated liver enzymes. Several other prospective studies also found no 
relationship between CYP2C19 genotype and toxicity ⁶¹,¹⁰²,¹⁰⁵,¹²³,¹²⁶,¹²⁸. However two studies 
done in Asian populations reported hepatotoxicity tended to occur with greater frequency in 
patients with voriconazole trough levels >4 mg/L and that carriers of the *2 or *3 alleles required 
either lower dosages of voriconazole to stay below trough concentrations of ≤4 mg/L or had 
higher trough levels than those without the *2 or *3 alleles ¹⁰²,¹⁰⁵.

Only one prospective study reported an association between CYP2C19 polymorphisms 
and voriconazole toxicity ¹²². In the study of 19 adult patients with hematological malignancies 
being treated for possible, probable, or proven IFIs, toxicity was higher in HEMs (3 of 5 HEMs, 
60%) than EMs (3 of 8 EMs, 42%) and URM (3 of 6 URM, 50%). None of the findings of the 
study were statistically significant.

2.4.3.3 Effect of CYP2C19 Genotype on Trough Levels and Time to 
Therapeutic Range

Of the studies reporting the association between CYP2C19 genotype and efficacy or safety, 
several have reported on the association between genotype and trough levels in patients ⁶¹,¹²²–
¹²⁴,¹²⁸. A recent prospective study found only EMs and URM had subtherapeutic levels at some 
point during voriconazole therapy ¹²⁹. This finding was echoed in another prospective study that 
reported EMs had significantly more out-of-range initial trough levels (46%) than HEMs (26%) 
and PMs (0%) (p=0.001) ¹²⁸. One study on Japanese children (n=37) found higher trough levels 
in those with *2 and *3 alleles than those heterozygous or homozygous for the *I or *I₇ allele. 
Several studies examining the effect of the *I₇ allele on voriconazole trough concentrations have
reported individuals homozygous or heterozygous for this variant to be associated with subtherapeutic voriconazole trough levels.

In a study in Caucasian cystic fibrosis lung transplant recipients, the proportion of out of range concentrations was significantly higher in CYP2C19*17 carriers and was associated with a higher rate of under-dosing than CYP2C19*1 carriers (37.9% vs 15.6%; p<0.01) 125. This study also reported that carriers of the *17 allele and *2 allele took the most number of days to reach therapeutic range (4 days for WT, 9.5 days for *17 allele carriers, and 11.5 days for *2 allele carriers) 125. In a small sample size prospective study, similar findings were made where HEMs (n= 5) needed on average more dose adjustments to reach therapeutic range and 2 EMs (n= 8) and 1 URM (n= 6) were given a CYP2C19 inhibitor to aid reaching therapeutic range.

Overall, studies that examined the association between CYP2C19 genotype and patient clinical outcome have essentially found little to no relationship for either efficacy or safety. However, it is important to note that TDM was conducted in these studies, and thus these studies are attempting to detect the difference in efficacy or toxicity between genotypes after dosage adjustments by TDM are made in attempt to bring patient plasma trough concentrations to therapeutic range. Thus the relationship between CYP2C19 genotype and i) initial voriconazole trough levels prior to dosage adjustments, ii) time to therapeutic range, iii) time within therapeutic range, and iv) number of dosage adjustments required, are the parameters that require further investigation. To our knowledge, there are a limited number of studies that have reported these relationships.

2.4.4 CYP2C19 Genotype Guided Dosing and Voriconazole Efficacy and Safety

One study was located that analyzed the effect of prospective CYP2C19-guided voriconazole dose selection 130,131 (see Table 2.4). In a pediatric hematopoietic stem cell transplantation (HSCT) population receiving voriconazole for prophylaxis, a prospective cohort of those who received initial voriconazole dosing based on their CYP2C19 genotype (n=20) 131 was compared with a retrospective cohort that initially received 5 mg/kg twice daily dosing regardless of genotype (n=25) 130. TDM was conducted for both cohorts and the targeted therapeutic range was 1.0-5.5 mg/L. Initial voriconazole dosages were 5 mg/kg BID for PMs, 6mg/kg for HEMs, and 7 mg/kg for EMs and URMs. Those who received dosing based on their CYP2C19 genotype
reached therapeutic concentrations faster than those who did not (median 6.5 days vs. median 29 days; p= 0.0001). The study also noted that of the cohort that did not receive genotype-guided dosing, HEMs took the longest time to reach therapeutic concentrations (median 56 days vs median 29 days for entire cohort). This finding was also reported in an Australian pilot study 122. The genotype guided cohort reported fewer children with elevated liver enzymes (5 vs. 1, 20% vs 5%) and fewer discontinuations due to toxicity (1 vs. 0; 4% vs 0%). There were also a fewer number of patients with elevated liver enzymes in the genotype guided cohort. Overall, there is limited evidence on the effect of CYP2C19 genotype-guided dosing on the efficacy and safety of voriconazole therapy and further research implementing CYP2C19 genotype-guided dosing in pediatric and adult patient populations is required.

2.4.5 Characteristics, Outcomes, and Results of Included Studies

Table 2.1 Studies on the relationship between voriconazole TDM and efficacy or toxicity
<table>
<thead>
<tr>
<th>Study</th>
<th>Study Design</th>
<th>n</th>
<th>Mean Age (range)</th>
<th>Underlying Condition</th>
<th>Indication</th>
<th>Ethnicity</th>
<th>Voriconazole Therapy</th>
<th>Target Range</th>
<th>Voriconazole Trough Concentrations</th>
<th>Toxicity Relationship</th>
<th>Efficacy Relationship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smith, 2006</td>
<td>Retrospective Study</td>
<td>28</td>
<td>NR; [0.8 – 70]</td>
<td>Lung transplant (n=4); Renal transplant (n=4); Liver Transplant (n=4); Heart transplant (n=2); BMT (n=5); Other (n=9)</td>
<td>IA (n=24); Scedosporium (n=1); Candida (n=1); Blastomycosis (n=1); Cryptococcus (n=1)</td>
<td>NR</td>
<td>All patients received a voriconazole loading dose and maintenance dosing of 200mg PO BID for at least 2 weeks.</td>
<td>NR</td>
<td>Range: 0.2–4.8 mcg/mL; Average: 1.10 ± 0.76 mcg/mL; Median: 1.05 mcg/mL</td>
<td>NR</td>
<td>A significant relationship between disease progression and voriconazole trough concentrations (P=0.025) Favorable responses were seen in 10/10 patients (100%) who had concentrations &gt;2.05 mcg/mL. Disease progression was observed in 8/18 (44%) of patients with concentrations &lt;2.05 mcg/mL.</td>
</tr>
<tr>
<td>Trifilo, 2007</td>
<td>Retrospective Study</td>
<td>71</td>
<td>NR; adults</td>
<td>HSCT recipients with hematological malignancies</td>
<td>Prophylaxis</td>
<td>NR</td>
<td>200mg PO BID, continued until 1 month beyond discontinuation of immunosuppressives or development of fungal infection requiring change in therapy. Average duration of therapy: 117 days (12-956 days)</td>
<td>NR</td>
<td>Voriconazole Levels: 53% of patients within 1.0-5.0mg/L; 32% of patients &lt;1.0mg/L; 10% of patients &gt;5.0mg/L</td>
<td>NR</td>
<td>Including the 4 zygomycosis cases, 6 candidiasis cases were seen among the 44 patients who had voriconazole levels ≤ 2mg/mL and none among the 27 patients who had levels &gt;2mg/mL. (P=0.049)</td>
</tr>
<tr>
<td>Pascual, 2008</td>
<td>Prospective Study</td>
<td>52</td>
<td>Median: 58.8 (23-78)</td>
<td>Hematologic malignancy (n=32); solid-organ transplant (n=6); abdominal surgery (n=3); chronic liver disease (n=3); other (13% n=16); none (8% n=4)</td>
<td>Proven or probable IA (50% n=26); proven or probable invasive candidiasis (15% n=8); other proven IFIs (4% n=2); possible IFIs (21% n=11); persistent fever during neutropenia (10% n=5)</td>
<td>Caucasian</td>
<td>Median Loading Dose: 12mg/kg/day PO or IV; Median Maintenance Dose: 8mg/kg/day IV (range: 6-11) or 6.5mg/kg/day PO (range: 2-11)</td>
<td>1-5.5 mg/L</td>
<td>Troughs &gt;1mg/L was observed in 75% (n=39) of patient, from which 44% had voriconazole troughs within the therapeutic range (1-5.5mg/L), and 31% had voriconazole troughs &gt;5mg/L. Of the 16 patients with troughs ≤5mg/L, 5 patients (31%) had encephalopathy vs no (0%) patients with levels ≤5.5mg/L present with encephalopathy (P=0.002) No significant association found between patients who had severe cholestatic hepatopathy (8% of patients with voriconazole troughs ≤5mg/L) and in 19% of patients with troughs &gt;5.5mg/L</td>
<td>NR</td>
<td>Lack of response was more frequently seen in patients who had voriconazole levels ≤1mg/L; (n=6/13, 43%) compared to patients who had levels &gt;1mg/L (n=15/39, 12%); P=0.02</td>
</tr>
<tr>
<td>Okuda, 2008</td>
<td>Retrospective Study</td>
<td>23</td>
<td>58.2 ±23.2 (18-85)</td>
<td>Various hematological malignancies</td>
<td>Probable or proven IFIs or PIF</td>
<td>Japanese</td>
<td>200-400mg PO or IV</td>
<td>1-4.5 mcg/mL</td>
<td>See table 4 page 1841 for individualized trough levels</td>
<td>Average trough concentrations in patients with ADR were significantly higher (7.64±2.84 mcg/mL) than those in patients without ADR(1.49±1.79 μg/mL). Liver dysfunction however was found at lower levels of voriconazole in 3 patients despite the low frequency (2.88, 4.21, 4.46 mcg/mL).</td>
<td>NR</td>
</tr>
<tr>
<td>Berge, 2009</td>
<td>Retrospective Study</td>
<td>35</td>
<td>24.9 ±4.4 (14-38)</td>
<td>LF lung transplant recipients</td>
<td>treatment (n=27) pre-emptive (n=8)</td>
<td>Primarily Caucasian</td>
<td>Initial Dosing: 400mg BID for 1 day and 1.5×4.5 - 4.0×1.0</td>
<td>0% of patients had very low levels &lt;0.5mg/L prior to adjustment. 27% patients (7%) experienced at least 1 ADR</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Author</td>
<td>Study Type</td>
<td>Sample Size</td>
<td>Patient Characteristics</td>
<td>Methodology</td>
<td>Outcomes</td>
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<tr>
<td>Ueda, 2009</td>
<td>Retrospective Study</td>
<td>34</td>
<td>Various hematological malignancies</td>
<td>Proven n=1; probable n=5; possible n=29; others n=14</td>
<td>Japanese Loading dose 6.0mg/kg and maintenance dose 4.0mg/kg</td>
<td>Median concentration was 3.01 mg/L (0.22-12.77)</td>
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<tr>
<td>Eiden, 2010</td>
<td>Retrospective Study</td>
<td>39</td>
<td>Various hematological malignancies</td>
<td>Indication: Proven IA (n=7); probable IA (n=12); proven candidiasis (n=1); other proven IFI (n=1); possible IFI (n=9); persistent fever during neutropenia (n=9)</td>
<td>NR</td>
<td>No correlation between toxicity and voriconazole trough concentration. One out of the 3 patients with neurotoxicity had a trough &gt;5.0mg/L (neurological ADR). In those with hepatotoxicity, voriconazole troughs were between 0.2 and 3.2 (mean: 1.38mg/L)</td>
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<tr>
<td>Miyaki, 2010</td>
<td>Retrospective Study</td>
<td>25</td>
<td>Various hematological malignancies</td>
<td>Proven [60%] or probable [40%] IFIs</td>
<td>NR</td>
<td>No linear correlation was found associating ADR occurrence to voriconazole trough concentrations. Patients with severe AEs had higher median voriconazole concentrations than the remaining cohort (2.38 vs. 1.30 mg/L; P&lt;0.04)</td>
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<tr>
<td>Brugge, 2011</td>
<td>Retrospective Study</td>
<td>18</td>
<td>Various hematological malignancies</td>
<td>Proposed Therapeutic Regimen: Patients ages 2-12: 7mg/kg BID IV or 200mg BID PO Patients &gt;12: used adult dose (including loading dose)</td>
<td>NR</td>
<td>Median concentration was 1.73 mg/L (range: 0-20mg/L)</td>
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</tr>
</tbody>
</table>

- **PO dosing** was associated with higher variability under-dosage compared to IV administration. Despite dose adjustment, trough levels remained <0.5mg/L in 20-25% of patients at 1 week.
- 12 patients experienced hepatotoxicity, but were not associated with higher voriconazole levels.
- Almost all patients reported visual disturbances during the early stages of therapy and were associated with the voriconazole loading dose.
- 13 patients were discontinued voriconazole therapy due to ADRs. 5 patients were discontinued therapy due to neurological disorders.

### Range of Actual Dosing:

- **Proposed Voriconazole Dosing:**
  - Patients 2-12 years in age (n=25) ranged from <0.1 mg/L to 6.0 mg/L (median 1.25mg/L). Those of patients >12 (n=14) ranged from <0.1mg/L to 9.6 mg/L (median 1.69mg/L).
  - Overall, 44% (8/18) of patients had their first trough level below either 1 or 2mg.
- Six patients reported AEs. No significant correlation was observed between plasma concentration and the occurrence of AEs (P=0.16). Two patients had plasma trough concentrations ≥6mg/L without any signs of toxicity.
- One patient had their therapy discontinued because they likely did not have a fungal infection.

### Dose Guidelines:

- **Dose Variations:**
  - 7mg/kg BID IV or 200mg BID PO
  - Varied dosing; average voriconazole dose was 6.7 mg/kg/day (24% received IV, 76% received PO)

### Median Trough Levels:

- **Initial Voriconazole Troughs:**
  - Median voriconazole trough: 1.4mg/L (IQR 0.5-2.6).
  - Trough was <1mg/L in 41% of cases and <0.5mg/L in 25% of cases.
- **Median Concentration:**
  - Median voriconazole concentration was 2.6 mg/L.
- **Median Trough Level:**
  - Median voriconazole trough: 1.73 mg/L (range: 0-20mg/L).
  - Median trough level 1.73 mg/L (range: 0-20mg/L).

### Correlation with Outcomes:

- **Correlation with Toxicity:**
  - No correlation was found associating ADR occurrence to voriconazole trough concentrations.
- **Correlation with Outcome:**
  - Patients with severe AEs had higher median voriconazole concentrations than the remaining cohort (2.38 vs. 1.30 mg/L; P<0.04).

### Other Findings:

- **Range of Actual Dosing:**
  - Range of Actual Dosing:
    - Median voriconazole trough: 1.73 mg/L (range: 0-20mg/L).
  - Median concentration was 2.6 mg/L.
  - Median trough level 1.73 mg/L (range: 0-20mg/L).

### Initial Voriconazole Troughs:

- **Initial Voriconazole Troughs:**
  - Median voriconazole trough: 1.4mg/L (IQR 0.5-2.6).
  - Trough was <1mg/L in 41% of cases and <0.5mg/L in 25% of cases.

### Median Concentration:

- **Median Concentration:**
  - Median voriconazole concentration was 2.6 mg/L.
- **Median Trough Level:**
  - Median voriconazole trough: 1.73 mg/L (range: 0-20mg/L).
  - Median trough level 1.73 mg/L (range: 0-20mg/L).

### Correlation with Toxicity:

- **Correlation with Toxicity:**
  - No correlation was found associating ADR occurrence to voriconazole trough concentrations.
- **Correlation with Outcome:**
  - Patients with severe AEs had higher median voriconazole concentrations than the remaining cohort (2.38 vs. 1.30 mg/L; P<0.04).

### Other Findings:

- **Range of Actual Dosing:**
  - Range of Actual Dosing:
    - Median voriconazole trough: 1.73 mg/L (range: 0-20mg/L).
  - Median concentration was 2.6 mg/L.
  - Median trough level 1.73 mg/L (range: 0-20mg/L).

### Correlation with Toxicity:

- **Correlation with Toxicity:**
  - No correlation was found associating ADR occurrence to voriconazole trough concentrations.
- **Correlation with Outcome:**
  - Patients with severe AEs had higher median voriconazole concentrations than the remaining cohort (2.38 vs. 1.30 mg/L; P<0.04).
| Dolton, 2012 | Retrospective Study | 201 | 54 (18-88) | Various hematological malignancies | Known/presumed fungal infection (n=170, 85%); Prophylaxis against fungal infection (n=31, 15%) | NR | NR | 1-5.5 mg/L | Median: 1.4 mg/L (range 0.14-3mg/L) | 10.5% of patients (n=21) had neurotoxic ADRs (visual/auditory hallucinations), 52% of them received IV while 48% received PO voriconazole. Trough levels was significantly higher than median voriconazole levels in patients who did not have neurotoxic AEs (median, 6.5 vs. 1.6 mg/L; P=0.01) | One patient switched to lipid amphotericin b due to persistent fever |
|---|---|---|---|---|---|---|---|---|---|---|
| Gomez-Lopez, 2012 | Retrospective Study | 14 | 46.8 (4-87) | Hematological malignancies n=6 (43%); SOT n=4 (29%); COPD n= 2, (14%); COPD: NR n=2, (14%) | Probable infection n=7 | Unknown | Dose range: 120 – 700mg/day | NR | Median Voriconazole Trough: 1.52 mg/L (IQR 0.29-2.43 mg/L) | NR | Therapeutic outcome was evaluable for 163 of the 170 patients who received voriconazole therapy for suspected/confirmed fungal infections. 15.3% (n=25/163) failed therapy with median voriconazole concentrations (0.9 mg/L) significantly lower than that of those who were treated successfully (2.1 mg/L), P <0.05. Higher therapeutic failure was seen in patients with proven/probable IFI. Voriconazole concentrations of ≤1.7 mg/L minimized the incidence of therapeutic failure. |
| Mitsani, 2012 | Prospective Study | 93 | 60 (20-74) | Lung transplant recipients | Prophylaxis | White n=85 Black n=7 Middle Eastern n=1 | Two IV loading doses of 6mg/kg BID followed by then 200mg PO BID | <4 mg/L | 23% of patients → all troughs ≤1mg/mL. 48% of patients → all troughs >1mg/mL | Associations between voriconazole trough and toxicity were not clear-cut. Trough cutoffs that identified patients with increased risk could not be assigned. Elevated LFTs were common but were in majority attributable to etiologies other than voriconazole therapy. 27% of patients were discontinued therapy due to drug toxicity (nausea/vomiting, hepatotoxicity, and CNS including visual disturbances and delirium). The median, |

| Median troughs were associated with |
| Positive cultures: 0.92mg/L |
| Negative cultures: 1.72mg/L |
| Positive cultures were significantly more likely with troughs ≤1.5mg/L, 60% of IFI and 70% of colonization events occurred at troughs of ≤1.5mg/L (vs. 46% of negative cultures) (P=0.01) |
| 10% of patients developed IFI with median trough levels of 0.99mcg/ml and 27% had fungi colonizing respiratory tracts with median trough level of 0.92 |
initial or maximum voriconazole trough levels were not significantly different from those of who did not experience drug toxicity.

µg/ml IFI or colonization were more likely with troughs of ≤1.5µg/ml (P=0.01) and among patients with no troughs >1.5µg/ml (P=0.007).

