The Novel Investigational Agent VT-1161 Displays Low *In Vitro* Potential for Emergence of Resistance

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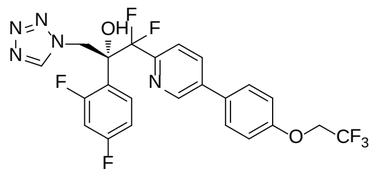
Introduction

Increase in drug resistance has been reported for the two major classes of antifungal drugs, the azoles which target CYP51 (1) and the echinocandins which target (1,3)-β-D-glucan synthase (2). VT-1161 is a novel CYP51 inhibitor (3) in clinical development and has shown a promising cross-resistant profile in initial *in vitro* and *in vivo* studies (4,5). To probe the ability of *C. albicans* to become resistant to VT-1161, in vitro passage studies were conducted, using a published system (6) that produces rapid elevation of MIC values when grown in the presence of fluconazole (FLU).

VT-1161 chemical structure:



Methods

Minimum inhibitory concentration (MIC) was determined at 50% inhibition of growth relative to no-drug control in YNB media as described (5) and in RPMI media (CLSI M27). Passage studies using *C. albicans* ATCC 36082 were conducted as described (5). Briefly, the passage system was validated by reproducing the published study, demonstrating rapid elevation of FLU's MICs (up to 256 µg/ml within 18 to 30 days of culture). This was followed by a side-by-side study where yeast was grown in YNB medium containing either VT-1161 or FLU at 4X, 8X, 16X, and ~128X the respective MIC value (the MIC determined in YNB medium). When cultures reached approximately 10^8 organisms/ml, an aliquot was collected for susceptibility testing and a separate aliquot was passed into fresh medium containing the same concentration of drug. To ascertain the stability of a resistant isolate, that isolate was grown in inhibitor-free YNB medium with MICs measured in isolates from each passage. The cross resistance of the resistant isolate was also determined for all major classes of antifungal drugs.

Results

Table1: MIC susceptibility against *C. albicans* ATCC 36082.

MIC(µg/ml)			
Agent	**N1**	**N2**	**N3**
	RPMI medium		
VT-1161	0.0037	0.0037	0.0037
Fluconazole	0.5	0.5	1
	YNB medium		
VT-1161	0.03	0.015	0.03
Fluconazole	2	1	1

Figure 1: Induction of *C. albicans* resistance upon growth in medium containing VT-1161 (top) or fluconazole (bottom). For each time course, the MIC was determined in both YNB and RPMI media. Note, for isolates from growth in 2 µg/ml VT-1161 for more than10 days, MIC could not be determined in RPMI due to lack of growth of those isolates in that medium.

