Contributions of the major *C. glabrata* triazole resistance determinants to the activity of the novel investigational tetrazoles VT-1598 and VT-1161

SG Whaley¹, NP Wiederhold², Q Zhang¹, CM Yates³, RJ Schotzinger³, EP Garvey³, PD Rogers¹

¹University of Tennessee Health Science Center, Memphis, TN; ²University of Texas Health Science San Antonio, San Antonio, TX; ³Viamet Pharmaceuticals, Durham, NC

BACKGROUND

- Among cases of invasive candidiasis in the United States, *Candida glabrata* is the second most common causative species.
- Resistance to the commonly used antifungal fluconazole is increasing in *C. glabrata* with reports of multidrug resistant isolates becoming a cause for concern.
- VT-1598 and VT-1161 are novel tetrazole CYP51 inhibitors that possess increased specificity for fungal CYP51 as compared to the human CYP450 enzymes. Previous studies have shown promising results against yeasts, endemics, and molds, including multiple species of *Candida*, often revealing greater potency than members of the azole class of antifungals.^{1,2}

PURPOSE

- In this study we assessed the activity of VT-1598 and VT-1161 against a panel of azole-resistant *C. glabrata* clinical isolates, as well as strains deleted for the genes encoding the key azole-resistance determinants Pdr1, Cdr1, Pdh1, Snq2, and Upc2A.

METHODS

- The SAT1 flipper method was used to construct all *C. glabrata* mutant strains described in Table 1. Parent isolates SM1 and SM3 are a previously characterized matched pair, isolated before and after fluconazole treatment.³
- Antifungal susceptibility testing was performed according to CLSI methods.

RESULTS

Table 1. Strains used in this study.

Strain	Parent	Genotype or description	Reference
SM1		Azole-SDD clinical isolate	(3)
SM3		Azole-resistant clinical isolate	(3)
SM1RPDR1(SM3)	SM1pdr1Δ	pdr1Δ::FRT-PDR1 ^{SM3}	(4)
SM1pdr1Δ	SM1	pdr1Δ::FRT	(4)
SM3pdr1Δ	SM3	pdr1Δ::FRT	(4)
cdr1Δ	SM1RPDR1(SM3)	pdr1Δ::FRT-PDR1 ^{SM3} /cdr1Δ::FRT	Unpublished
pdh1Δ	SM1RPDR1(SM3)	pdr1Δ::FRT-PDR1 ^{SM3} /pdh1Δ::FRT	Unpublished
snq2Δ	SM1RPDR1(SM3)	pdr1Δ::FRT-PDR1 ^{SM3} /snq2Δ::FRT	Unpublished
cdr1Δpdh1Δ	cdr1Δ	pdr1Δ::FRT-PDR1 ^{SM3} /cdr1Δ::FRT/pdh1Δ::FRT	Unpublished
cdr1Δsnq2Δ	cdr1Δ	pdr1Δ::FRT-PDR1 ^{SM3} /cdr1Δ::FRT/snq2Δ::FRT	Unpublished
pdh1Δsnq2Δ	snq2Δ	pdr1Δ::FRT-PDR1 ^{SM3} /snq2Δ::FRT/pdh1Δ::FRT	Unpublished
cdr1Δpdh1Δsnq2Δ	cdr1Δpdh1Δ	pdr1Δ::FRT-PDR1 ^{SM3} /cdr1Δ::FRT/snq2Δ::FRT	Unpublished
SM1upc2aΔ	SM1	upc2aΔ::FRT	(5)
SM3upc2aΔ	SM3	upc2aΔ::FRT	(5)

Figure 1. Antifungal susceptibility data for a collection of fluconazole resistant (orange) *C. glabrata* isolates compared to a susceptible dose dependent clinical isolate (green). Values on the y-axis are µg/ml drug concentrations.

Figure 2. Susceptibility data for constructed *PDR1* deletion strains compared to the matched clinical pair parent isolates.

Figure 3. Susceptibility data for constructed ABC transporter single deletion strains compared to the parent strain.

Figure 4. Susceptibility data for constructed ABC transporter double and triple deletion strains compared to the parent strain.

Figure 5. Susceptibility data for constructed *UPC2A* deletion strains compared to the matched clinical pair parent isolates.

CONCLUSIONS

- As with fluconazole, activating mutations in *PDR1* leading to overexpression of *CDR1*, *PDH1*, and *SNQ2* reduces susceptibility of *C. glabrata* to VT-1598 and VT-1161.
- Likewise, deletion of *UPC2A* results in increased susceptibility of *C. glabrata* to VT-1598 and VT-1161 suggesting a strategy for improving the activity of this novel antifungal against fluconazole resistant *C. glabrata*.

REFERENCES

- Wiederhold NP, Patterson HP, Tran BH, Yates CM, Schotzinger RJ, Garvey EP. Fungal-specific Cyp51 inhibitor VT-1598 demonstrates in vitro activity against *Candida* and *Cryptococcus* species, endemic fungi, including *Coccidioides* species, *Aspergillus* species and *Rhizopus arrhizus*. *J Antimicrob Chemother*. 2018 Feb 1;73(2):404-408.
- Warrilow AG, Hull CM, Parker JE, Garvey EP, Hoekstra WJ, Moore WR, Schotzinger RJ, Kelly DE, Kelly SL. The clinical candidate VT-1161 is a highly potent inhibitor of *Candida albicans* CYP51 but fails to bind the human enzyme. *Antimicrob Agents Chemother*. 2014 Dec;58(12):7121-7.
- Magill SS, Shields C, Sears CL, Choti M, Merz WG. 2006. Triazole cross-resistance among *Candida* spp.: case report, occurrence among bloodstream isolates, and implications for antifungal therapy. *J Clin Microbiol*, 2006 44:529-535.
- Caudle KE, Barker KS, Wiederhold NP, Xu L, Homayouni R, Rogers PD. Genomewide expression profile analysis of the *Candida glabrata* Pdr1 regulon. *Eukaryot Cell*, 2011 10:373-383.
- SG Whaley, KE Caudle, JP Vermitsky, SG Chadwick, G Toner, KS Barker, SE Gyax, PD Rogers. *UPC2A* is required for high-level azole antifungal resistance in *Candida glabrata*. *Antimicrob Agents Chemother*, 2014 Aug;58(8):4543-54.

This work was supported by NIH grants R01AI058145 and R01AI131620 awarded to PDR.