<table>
<thead>
<tr>
<th>Study</th>
<th>TDM</th>
<th>Non-TDM Group</th>
<th>Median F/T Level</th>
<th>TDM Group</th>
<th>Non-TDM Group</th>
<th>Median F/T Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Park 2012</td>
<td>RCT</td>
<td>108 TDM Group: n=55; Non-TDM Group: n=53</td>
<td>55 ± 15 years</td>
<td>TDM Group: Underlying Conditions: 80% (n=44) hematological disease; 7% (n=4) steroid use; 40% (n=22) others</td>
<td>Non-TDM Group: Underlying Conditions: 74% (n=39) hematological disease; 11% (n=6) steroid use; 15% (n=8) others</td>
<td>Initial dosing for both trial arms: LD of 6mg/kg BID, MD of 4mg/kg BID</td>
</tr>
<tr>
<td>Korean</td>
<td>1.0–5.3 mg/L</td>
<td>TDM Group: Initial voriconazole trough levels &gt; 5.5 mg/L were observed in 40% (n=21) of patients. Initial voriconazole trough levels &lt;1 mg/L were observed in 9% (n=5) of patients. Mean final trough levels were 3.2±2.1 mg/L.</td>
<td>Non-TDM Group: Initial voriconazole trough levels &gt; 5.5 mg/L were observed in 37% (n=18) of patients. Initial voriconazole trough levels &lt;1 mg/L were observed in 12% (n=6) of patients. Mean final trough levels were 4.3±3.1 mg/L.</td>
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</table>

Pieper 2012 | Retrospective Study (Non Comparati ve Cohort) | 74 | 10.2 (0.2–18) | Hematological malignancies (66.2%), or BM failure syndrome (14.9%). | Treatment for possible (n=7), probable (n=13), prophylaxis (n=79), empirical therapy (n=2) | Caucasian (93%) Other (7%) | Median Maintenance Dose: 4.8mg/kg BID (range: 2.2–17.4) | Median Maintenance Dose: 195.9 mg BID (range: 35–500) | Upper limit threshold of 5.5 mg/L | Initial and subsequent trough levels measured at median 32 and 68 days. Trough plasma concentrations at steady state ranged from 0.2 – 14.9 mg/L (15.9±2.17), with high intra- and inter- individual variability and no apparent relationship to dose (P=0.074, ANOVA) | The difference between mean final trough levels in the TDM vs. non-TDM group was P=0.10. | AEs occurred in 76.2% courses and in 9.9% of courses lead to discontinuation of voriconazole therapy. There were no consistent correlations between dose, trough concentration, and AEs. | Treatment success was observed in 40% of patients with IFDs and in 82.7% of those who received empirical therapy or prophylaxis. There were no consistent correlations between dose, trough concentrations, and therapeutic response. |
<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>Median Age</th>
<th>Underlying Conditions</th>
<th>Underlying Conditions</th>
<th>Median Dose (mg/kg/d)</th>
<th>Vori dose (mg/kg/d); median (range)</th>
<th>pH Drug</th>
<th>NR</th>
<th>Correlations between voriconazole concentrations and total serum protein, serum albumin, ALT, ALP, and serum ammonia with P values 0.249, 0.462, 0.3, 0.054, and 0.987, respectively, were not statistically significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Racil 2012</td>
<td>Retrospective Study</td>
<td>264</td>
<td>Median 51 (18-77)</td>
<td>AML (n=118, 44.7%); AML (n=30, 11.4%); NHL (n=25, 9.8%); CML (n=16, 6.1%); CLL (n=15, 5.7%); MM (n=12, 4.9%); other (n=46, 17.4%)</td>
<td>58</td>
<td>2.2-27.3</td>
<td>4.52 mcg/ml</td>
<td>No statistically significant relationship (P=0.3258) was found between plasma concentration and overall survival</td>
<td></td>
</tr>
<tr>
<td>Aouinti 2012</td>
<td>Prospective Study</td>
<td>30</td>
<td>Median: 10 (1 month-17 years)</td>
<td>Haematological malignancies (53.3%) the most common underlying conditions</td>
<td>63% of patients had proven/probable IFI</td>
<td>White (70%) Asian (13.3%) Maghreb (13.3%) African Black (3.3%) S. American (10%)</td>
<td>20mg/kg/d</td>
<td>1.5-5.5 mg/L</td>
<td>50% of samples were &lt;1mg/L, 84% of samples were within target range, 14% of samples were &gt;5.5mg/L</td>
</tr>
<tr>
<td>Bartellink, 2013</td>
<td>Retrospective Study</td>
<td>54</td>
<td>Median: 29 (4-61)</td>
<td>Allogenic HSCT recipients</td>
<td>61% of patients had been given voriconazole for IA</td>
<td>North African (4.6%)</td>
<td>2-15mg/kg/day</td>
<td>2-15mg/kg/day</td>
<td>12 AEs occurred in 8 patients</td>
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<td></td>
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<td>Significant relationships were found between supratherapeutic concentrations above 5.5 mg/L, and neurological and cutaneous toxicities (P=0.0001). No relationships between trough concentrations and hepatotoxicity was established.</td>
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<td></td>
<td>Measured voriconazole concentrations did not correlated (not statistically significant) with treatment outcome in patients with IA</td>
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<td></td>
<td></td>
<td>Measured voriconazole concentrations did not correlated (not statistically significant) with treatment outcome in patients with IA</td>
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</tbody>
</table>

**Notes:**
- **Median Dose:** Dosing varies depending on age and weight.
- **Vori dose:** seen as therapeutic range.
- **Medicine:** Recommended dosing is based on age, weight, and underlying conditions.
- **Correlations:** Significant relationships found between plasma concentrations and overall survival.
- **AEs:** Significant AEs occurred in 8 patients.
- **Supratherapeutic:** Levels were a source of toxicity.
- **Hepatotoxicity:** Patients had liver dysfunction or disturbance in liver function and auditory hallucination.
- **Dose:** Doses as high as 70mg/kg/d were given to patients based on TDM.
<table>
<thead>
<tr>
<th>Study &amp; Year</th>
<th>Study Type &amp; Cohort</th>
<th>Median LD (mg)</th>
<th>Median MD (mg)</th>
<th>First levels: (%)</th>
<th>Trough levels associated with...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choi, 2013</td>
<td>Retrospective Study</td>
<td>12.2 (1.2-18.9)</td>
<td>200mg/12h</td>
<td>1-6 mcg/mL</td>
<td>Treatment Success:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Possible (n=7); probable (n=12); proven (n=8)</td>
<td></td>
<td></td>
<td>&lt;1mcg/mL (n=13, 19.7%); ≥1mcg/mL (n=53, 80.3%)</td>
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<tr>
<td></td>
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<td>South Korean</td>
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<td>Treatment Failure:</td>
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<td></td>
<td>Median duration of therapy was 60 days (10-350days)</td>
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<td></td>
<td>&lt;1mcg/mL (n=16, 42.1%); ≥1mcg/mL (n=22, 57.9%)</td>
</tr>
<tr>
<td>Chu, 2013</td>
<td>Retrospective Cohort</td>
<td>Median: 53 (IQR:38-64)</td>
<td></td>
<td>Possible (n=7); probable (n=12); proven (n=8)</td>
<td>Voriconazole trough levels</td>
</tr>
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<td></td>
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<td>Underlying diseases: HSCT (n=47); hematological malignancy without a stem cell transplant (n=37); solid organ transplantation (n=10); other diseases (n=9); none (n=8)</td>
<td></td>
<td></td>
<td>&lt;1mcg/mL were more frequently seen in those with treatment failure at 6 weeks (success vs. failure was 19.7% vs. 42.1%; p=0.012)</td>
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<td></td>
<td></td>
<td>Voriconazole Indication: IA pulmonary (n=87); IA sinus or CNS (n=5); invasive candida (n=9); other IFI (n=13); febrile neutropenia(n=9)</td>
<td></td>
<td></td>
<td>No significant relationship between achieving therapeutic levels and clinical response was observed upon follow up at 12 weeks in patients with proven/probable IFI</td>
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<td>White (n=85); Asian (n=7); African-American (n=5)</td>
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<td>At 6 weeks, a significant association was observed between sub-therapeutic levels and clinical response</td>
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<td>Median LD (n=59) was 400mg/dose; median maintenance dose (n=107) was 260mg/dose</td>
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<td></td>
<td>Low voriconazole levels were independent predictors of clinical failure of voriconazole therapy/prophylaxis (P=0.041; OR=2.92, 95% CI: 1.05-8.14)</td>
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<tr>
<td></td>
<td></td>
<td>Loading Dose: (n=59) median 400mg/dose [IQR: 350,480]</td>
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<td></td>
<td>Voriconazole levels were independent predictors of clinical failure of voriconazole therapy/prophylaxis (P=0.041; OR=2.92, 95% CI: 1.05-8.14)</td>
</tr>
<tr>
<td>Hoening 2013</td>
<td>Prospective Cohort</td>
<td>Median: 51.55 (18.73-81.27)</td>
<td></td>
<td></td>
<td>Voriconazole levels were independent predictors of clinical failure of voriconazole therapy/prophylaxis (P=0.041; OR=2.92, 95% CI: 1.05-8.14)</td>
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<td>Adult patients treated with voriconazole who either had underlying hematologic malignancies (n=40) or were admitted to the ICU (n=21)</td>
<td></td>
<td></td>
<td>Voriconazole levels were independent predictors of clinical failure of voriconazole therapy/prophylaxis (P=0.041; OR=2.92, 95% CI: 1.05-8.14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n= 20 (33%) for prophylaxis, n= 41 (67%) for therapy</td>
<td></td>
<td></td>
<td>Voriconazole levels were independent predictors of clinical failure of voriconazole therapy/prophylaxis (P=0.041; OR=2.92, 95% CI: 1.05-8.14)</td>
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<tr>
<td></td>
<td></td>
<td>Unknown</td>
<td></td>
<td></td>
<td>Voriconazole levels were independent predictors of clinical failure of voriconazole therapy/prophylaxis (P=0.041; OR=2.92, 95% CI: 1.05-8.14)</td>
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<tr>
<td></td>
<td></td>
<td>N=18 samples, potentially toxic voriconazole levels were obtained</td>
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<td></td>
<td>Voriconazole levels were independent predictors of clinical failure of voriconazole therapy/prophylaxis (P=0.041; OR=2.92, 95% CI: 1.05-8.14)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Voriconazole levels were significantly higher (median 4.7mg/L) in patients who had an AE (P&lt;0.001; IQR: 4.2-5.1)</td>
<td></td>
<td></td>
<td>Voriconazole levels were independent predictors of clinical failure of voriconazole therapy/prophylaxis (P=0.041; OR=2.92, 95% CI: 1.05-8.14)</td>
</tr>
</tbody>
</table>

Note: IQR = Interquartile Range.
| Lee 2013 | Retrospective Study | 52 | Median: 51 (21-67) | Adult patients with hematologic malignancies (AML 55%, ALL 9%, Myelodysplastic syndrome 18%, other 18%) | Proven, probable, or possible IA. | South Korean | 81% of patients received LD: 6mg/kg Q12h IV MD: 4mg/kg Q12h IV | 19% of patients received LD: 400mg PO Q12h MD: 200mg PO Q12 | 2 - 6 mg/L | ≤0.5mg/L (n=5) | ≤1mg/L (n=1) | ≤2mg/L (n=5) | ≤3mg/L (n=6) | ≤4mg/L (n=29) | Trough concentrations were ≤2 mg/L in 11 cases (21%); >2 mg/L in 41 cases (79%). | NR | At the 2 week response assessment, patients with initial voriconazole troughs <2mg/L were less likely to have a successful response compared to patients with levels ≥2mg/L (45% vs. 51%) but this difference was not statistically significant (P=0.73). Did find that initial voriconazole trough levels were associated with successful responses (tough levels may differ in successive measurements) |
|---------|------------------|----|-------------------|---------------------------------|--------------------------|---------------|-------------------------------------------------|-----------------------------|-----------------|----------------|----------------|----------------|----------------|-------------------------------------------------|------|--------------------------------------------------|
| Suzuki 2013 | Retrospective Cohort | 39 | Total: 55.9s (19.5 - 84) | Japanese in-patients who received voriconazole and for whom TDM data was available | NR | Japanese | Hepatotoxic: PO/IV (5.8) | Initial dose: 6.2±1.5 mg/kg/d (3.5-8.3) | No Hepatotoxicity: PO/IV (11.7) | Initial dose: 6.3±2.5 mg/kg/d (0.9-10.6) | 1-4 mcg/mL | Group A: first voriconazole TDM <4mg/mL (n=25) | Group B: first voriconazole TDM >4mg/mL, but second level <4mg/mL (n=8) | Group C: in first & second TDMs levels >4mcg/mL (n=6) | Incidence of hepatotoxicity: Group A =16.0%; B=25.0%; C=83.3% | Significant differences were observed between groups A&B (P=0.003) and between groups B&C (P=0.025). | NR | | |
| Saini, 2014 | Prospective Study | 69 | Median: 58 (19-80) | Adult patients with hematologic malignancies (mostly AML) who received voriconazole for proven (n=3), probable (n=9), and possible (n=50) IFI. | Proven = 3 (4.2%) | Probably = 9 (12.7) | Possible = 30 (41.3) | Most patients receive 200mg BID following loading doses, with median voriconazole doses of 2.95 mg/kg BID (range: 1.7-5.0 mg/kg BID) | Unknown | 1.4-8.3 mcg/mL (range: 0.1-8.9); p=0.03 | Group A: first voriconazole TDM <4mg/mL (n=12) | Group B: first voriconazole TDM >4mg/mL, but second level <4mg/mL (n=28) | Mean first voriconazole trough: 3.6±2.3 mcg/mL (range: 0.1-8.9); p=0.03 | Patients with abnormal bilirubin (>1ULN) had significantly higher median voriconazole levels at both the day 6 and 8 liver enzyme assessments compared to those with normal bilirubin (3.5mcg/ml vs. 2.4mcg/ml; P=0.03), and the day 14 and 16 liver enzyme assessments (3.5mcg/ml vs. 2.1mcg/ml; P=0.026). 4 of 15 patients with steady state voriconazole levels >5mcg/ml had elevated bilirubin (>1ULN) at day 6-8 and at day 14-16. | NR | | |
| Kang, 2015 | Retrospective Cohort | n=61 | TDM/Non-TDM n=31 | n=30 | TDM Group: 8.7±6.3 Non-TDM Group: 8.3±5.0 | Children with hematologic, oncologic and solid organ transplants who received voriconazole therapy | TDM Group: 87.8% (n=21) proven/probable IFI; 85.7% IA; the rest had candida | Non-TDM Group: 46.6% (n=14) proven/probable IFI; 78.6% IA; the rest Candida | Korean | All patients had LD of 6mg/kg Q12H and MD of 4mg/kg Q12H | TDM Group: | Dose adjustments were made if plasma levels were below therapeutic range (increased dose by 20%) | If levels were extremely above the therapeutic range, 1 dose was | 1.5-5 mg/L | Only 36.6% of initial trough levels were within therapeutic range at the first monitoring. Of 271 trough level measurements, only 20.7% (n=56) had levels in toxic range; 29.8% (n=83) had troughs below therapeutic range; 49.4% (n=134) had their levels within therapeutic range. | Mild to severe ADRs occurred in 74.2% (23/31) of TDM patients compared to 43.3% (13/30) of non-TDM patients (P=0.021). There were no significant differences in the incidence of each ADR between patients in TDM group vs. those in the non-TDM group. Discontinuation of voriconazole therapy occurred in Only patients with proven/probable IFI were included in the analysis of response to therapy. | At 12 weeks, there was a significantly higher rate of therapeutic failure in non-TDM patients (78.8%) vs. TDM group patients (40%); P=0.038. At 6 weeks post-start therapy: 75% of patients who had levels <3mg/L, for at least half the duration of therapy experienced | |
20% possible invasive infections (n=4); 80% probable/proven infections (n=16); 95% pulmonary aspergillosis (n=19); 5% candidaemia (n=1)

Japanese

LD: 6-8 mg/kg/dose BID MD: 4-6 mg/kg/dose BID 1.0 ≥ and <5.0 mg/L ≤5mcg/ml (p=0.01)

Next 18 patients required increased doses based on TDM data during their course of therapy. 11 of 20 of patients did not achieve adequate concentrations after initial dosing: 6/8 of children ≤5y; 3/7 of children 6-12y; and 2/5 of children ≥13y

Patients ≤5y; the median concentration of IV voriconazole was 1.7mg/L vs. 0.4mg/L in PO voriconazole; P<0.001 Patients 6-12y; the median concentration of IV voriconazole was 2.6mg/L vs. 0.6mg/L in PO voriconazole; P<0.001 Patients ≥13y; No difference in trough concentrations between IV and PO formulations

Mean voriconazole concentrations at first monitoring were significantly lower in those with unfavorable responses than those with favorable responses (0.2 vs. 1.7 mg/L, P= 0.001)

2.36± 1.67mcg/mL (p<0.01). Unclear (trough values were presented in line graph, but they are not mentioned in the text and one cannot accurately determine values numerically)

Trough levels in patients with and without hepatotoxicity were 5.5±2.73mcg/mL and 2.36±1.67mcg/mL (p=0.01). Hepatotoxicity was more frequently observed in 90% of those with trough concentrations >5 mcg/ml than in 31.6% of patients with trough concentrations ≤5mcg/mL (p=0.01).

