Activity of VT-1129 against *Cryptococcus neoformans* Clinical Isolates with High Fluconazole MICs

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Abstract

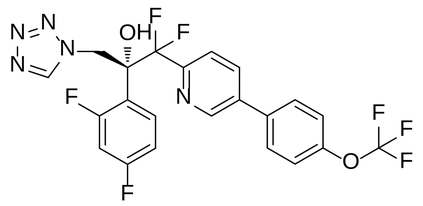
Background: Although antifungal drug resistance in the human fungal pathogen *Cryptococcus neoformans* is relatively uncommon, fluconazole-resistant strains have been identified and can be problematic in the context of both preemptive treatment in at-risk patient populations or during consolidation therapy for cryptococcal meningitis in persons living with AIDS. We determined the activity of VT-1129, an orally-available and selective inhibitor of fungal CYP51, in *Cryptococcus* isolates with high fluconazole minimum inhibitory concentrations (MICs).

Methods: Using broth microdilution assays, susceptibility to VT-1129 (1 to 0.0037 μg/mL) and fluconazole (64 to 0.25 μg/mL) was determined using a subset of 52 Ugandan clinical isolates of *Cryptococcus neoformans* that were previously shown to be FLU susceptible (MIC <16 μg/mL; N=18), dose-dependent susceptible (MIC=16-32 μg/mL; N=28), or resistant (MIC=64 μg/mL; N=6), with KN99α as a reference isolate. Growth inhibition at 72 hours of 50% and 100% was determined via spectrophotometric reading at 600nm.

Results: For the 51 isolates examined, the VT-1129 MIC range was 0.0038 to >1 μg/mL, the MIC_{50} was 0.025 μg/mL, and the MIC_{90} was 0.25 μg/mL, compared to fluconazole's MIC range of 0.5 to 64 μg/mL, and MIC_{50} and MIC_{90} values of 16 and 32 μg/mL, respectively. Among the 33 dose-dependent susceptible and resistant isolates, the MIC_{50} and MIC_{90} for VT-1129 were 0.05 and 0.5 μg/mL, respectively, only slightly higher than for susceptible strains. For these 33 isolates, the MIC_{50} and MIC_{90} for FLU were 16 and 64 μg/mL, respectively.

Conclusions: VT-1129 has robust activity against *Cryptococcus* isolates with elevated fluconazole MICs and may be an option in persons infected with such strains. Phase I studies for Viamet-1129 are expected to start in late 2015.

Fig 1: Chemical Structure of VT-1129



Introduction

Cryptococcal meningitis is the most common cause of adult meningitis in Sub-Saharan Africa and accounts for 15% of HIV/AIDS-related mortality. Treatment is limited due to the small number of available antifungals able to penetrate the central nervous system, and thus is impacted by rises in antifungal drug resistance. In two in vitro studies with large cohorts of clinical isolates, the new antifungal drug VT-1129 is highly effective against *Cryptococcus neoformans* and *C. gatti* with MICs against most isolates ≤0.03 μg/mL. To compare the potency of VT-1129 on *C. neoformans* isolates with dose-dependent susceptibility or resistance to fluconazole, we determined the MIC_{50} and MIC_{90} of VT-1129 on clinical isolates previously assayed for fluconazole susceptibility or resistance.

Materials & Methods

Clinical Isolates: *Cryptococcus neoformans* isolates were prospectively collected from 198 cerebrospinal fluid (CSF) cultures of research participants enrolled in the Cryptococcal Optimal ART Timing (COAT) trial in Uganda during 2010-12. A subset of 52 isolates were selected for testing, of which 34 had reduced fluconazole susceptibility.

52 clinical isolates of *C. neoformans* previously shown to be fluconazole susceptible (MIC ≤ 8 μg/mL, n=18 strains), dose-dependent (MIC 16 or 32 μg/mL, n=28 strains), or resistant (MIC ≥64 μg/mL, n=6 strains) were assayed for VT-1129 susceptibility.