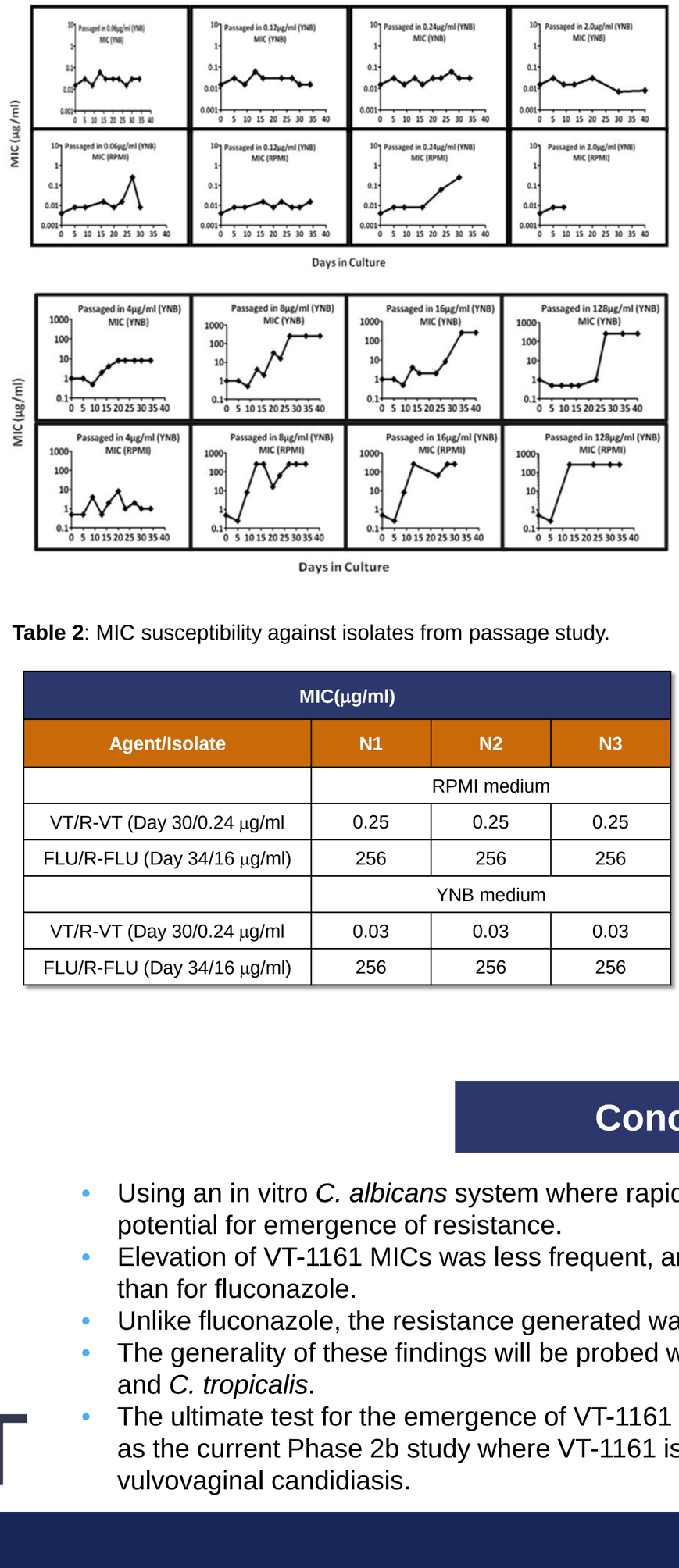


Table 2: MIC susceptibility against isolates from passage study.

MIC(µg/ml)			
Agent/Isolate	**N1**	**N2**	**N3**
	RPMI medium		
VT/R-VT (Day 30/0.24 µg/ml	0.25	0.25	0.25
FLU/R-FLU (Day 34/16 µg/ml)	256	256	256
	YNB medium		
VT/R-VT (Day 30/0.24 µg/ml	0.03	0.03	0.03
FLU/R-FLU (Day 34/16 µg/ml)	256	256	256

Figure 2 Stability of *C. albicans* resistance upon growth in absence of VT-1161 (top) or fluconazole (bottom).). For each time course, the MIC was determined in both YNB and RPMI media.

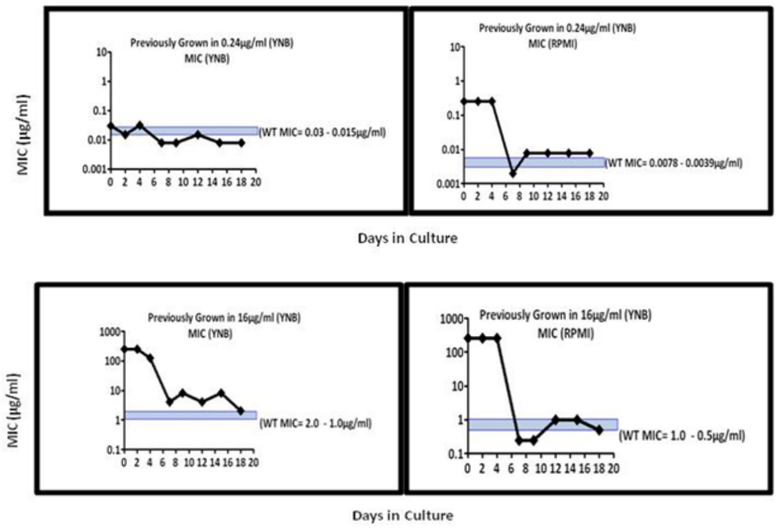


Table 3: MIC susceptibility of antifungals from different drug classes against C. albicans 36082 and isolates from passage study.

MIC (µg/ml) (in RPMI medium)					
Isolate	VT-1161	FLU	VOR	AMB	CAS
C. albicans ATCC 36082	0.0039	0.5	0.031	0.5	0.25
R-VT (Day 30/0.24 µg/ml	0.25	0.5	0.031	0.5	0.25
R-FLU (Day 34/16 µg/ml)	0.0019	256	4	0.5	0.25

References

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5. Schell, WA, et al. Investigational CYP51 Inhibitors VT-1161 and VT-1129 Show Strong *In Vitro* Activity Against *Candida glabrata* Isolates Clinically Resistant to Azole and Echinocandin Compounds. Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) 2015, San Diego, CA. F 740.
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Conclusions

- Using an in vitro *C. albicans* system where rapid resistance to fluconazole occurs, VT-1161 showed low potential for emergence of resistance.
- Elevation of VT-1161 MICs was less frequent, and the magnitude of MIC increase was less pronounced than for fluconazole.
- Unlike fluconazole, the resistance generated was not cross resistant with voriconazole.
- The generality of these findings will be probed with other *C. albicans* isolates and also with *C. glabrata* and *C. tropicalis*.
- The ultimate test for the emergence of VT-1161 resistance will be long-term clinical use of VT-1161, such as the current Phase 2b study where VT-1161 is dosed for up to 6 months in treatment of recurrent vulvovaginal candidiasis.

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