2.36± 1.67mcg/mL (p<0.01). Unclear (trough values were presented in line graph, but they are not mentioned in the text and one cannot accurately determine values numerically)

Trough levels in patients with and without hepatotoxicity were 5.5±2.73mcg/mL and 2.36±1.67mcg/mL (p=0.01). Hepatotoxicity was more frequently observed in 90% of those with trough concentrations >5 mcg/ml than in 31.6% of patients with trough concentrations ≤5mcg/mL (p=0.01). Hepatotoxicity was more frequently observed in 90% of those with trough concentrations >5 mcg/ml than in 31.6% of patients with trough concentrations ≤5mcg/mL (p=0.01).
| Tucker, 2015 | Pediatric oncology and/or bone marrow transplant patients with documented or suspected IFI | 11 | Median: 8 (0.8-14.8) | Pediatric oncology and/or bone marrow transplant patients with documented or suspected IFI | Proven IFI: n= 6 | Probable IFI: n= 5 | Unknown | Median dosage: 6 mg/kg (5.8-7.7 mg/kg) given Q12H (n= 5) or Q24H (n = 6) | Media dosage required to reach therapeutic range: 10mg/kg/day (6-26 mg/kg/day) | 1-6mg/L | Average: 2.85 mg/L (range: 0.61-5.32 mg/L) | Trough concentration: <1mg/L (n=1); >2mg/L (n=7) | All but 1 child (0.61 mg/L) achieved trough levels above 1 mg/L. 7 children had trough concentrations above 2 mg/L. | 3 patients experienced AEs/ADRs potentially related to voriconazole therapy (increased LFTs, QTc prolongation) | Voriconazole was discontinued in 2/3 patients. The patient with increased LFTs had a voriconazole trough of 12.14mg/L, and the patient with QTc prolongation had a trough of 8.52mg/L. | 3/4 patients who had therapeutic failure also had voriconazole trough levels <2mg/L (not statistically significant) | 4 patients experienced therapeutic failure (3 of them were from 4 youngest patients). Their trough levels were 1.91, 4.39, and 3.58 mg/L. All patients who experienced treatment failures died. | Age was the only available factor that was significantly associated with clinical efficacy (younger patients were more likely to have therapeutic failure and mortality). |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TDM | N= 14 | TDM Group: 63.4 (49-83) | 33% other solid tumors | TDM Group: 47% acute leukemia; 27% chronic leukemia; 7% lymphoma; 14% other solid tumors | 64% possible IA; 21% probable IA; 7% proven IA | TDM Group: 100% white, 0% Hispanic | Starting Dose for Standard Dosing Group: 2.2-9.1 mg/kg (mean: 5.6) | 77% received PO, 5% received IV voriconazole therapy | Starting Dose for TDM Group: 2.3-4.5 mg/kg (mean: 3.9) | 43% received PO, 57% received IV voriconazole therapy. | Dose was increased by 1mg/kg for those with levels <1 and decreased by 1mg/kg for those with levels >5 µg/ml. | Proven IFI: n= 6 | Probable IFI: n= 5 | Unknown | Median dosage: 6 mg/kg (5.8-7.7 mg/kg) given Q12H (n= 5) or Q24H (n = 6) | Media dosage required to reach therapeutic range: 10mg/kg/day (6-26 mg/kg/day) | 1-6mg/L | Average: 2.85 mg/L (range: 0.61-5.32 mg/L) | Trough concentration: <1mg/L (n=1); >2mg/L (n=7) | All but 1 child (0.61 mg/L) achieved trough levels above 1 mg/L. 7 children had trough concentrations above 2 mg/L. | 3 patients experienced AEs/ADRs potentially related to voriconazole therapy (increased LFTs, QTc prolongation) | Voriconazole was discontinued in 2/3 patients. The patient with increased LFTs had a voriconazole trough of 12.14mg/L, and the patient with QTc prolongation had a trough of 8.52mg/L. | 3/4 patients who had therapeutic failure also had voriconazole trough levels <2mg/L (not statistically significant) | 4 patients experienced therapeutic failure (3 of them were from 4 youngest patients). Their trough levels were 1.91, 4.39, and 3.58 mg/L. All patients who experienced treatment failures died. | Age was the only available factor that was significantly associated with clinical efficacy (younger patients were more likely to have therapeutic failure and mortality). |
| IFI, Invasive fungal infection; IFD, Invasive fungal disease; AML, Acute myelogenous leukemia; ALL, Acute lymphoblastic leukemia; NHL, Non-hodgkin lymphoma; CML, Chronic myelogenous leukemia; CLL, Chronic lymphocytic leukemia; MM, Multiple myeloma |

TDM, Therapeutic drug monitoring; 2/14 (14.3%) died (with P=0.6 compared to the other arm); 1/14 (7.1%) had failed therapy (with P=0.17 compared to the other arm); 12/14 (85.7%) had positive outcomes (stable, partial, or complete response) (with P=0.05 compared to the other arm)
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>n</th>
<th>Age</th>
<th>Patient Population</th>
<th>Ethnicity</th>
<th>Voriconazole Dose</th>
<th>CYP2C19 Genotype</th>
<th>C_{min} (trough*)</th>
<th>T_{max}</th>
<th>AUC(0-24h)</th>
<th>AUC(0-∞)</th>
<th>CL</th>
<th>t_1/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ikeda, 2004</td>
<td>Prospective Study</td>
<td>12</td>
<td>Unknown</td>
<td>Healthy Japanese Volunteers</td>
<td>Japanese</td>
<td>Voriconazole 200mg PO BID x10 days</td>
<td>EM (n=3)</td>
<td>Trough concentrations suggest that PMs were exposed to higher concentrations of voriconazole than EM.</td>
<td>3.8 x↑ (vs. EM)</td>
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<td>PM (n=1)</td>
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<td>HEM (n=2)</td>
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<td>Voriconazole 300mg PO BID x10 days</td>
<td>EM (n=3)</td>
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<td>PM (n=1)</td>
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<td>HEM (n=2)</td>
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</tr>
<tr>
<td>Shcola, 2009</td>
<td>Prospective Cohort</td>
<td>20</td>
<td>(20-38)</td>
<td>Healthy volunteers</td>
<td>Caucasian</td>
<td>Voriconazole 400mg PO or IV single dose followed by 14 day washout before crossover of voriconazole 400mg PO or IV</td>
<td>EM = *1/<em>1</em>7 (n=8)</td>
<td>IV: 1.13, 6.19, 3.59*2 (μg/ml)</td>
<td>1.13, 6.19, 3.59*2 (μg/ml)</td>
<td>146.70*2</td>
<td></td>
<td></td>
<td>IV: 7.8±2.4 h PO: 7.5±1.2</td>
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<td>HEM = *1/*2; *1/<em>3</em>7 (n=8)</td>
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<td>PM*: *2/*2; *2/*3; *3/<em>3</em>7 (n=4)</td>
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<tr>
<td>Wang, 2009</td>
<td>Prospective Study</td>
<td>20</td>
<td>21±2</td>
<td>Healthy males from Hunan Province</td>
<td>Chinese</td>
<td>Single PO dose voriconazole 200mg</td>
<td>*1/*17 (n=4)</td>
<td>1.19 ±0.98 h</td>
<td>6.92 ±1.71</td>
<td>5.26 ±1.18</td>
<td>6.96 ±1.71</td>
<td>7.19h</td>
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<td>*the C_{max} of UMs was statistically significant different from that of EMs (P=0.036) and PMs (P=0.035)</td>
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<td>*T_{1/2} in UMs was 87% (P=0.58) of that of EMs and 51% (P=0.002) of that of PMs</td>
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<td></td>
<td>*P&lt;0.05 for URM vs. EM **P&lt;0.05 for PM vs. EM</td>
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<tr>
<td>Weiss, 35</td>
<td></td>
<td>19.37</td>
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<td></td>
<td>*1/*17 (n=8)</td>
<td>1.19 ±0.98 h</td>
<td>6.92 ±1.71</td>
<td>5.26 ±1.18</td>
<td>6.96 ±1.71</td>
<td>7.19h</td>
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</tr>
</tbody>
</table>

*Values presented are mean ± standard deviation (SD) unless otherwise stated.

**P<0.05 for URM vs. EM **P<0.05 for PM vs. EM
2009 Prospective Study

Healthy non-smokers 32 White; 2 Asian; 1 South American

Single PO dose of voriconazole 400mg with 200mL mineral water

<table>
<thead>
<tr>
<th>*2/*17 (n=2)</th>
<th>*1/*1 (n=9)</th>
<th>*1/*2 (n=11)</th>
<th>*2/*2 (n=4)</th>
<th>*2/*2/*17 (n=1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value</td>
<td>0.3675</td>
<td>0.0006</td>
<td>0.0006</td>
<td>0.0092</td>
</tr>
</tbody>
</table>

Lee Prospective Study 2012

Healthy male volunteers Korean

EM= *1/*1 (n=6)

EM= *1/*2; *1/*3 (n=6)

HEM= *1/*2; *1/*3 (n=6)

PM= *2/*2; *2/*3 (n=6)

<table>
<thead>
<tr>
<th>EM= *1/*1 (n=6)</th>
<th>EM= *1/*2; *1/*3 (n=6)</th>
<th>HEM= *1/*2; *1/*3 (n=6)</th>
<th>PM= *2/*2; *2/*3 (n=6)</th>
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<tbody>
<tr>
<td>P value</td>
<td>0.3675</td>
<td>0.0006</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

54 Mean: 47 ± 14 Patients with hematological Thai

IM 42%
| Chayakul, 2014 | Prospective Study | diseases (80%) in which 70% were acute leukemia, who had invasive aspergillosis (IA was diagnosed in 19%; probable IA 52%; possible IA 29%) | Voriconazole 400mg PO BID x2 (loading dose), then 200mg PO BID | Day | 3 | 7 | 14 | 28 | ---- | ---- | ---- | ---- | ---- |
|---------------|------------------|-------------------------------------------------|-------------------------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|               |                  | EM 49%                                          | 7.1mcg/ml                                       | 7.2 | 3.6 | 2.3 | ---- | ---- | ---- | ---- | ---- | ---- | ---- |
|               |                  | PM 9%                                           | 7.7                                            | 7.6 | 7.0 | 6.2 | (P<0.05) | ---- | ---- | ---- | ---- | ---- | ---- | ---- |

**Chawla, 2015**  
Retrospective Study  
55 Sub-therapeutic levels (n=20):  
Therapeutic levels (n=28):  
Toxic levels (n=27):  
Patients who had IFI or were on empirical voriconazole therapy for at least 4 days. Conditions: n=12 (22%) cancer; n=10 (18%) solid-organ transplant; n=14 (27%) other; n=14 had IFI  
Indication for Voriconazole Therapy: Prophylaxis (n=9); therapy for proven/probable infections  
*1/*1: (n=14)  
*1/*2: (n=17)  
*2/*2: (n=4)  
*1/*17: (n=10)  
*2/*17: (n=8)  
*17/*17: (n=1)  
*1/*3: (n=1)  
*17/*17 (n=4)  
*12y: (n=2)  
*12y: (n=2)  
*1/*17 (n=8)  
*12y: (n=6)  
*12y: (n=2)

**Hicks, 2015**  
Retrospective Study  
33 Median 9 years  
Immunocompromised pediatric patients (36.4% ALL; 19.4% AML; 9.1% non-Hodgkin lymphoma; 15.1% other) who received oral voriconazole therapy for prophylaxis or treatment of IFI.  
African (n=6); European (n=23); Hispanic (n=2); Multiracial (n=2)  
Initial recommended maintenance dose:  
≥12 years: 200mg PO BID  
<12 years: 7mg/kg PO BID  
*17/*17 (n=4)  
*12y: (n=2)  
*12y: (n=2)  
*1/*17 (n=8)  
*12y: (n=6)  
*12y: (n=2)

Indian Sub-therapeutic levels: median 400mg/day (200-500)  
Therapeutic levels: median 400mg/day (200-600)  
Toxic levels: median 400mg/day (400-8000)  
*1/*17 patients had median voriconazole levels that were 1.3 times higher than those of EM patients. *2/*2 patients had levels 1.9 times higher than those of EM patients.  
Low drug levels were seen in *1/*17 and *2/*17 patients.  
From Figure 2a in the paper, *2/*2 patients had the highest voriconazole levels.  
Voriconazole levels from high to low: *2/*2, followed by *1/*2, then *1/*1, followed by *2/*17, and finally *1/*17  
Voriconazole levels in these patients showed a similar genotypic influence, but, a statistical significant difference was noted in only the *2/*2 genotype (p = 0.01); while others *1/*2 (p = 0.23), *2/*17 (p = 0.57) and *1/*17 (p = 0.97) were not statistically significant.

---

*1/*2 patients had median voriconazole levels that were 1.3 times higher than those of EM patients. *2/*2 patients had levels 1.9 times higher than those of EM patients.

Low drug levels were seen in *1/*17 and *2/*17 patients.

From Figure 2a in the paper, *2/*2 patients had the highest voriconazole levels.

Voriconazole levels from high to low: *2/*2, followed by *1/*2, then *1/*1, followed by *2/*17, and finally *1/*17.

Voriconazole levels in these patients showed a similar genotypic influence, but, a statistical significant difference was noted in only the *2/*2 genotype (p = 0.01); while others *1/*2 (p = 0.23), *2/*17 (p = 0.57) and *1/*17 (p = 0.97) were not statistically significant.
<table>
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</thead>
<tbody>
<tr>
<td></td>
<td>Indication for Voriconazole Therapy:</td>
<td>6% proven IFI (n=2); 46% probable IFI (n=15); 42% possible IFI (n=14); 6% not determined (n=2)</td>
<td>After treatment initiation (without a LD), or dose adjustment, a 3 day period was allowed before Cmin steady state levels was taken</td>
</tr>
<tr>
<td></td>
<td>Underlying Hematological Conditions:</td>
<td>67% AML (n=22); 15% ALL (n=5); 9% Non-Hodgkin lymphoma (n=3); 6% CLL (n=2); 3% myelodysplastic syndrome (n=1)</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

*target therapeutic range: 1-6mg/L

<table>
<thead>
<tr>
<th>*1/1² (n=11)</th>
<th>&lt;12yo: 0.82 mg/L (0.03-20.17)</th>
<th>≥12yo: 1.39 mg/L (0.05-3.57)</th>
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</tr>
</thead>
<tbody>
<tr>
<td>&lt;12y: (n=8)</td>
<td>≥12yo: 1.39 mg/L (0.05-3.57)</td>
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<tr>
<td>≥12y: (n=3)</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>*1/2A or B (n=9)</th>
<th>&lt;12yo: 2.71 mg/L (0.20-8.46)</th>
<th>≥12yo: 1.64 mg/L (0.03-11.21)</th>
<th>---</th>
<th>---</th>
<th>---</th>
<th>---</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12y: (n=3)</td>
<td>≥12yo: 1.64 mg/L (0.03-11.21)</td>
<td>---</td>
<td>---</td>
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<td>---</td>
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<tr>
<td>≥12y: (n=6)</td>
<td>---</td>
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<td>---</td>
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<td>---</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><em>2A</em>/2A (n=1)</th>
<th>&lt;12yo: (N/A)</th>
<th>≥12yo: 3.69 mg/L (2.97-3.86)</th>
<th>---</th>
<th>---</th>
<th>---</th>
<th>---</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12y: (n=0)</td>
<td>≥12yo: 3.69 mg/L (2.97-3.86)</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>≥12y: (n=1)</td>
<td>---</td>
<td>---</td>
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<td>---</td>
</tr>
</tbody>
</table>

*only 88% of the subjects were genotyped

<table>
<thead>
<tr>
<th>URM (37%, n=11)</th>
<th>---</th>
<th>---</th>
<th>---</th>
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<th>---</th>
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</tr>
</thead>
<tbody>
<tr>
<td>EM (34%, n=10)</td>
<td>---</td>
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</tr>
<tr>
<td>IM (28%, n=8)</td>
<td>---</td>
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</tr>
</tbody>
</table>

39% of initial trough levels were outside the therapeutic range. 33% were ≤1mg/L, 6% were ≥5mg/L

2C19 phenotype affected initial voriconazole Cmin/Dose (P=0.04), with a higher initial voriconazole Cmin/D in IM vs. URM (P=0.007)
<table>
<thead>
<tr>
<th>Yamada, 2015</th>
<th>Prospective Study</th>
<th>Median: 70 [64-75]</th>
<th>Adult patients who were receiving voriconazole therapy</th>
<th>Japanese</th>
<th>After treatment initiation (without a loading dose), or dose adjustment, a 3-day period was allowed in order to obtain ( C_{\text{min}} ) at steady state</th>
<th>URM (n=11; 37%)</th>
<th>EM (n=10; 34%)</th>
<th>IM (n=8; 28%)</th>
<th>39% of initial trough concentrations were outside the therapeutic range.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Underlying Conditions: pulmonary aspergillosis (n=25); AML (n=10); possible fungal infection (n=7); lymphoma malignum (n=6); candida esophagitis (n=3); others (n=7)</td>
<td></td>
<td></td>
<td>EM (n=10; 34%)</td>
<td>IM (n=8; 28%)</td>
<td></td>
<td>33% were ( \leq 1 ) mg/L; 6% were &gt;5 mg/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>--- only 88% of patients were genotyped---</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2C19 phenotype affected initial voriconazole ( C_{\text{min}} ) adjusted on voriconazole dose ( (C_{\text{min}}/D) ) ( [P=0.04] ); with a higher initial voriconazole ( C_{\text{min}}/D ) in IMs compared to UMs ( (P=0.007) )</td>
</tr>
</tbody>
</table>

| Lamoureux, 2016 | Retrospective Study | 35 | Adult patients who received PO voriconazole for the treatment of suspected or proven IFI between 2012 and 2014, for whom TDM was available. | Unknown | Some patients received a LD of 400 mg PO Q12H for the first day. The initial recommended voriconazole maintenance dose was 200 mg BID in accordance with the drug label. Subsequent dosage adjustments were performed after TDM and/or CYP2C19 genotyping at the discretion of the attending MD. | Genotype information available for use by clinicians (n=35 patients) | Patients with *1/*17 and *17/*17 had significantly lower concentrations than those with *1/*1 \( (P>0.001) \). Patients with *2/*2 had significantly higher concentrations than those with *1/*1 \( (P=0.00626) \) The *2 allele was associated with higher trough levels than *1/*1 |
|---|---|---|---|---|---|---|---|---|---|
| | | | *1/*1: n=11 | | | | | | |
| | | | *1/*17: n=13 | | | | | | |
| | | | *17/*17: n=4 | | | | | | |
| | | | *1/*2: n=6 | | | | | | |
| | | | *2/*2: n=1 | | | | | | |

TDM, Therapeutic drug monitoring; URM, Ultra-rapid metabolizer, EM, extensive metabolizer, HEM, Heterozygous Extensive Metabolizer, PM, Poor Metabolizer; Invasive fungal infection; \( C_{\text{min}} \) (trough), Trough Level, \( T_{\text{max}} \), Time to Peak Concentration, \( \text{AUC}_{0-24} \), 24-hr Exposure, \( \text{AUC}_{0-\infty} \), Total Exposure, CL, Clearance, \( t_{1/2} \), half-life
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>n</th>
<th>Age</th>
<th>Patient Population</th>
<th>Ethnicity</th>
<th>Voriconazole Dose</th>
<th>CYP2C19 Genotype</th>
<th>PK*</th>
<th>Efficacy</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levi,</td>
<td>Retrospective Study (prospective genotyping)</td>
<td>86</td>
<td>17-84</td>
<td>Patients with hematological malignancy given voriconazole between Nov. 2002 and Jan. 2005</td>
<td>Average: 56</td>
<td>Not Reported</td>
<td>Wild-type (n=63), Non-wild-type (n=23)</td>
<td>CYP2C19 was scored as wild-type' if no <em>2 or</em>3 alleles were detected</td>
<td>NR</td>
<td>No significant relationship was found between liver enzymes (hepatotoxicity) and CYP2C19 polymorphism.</td>
</tr>
<tr>
<td></td>
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<td>Voriconazole indication:</td>
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<td>12 patients were given standard 7 day IV and sequential PO administration of voriconazole.</td>
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<td></td>
<td>Possible IA = 45%; probable IA = 38%; proven IA = 1%; invasive candida = 9%; other = 6%</td>
<td></td>
<td>All other patients were given voriconazole PO:</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6mg/kg BID x24h</td>
<td></td>
<td>then 4 mg/kg PO BID days 2-7</td>
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<td></td>
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<td></td>
<td>then 200mg BID thereafter</td>
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<td></td>
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</tr>
<tr>
<td>Matsumoto,</td>
<td>Prospective Study</td>
<td>29</td>
<td>57.3±19.3</td>
<td>Patients who received voriconazole for treatment of fungal infection</td>
<td>Japanese</td>
<td>Voriconazole 6mg/kg BID x 1 day</td>
<td>WT *1/*1 (n=10) Non-WT (n=19)</td>
<td></td>
<td>Therapeutic Range</td>
<td>There was no significant correlation between the voriconazole trough concentration and efficacy.</td>
</tr>
<tr>
<td>2009</td>
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<td></td>
<td>Trough concentrations in the effective group (21 of 29 patients) were ≥1.2 mg/L, which were consistent with the proposed target trough concentration of 2 mg/L for efficacy</td>
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<tr>
<td></td>
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<td></td>
<td>then 3.6 ± 0.8 mg/kg BID</td>
<td>wild-type: no *2 or *3 alleles</td>
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</tr>
<tr>
<td>Brugmann,</td>
<td>Prospective Study</td>
<td>10</td>
<td>Mean: 49 (28-60)</td>
<td>Prophylaxis antifungal therapy was given to allogeneic HSCT recipients</td>
<td>Dutch</td>
<td>Voriconazole therapy was initiated 1 week before HSCT.</td>
<td>CYP2C19 *1/*1 (n=4) CYP2C19 *1/*2 (n=6)</td>
<td></td>
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<tr>
<td>2010</td>
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<td></td>
<td></td>
<td>2LD of 6mg/kg Q12h</td>
<td></td>
<td>-No significant difference between groups with regards to CL On day 7 of therapy</td>
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<td></td>
<td>then 4mg/kg Q12h x 12 days</td>
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<td>-*1/*1 → CL =15.52 L/h</td>
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<td></td>
<td>-*1/*2 → CL =8.49 L/h (P=0.257) On day 14 of therapy</td>
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<td>-*1/*1 → CL =14.15 L/h</td>
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<td></td>
<td></td>
<td>-*1/*2 → CL =9.71 L/h (P=0.762)</td>
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<td></td>
<td></td>
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<td></td>
<td>-No correlation between CYP2C19 genotype &amp; toxicity</td>
</tr>
</tbody>
</table>

**Table 2.3 Studies on the CYP2C19 polymorphisms and their efficacy and toxicity relationship**
| Berge, 2011 | Case series | 24 | Mean: 26±7 (15-40) | Caucasian | Standard LD (6mg/kg x2, Q12h) followed by maintenance dosing (4mg/kg Q12h or 200mg PO BID for patients > 40kg adjusted for route & body weight) | PPI Use: pantoprazole (n=7); omeprazole (n=13); esomeprazole (n=1); rabeprazole (n=1); no PPI (n=2) | Doses after TDM | CYP2C19 *1/*1= 633±197 mg | CYP2C19 *1/*2= 440±107 mg | CYP2C19 *1/*17; *17/*17= 600±193 mg | Case | CYP2C19 *1/*17; *17/*17 had less exposure vs. *1/*1 | CYP2C19 *1/*2 had more exposure vs. *1/*1 | Time to Therapeutic Range | *1/*2= 11.5 days | *1/*1= 4 days | *17/*17= 9.5 days | *17/*17= 15 days | Target therapeutic range was 1-2mg/L | No significant relationship was observed between genotypes and toxicity (P=0.40). | n=5 patients with *1/*1 (83.3%), n=5 with *1/*17 (50%), and n=5 (62.5%) with *1/*2 alleles had ADRs. |
| Kim SH, 2011 | Prospective Study | 25 | Mean: 45 (38-54) | Korean | Voriconazole IV LD of 6 mg/kg BID on day 1 followed by 4mg/kg BID whenever possible according to manufacturer’s recommendations | EM= *1/*1 (n=6) | Overall, no relationship was found between CYP 2C19 genotyping and trough concentrations (P=0.859) | Average Trough Concentrations: | EM 2.12 mg/L (1.70-5.68) | HEM 3.76 mg/L (0.92-6.96) | PM 2.75 mg/L (2.35-2.94) | No significant relationship was observed between CYP 2C19 genotypes and SAEs. | Troughs ≥ 5.83 mg/L were independently associated with SAE (P=0.043). |
| Kim, 2013 | Prospective Study | 104 | 53 ±13 | Korean | 6mg/kg IV BID on day 1 followed by 4mg/kg BID or 200mg PO BID | EM=*1/*1 or *1/*17 (n=39) | Percentage of patients with out-of-range initial trough levels: | EM 46% vs. HEM 26% vs. PM 0% (P=0.001) | Below range levels were most often observed in EMs (33%), HEMs (12%), PMs (0%); P=0.005 | 31% of patients experienced treatment failure: | HEM 28% (N=14) | EM 39% (N=15) | PM 20% (N=3) | Adverse events did not differ significantly between the 3 genotype groups (P=0.518) | **Under routine voriconazole TDM, there was no significant relationship between CYP2C19 genotype and efficacy or toxicity of voriconazole** |
| Study | Median | Japanese children with ALL (n=18); AML (n=10); neuroblastoma (n=3); others (n=6) | Loading dose: NR | NM (normal metabolizer) = *1/*1 (N=12), IM (intermediate metabolizer) = *1/*2 or *1/*3 allele (N=16) PM (poor metabolizer) = *2/*2 or T2/*3 or *3/*3 (N=7), HM (hypermetabolizer) = *1/*17 or *11/*17 (N=2) | All patients with high voriconazole concentrations (> target range of 5mg/dL) were either PMs or IMs.  
Logistic regression recommended target trough range of 1.5-4.0mg/L.  
Best efficacy was observed when the targeting therapeutic range was 1.5-4mg/L. | No association between genotype and toxicity  
One patient [PM *2/*3] developed hyponatremia /SIADH (voriconazole was immediately discontinued and patient was treated for hyponatremia). This patient had very high trough voriconazole levels (6.9mg/L).  
One patient (NM *1/*1) developed ventricular tachycardia, TdP & HF / QT prolongation 24 days after starting voriconazole. The trough level was 3.8mg/L. | Narita, 2013 |
|---|---|---|---|---|---|---|---|
| Wang 2014 | Mean: 60.6 (18-99) | Adult patients with proven/probable IFI receiving >14 days of voriconazole therapy for which at least one plasma voriconazole Cmin was available.  
*Excluded patients <18 years, those who lacked compliance with regimen or used additional antifungal agents | PM Dose: 200 mg IV/PO BID  
Non-PM Dose: 200 mg IV BID  
Or 300mg PO BID  
(to help achieve minimum therapeutic target ASAP) | Logistic regression recommended target trough range of 1.5-4.0mg/L.  
Best efficacy was observed when the targeting therapeutic range was 1.5-4mg/L. | No relationship was observed between voriconazole hepatotoxicity and different genotypes.  
Hepatotoxicity occurred more frequently in patients with voriconazole levels >4mg/L (17/20; 35% frequency) than those with levels <4mg/L (11/24; 8.9% frequency); P <0.05 | Wang 2014 |

**Target therapeutic range:** 1.5-5.5mg/L  
**Upper limit of target range:** 5mg/dL
in order to improve efficacy & survival
Optimum therapeutic range was 1.5-4.0 mg/L

| Zonis, 2014 | Mean: 43 (13-76) | Voriconazole was given for empirical or prophylactic therapy to patients with hematologic malignancies (n=44; post-allogenic stem cell transplant (n=33)); aplastic anemia (n=18); Post-allogenic stem cell transplant (n=11); solid tumor with autologous stem cell transplant (n=13); others (n=20) | White 59%; White Latino 22%; African American 16%; Asian 3%
recommended dose of 200mg PO BID in 11 of the assays
A mean dose of 3.2mg/kg (range 2.6-4.7mg/kg) was given PO in 9 patients and IV in 1 patient.
PO dose was 200mg BID for 11 of the assays.
CYP2C19 *1/*1 (n=63)
CYP2C19 *2/*2 (n=4)
CYP2C19 *1/*2 (n=18)
CYP2C19 *1/*3 (n=1)
CYP2C19 *1/*9 (n=1)
CYP2C19 *1/*11 (n=1)
CYP2C19 *1/*15 (n=1)
CYP2C19 "new" (n=3)
A clear trend was observed that voriconazole levels tended to be higher in *2/*2 patients. There was a statistically significant difference between voriconazole levels of *1/*1 and *2/*2 patients (P=0.0029).
Prolonged therapy did NOT cause auto-induction of voriconazole metabolism.

| Trubiano 2015 | Median: 64 (60-66) | Adult patients with underlying hematologic malignancy actively receiving chemotherapy, with possible, probable, or proven IFD, receiving presumptive/definitive therapeutic dose voriconazole and have at least 1 trough level as part of routine clinical care. | Caucasian Australian (n=14; 74%)
Caucasian European (n=3; 16%)
African/Asian (n=2; 10%)
LD of 6 mg/kg BID IV for ≥2H
× 4d, then maintenance dose of 4 mg/kg BID.
Subsequent dosing adjustments were made by the treating clinician based on TDM voriconazole levels following the initial 4 mg/kg maintenance dose.
*target therapeutic range: 1.5-5.5mg/L
EM= *1/*1 (n=8)
URM= *17/*17; *1/*17 (n=4)
IM= *2/*2; *1/*2; *1/*3; *2/*17 (n=5)
The highest first trough levels was observed in the IM group (median 4.8 mg/L) and was lowest in the URM group (median 2.2 mg/L).
Only EMs or URM s had a therapeutic level of <1 mg/L at some stage of therapy. A CYP2C19 inhibitor was administered to some EMs and URM s due to refractory subtherapeutic levels.
Time to therapeutic range was highest for IMs.

TDM, Therapeutic drug monitoring; URM, Ultra-rapid metabolizer, EM, extensive metabolizer, HEM, Heterozygous Extensive Metabolizer, PM, Poor Metabolizer
Invasive fungal infection; C_{min} (troughs), Trough Level, T_{max}, Time to Peak Concentration, AUC_{0-24h}, 24-hr Exposure, AUC_{0-∞}, Total Exposure, CL, Clearance, t_{1/2}, Half-life

*Trough Concentration, Target Therapeutic Range & Time to Range
**Table 2.4 Studies on CYP2C19 genotypic dosing and voriconazole efficacy and/or toxicity in patients**

<table>
<thead>
<tr>
<th>Study</th>
<th>Study type</th>
<th>n</th>
<th>Patient Population</th>
<th>Demographics</th>
<th>Genotype</th>
<th>Voriconazole Dose</th>
<th>Target Therapeutic Range</th>
<th>Efficacy</th>
<th>Toxicity</th>
<th>Dosing Algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teusink, et al. 2013</td>
<td>Prospective Study</td>
<td>25</td>
<td>Undergoing HCT at Cincinnati Children’s Hospital and receiving prophylaxis</td>
<td>All patients at Cincinnati Children’s Hospital</td>
<td>CYP2C19 wildtype (n=17) CYP2C19 heterozygous (n=3) CYP2C19 variant (n=2)</td>
<td>4mg/kg/dose Q12h Dosage was adjusted until voriconazole levels were between 1-5.5 mg/L 5mg/kg/dose Q12h</td>
<td>1-5.0mg/L</td>
<td>Voriconazole exposure was influenced by genotype. Larger interpatient variability in voriconazole levels was seen in wild-types.</td>
<td>1 patient showed clinical adverse effects.</td>
<td></td>
</tr>
<tr>
<td>Teusink, et al. 2014</td>
<td>Prospective Cohort Study</td>
<td>20</td>
<td>Undergoing HSCT at Cincinnati Children’s Hospital and receiving prophylactic voriconazole therapy</td>
<td>10 months – 26 years Grouped into: Poor metabolizers (*2/*2) Intermediate metabolizers (*1/*2) Extensive, ultra-rapid metabolizers (*1/*1, *1/*17)</td>
<td>Individualized dose based on CYP19 genotype, given Q12h Initial dosing: Poor metabolizers: 5mg/kg Q12h Intermediate: 6mg/kg Q12h Extensive/ultra-rapid: 7mg/kg Q12h</td>
<td>1-5.0mg/L</td>
<td>Time to reach the target level significantly improved when dosed according to genotyping (vs. pilot study: 8 days vs. 30 days)</td>
<td>No adverse effects to voriconazole therapy (as opposed to 2013 study where 1 patient experienced adverse effects)</td>
<td>Poor metabolizers (*2/*2): start 5mg/kg Q12 Intermediate metabolizers (*1/*2): start 6mg/kg Q12 Extensive, ultra-rapid metabolizers (*1/*1, *1/*17) start 7mg/kg Q12</td>
<td>If trough concentrations &lt; 0.1mg/L: Increase dose by 50%, recheck level prior to 9th new dose If trough concentrations ≥0.1mg/L, ≤1.0mg/L: Increase dose by 50%, recheck level prior to 9th new dose If trough concentrations ≥1mg/L, ≤5.0mg/L: Maintain dose, recheck level in 1 week If trough concentrations &gt;5.0mg/L: Hold 2 doses and restart at lower dose (25-50% dose reduction)</td>
</tr>
</tbody>
</table>

TDM, Therapeutic drug monitoring; URM, Ultra-rapid metabolizer; EM, extensive metabolizer, HEM, Heterozygous Extensive Metabolizer, PM, Poor Metabolizer Invasive fungal infection; Cmin (trough), Trough Level, Tmax, Time to Peak Concentration, AUC0–24h, 24-hr Exposure, AUC0–∞, Total Exposure, CL, Clearance, t1/2, Half-life
2.5 Discussion

Voriconazole is the primary therapy for the treatment of IA, candidaemia in neutropenic patients, and other fungal infections that are a significant cause of mortality. Given its important role in the critically ill patient populations susceptible to such fungal infections, early initiation of accurate dosing is paramount. This review critically examined the evidence on implementing \textit{CYP2C19} genotyping in conjunction to voriconazole’s existing dosing strategies, specifically TDM.

The effect of TDM on treatment outcomes in voriconazole therapy has been extensively studied by numerous retrospective and prospective studies. To our knowledge, few studies had a non-TDM comparison cohort. Only one single center randomized controlled trial \cite{132}, one prematurely ended multi-center randomized controlled trial \cite{99}, and one retrospective cohort study were published \cite{133}. Cumulatively, with the numerous retrospective and prospective observational studies located, a concentration-efficacy relationship and some concentration-toxicity associations were able to be established. Specifically, a concentration-neurotoxicity relationship has been well defined but a trough concentration-hepatotoxicity relationship has been less well characterized. Other rarer adverse effects of voriconazole (i.e. arrhythmias and QT prolongation) have been infrequently reported in these studies due to insufficient sample sizes and associations with voriconazole concentrations are unable to be made.

Aside from a few studies with a non-TDM comparison cohort, there have only been descriptive case series studies supporting the usage of TDM. Thus the strength of the evidence supporting the usage of TDM in voriconazole therapy is moderate due to the lack of large sample, multi-center, controlled clinical trials. Nonetheless, given the establishment of voriconazole concentration and efficacy and toxicity relationships, the proportion of studies supporting the usage of TDM, and its effectiveness in reducing the incidence of discontinuations of voriconazole therapy due to drug-related adverse effects, we recommend usage of TDM in voriconazole therapy, especially in pediatric populations where there is evidence that current voriconazole dosing guidelines are insufficient.

What is lacking however, is a consensus voriconazole target therapeutic range to maximize efficacy while minimizing toxicity and validated dosage adjustment guidelines for out-of-range trough levels. Two recently published meta-analyses on the relationship between
voriconazole therapeutic range with and the efficacy and safety of voriconazole have found, respectively, that lower thresholds of >0.5mg/L and >1.0mg/L are associated with greater efficacy and higher thresholds of >3.0 mg/L and >6.0 mg/L and are associated with toxicity. Based on the systematic review of the literature, where no additional efficacy advantage has been reported with trough concentrations >4.0 mg/L, we support a minimum threshold of ≥1.0mg/L for increased efficacy and a maximum threshold of ≤4.0 mg/L to minimize toxicity. However, given the findings of several studies that showed that trough levels of ≥1.5 mg-2.2 mg/L were significantly associated with increased efficacy, and that no studies to our knowledge have reported associations of toxicity with trough levels <2.5 mg/L, further investigation of the voriconazole concentration-efficacy relationship at higher thresholds of ≥1.5 mg/L is warranted.

CYP2C19 polymorphisms significantly affect voriconazole metabolism and concentrations and multiple fold differences have been found between PMs and URMs in several pharmacokinetic parameters including voriconazole AUC, Cmin, CL/F, and t1/2. The clinical implications of these differences between CYP2C19 genotypes has yet to be established. Studies investigating the relationship between CYP2C19 genotype and efficacy and safety have generally found no associations or no statistically significant findings. However, there are a number of limitations that may explain why there was no relationship observed by these studies: i) a significant proportion of studies did not investigate the more recently discovered *17 allele; ii) TDM was conducted in most of these studies and differences in efficacy and safety between genotypes may have been minimized; iii) a majority of the studies, due to their geographic location were only able to recruit primarily one ethnicity which underrepresents ethno-specific alleles; and iv) small sample sizes compound the previous two issues, and can potentially mean these studies were greatly underpowered. Clearly, further studies with larger sample sizes including an adequate number of carriers of all 4 alleles of interest are needed to investigate the relationship between CYP2C19 genotype and voriconazole efficacy and safety.

There was very little evidence on the effect of prospective CYP2C19 genotyping to aid initial voriconazole dosing. The only study that we are aware of, found CYP2C19 genotype-guided dosing significantly reduced the amount of time required to reach therapeutic concentrations. However, CYP2C19 guided dosing did not significantly affect the incidence
of toxicity or therapy discontinuation. No significant associations to efficacy were reported likely because patients received voriconazole for prophylaxis.

Dosing recommendations for CYP2C19-guided dosing for voriconazole are lacking. To our knowledge, only three studies have put forth dosage recommendations based on CYP2C19 metabolizer status and only the one has been used in a trial. One study recommended WTs be dosed at 7.2 mg-8.9 mg/kg/day and carriers of either the *2 or *3 alleles to be dosed at 4.4-6.5mg/kg/day. Another study recommended that non-PMs be given voriconazole 300 mg orally twice daily or 200mg intravenously twice daily and PMs be given voriconazole 200mg orally or intravenously twice daily. The third study put forth dosage recommendations for a pediatric patient population and dosed PMs at 5 mg/kg Q12, HEMs at 6mg/kg Q12, and EMs or URMs at 7 mg/kg Q12. Further validation of these dosage recommendations is required.

Use of CYP2C19 genotype to aid voriconazole dosing has been considered by several investigators and reviewers, but there may be a more passive role for prospective CYP2C19 genotyping. It is feasible that CYP2C19 genetic status is provided to clinicians with the intent to simply increase monitoring of select patients; such as higher liver enzyme test frequencies for PMs (for potentially higher risk of hepatotoxicity) or more frequent and regular TDM for HEMs (for potentially longer time to therapeutic range).

CYP29C19 genotyping is becoming increasingly feasible for implementation with voriconazole therapy. The cost of CYP2C19 genotyping has decreased significantly in recent years at <$150CAD/person and turn-around-times have decreased to ~1 hour with recently approved point-of-care medical devices such as the Spartan RX CYP2C19 system (Spartan Bioscience Inc., Ottawa, Canada). CYP2C19 real-time genotyping has also seen increasing validation for use in acute coronary syndromes and individuals prescribed clopidogrel therapy. The greatest barrier to implementation of CYP2C19 pharmacogenetics in voriconazole therapy is the lack of methodologically strong studies showing a therapeutic effect and prevention of toxicity. Overall, in agreement with several other reviews, there needs to be further research on the role of prospective CYP2C19 genotyping for voriconazole therapy.
3  Chapter III: Methods

3.1  Study Overview

A single-blinded, block-randomized, controlled pilot study was conducted investigating the impact of prospective cytochrome P450 2C19 genotype-specific dosing used in conjunction with TDM vs. TDM alone, on achieving therapeutic voriconazole plasma concentration levels. Eligible and consenting participants were block-randomized and allocated to one of two study arms: the (1) TDM + Genotyping trial arm, where patients were prospectively genotyped and received an interventional dose of voriconazole depending on their genotype until TDM was conducted; or the (2) TDM trial arm, where patients received prospective genotyping and TDM but did not receive genotype-guided dosing.

3.2  Patient Recruitment

3.2.1  Inclusion Criteria:

All SOT patients ≥ 18 years of age that were prescribed voriconazole for empirical therapy, prophylaxis, possible, probable or proven IFIs at the Toronto General Hospital from Dec 1, 2015 to July 1, 2016 were considered for study recruitment. Voriconazole is currently being used at this centre for empiric, pre-emptive, or targeted therapy for possible, probable or proven IA infections, according to definitions of the Invasive Fungal Infection Group of the European Organization for Research and Treatment of Cancer and Mycoses Study Group of the National Institute of Allergy and Infectious Diseases⁸.

3.2.2  Exclusion Criteria:

Patients that previously took voriconazole within the 2 weeks prior to potential study recruitment were ineligible.

3.2.3  Sampling

Patients were recruited by convenience sampling. Pharmacists in the Multi-Organ Transplant program at UHN notified the study researchers when a new-start voriconazole was prescribed. The graduate student coordinated with the pharmacists regarding patient enrollment status, delivery of the intervention, and scheduling of TDM.
3.3 Study Procedures

3.3.1 Prior to Randomization and Allocation

The study was approved by the Research Ethics Board of University Health Network, REB #15-9226-B on October 6, 2015. Prior to commencement of the trial, physicians and pharmacists in the Multi-Organ Transplant program were aware of the trial and provided feedback via presentations given by the graduate student. Pharmacists notified the graduate student when patients were prescribed voriconazole. Eligible participants were screened and recruited by the graduate student. All enrolled patients provided informed consent including consent to genetic testing. Patient genotypes were procured within 24 hours of obtaining consent via buccal swab samples and typically within 48 hours within the start of voriconazole therapy. Patients did not know their genotyping results and were blinded to which trial arm they were randomized to.

3.3.2 Patient Randomization and Allocation

Upon determination of genetic status, patients were block-randomized (with a block size of 6) with a 1:1 ratio and allocated to either the TDM (control) or TDM + Genotyping (treatment) trial arm. Patients with URM or EM genotypes underwent separate computerized block randomization (block size of 6) from patients with HEM or PM genotypes based on existing literature that recommended HEMs and PMs be dosed with specific dosages. In order to ensure a relatively heterogeneous mixture of genotypes in each trial arm, URM and EMs were separately block-randomized from HEMs and PMs. The randomization scheme was kept hidden from the study researchers via consecutively numbered, locked digital files (akin to sealed envelopes) in order to minimize bias.

3.3.3 Trial Arms

For all patients, the route of administration of voriconazole therapy was intravenous or oral and was determined by the physician. Dosages adjustments were not made when patients switched to or from intravenous or oral formulations. Oral formulations were rounded to the nearest 50 mg while IV dosing was not rounded. When voriconazole steady-state concentrations were reached (≥ day 4 post-start voriconazole), all patients reverted back to the standard of care and received TDM regardless of trial arm.
3.3.3.1 TDM arm:

After prospective genotyping, patients in the control arm received the standard of care, where a loading dose of 6 mg/kg twice daily was given for one day followed by a maintenance dose of 4 mg/kg twice daily, unless ordered otherwise by the attending physician (see table 3.1). Steady state voriconazole plasma trough concentrations were taken at least 4 days and up to 7 days post-start voriconazole. Trough concentrations were drawn no more than 1 hour before the next scheduled voriconazole administration which was given at 10 am and 10 pm daily. Voriconazole dosage adjustments were made according to the results of voriconazole plasma trough levels.

3.3.3.2 TDM + Genotyping arm:

Patients in the TDM + Genotyping arm underwent prospective genotyping and those with genotypes reflecting a PM or HEM phenotype received an experimental maintenance dosage of voriconazole of 2 mg/kg voriconazole twice daily (see Table 3.1). This was based on prior studies that identified that HEMs and PMs were more likely to develop toxicity at the standard 200 mg twice daily dosing. Loading dosages were kept at 6 mg/kg twice daily unless ordered otherwise by the attending physician. TDM was conducted in a similar fashion as the control group, where steady state voriconazole plasma trough levels were taken within 1 hour before scheduled voriconazole administrations at 10 am and 10 pm, and at least 4 days and up to 6 days after voriconazole initiation. Although pharmacists were aware of patients’ genotype, dosage adjustments were based only on plasma trough level results without consideration of patient genotype. The graduate student regularly verified that patients were given dosage adjustments in accordance with the dosing guidelines presented in the tables below.
Table 3.1 Dosing Algorithm for standard of care (TDM) and intervention (TDM + Genotyping) arms

<table>
<thead>
<tr>
<th>TDM arm</th>
<th>TDM + Genotyping arm</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Loading Dose:</strong></td>
<td><strong>Loading Dose:</strong></td>
</tr>
<tr>
<td>6 mg/kg voriconazole twice on the first day, followed by:</td>
<td>6 mg/kg voriconazole twice on the first day, followed by:</td>
</tr>
<tr>
<td><strong>Maintenance Dose:</strong></td>
<td><strong>Maintenance Dose:</strong></td>
</tr>
<tr>
<td>4 mg/kg twice daily until first trough level measurement</td>
<td>2 mg/kg twice daily until first trough level measurement</td>
</tr>
</tbody>
</table>

| Extensive Metabolizers | Heterozygous Extensive or Poor Metabolizers |

3.3.4 Post-TDM

Following TDM, all patients in the trial received dosage adjustments, if necessary, based on trough level concentrations. Dose adjustments for all patients adhered to the following strategy in order to reach the target therapeutic window of 1.0-5.0 mg/L (see Tables 3.2 and 3.3): The dosage was increased by 100% if the trough level was <1.0 mg/L. The dosage was lowered by 50% if the trough level was >5.5 mg/L and in the absence of voriconazole-related AEs. If trough levels were >10.0 mg/L or if AEs occurred with trough levels >5.5 mg/L, the next dose was skipped and subsequent doses were reduced by 50% 128. When a dose adjustment was made, TDM was conducted again between 4-7 days after the dosage adjustment occurred. The pharmacists were made aware of these dosing guidelines and the graduate student conducted regular follow-ups.

Table 3.2 Voriconazole target trough ranges

<table>
<thead>
<tr>
<th>Prophylaxis</th>
<th>Treatment</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥0.5 mg/L</td>
<td>1.5-5.0 mg/L</td>
<td>&lt;5.5 mg/L</td>
</tr>
</tbody>
</table>
Table 3.3 Voriconazole dosage adjustment strategy

<table>
<thead>
<tr>
<th>Measured Level (mg/L)</th>
<th>Dose adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.5</td>
<td>Increase by 50%</td>
</tr>
<tr>
<td>≥0.5 &lt;1.5</td>
<td>Increase by 25%</td>
</tr>
<tr>
<td>≥1.5 &lt;5.5</td>
<td>No adjustment</td>
</tr>
<tr>
<td>≥5.5 and drug-related toxicities</td>
<td>Decrease by 25%</td>
</tr>
</tbody>
</table>

3.4 CYP2C19 Genotyping

3.4.1 Sample Collection

Upon obtaining patient informed consent (see Appendix E), buccal swabs were collected by the graduate student using buccal sample collection kits supplied by Spartan Bioscience Inc. (Ottawa, ON). Each patient required 3 buccal swabs for the 3 alleles (*2, *3, and *17) to be detected. Buccal swab samples were transported with frozen cold blocks to Haber’s Compounding Pharmacy (Toronto, ON) and analysed using the Spartan RX CYP2C19 Assay manufactured by Spartan Bioscience Inc. (Ottawa, ON) which was located in the pharmacy. Collection kits were stored at -20°C at Toronto General Hospital prior to sample collection.

3.4.2 CYP2C19 Genotyping

Analysis was conducted using the Spartan RX CYP2C19 system which is an in vitro diagnostic polymerase chain reaction (PCR) based test capable of identifying CYP2C19 *2, *3, and *17 alleles by respectively recognizing the 19154G>A, 17948G>A, and -806C>T SNPs. The assay operates by extracting genomic DNA from buccal swab samples. The wild type CYP2C19 *1 allele is assumed by the Spartan RX CYP2C19 assay if the *2, *3, and *17 alleles are absent, which is a minor limitation of the assay given the relative rarity of the non-detected alleles (i.e. *4-8). The automated assay protocol consists of DNA extraction, PCR amplification of extracted DNA, detection of amplified PCR products via fluorescent oligonucleotide probes, and detection and analysis of the fluorescent signal. An internal positive control for the assay exists to ensure that the assay’s protocols succeeded.
3.4.3 CYP2C19 Genotype Categorization

All subjects were categorized based on their CYP2C19 genotype into either the URM, EM, HEM, or PM phenotypic categories by the CYP2C19 genotype grouping strategy previously discussed in Chapter I. Patients with the wild-type genotype *1/*1 were categorized as EMs, patients with only one copy of either the CYP2C19*2 or *3 alleles were categorized as HEMs, patients with two copies of either the CYP2C19*2 or *3 alleles were categorized as PMs and patients with at least one copy of the *17 allele were categorized as URMs. There were no patients having the combination of a copy of the *17 allele in conjunction with the *2 or *3 loss-of-function allele.

3.5 Study Endpoints

3.5.1 Reaching Therapeutic Concentrations

The primary study endpoint was to determine if genotype-guided dosing increases the proportion of patients who reach therapeutic concentrations of voriconazole at the initial trough level measurement. Outcomes had a binary categorization as either success (target range reached) or failure (concentration of voriconazole was not within target range).

3.5.2 Efficacy and Toxicity

Secondary endpoints were related to clinical efficacy and toxicity and also had a binary categorization as either success (complete or partial response) or failure (stable response, progression of disease, or death attributed to IA). Discontinuation of voriconazole (due to breakthrough of IFI or voriconazole-related AEs) were also regarded as treatment failures. Each patient was assessed by the infectious disease specialist on a composite of clinical (fever, signs/symptoms of infection), radiological (CT), and mycological findings (culture and microscopic findings) \(^8\).

3.6 Sample Size Calculation

Sample size was calculated using a binary outcome, parallel group, non-inferiority trial design (two-tailed chi-squared statistic) for comparing two proportions. A non-inferiority trial design was chosen over a superiority trial design in order to address the possibility that TDM in
conjunction with genotyping is less beneficial than TDM alone. This is possible if HEMs and PMs randomized to the Genotyping + TDM arm respond poorly to their experimental dosages.

The null hypothesis is that a greater proportion of those receiving the standard of care (TDM arm) will achieve targeted voriconazole therapeutic trough concentrations at steady-state than those receiving prospective genotyping with voriconazole (Genotyping + TDM arm) by more than 5% (non-inferiority margin).

\[ H_0: p_{Genotyping + TDM} - p_{TDM} \leq -0.05 \]

The alternative hypothesis is the proportion of patients who achieve therapeutic voriconazole trough concentrations at steady-state concentrations in the Genotyping + TDM cohort will be greater than the said proportion in the TDM cohort or at least within 5% of that proportion.

\[ H_A: p_{Genotyping + TDM} - p_{TDM} > -0.05 \]

The expected proportion of patients achieving therapeutic concentrations in the Genotyping + TDM cohort was 0.80 and 0.50 in the TDM group, thus we expected to see a difference of 30% between groups. The significance level (\( \alpha \)) was set to 0.05, the power (1 – \( \beta \)) was set to 0.20, and the non-inferiority margin (\( \Delta \)) was set to 0.05. The computed required sample size was found to be 21 participants per trial arm.

The sample size was calculated using the statistical computer language and software R and the package TrialSize (see Appendix G for R code) the formulas used by the software to calculate sample size and power are:

\[ n_A = \kappa n_B \quad \text{and} \quad n_B = \left( \frac{p_A(1 - p_A)}{\kappa} + p_B(1 - p_B) \right) \left( \frac{z_{1-\alpha} + z_{1-\beta}}{p_A - p_B - \delta} \right)^2 \]

\[ 1 - \beta = \Phi \left( z - z_{1-\alpha/2} \right) + \Phi \left( -z - z_{1-\alpha/2} \right), \quad z = \frac{p_A - p_B - \delta}{\sqrt{\frac{p_A(1-p_A)}{n_A} + \frac{p_B(1-p_B)}{n_B}}} \]
Where $\kappa = n_A/n_B$ is the allocation ratio, $\Phi$ is the standard Normal distribution function, $\Phi^{-1}$ is the standard Normal quantile function, $\alpha$ is the probability of a Type I error, $\beta$ is the probability of a Type II error, and $\delta$ is the non-inferiority margin $^{141}$.

### 3.7 Assessments and Evaluations

Each patient was followed for the duration of voriconazole therapy. Initial baseline, TDM, efficacy, and safety data were recorded throughout the patient’s voriconazole therapy. The length of voriconazole treatment was determined and assessed regularly by an infectious disease specialist.

#### 3.7.1 Initial Assessments:

Baseline data was collected shortly after recruitment using the data collection forms (see Appendix F) Basic demographic data, voriconazole indication, start date, initial loading and maintenance dosages, concomitant medications, details of IFI, co-morbidities and $CYP2C19$ genetic status were recorded. Basic demographic information consisted of gender, age, ethnicity, and body weight (kg).

#### 3.7.2 Therapeutic Drug Monitoring Assessments:

Voriconazole trough level results were typically available within 24 hours of being drawn and were recorded shortly thereafter. Upon each trough level collection, the following was recorded (see data collection forms in supplemental data): trough level (mg/L), voriconazole dosage adjustment (Y/N), prior dosage and route, and new dosage and route, disease progression (Y/N), if patient received loading dose (Y/N), voriconazole dose adjustment (Y/N), initial voriconazole dosage, current voriconazole dosage, number of days post-start of voriconazole treatment, rate of invasive fungal infection, patient response to voriconazole therapy, AEs during the study period, and time when trough level was drawn.

#### 3.7.3 Efficacy Assessments

Response to voriconazole therapy was assessed by clinicians through clinical signs and symptoms (i.e. resolution of fever), mycological findings (negative for galactomannan), and radiological findings (CT scans). Stable response, resolution of fungal infection, and no breakthrough fungal infections was considered therapy success.
3.7.4 Toxicity Assessments:

Voriconazole-related hepatotoxicity, neurotoxicity, cutaneous toxicity, and drug-related discontinuations, were recorded from patient electronic medical records. Patients were discontinued from voriconazole treatment at the physician’s discretion. For evaluation of hepatotoxicity: Total bilirubin (T-Bil), alkaline phosphatase (ALP), γ-glutamyltranspeptidase (GGT), serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) levels were recorded and compared to the values established by the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE version 4.0). The values are as follows:

For T-Bil: grade 0, none; grade 1, >1.0-1.5×ULN (upper limit of normal); grade 2, >1.5–3.0×ULN; grade 3, >3.0–10.0×ULN; grade 4, >10.0×ULN

For ALP, GGT, AST and ALT: grade 0, none; grade 1, 1.0–2.5×ULN; grade 2, >2.5–5.0×ULN; grade 3, >5.0–20.0×ULN; grade 4, >20.0×ULN.

The ULN values used are: 1.2 mg/dL, 359 U/L, 47 U/L, 33 U/L and 27 U/L for bilirubin, ALP, GGT, AST and ALT respectively.

3.8 Data Management

Baseline data were collected from patient medical charts and electronic personal records (EPR) by the candidate. All data collected were documented on forms created by Microsoft Word that had no patient identifiers (see Appendix F). Participants were given a unique study identification number which was the identifier provided to Haber’s Compounding Pharmacy and the link between patient identity and data forms. The patient’s study enrollment date was used as a secondary identifier that was linked to the unique study identification number so that in the event of errors, the patient would still be identifiable.

3.9 Data Analyses

Descriptive statistics were performed on collected data to determine medians and interquartile ranges (IQR) for continuous variables, and counts and proportions for binary variables. Inferential statistics were applied where possible in order to derive correlational or causal
relationships in order to satisfy primary and secondary endpoints. All statistical analyses were conducted by the candidate using the statistical computer language and software R\textsuperscript{139}.

Upon reaching the expected sample size, Fisher's exact test of independence will be used to test for statistical significance for proportions calculated from contingency tables. The Mann–Whitney U test (also called the Mann–Whitney–Wilcoxon (MWW), will be used to test if there is a statistically significant difference between medians.
Chapter IV: Results

Included in this chapter are results of the randomized, controlled, pilot trial. The recruitment process, patient characteristics, and results of the intervention are provided. All patients received \textit{CYP2C19} genotyping, however, only one participant received an experimental dose of voriconazole based on their \textit{CYP2C19} metabolizer status. Since this pilot trial had a limited sample size, descriptive statistics will be the focus of the results. The results associated with the outlined primary, secondary, and exploratory outcomes are reported.

4.1 Recruitment

Recruitment took place from December 1\textsuperscript{st} 2015 to July 15\textsuperscript{th}, 2016. Fifteen SOT recipients who were prescribed a new start voriconazole were approached for participation in this study. A total of 14 patients consented and were enrolled into the study. Although it was estimated that a total of 42 participants would be required to adequately measure primary outcomes, this was a pilot trial and further patient recruitment is expected. A summary of the participant flow is depicted in Figure 4.1 below.
4.2 Baseline Characteristics

Of the 15 participants, the average age was 43, with 18 years of age being the youngest, and 78 being the oldest. The majority of participants were of Caucasian ethnicity (n= 11, 79%), followed by one Asian (7%), one Middle-easterner (7%), and one South American (7%) self-identifying as Guyanese. Twelve of the participants were lung transplant recipients (86%), while 1 (7%) was a renal transplant and 1 (7%) received a liver transplant. Five participants (36%) in the sample had cystic fibrosis (CF). The patient characteristics can be seen in Table 4.1 below.
<table>
<thead>
<tr>
<th>Patient #</th>
<th>Sex</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Weight (kg)</th>
<th>Transplant Type</th>
<th>Indication for Voriconazole</th>
<th>Underlying Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>36</td>
<td>Caucasian</td>
<td>115.0</td>
<td>Double lung and heart</td>
<td>BAL: positive galactomannan</td>
<td>Eisenmenger's syndrome</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>78</td>
<td>Caucasian</td>
<td>53.2</td>
<td>Double lung</td>
<td>A. fumigatus isolated from sputum</td>
<td>Chronic obstructive pulmonary disease (COPD)</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>58</td>
<td>Caucasian</td>
<td>77.2</td>
<td>Left Lung</td>
<td>Empiric</td>
<td>Asthma and emphysema</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>22</td>
<td>Caucasian</td>
<td>52.6</td>
<td>Left Lung</td>
<td>BAL: A. fumigatus isolated (pre-op)</td>
<td>Cystic fibrosis (CF)</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>18</td>
<td>Caucasian</td>
<td>55.2</td>
<td>Double lung</td>
<td>Empiric</td>
<td>Cystic fibrosis (CF)</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>63</td>
<td>Caucasian</td>
<td>48.5</td>
<td>Double lung</td>
<td>Oral invasive non-albicans Candida</td>
<td>Interstitial lung disease (ILD)</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>54</td>
<td>Guyanese</td>
<td>49.5</td>
<td>Double lung</td>
<td>A. fumigatus isolated from sputum (pre-op)</td>
<td>Chronic myeloid leukemia (CML)</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>30</td>
<td>Caucasian</td>
<td>46.4</td>
<td>Double lung</td>
<td>BAL: A. fumigatus isolated (pre-op)</td>
<td>Cystic fibrosis (CF)</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>62</td>
<td>Caucasian</td>
<td>89.5</td>
<td>Double lung</td>
<td>BAL: positive galactomannan</td>
<td>Idiopathic pulmonary fibrosis</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>39</td>
<td>Middle Eastern</td>
<td>36.2</td>
<td>Double lung</td>
<td>Bronchoscopy: A. terreus isolated BAL: positive galactomannan</td>
<td>Interstitial lung disease (ILD)</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>62</td>
<td>Asian</td>
<td>107</td>
<td>Liver</td>
<td>Thoracic CT: cavity formation and clusters of nodules (fungal infection not ruled out)</td>
<td>Alcoholic cirrhosis and hepatocellular carcinoma</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>28</td>
<td>Caucasian</td>
<td>41.2</td>
<td>Double lung (two times)</td>
<td>A. fumigatus in aortic tissue</td>
<td>Cystic fibrosis (CF)</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>35</td>
<td>Caucasian</td>
<td>75.4</td>
<td>Renal</td>
<td>Empiric</td>
<td>Alport's syndrome</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>21</td>
<td>Caucasian</td>
<td>61.9</td>
<td>Double lung</td>
<td>BAL: A. fumigatus isolated (pre-op)</td>
<td>Cystic fibrosis (CF)</td>
</tr>
</tbody>
</table>

BAL, bronchoalveolar lavage
Table 4.2 Summary Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>TDM arm (%)</th>
<th>Genotyping + TDM arm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>4 (50)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Female</td>
<td>4 (50)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>Median Age</td>
<td>45.4 [IQR: 22-62]</td>
<td>40.5 [IQR: 25.5-60.5]</td>
</tr>
<tr>
<td>Voriconazole-naïve</td>
<td>3 (38)</td>
<td>4 (67)</td>
</tr>
<tr>
<td>Lung Transplant</td>
<td>7 (88)</td>
<td>5 (83)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>5 (63)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (13)</td>
<td>0</td>
</tr>
<tr>
<td>Middle Eastern</td>
<td>1 (13)</td>
<td>0</td>
</tr>
<tr>
<td>South American</td>
<td>1 (13)</td>
<td>0</td>
</tr>
<tr>
<td>Ultra-rapid Metabolizer</td>
<td>1 (13)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>Extensive Metabolizer</td>
<td>4 (50)</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Heterozygous Extensive Metabolizer</td>
<td>1 (13)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>Poor Metabolizer</td>
<td>2 (25)</td>
<td>0</td>
</tr>
<tr>
<td>*2 carriers</td>
<td>3 (38)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>*3 carriers</td>
<td>1 (13)</td>
<td>0</td>
</tr>
<tr>
<td>*17 carriers</td>
<td>1 (13)</td>
<td>2 (33)</td>
</tr>
</tbody>
</table>

TDM, therapeutic drug monitoring; IQR, interquartile range
Values in parentheses are percentages

4.3 Genotyping and Randomization Results

Buccal swab samples from 15 patients were collected and DNA was successfully genotyped for CYP2C19*1, CYP2C19*2, CYP2C19*3, and CYP2C19*17. Of the 3 (21%) URMs, all were heterozygous for the *17 allele. Fifty percent (n= 7) of the patients were EMs and had the WT genotype. There were 2 (14%) HEMs in the sample and both were heterozygous for the *2 allele. There were 2 PMs (14%) in the sample, one who was homozygous for *2 and one had the *2/*3 genotype.

Of the 8 participants in the control TDM arm, 5 (63%) were EMs, 2 (25%) were PMs, and 1 (13%) was a HEM. Of the 6 participants randomized to the TDM + Genotyping arm, 3 were URMs, 2 were EMs, 1 was a HEM and there were no PMs. Since only carriers of either the *2 or *3 in the TDM + Genotyping Arm were to receive a genotype-specific dose of voriconazole, there was only one patient who received the genotype-guided dosing intervention.

Table 4.2 outlines each patient’s genotype, phenotype, trial arm allocation, if they received genotype-guided dosing, if they previously were prescribed voriconazole at Toronto
General Hospital, the final voriconazole dosage they were prescribed, and their initial trough level (first TDM results).

Table 4.3 Patient Genotype, Intervention, and Trough

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Genotype</th>
<th>Phenotype</th>
<th>Trial Arm</th>
<th>Received Experimental Dosing</th>
<th>VRC Naive</th>
<th>Final Maintenance Dosage (mg/kg)</th>
<th>Initial Trough Levels (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>*1/*1</td>
<td>EM</td>
<td>TDM + Genotyping</td>
<td>N</td>
<td>N</td>
<td>400 mg PO BID (3.5)</td>
<td>2.2</td>
</tr>
<tr>
<td>2</td>
<td>*1/*1</td>
<td>EM</td>
<td>TDM</td>
<td>N</td>
<td>N</td>
<td>200 mg PO BID (3.8)</td>
<td>5.4</td>
</tr>
<tr>
<td>3</td>
<td>*1/*1</td>
<td>EM</td>
<td>TDM + Genotyping</td>
<td>N</td>
<td>N</td>
<td>200 mg PO BID (2.6)</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>*1/*1</td>
<td>EM</td>
<td>TDM</td>
<td>N</td>
<td>Y</td>
<td>250 mg PO BID (3.8)</td>
<td>0¹</td>
</tr>
<tr>
<td>5</td>
<td>*1/*1</td>
<td>EM</td>
<td>TDM</td>
<td>N</td>
<td>N</td>
<td>330 mg IV BID (6.0)</td>
<td>4.3</td>
</tr>
<tr>
<td>6</td>
<td>*1/*1</td>
<td>EM</td>
<td>TDM + Genotyping</td>
<td>N</td>
<td>Y</td>
<td>200 mg IV BID (4.1)</td>
<td>3.5</td>
</tr>
<tr>
<td>7</td>
<td>*1/*1</td>
<td>EM</td>
<td>TDM</td>
<td>N</td>
<td>N</td>
<td>200 mg PO BID (4.0)</td>
<td>2.4</td>
</tr>
<tr>
<td>8</td>
<td>*1/*2</td>
<td>HEM</td>
<td>TDM + Genotyping</td>
<td>Y (2.2 mg/kg)</td>
<td>Y</td>
<td>300 mg PO BID (6.5)</td>
<td>0¹</td>
</tr>
<tr>
<td>9</td>
<td>*1/*2</td>
<td>HEM</td>
<td>TDM</td>
<td>N</td>
<td>Y</td>
<td>200 mg PO BID (2.2)</td>
<td>2.2</td>
</tr>
<tr>
<td>10</td>
<td>*2/*2</td>
<td>PM</td>
<td>TDM</td>
<td>N</td>
<td>N</td>
<td>150 mg PO BID (4.1)</td>
<td>3.9</td>
</tr>
<tr>
<td>11</td>
<td>*2/*3</td>
<td>PM</td>
<td>TDM</td>
<td>N</td>
<td>Y</td>
<td>300 mg PO BID (2.8)</td>
<td>3.1</td>
</tr>
<tr>
<td>12</td>
<td>*1/*17</td>
<td>URM</td>
<td>TDM</td>
<td>N</td>
<td>N</td>
<td>250 mg PO BID (6.1)</td>
<td>4.1</td>
</tr>
<tr>
<td>13</td>
<td>*1/*17</td>
<td>URM</td>
<td>TDM + Genotyping</td>
<td>N</td>
<td>Y</td>
<td>300 mg PO BID (4.0)</td>
<td>2.1</td>
</tr>
<tr>
<td>14</td>
<td>*1/*17</td>
<td>URM</td>
<td>TDM + Genotyping</td>
<td>N</td>
<td>Y</td>
<td>250 mg PO BID (4.0)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

TDM, therapeutic drug monitoring; VRC, voriconazole; URM, ultra-rapid metabolizer; EM, extensive metabolizer; HEM, heterozygous extensive metabolizer; PM, poor metabolizer

¹voriconazole concentration was too low to be detectable (< 0.2 mg/kg)

Values in brackets dosages in mg/kg
4.4 Primary Objectives

4.4.1 Proportion of Patients within Therapeutic Range

The primary objective of this study was to determine if the proportion of patients who achieved therapeutic voriconazole trough concentrations at steady-state through genotype-guided dosing in conjunction with TDM was greater than the proportion of patients who obtain therapeutic voriconazole concentrations via TDM alone. The hypothesis was that of patients who receive genotype-guided dosing in conjunction with TDM, a greater proportion will reach target voriconazole trough concentrations at the initial trough level measurement compared to patients who receive solely TDM.

The proportion of patients in the TDM group that were within target therapeutic range at the initial trough level was 0.75 (6 of 8 patients) while the proportion of patients that were within therapeutic range in the Genotyping + TDM group was 0.83 (5 of 6 patients). This result can be found in Table 4.4. For both trial arms, the proportion of patients that reached therapeutic range at the initial trough level measurement was higher than expected. A likely explanation may be that 7 (50%) patients had previously been administered voriconazole at Toronto General Hospital and clinician’s notes detailing prior experience with the drug were available for these patients. Thus, clinicians may have been able to more accurately select the initial voriconazole dosage for these individuals. Of the 7 patients who previously received voriconazole at Toronto General Hospital, 6 were within target range while of the 7 voriconazole-naïve patients, 5 were within target range (86% vs. 71%,) (see Table 4.5).

A sub-group analysis of the primary outcome measure was conducted on those who were voriconazole-naïve. The proportion of patients in the TDM group that were within range at the initial trough level was 0.67 (2 of 3 patients) while the proportion of patients that were within therapeutic range in the Genotyping group was 0.75 (3 of 4 patients). The result can be found in Table 4.4.

Overall, because of the small sample size where only one patient received genotype-guided dosing, further study enrollment is required to accept or reject the hypothesis that patients who receive genotype-guided dosing in conjunction with TDM will reach target voriconazole
trough concentrations in greater proportion at the initial trough concentration measurement, compared to patients who receive solely TDM.

4.4.2 Dosage Adjustments Necessary to Reach Therapeutic Range

Another primary objective of the study was to determine if genotype-guided dosing in conjunction with TDM will result in a fewer number of dose adjustments needed to achieve therapeutic voriconazole levels, compared to TDM alone.

A further analysis was conducted to see if there were differences the median number of trough level drawings received by those in the TDM arm versus those in the Genotyping + TDM arm. Those in the TDM arm received a median of 2.0 trough level measurements while those in the Genotyping + TDM arm also received a median of 2.0 trough level measurements. Evaluating only those who were voriconazole-naïve, patients in the TDM arm and in the Genotyping + TDM arm received a median of 2.0 and 1.5 trough level measurements respectively (see Table 4.4).

Overall, the hypothesis that patients who receive genotype-guided dosing in conjunction with TDM will require a fewer number of dosage adjustments to reach therapeutic voriconazole range than patients who receive solely TDM cannot be accepted at this time. Further study enrollment is required to accept or reject this hypothesis.

Table 4.4 Patients receiving TDM vs Genotyping + TDM

<table>
<thead>
<tr>
<th></th>
<th>TDM</th>
<th>Genotyping + TDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage Within TR at Initial Trough Level</td>
<td>75% (6/8)</td>
<td>83.3% (5/6)</td>
</tr>
<tr>
<td>Percentage of VRC-naïve Patients Within TR at Initial Trough Level</td>
<td>66.7% (2/3)</td>
<td>75% (3/4)</td>
</tr>
<tr>
<td>Median number of trough level measurements per patient</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Median number of trough level measurements per VRC-naïve patient</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Patients who required no dosage adjustments</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Patients who required 1 dosage adjustment</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
Patients who required more than 1 dosage adjustment | 0 | 1
---|---|---
TR, target therapeutic range; TDM, therapeutic drug monitoring; VRC, voriconazole
Values in brackets are number of patients

**Table 4.5 Percentage of Patients Within VRC TR at Initial Trough Level (VRC-experienced vs VRC-naïve)**

<table>
<thead>
<tr>
<th>Percentage Within VRC TR at Initial Trough Level</th>
<th>VRC-experienced</th>
<th>VRC-naïve</th>
</tr>
</thead>
<tbody>
<tr>
<td>86% (6/7)</td>
<td>71.4% (5/7)</td>
<td></td>
</tr>
</tbody>
</table>
TR, target therapeutic range; TDM, therapeutic drug monitoring; VRC, voriconazole
Values in brackets are number of patients

**4.4.3 Suggested Genotype-guided Dosages**

The third primary objective of the study was to evaluate the genotype-guided dosages suggested for the genotyping plus TDM trial arm. There was only one patient who received the genotype guided dose of 2 mg/kg. This voriconazole-naïve patient received 100 mg of voriconazole from day 2–4 of her post start of her voriconazole therapy. The patient started her voriconazole therapy with a loading dosage of 400mg PO b.i.d twice daily for one day. The patient was initially administered a maintenance dosage of oral voriconazole 200mg b.i.d. for one day prior to availability of genotyping results. Genotyping results were available on the third day of voriconazole therapy and the patient was administered oral voriconazole 100 mg b.i.d. from day 3 to day 7, taking a total of 10 doses. On day 7 post-start voriconazole, TDM was conducted and found undetectable amounts of voriconazole. The patient’s voriconazole dose was escalated to 200 mg. Two more dose adjustments were made, eventually increasing her dose to 300 mg.

This voriconazole-naïve patient’s initial and final maintenance dosages, initial trough concentration, number of dosage adjustments and number of trough level measurements were compared with the other voriconazole-naïve patients in the study. The findings are reported in Table 4.6.

**Table 4.6 Genotype-Guided Dosing Patient vs VRC-naïve Patients**

<table>
<thead>
<tr>
<th>Initial Maintenance Dose (mg/kg)</th>
<th>Final Maintenance Dose (mg/kg)</th>
<th>Initial Trough Level (mg/L)</th>
<th>Dosage Adjustments (#)</th>
<th>Trough Level Measurements (#)</th>
</tr>
</thead>
</table>
The single patient who received a genotype-guided dosage required a higher final maintenance dosage, number of dosage adjustments, and number of trough level measurements and lower initial trough level compared to other voriconazole-naïve patients. The final maintenance dose required by the patient was higher compared to the median final maintenance dose required by other voriconazole-naïve patients (6.5 vs 4.0 mg/kg). The intervention patient’s initial trough level was subtherapeutic and reported to be ≤0.20 mg/L, compared to the median 2.15 mg/L. The patient also required 3 dosage adjustments compared to the median 0 dosage adjustments required by those who were voriconazole-naïve. Lastly the intervention patient received 7 trough level measurements compared to the median 1.5 trough level measurements received by those who were voriconazole-naïve. It is unknown if these findings can be attributed to the intervention or if other factors such as the patient’s HEM phenotype are involved. An exploratory analysis of this follows in the next section.

The hypothesis was that the suggested genotype-guided dose of 2 mg/kg would benefit PMs the most. From this singular HEM patient, the benefit of a lowered dose conveyed to PMs cannot be evaluated and it is unknown if the suggested genotype-guided dosage for HEMs may be appropriate. This HEM patient displayed interesting characteristics: the initial trough level was lower than the initial trough level reported by other voriconazole-naïve patients, which can be explained by the lowered maintenance dose the patient received. The patient also required a higher final maintenance dosage to stay within therapeutic ranges compared to other voriconazole-naïve patients which was unexpected given the patient’s metabolizer status. More importantly, the patient was more difficult to accurately dose for and required more time to reach therapeutic range. Three other studies also reported that HEMs took longer and required more monitoring to reach therapeutic range. Further analyses on the effect of genotype on
voriconazole dosing parameters may be beneficial in evaluating the role of genotype-guided
dosing in voriconazole therapy. These analyses follow in next section.

4.5 Exploratory Outcomes

4.5.1 Effect of CYP2C19 Genotype

The effect of CYP2C19 genotype on the mean number of trough level drawings, dosage
adjustments and proportion of patients that are within therapeutic range at the initial trough level
was analyzed and the results are in Table 4.7. Given the small sample size, no associations could
be determined.

Table 4.7 TDM, Dosage Adjustments, and Trough Level Measurements by
CYP2C19 Phenotype

<table>
<thead>
<tr>
<th></th>
<th>URM</th>
<th>EM</th>
<th>HEM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median TDMs per patient</td>
<td>2</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Median dosage adjustments per patient</td>
<td>0</td>
<td>0</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>Proportion of Patients that reached Target Therapeutic Range at the first TDM</td>
<td>1</td>
<td>0.86</td>
<td>0.5</td>
<td>1</td>
</tr>
</tbody>
</table>

TDM, therapeutic drug monitoring; URM, ultra-rapid metabolizer; EM, extensive metabolizer; HEM, heterozygous extensive metabolizer; PM, poor metabolizer.

4.5.2 CYP2C19 Genotype on Voriconazole Trough Levels

The effect of CYP2C19 metabolizer status and voriconazole initial trough levels was plotted and
analyzed and a subgroup analysis was conducted for voriconazole-naïve patients. Visually, PMs
displayed higher initial trough levels compared to other phenotypes.

A similar analysis was conducted examining the effect of CYP2C19 metabolizer status
and final maintenance dosages of voriconazole therapy. Importantly, the standard 4.0 mg/kg
seemed appropriate for EMs and HEMs. URM and PMs may benefit from a higher or lower
dosage of voriconazole respectively. More URM and PMs will need to be enrolled to
adequately assess this finding.
Figure 4.2 *CYP2C19* Phenotype and Voriconazole Initial Trough Level

![Bar chart showing median initial trough levels for different CYP2C19 phenotypes.](chart1.png)

VRC, voriconazole; URM, ultra-rapid metabolizer; EM, extensive metabolizer; HEM, heterozygous extensive metabolizer; PM, poor metabolizer

Figure 4.3 *CYP2C19* phenotype and voriconazole maintenance dose

![Bar chart showing median final maintenance doses for different CYP2C19 phenotypes.](chart2.png)

VRC, voriconazole; URM, ultra-rapid metabolizer; EM, extensive metabolizer; HEM, heterozygous extensive metabolizer; PM, poor metabolizer

4.5.3 *CYP2C19* Genotyping and Voriconazole Efficacy and Toxicity

Our secondary outcome was to determine if genotyping could increase voriconazole treatment success, predict the incidence of voriconazole-related AEs, and reduce discontinuations of
voriconazole therapy due to toxicity. There was limited data to evaluate efficacy and safety of voriconazole therapy. Out of 14 patients, (i) only 1 patient received the experimental maintenance dosage of 2 mg/kg; (ii) 3 patients were enrolled recently and there was insufficient follow-up time to determine efficacy or toxicity; (iii) 3 other patients received empiric therapy where voriconazole was discontinued shortly after the patient tested positive for other pathogens.

Of the 8 patients for whom efficacy and toxicity could be evaluated, two developed hepatotoxicity and both patients were URMs. One received a dosage reduction and the other discontinued therapy due to hepatotoxicity. The patient that received a dosage reduction displayed an initial trough level of 4.1 mg/L while receiving 7.3 mg/kg voriconazole twice daily. A dosage reduction to 6.1 mg/kg twice daily alleviated the hepatotoxicity and resulted in a trough level of 3.4 mg/L. The patient that was discontinued voriconazole had a trough level of 1.0 mg/L while receiving 4.0 mg/kg twice daily. The single patient who received genotype-guided dosing reported photosensitivity although no trough level measurements exceeded 1.6 mg/L.

Voriconazole was efficacious in all 8 patients for whom efficacy and toxicity data were available. Patients who were prescribed voriconazole based on positive mycological or radiological findings (n = 6) showed no progression of disease (stable response) or resolution of disease. Of the 7 patients, 6 had initial trough levels within therapeutic range. The patient who received genotype-guided dosing was prescribed voriconazole for prophylaxis and was not within therapeutic range for 5 of the 7 trough level measurements. No breakthrough fungal infections were seen in this patient. Of the 7 patients, 1 was an URM, 2 were EMs, 2 were HEMs, and 2 were PMs.

Overall, because of the small sample size, no association could be determined between voriconazole-related toxicity or discontinuation of therapy and voriconazole trough concentrations. No associations between efficacy and trough concentrations could be determined either, although 6 of the 7 patients who showed stable response had trough levels within therapeutic range.
5 Chapter V: Discussion and Conclusion

5.1 Summary of Results

To our knowledge, this is the first randomized controlled trial investigating the effect of \textit{CYP2C19} genotype on voriconazole therapy. Only one other cohort study also investigated the effect of genotype-guided dosing for voriconazole therapy. This pilot study’s aim was to examine the effect of genotype-guided dosing on achieving therapeutic voriconazole concentrations. Since this study is the first to use real-time \textit{CYP2C19} dosing in voriconazole therapy, it demonstrated the practical possibility of incorporating \textit{CYP2C19} pharmacogenomics in voriconazole therapy.

5.1.1 Effect of Genotype-guided Dosing on Reaching Therapeutic Range

The study’s primary aim was to determine if \textit{CYP2C19} genotype-guided dosing would increase the proportion of patients within therapeutic range at the initial trough level measurement at steady-state concentrations. Another primary outcome was to determine if genotype-guided dosing would decrease the number of dosage adjustments required for patients to reach therapeutic range. Secondary outcomes involved determining the impact of genotype-guided dosing on voriconazole efficacy and toxicity.

Only 1 patient received genotype-guided dosing. As per the protocol, only HEMs and PMs enrolled in the Genotyping + TDM arm would receive the intervention. Of the 14 participants, 2 HEMs and 2 PMs were recruited into the study. Although a 1:1 allocation ratio was used, the block randomization scheme resulted in 3 of the HEM/PMs being allocated to the standard of care arm and only 1 HEM due to block randomization for the trial arms.

We report that the intervention patient was not within target range at the first trough level measurement and differed from other enrolled patients in several parameters. The patient required more dosage adjustments and trough level measurements to reach target range, and required a higher final maintenance dosage to reach therapeutic levels. Since no other patients received the intervention, it is unknown what role the intervention played in these findings or if other factors such as the patient’s HEM phenotype contributed. Overall, the effectiveness of genotype guided dosing cannot be determined and further enrollment of HEM and PM patients
are required to investigate these associations between genotype-guided dosing and reaching voriconazole therapeutic concentrations.

5.1.2 Effect of Genotype-guided Dosing on Efficacy and Toxicity

The study’s secondary outcomes involved investigating the effect of genotype-guided dosing on the efficacy and/or toxicity of voriconazole therapy. There was limited data to evaluate efficacy and safety of voriconazole therapy because only one patient received genotype-guided dosing. Given the small sample, we were unable to determine any association between genotype guided-dosing and response to therapy, incidence of voriconazole-related AEs, or discontinuations of therapy due to toxicity.

5.1.3 Effect of CYP2C19 Genotype on Reaching Therapeutic Range

CYP2C19 polymorphisms have been reported by other studies to significantly affect voriconazole metabolism and concentrations where multiple fold differences have been found between PMs and URMs in several pharmacokinetic parameters including voriconazole AUC, $C_{\text{min}}$, CL/F, and $t_{1/2}$. However, the clinical implications of these differences between CYP2C19 genotypes have yet to be established. Thus we conducted exploratory analyses on the effects of CYP2C19 genotype on voriconazole therapy.

We evaluated if there were associations between genotype and number of trough level measurements, initial trough level concentrations, number of dosage adjustments and final maintenance dosage needed for patients to achieve target range. We found that HEMs received a higher number of mean trough level drawings compared to EMs. Two other studies also reported that HEMs, compared to other phenotypes, received the most number of trough level measurements and also took the longest time to reach target range. Due to the small sample size, we were unable to find any associations between CYP2C19 genotype and the mean number dosage adjustments or proportion of patients within therapeutic range at the initial trough level measurement. The patient who received genotype-guided dosing was included in this analysis and may have had a disproportionate effect as this patient needed more dosage adjustments, compared to other patients.
5.1.4 Dosing Recommendations for CYP2C19 Genotype

In our analysis of the effect of CYP2C19 phenotype and the final maintenance dose the patient received, URMs required on average 4.7 mg/kg while PMs required 3.5 mg/kg to reach therapeutic range. This finding implies our currently suggested dosage of 2 mg/kg for HEMs and PMs may need an upwards dosage adjustment and that URMs may benefit from an increased dosage. Our suggested dosage of 2 mg/kg for HEMs and PMs was derived from genotype-specific dosing recommendations put forward by previous investigators \(^{102,105}\) and based on the clinical experience from our study team.

Currently, dosing recommendations for CYP2C19 genotypes are lacking and only three studies have put forth suggestions \(^{102,105,133}\). One study recommended WTs be dosed at 3.6-4.45 mg/kg and HEMs and PMs be dosed at 2.2-3.25 mg/kg. \(^{105}\). Another study suggested that PMs should receive voriconazole 200mg and non-PMs be given 300 mg oral voriconazole or 200mg voriconazole i.v. \(^{102}\). The third study suggested and tested dosing recommendations in a pediatric population which is known to exhibit vastly different voriconazole pharmacokinetics from adult populations \(^{133}\). From our findings and what has been published in the literature, URMs may benefit from receiving 5 mg/kg twice daily while HEMs and PMs may benefit from being dosed at 3 mg/kg twice daily. Further validation of these suggested genotype-guided dosages is required.

5.2 Strengths and Limitations of the Study

5.2.1 Strengths

5.2.1.1 Randomization

This pilot study is a parallel two-group block-randomized controlled trial which is considered to be the least susceptible to bias compared to other study designs \(^{142}\). The study used block randomization with randomly selected block sizes of 2, 4, or 6 which reduces the risk of selection bias. A fixed block size can allow the researcher to predict the trial arm a participant will be allocated to depending on the randomization sequence of prior participants \(^{143}\).
5.2.1.2 Allocation Concealment

The randomization scheme was hidden from the candidate who was in charge of patient recruitment. Treatment allocation was also concealed to the candidate using sealed digital files (as an alternative to sequentially numbered sealed envelopes) prior to patient genotyping and randomization. This ascertained that the candidate did not know which trial arm the patient would be allocated to prior to obtaining informed consent. Evidence exists that the effects of treatment interventions can be exaggerated if the randomization sequence is not hidden from investigators during the patient recruitment process due to denying enrollment of participants who may not respond to the treatment\textsuperscript{144, 142}.

5.2.1.3 Participant Blinding

Participants were blinded to their group allocation and if they received genotype-guided dosing. Their $CYP2C19$ genotype was not disclosed to them either. This reduced the risk of reporting bias where patients who are aware of their metabolizer status may be more or less likely to report adverse events.

5.2.2 Limitations

5.2.2.1 Small sample

Enrollment for this study began in December, 2015 and further patient recruitment is required. Currently, the sample is insufficiently large to calculate the primary outcome with statistical significance. There are currently 8 patients in the standard of care arm and 6 patients in the intervention arm. Based on our estimation that the genotype-guided trial arm would have 30% more patients within therapeutic range than those in the standard of care trial arm we calculated that and a sample size of 21 participants per trial arm would be able detect this difference with a power of 0.8 and significance level of 0.05. Further study recruitment with attention to the other limitations listed below is anticipated to allow adequate statistical power to answer the study’s primary research question.

5.2.2.2 Overestimation of Treatment Effect

In the sample size calculation, it was estimated that 80% of patients in the Genotyping + TDM arm would be able reach therapeutic range at the first trough level measurement. Thus, genotype-
guided dosing would be able to increase the proportion of patients within therapeutic range by 30%, compared to the 50% for patients receiving the standard of care. In our patient sample, 85% of patients reached therapeutic range by the initial trough level measurement which greatly exceeded our expected proportion.

In addition, only a small proportion of patients in the Genotyping + TDM arm received the experimental dosage. Only HEMs and PMs were recommended genotype-specific dosages in our study and only 29% of the participants were carriers of either the *2 or *3 allele. In addition, due to the block-randomization scheme, only 1 patient in the study received the intervention. Overall to increase the detection of the treatment effect, a larger sample of heterogeneous genotypes needs to be enrolled and the possibility of genotype-guided dosing for URMs should be investigated.

5.2.2.3 Voriconazole-experienced Patients

For both trial arms, the proportion of patients who were within therapeutic range at the initial trough level measurement was significantly higher than what was anticipated. We expected that 50% of participants would be within therapeutic range at the first TDM event, based on a randomized controlled trial investigating voriconazole TDM \(^{42}\). Other observational studies reported similar percentages (53-70%) of patients reaching therapeutic level at the first trough level measurement \(^{61,128,145}\).

Excluding patient #8 in Table 4.2 who received genotype-guided dosing, we unexpectedly found that the proportion of patients whose initial trough levels were within voriconazole therapeutic range was 0.85 (11 out of 13 patients). This was 0.35 over our anticipated proportion and has implications for our primary research question and for our sample size calculations. To investigate this further, we calculated the statistical significance of this suggest a potential reason that could explain this deviation.

We compared the proportion of those within therapeutic range at Toronto General Hospital at the initial trough level measurement with data reported from a randomized controlled trial using a similar weight-based voriconazole dosing algorithm and target therapeutic range \(^{42}\). Fisher’s exact test was employed as it is used in the analysis of contingency tables and is valid when sample sizes are small \(^{146}\). We found that there was a statically significant difference in the
proportion of patients who reach therapeutic range at the initial trough level measurement (85% of 13 patients vs. 51% of 101 patients, p= 0.035). This suggests that there may be a confounder that explains why at Toronto General Hospital, there is a high proportion at patients who reach therapeutic range at the first trough level measurement. See Appendix G for details of the statistical analysis.

During review of patient medical records, it was found that half of the patients recruited (7 of the 14 patients) were previously administered voriconazole at the Toronto General Hospital. Clinician notes often described the outcome of voriconazole therapy for these patient and which dosages were used to reach therapeutic levels and achieve clinical success. Some patients were not administered voriconazole using the standard weight-based dosing algorithm and it is unclear how much of a role clinician-driven dosing played in patients reaching therapeutic range.

Overall, a significantly large proportion of patients were within therapeutic range at the first trough level measurement which may have impacted results. We hypothesize this is due to clinician-driven dosing for voriconazole-experienced patients. When appropriate, sub-group analyses with voriconazole-naïve patients was conducted to formulate our results. Although this limited the potential confounder, sample size was adversely affected. With regards to further study enrollment, it may be beneficial to screen for voriconazole-naïve patients.

5.3 Future Directions for the Study

Since only 14 patients have been enrolled, further patient recruitment should continue. However, it may be useful to adjust some aspects of the protocol to address the limitations of the study listed above.

5.3.1 Genotype-guided Dosing Recommendations for URMs

Thus far, 50% of the participants have been EMs, 21% of participants have been URMs and 29% of participants have been either HEMs and PMs. Assuming future patient enrollment will not be substantially different in terms of genetic make-up, only a portion of the patients in the Genotyping + TDM trial arm will actually receive the intervention. In order to increase the treatment effect seen in the intervention trial arm, dosage adjustments for URMs can potentially be implemented.
Currently, there are no existing dosage recommendations for adult URMs. The only other genotype-guided dosing study was conducted in a pediatric patient population where URMS were dosed at 7 mg/kg twice daily\textsuperscript{133}. Our study found that the average dose required for URMs to be within therapeutic range was 4.7 mg/kg. Several studies examining the effect of the *I7 allele on voriconazole trough concentrations found URMs to be associated with subtherapeutic voriconazole trough levels, longer times to therapeutic range, and a higher under-dose rate \textsuperscript{122,124,125}. Potentially, URMs that are allocated to the Genotyping + TDM arm can be dosed at 5.0 mg/kg.

5.3.2 Additional Exclusion Criteria

The study may also benefit from adding additional exclusion criteria to reduce potential confounders in the study. Those that have previously taken voriconazole at Toronto General Hospital and other patients that received clinician-driven dosing could be potentially excluded. Furthermore, patients who are recipients of liver transplants may be excluded in the future since they would effectively have two \textit{CYP2C19} genotypes. In the current patient population, one liver transplant recipient was prescribed voriconazole after receiving a living donor transplant from their biological brother. The patient was found to be a HEM after consenting to a buccal swab and genotyping. We were unable to determine the donor’s genotype. The patient was not excluded due the expression of \textit{CYP2C19} in the small intestine\textsuperscript{147,148}, effectively providing the patient with dual \textit{CYP2C19} genotypes. However, because the expression of \textit{CYP2C19} in the small intestine is minimal relative to the level of expression in the liver, future liver transplant recipients should be excluded from the study.

5.3.3 Superiority Trial Design

The initial trial design chosen for the study was a non-inferiority trial design. A superiority trial would be a more logical choice given the relatively high cost of \textit{CYP2C19} genotyping. Sample size was re-calculated using a binary outcome, parallel group, superiority trial design (two-tailed chi-squared statistic) for comparing two proportions.

The null hypothesis is that a greater proportion of those receiving the standard of care (TDM arm) will achieve targeted voriconazole therapeutic trough concentrations at steady-state than those receiving prospective genotyping with voriconazole (Genotyping + TDM arm) by at
least 5% (superiority margin). The expected proportion of patients achieving therapeutic concentrations in the Genotyping + TDM was set to 0.80 and 0.50 in the TDM group, the significance level (α) to 0.05, the power (1-β) to 0.20, and the superiority margin (Δ) to 0.05. The computed required sample size was found to be 41 participants per trial arm.

5.4 Implementation Real-time Genotyping

The possibility of CYP2C19 real-time genotyping has increased substantially in recent years and there is increasing implementation of CYP2C19 pharmacogenomics for individuals prescribed clopidogrel therapy for acute coronary syndromes 135–138. Until recently, usage of pharmacogenomics was not possible in the management of invasive aspergillosis 55. Long turn-around times and prohibitive costs presented significant challenges. With regards to initial voriconazole dose selection based on CYP2C19 genotype, a quick turn-around time is critical given its primary purpose of increasing the likelihood of achieving therapeutic voriconazole concentrations upon initial trough measurements. Currently patient CYP2C19 genotype and phenotype can be determined within <2 hours of sample collection and cost <$150CAD/per patient.

The main barrier to the implementation of CYP2C19 pharmacogenetics for voriconazole therapy is the lack of evidence demonstrating a relationship between CYP2C19 genotype on voriconazole efficacy and toxicity. Thus, before CYP2C19 genotyping can be widely used in clinical settings, large, high-volume clinical trials need to be conducted showing a therapeutic benefit for CYP2C19 genotype-guided dosing. In addition, there needs to be establishment of recommended dosages for specific CYP2C19 genotypes.

5.5 Conclusion

Voriconazole is the primary therapy for the treatment of IA. Dose optimization is difficult in part due to the high frequency of genetic polymorphisms in CYP2C19 that significantly affect the metabolism of voriconazole. Recommendations of weight-based dosing in oral voriconazole therapy and usage of TDM have been beneficial in aiding patients in reaching therapeutic range. However, there is an inherent delay of TDM because trough levels can only be drawn after voriconazole steady state concentrations are reached. Early initiation of accurate dosing is vital in critically ill patient populations yet only ~50-70% of patients are within therapeutic range.
when initial trough levels are drawn. It is plausible that using \textit{CYP2C19} genotype to guide initial voriconazole dose selection, in conjunction to TDM may be therapeutically beneficial. This is the first randomized controlled trial that investigated the use of \textit{CYP2C19} genotype to guide initial voriconazole dosing. The aim of this pilot randomized controlled trial was to determine if genotype-guided dosing increases the proportion of patients within voriconazole therapeutic levels at the first trough level measurement.

Fourteen patients were recruited and all were genotyped in real-time. The proportion of patients who were within therapeutic range at the initial trough level was 0.85. Only one patient received genotyped-guided dosing and was found to have a significantly lower initial trough level, greater number of dosage adjustments, greater number of trough level measurements and greater final maintenance dosage than other patients who were voriconazole-naïve. Further enrollment of HEM and PM patients are required to further investigate these associations between genotype-guided dosing and reaching voriconazole therapeutic concentrations.

Further patient enrollment is anticipated for the trial. Currently only a small proportion of patients in the intervention arm receive genotype-guided dosing. Genotype-guided dosing for URMs can potentially be implemented to increase the proportion of patients who will receive the intervention. Half of the current patient population had previously been administered voriconazole by clinicians in Toronto General Hospital and clinician-driven dosing may be a potential confounder. Establishing further exclusion criteria may be able to limit possible confounders on the effects of \textit{CYP2C19} genotype-guided dosing. Exempting those receiving clinician-driven dosing and liver-transplant recipients, whose \textit{CYP2C19} genotype cannot be fully elucidated, may be appropriate.

This pilot trial demonstrated the practical implementation of \textit{CYP2C19} genotype-guided dosing. Due to the small sample enrolled, no determination of the clinical benefit of \textit{CYP2C19} genotype-guided dosing could be made. Overall, the greatest barrier to implementation of \textit{CYP2C19} pharmacogenetics in voriconazole therapy is the lack of methodologically strong studies showing a therapeutic effect and prevention of toxicity. Further research with larger sample sizes including a genetically heterogeneous patient population of carriers of all 4 alleles of interest is required to investigate the impact of \textit{CYP2C19} genotype-guided dosing.
References


109. Espinel-Ingroff A. In vitro activity of the new triazole voriconazole (UK-109,496) against


140. Zhang E, Qian VW, Chow S-C, Zhang HG. TrialSize: R functions in Chapter 3,4,6,7,9,10,11,12,14,15No Title. 2013. https://cran.r-project.org/package=TrialSize.


Appendices

Appendix A: Search Terms

Voriconazole and TDM Search Terms - EMBASE

Voriconazole
1. exp Voriconazole/
2. vfend*.af.
3. voriconazole*.af.
4. JFU09I87TR.af.
6. uk??109496.af.
7. (apo-voriconazole or sandoz-voriconazole or teva-voriconazole).af.

Therapeutic Drug Monitoring
9. exp drug monitoring/
10. exp physiologic monitoring/
11. ((drug or medic* or therap* or physiol*) adj2 monitor*).af.
12. (therapeutic adj2 range).af.
15. tdm.af.

Voriconazole and TDM Search Terms - Medline

Voriconazole
1. exp Voriconazole/
2. vfend*.af.
3. voriconazole*.af.
4. JFU09I87TR.af.
6. uk??109496.af.
7. (apo-voriconazole or sandoz-voriconazole or teva-voriconazole).af.
Therapeutic Drug Monitoring
23. exp Drug Monitoring/
24. (therapeutic adj2 monitor*).af.
25. (drug* adj2 monitor*).af.
27. exp Monitoring, Physiologic/
28. tdm.af.
29. tdm.ti.
30. 23 or 24 or 25 or 26 or 27 or 28 or 29
31. 8 and 30

Voriconazole and Genetics Search Terms - EMBASE

Voriconazole
1. exp Voriconazole/
2. vfend*.af.
3. voriconazole*.af.
4. JFU09I87TR.af.
6. uk??109496.af.
7. (apo-voriconazole or sandoz-voriconazole or teva-voriconazole).af.

Genetic Polymorphisms
10. (individual* adj3 medicine*).tw.
12. (pharmacogenomic* or pharmacogenetic*).af.
13. exp Pharmacogenetics/
15. (genetic adj3 variation*).af.
17. exp genetic heterogeneity/
18. genetic polymorphism/
19. exp genetic screening/
20. exp genetic variability/
22. p-450*.af.
23. exp cytochrome P450/
24. exp cytochrome P450 2C19/
25. CYP2C19.af.
26. cytochrome P450 2C19.af.
27. P 450 2C19.af.
29. cyp 2c19.af.
Voriconazole and Genetics Search Terms - Medline

Voriconazole
1. exp Voriconazole/
2. vfend*.af.
3. voriconazole*.af.
4. JFU09I87TR.af.
6. uk??109496.af.
7. (apo-voriconazole or sandoz-voriconazole or teva-voriconazole).af.

Genetic Polymorphisms
9. exp Polymorphism, Genetic/
12. exp Genetic Testing/
14. (individual* adj3 medicine*).tw.
15. (personal* adj3 medicine).af.
16. (pharmacogenomic* or pharmacogenetic*).af.
17. exp Pharmacogenetics/
18. (genetic adj3 polymorphism*).af.
19. exp Genetic Variation/
20. (genetic adj3 variation*).af.
22. CYP2C19.af.
23. cytochrome P450 2C19.af.
26. cyp 2c19.af.
27. exp Cytochrome P-450 CYP2C19/
Appendix B: Data Extraction Forms

Studies on the relationship between TDM and efficacy or safety

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<th>Population</th>
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<th>Target Therapeutic Range</th>
<th>Trough Concentrations</th>
<th>Efficacy Relationship</th>
<th>Toxicity Relationship</th>
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</table>

Studies on the pharmacokinetics of voriconazole and CYP2C19 polymorphisms

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<th>Patient Pop.</th>
<th>Voriconazole Dosage</th>
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Studies on the CYP2C19 polymorphisms and their efficacy and toxicity relationship

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<th>Ethnicity</th>
<th>Population</th>
<th>VRC Dosage Regimen</th>
<th>n</th>
<th>Genotype</th>
<th>Trough Concentrations</th>
<th>Target Therapeutic Range and Time to Range</th>
<th>Efficacy</th>
<th>Toxicity</th>
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Studies on CYP2C19 genotypic dosing and voriconazole efficacy and/or toxicity in patients

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<th>Target Therapeutic Range</th>
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Appendix C: Quality Assessments Forms

Randomized Controlled Trials

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Reference:  
Decision:  
Study Design:  
Rationale for Exclusion:

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<th>Rationale</th>
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<td>Allocation concealment</td>
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<td>Blinding study participants and personnel</td>
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<td>Blinding outcome assessors</td>
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<td>Selective outcome reporting</td>
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## Cohort Studies

**Reviewer:**

**Reference:**

**Decision:**

**Study Design:**

**Rationale for Exclusion:**

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<td>Representativeness of exposed cohort (*)</td>
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<td>Selection of non-exposed cohort (*)</td>
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<td>Ascertainment of Exposure (*)</td>
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<td>Outcome of interest not present at start of study (*)</td>
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<td>Comparability of cohorts (**)</td>
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<td>Assessment of outcome (*)</td>
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Case Series

Reviewer: 

Reference: 

Decision: 

Study Design: 

Rationale for Exclusion: 

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<tr>
<td>Were confounding factors identified and strategies to deal with them stated?</td>
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<tr>
<td>Were outcomes assessed using objective criteria?</td>
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<tr>
<td>If comparisons are being made, was there sufficient descriptions of the group?</td>
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<tr>
<td>Was follow-up carried out over a sufficient time period?</td>
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<tr>
<td>Were the outcomes of people who withdrew described and included in the analysis?</td>
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<tr>
<td>Were outcomes measured in a reliable way?</td>
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<td>Was appropriate statistical methods used?</td>
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Appendix D: Quality Assessments

Case Series
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<th>If comparisons being made; were groups sufficiently described?</th>
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</tr>
<tr>
<td>Racil, 2012</td>
<td>Prospective</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Not Applicable No comparisons were made</td>
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<td>Yes</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Not Applicable No patients withdrew</td>
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<td>Not Applicable No patients withdrew</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No comparisons made</td>
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<td>Not Applicable No patients withdrew</td>
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<td>No Comparisons made</td>
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<tr>
<td>Wang, 2009</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Lee, 2012</td>
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<td>Yes</td>
<td>No</td>
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<td>No patients withdrew</td>
<td>Yes</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<td>Study Type</td>
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<td>Data Bias</td>
<td>Comparisons made</td>
<td>Different between CYP2C19 genotypes</td>
<td>Statistical analysis conducted</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No statistical analysis conducted</td>
<td>Low</td>
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<td>Retrospective</td>
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## Cohort Studies

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<td>*</td>
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<td>Scholz 2009</td>
<td>*</td>
<td>*</td>
<td>*</td>
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<tr>
<td>Suzuki 2013</td>
<td>* Insufficient information given about patient population regarding underlying disease, and indication for voriconazole</td>
<td>** Insufficient evidence that outcome of interest was not present at start of study</td>
<td>*</td>
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<td>Hoenigl 2013</td>
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<td>*</td>
<td>*</td>
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<td>Chu 2013</td>
<td>*</td>
<td>*</td>
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<td>Kang, 2015</td>
<td>*</td>
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## Randomized Controlled Trials

<table>
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<th>Study</th>
<th>Sequence Generation</th>
<th>Allocation Concealment</th>
<th>Blinding Study (participants &amp; researchers)</th>
<th>Blinding Outcome Assessors</th>
<th>Incomplete Outcome Data</th>
<th>Selective Outcome Reporting</th>
<th>Other Sources of Bias</th>
<th>Overall Risk of Bias Assessment</th>
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<td>Park 2012</td>
<td>Low</td>
<td>Unclear</td>
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<td>Low</td>
<td>Low</td>
<td>High</td>
<td>Low</td>
<td>High Risk</td>
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<tr>
<td></td>
<td></td>
<td>Not specified</td>
<td>Only assessors of adverse events were blinded</td>
<td>Assessor blinded, and analyzing objective outcomes</td>
<td>Only 2 subjects withdrew from the study (1.81% and less than 10%). No ITT was reported.</td>
<td></td>
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<tr>
<td>Neofytos 2015</td>
<td>Low</td>
<td>Unclear</td>
<td>Medium</td>
<td>Low</td>
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<td></td>
<td></td>
<td>Not specified</td>
<td>Study was non-blinded. Outcomes assess were primarily quantitative.</td>
<td>Assessors were not blinded, but objective outcomes were measured.</td>
<td>Study was terminated prematurely after 29 patients were enrolled.</td>
<td>No discrepancies were present between the methods and results sections in the study report.</td>
<td></td>
<td>Medium Risk</td>
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</table>
Appendix E: Informed Consent Form

Consent to Participate in a Research Study

A Randomized Controlled Trial of the Effect of Cytochrome P450 2C19 Genotype-Specific Dosing Plus TDM vs. TDM Alone on Reaching Therapeutic Voriconazole Blood Levels in Solid Organ Transplant Patients with Aspergillosis

Study Site Address: University Health Network (Toronto General Hospital)
200 Elizabeth St, Toronto, ON M5G 2C4

Investigators: Marissa Battistella (PI) Tel: 416-340-4800 x3207.
Joseph Lee (MSc Student)

This Informed Consent Form has:

- An Information Section which will inform you about the study
- A Consent and Signature Section for you to sign upon your agreement to participate in the study

This Informed Consent Form will describe the purpose, procedures, risks and benefits of this research study to you. A copy of this Informed Consent Form will be provided to you:

Before you make a decision to take part in this study, it is paramount that you understand the objective of the research and what it will involve. Please read the following information before giving your consent.
Introduction:
You are being asked to take part in this research study because you received a transplanted liver kidney heart or lung (solid organs) and will be receiving an agent to treat your fungal infection, called voriconazole. Please read this explanation about the study and its risks and benefits in order to decide if you would like to take part in it. You should take as much time as you need to make your decision. You should ask the study doctor or study staff to explain anything that you do not understand and make sure that your questions have been answered before signing this consent form. Your participation in this study is voluntary.

Purpose of Research
Invasive aspergillosis (IA) is a fungal infection which left untreated, can cause dangerous complications and death. Transplant patients who have weakened immune systems are at higher risk of getting IA. Voriconazole is a medication that is prescribed for IA. In order for voriconazole to work, it is important to maintain the right amount of drug in the blood. We check for the right amount of voriconazole in the blood by collecting blood samples. This process is called therapeutic drug monitoring (TDM) and usually done for transplant patients with weakened immune systems. However, maintaining the right amount of drug in the blood is still difficult since voriconazole is absorbed and excreted by the body differently for each person, depending on their genetic make-up. This study will try to understand if genetic testing early on in addition with TDM will be able to provide us with doses tailored to each individual. Participating in this study will not take away from the treatment you would have received otherwise.

Study Design:
Over the course of one year, patients receiving voriconazole therapy for IA will be randomly split into one of two groups. The control group will receive voriconazole dosages and then TDM according to the usual treatment for your condition. A treatment group will receive altered dosages that are specific to their genetic make-up for the first four days of treatment and will then receive routine TDM. This altered dosing for the treatment group is experimental and is not routinely used in patients' care. TDM will take place at approximately the fourth day after starting voriconazole for all patients, and from this point on the usual treatment will resume for all patients.

Study Participants
The study will recruit approximately 30 patients with solid organ transplants.

Study Visits and Procedures:
Your participation in the study will be for the entire amount of time you are taking voriconazole. The study staff will meet with you once, provided you meet the study criteria. This will be the only visit from the study staff. This visit will take place immediately after being prescribed voriconazole and is estimated to take 1-2 hours.

Should you decide to participate in the study, basic demographic information will be collected at this time and your medical records will be accessible to the study staff. One saliva sample will be collected from you at this time for the genetic testing portion of the study. The genetic testing used in this study will be
specific to looking at the way your body handles voriconazole and will not inform us of any other potential condition. No incidental findings will be discovered. This procedure will be done by Spartan Bioscience, a third party company. Your saliva sample will be sent with a code and will not show your name or address, or any information that directly identifies you. Your saliva sample will be immediately destroyed after the genetic testing component has been completed. Your genetic information will remain confidential as no research data will be shared with Spartan Bioscience or anyone outside the study staff. No additional procedures outside of routine care will be done. As part of routine care, your treatment will be regularly be assessed by a physician.

**Side Effects and Risks**
Taking part in this study may alter your treatment plan from the routine standard of care given at this institution. Your voriconazole dosage may change to suit your genetic make-up. These altered dosages have been determined by the study staff. This may increase or decrease the effectiveness of voriconazole therapy as well as increase or decrease the frequency and/or severity of the side effects voriconazole can cause. Visual disturbances and skin rashes are common side effects associated with voriconazole. Although more rare, voriconazole can impair your liver, brain, and cardiovascular system. For your safety, the study staff will monitor your progress in the study. During the study, it is paramount that you notify the study staff about any discomfort you experience. For your safety, if the study staff believes that your safety is compromised at any point in the study, you will be withdrawn.

**Risks Associated with Saliva and Blood Sampling:**
There will be saliva sampling for the genetic component of this study. This will be done by using a cheek swab. There are no risks associated with collecting saliva through cheek swabs. There are no risks associated with the genetic testing component of the study since no potential risks related to genetic information will be discovered. The blood sampling that is required for this study is part of the routine standard of care so there are no additional risks due to blood sampling.

**Alternatives to Being in the Study**
You do not have to join this study to receive treatment for your condition. Voriconazole with TDM is the approved treatment for your condition.

**Voluntary Participation**
Your participation in this study is entirely voluntary. You may decide not to be in this study, or to be in the study now, and then change your mind later. You may leave the study at any time without affecting your care. We will give you new information that is learned during the study that might affect your decision to stay in the study.

**Withdrawal from Study:**
The Researchers can take you off voriconazole early for safety reasons. Voriconazole can have severe side effects in some individuals and patients may be withdrawn from voriconazole treatment and the study for safety reasons at the discretion of their physician. If you decide to leave the study, the information that was collected before you left the study will still be used in order to help answer the research question. No new information will be collected without your permission. The saliva sample collected from you and the records linking your identity to your sample are destroyed immediately after
the genetic testing portion is concluded. Any research results that we obtained prior to your withdrawal of consent will be kept.

**Benefits of the Study to You**
You may be receiving a dose of voriconazole that is more tailored to your genetic make-up. The study results collected in this research may benefit others in the future because this study may help improve guidelines for the usage of voriconazole for the treatment of invasive aspergillosis.

**Confidentiality**
If you agree to join this study, the study team will look at your personal health information and collect only the information they need for the study. Personal health information is any information that could be used to identify you and includes your:

- name and initials
- UHN Medical Record Number
- date of birth
- new or existing medical records that include types, dates, and results of medical tests or procedures

Your participation in this study will also be recorded in your medical record at this hospital. This is for clinical safety purposes.

**Research Information in Shared Clinical Records**
If you participate in this study, information about you from this research project may be stored in your hospital file and in the UHN computer system. The UHN shares the patient information stored on its computers with other hospitals and health care providers in Ontario so they can access the information if it is needed for your clinical care. The study team can tell you what information about you will be stored electronically and may be shared outside of the UHN. If you have any concerns about this, or have any questions, please contact the UHN Privacy Office at 416-340-4800, x6937 (or by email at privacy@uhn.ca).

The following people may come to the hospital to look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study is following proper laws and guidelines:

- Representatives of the University Health Network (UHN) including the UHN Research Ethics Board

The study doctor will keep any personal health information about you in a secure and confidential location for 10 years. A list linking your study number with your name will be kept by the study doctor in a secure place, separate from your study file.

All information collected during this study, including your personal health information, will be kept confidential and will not be shared with anyone outside the study unless required by law. You will not be named in any reports, publications, or presentations that may come from this study.

**Rights as a Participant:**
If you are harmed as a direct result of taking part in this study, all necessary medical treatment will be made available to you at no cost.

By signing this form you do not give up any of your legal rights against the investigators, sponsor or involved institutions for compensation, nor does this form relieve the investigators, sponsor or involved institutions of their legal and professional responsibilities.

**Costs and Reimbursement**
You will not have to pay for any of the procedures involved with this study. This applies to both the routine care given for your condition and for any study-related interventions. The genetic testing will not reveal any incidental findings and will not incur any additional expenses.

**Reminders and Responsibilities**
You will need to:
- Inform your study doctor if you wish to withdraw from the study at any point
- Inform your study doctor if you are pregnant or plan on becoming pregnant during the study
- Notify your study doctor if you have taken or will be taking any other medications, supplements or natural health products within 14 days before the start of the study and throughout the entire duration of the study
- Refrain from taking any illegal drugs, narcotics, or any medication not prescribed to you
- Refrain from participating in any other clinical studies

**Conflict of Interest**
Researchers have an interest in completing this study. Their interests should not influence your decision to participate in this study. The researchers involved in this study have no other conflicts of interest.

**Questions about the Study:**
If you have any questions, concerns or would like to speak to the study team for any reason, you can contact Marisa Battistella at 416-340-4800 x 3207. If you have any questions about your rights as a research participant or have concerns about this study, call the Chair of the University Health Network Research Ethics Board (UHN REB) or the Research Ethics office number at 416-581-7849. The REB is a group of people who oversee the ethical conduct of research studies. The UHN REB is not part of the study team. Everything that you discuss will be kept confidential. You will be given a signed copy of this consent form.
**Consent:**
This study has been explained to me and any questions I had have been answered. I know that I may leave the study at any time. I agree to take part in this study.

<table>
<thead>
<tr>
<th>Name of Study Participant (Please Print)</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

My signature means that I have explained the study to the participant named above. I have answered all questions.

<table>
<thead>
<tr>
<th>Name of Person Obtaining Consent (Please Print)</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

Was the participant assisted during the consent process?  
☐ Yes  ☐ No
If Yes, please check the relevant box and complete the signature space below:

☐ The person signing below acted as a translator for the participant during the consent process and attests that the study as set out in this form was accurately translated and has had any questions answered.

<table>
<thead>
<tr>
<th>Name of Interpreter (Please Print)</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

Language

☐ The consent form was read to the participant. The person signing below attests that the study as set out in this form was accurately explained to, and has had any questions answered.

<table>
<thead>
<tr>
<th>Full Name of Witness (Please Print)</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

☐ Your signature on this form indicates that you are acting as a substitute decision maker for the participant and the study has been explained to you and all your questions have been answered to your satisfaction. You agree to allow the person you represent to take part in the study. You know that the person you represent can leave the study any time.

<table>
<thead>
<tr>
<th>Full Name of Witness (Please Print)</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>

**Relationship to Participant**
### Appendix F: Data Collection Form

#### Voriconazole Study Data Collection Form

<table>
<thead>
<tr>
<th>Study #</th>
<th>Sex</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Weight</th>
<th>BMI</th>
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<tbody>
<tr>
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<table>
<thead>
<tr>
<th>VRC Start Date</th>
<th>Loading Dose</th>
<th>Maintenance Dose</th>
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<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Trial Arm</th>
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<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Indication for VRC</th>
<th>Co-morbidities</th>
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</thead>
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**Medical History**

**Study Notes & Details**

**Concomitant Medications**

<table>
<thead>
<tr>
<th>TDM #1 Date</th>
<th>Trough Level</th>
<th>Prior Dosage</th>
<th>New Dosage</th>
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</thead>
<tbody>
<tr>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>TDM #2 Date</th>
<th>Trough Level</th>
<th>Prior Dosage</th>
<th>New Dosage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>TDM #3 Date</th>
<th>Trough Level</th>
<th>Prior Dosage</th>
<th>New Dosage</th>
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<td></td>
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</table>

<table>
<thead>
<tr>
<th>TDM #4 Date</th>
<th>Trough Level</th>
<th>Prior Dosage</th>
<th>New Dosage</th>
</tr>
</thead>
<tbody>
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<table>
<thead>
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<th>TDM #5 Date</th>
<th>Trough Level</th>
<th>Prior Dosage</th>
<th>New Dosage</th>
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<tr>
<td>Liver Enzymes (Baseline)</td>
<td>Liver Enzymes</td>
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<td></td>
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<tr>
<td>---------------------------------</td>
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<tr>
<td>Date:</td>
<td>Date:</td>
<td></td>
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<tr>
<td>Liver Enzymes</td>
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<tr>
<td>Date:</td>
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<tr>
<td>Liver Enzymes</td>
<td>Liver Enzymes</td>
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<tr>
<td>Date:</td>
<td>Date:</td>
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<tr>
<td>Liver Enzymes</td>
<td>Liver Enzymes</td>
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<td>Date:</td>
<td>Date:</td>
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<td>Date:</td>
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<tr>
<td>CNI Level &amp; Dosage</td>
<td>CNI Level &amp; Dosage</td>
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<td>Date:</td>
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<td>Adverse Events</td>
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<td>Date:</td>
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</tr>
<tr>
<td>Response to Therapy</td>
<td>Response to Therapy</td>
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<tr>
<td>Date:</td>
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<tr>
<td>Mycological Findings</td>
<td>Mycological Findings</td>
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<td>Date:</td>
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<td>Mycological Findings</td>
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<td>Date:</td>
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<tr>
<td>Radiological Findings</td>
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<tr>
<td>Clinical Findings</td>
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<tr>
<td>Other Notes</td>
<td>Other Notes</td>
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</tr>
<tr>
<td>Date:</td>
<td>Date:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix G: R Code

Sample Size Calculation

```r
library(TrialSize)
TwoSampleProportion.NIS(alpha=0.05, beta=0.2, p1=0.50, p2=0.8, k=1, delta=0.3, margin=0.05)
```

Effect of Genotyping

```r
#Proportion Within TR at Initial Trough Level
TR.TDMvsGenotyping=prop.test(x=c(6,5), n=c(8,6), correct=FALSE)
fisher.test(matrix(c(6, 8-6, 5, 6-5), ncol=2))

#Proportion within TR at Initial Trough Level (TGH vs Proportion Reported in Park 2012)
TR.TGHvsPark2012=prop.test(x=c(52,11), n=c(102,13), correct=FALSE)
fisher.test(matrix(c(52, 102-52, 11, 13-11), ncol=2))

#Proportion Within TR at Initial Trough Level (VRC-experienced vs VRC-naïve)
TR.VRC.naive=prop.test(x=c(6,5), n=c(7,7), correct=FALSE)
fisher.test(matrix(c(6, 7-6, 5, 7-5), ncol=2))

#Proportion of VRC-naïve Patients Within TR at Initial Trough Level
TR.TDMvsGenotyping.naive=prop.test(x=c(2,3), n=c(3,4), correct=FALSE)
fisher.test(matrix(c(2, 3-2, 1, 1-3), ncol=2))

#Mean dosage adjustments per patient
DA.TDMvsGenotyping=t.test(x=c(0,1,0,0,1,0,0,3), y=c(0,0,0,0,0,3), var.equal = TRUE)

#Mean dosage adjustments per VRC-naïve patient
DA.TDMvsGenotyping.naive=t.test(x=c(0,0,1), y=c(0,0,0,3), var.equal = TRUE)
#DA.InterventionvsVRCNaive=t.test(x=c(0,0,1,0,0,0), y=c(3), var.equal = TRUE)

#Mean trough level measurements per patient
TDMs.TDMvsGenotyping=t.test(x=c(1,3,1,3,3,2,2,1), y=c(2,2,2,1,1,7), var.equal = TRUE)
#TDMs.InterventionvsVRCNaive=t.test(x=c(1,3,2,2,1,1), y=c(7), var.equal = TRUE)

#Mean trough level measurements per VRC-naïve patient
TDMs.TDMvsGenotyping.naive=t.test(x=c(1,3,2), y=c(2,1,1,7), var.equal = TRUE)

Effect of Genotype

```r
t.test(x=c(0.5,2.2,3.1), y=c(2.1,1,0,3.5))
```
Appendix H: Exploratory Calculations on Reaching Therapeutic Range

Percentage of Patients Within VRC TR at Initial Trough Level (TGH vs Percentage Reported in Park 2012)

Fischer’s exact test was used to calculate significance.

<table>
<thead>
<tr>
<th></th>
<th>Park 2012</th>
<th>TGH</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage within VRC TR at Initial Trough Level</td>
<td>51% (52/102)</td>
<td>84.6% (11/13)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

TR, target therapeutic range; TDM, therapeutic drug monitoring; VRC, voriconazole
Values in brackets are number of patients