Susceptibility Testing: Broth microdilution assays were performed according to CLSI guidelines using a cell concentration of $2.5x10^3$. Spectrophotometric analysis of well turbidity at 600 nm was used to determine the MIC for each strain. Plates were scanned in a Biotek Synergy H1 Hybrid reader (Winooski, VT) prior to and after 72 hours incubation at 35ºC.

VT-1129 MIC was determined using both a 50% reduction in growth and 100% inhibition of growth. The 100% inhibition in growth was defined as the drug concentration at which no growth was observed at 72 hours. A 50% reduction in growth was defined as the drug concentration at which well turbidity was less than or equal to 50% of the no drug control.

Results

In 52 *Cryptococcus neoformans* clinical isolates, the VT-1129 MIC was determined for 18 strains with fluconazole MIC ≤8 μg/mL, 28 strains with MIC of 16 or 32 μg/mL, and 6 strains with MIC ≥64 μg/mL (**Table 1**). VT-1129 MIC distribution using both 50% reduction in growth and 100% growth inhibition are presented in **Table 2**. The VT-1129 MIC_{50} and MIC_{90} differed slightly depending on whether 50% or 100% growth inhibition determined the MIC. Interestingly, 2 of 6 fluconazole resistant strains (MIC ≥64 μg/mL), 11 of 28 strains with dose-dependent susceptibility to fluconazole (MIC 16 or 32 μg/mL), and 13 of 18 strains susceptible to fluconazole (MIC ≤8 μg/mL) had VT-1129 MICs at or below the previously reported VT-1129 MIC_{50} for *C. neoformans* of 0.03 μg/mL (**Table 3**).

Table 1. Fluconazole MIC Distribution

Fluconazole Concentration (μg/mL)	MIC Distribution 50% growth inhibition	Overall Population MIC Distribution N=198
0.5	2 (4%)	8%
1	2 (8%)	16%
2	6 (19%)	25%
4	6 (31%)	44%
8	2 (35%)	69%
16	20 (73%)	87%
32	8 (88%)	97%
64	6 (100%)	100%

Table 2. VT-1129 MIC Distribution

VT-1129 Concentration (μg/mL)	Distribution at 50% growth inhibition	Distribution at 100% growth inhibition
0.00375	5 (10%)	4 (8%)
0.0075	12 (33%)	6 (19%)
0.015	9 (**50**%)	11 (40%)
0.025	7 (63%)	9 (**58**%)
0.05	6 (75%)	4 (65%)
0.1	7 (88%)	3 (71%)
0.25	3 (**94**%)	10 (**90**%)
0.5	1 (96%)	2 (94%)
1.0	2 (100%)	0 (94%)
>1.0		3 (100%)

MIC_{50}

MIC_{90}

Table 3. Line Listing of MICs (μg/mL)

Cryptococcus neoformans Isolate	Fluconazole MIC 50% growth inhibition	VT-1129 MIC 50% growth inhibition	VT-1129 MIC 100% growth inhibition
10005	0.5	0.025	0.025
10069	0.5	0.015	0.015
10030	1	0.0075	0.015
10104	1	0.0075	0.025
10049	2	0.0075	0.015
10052	2	0.015	0.015
10066	2	0.015	0.015
10107	2	0.0075	0.015
10256	2	0.015	0.015
10246	2	0.0075	0.0075
10018	4	0.0075	0.0075
10092	4	0.015	0.025
10112	4	0.0075	0.015
10165	4	0.00375	0.00375
10222	4	0.25	0.25
10246	4	0.015	0.015
10109	8	0.025	0.025
10199	8	0.00375	0.00375
10011	16	1	>1
10040	16	0.0075	0.0075
10051	16	0.025	0.025
10054	16	0.025	0.025
10062	16	0.00375	0.00375
10067	16	0.0075	0.0075
10072	16	0.015	0.5
10083	16	0.1	0.5
10114	16	0.0075	0.0075
10118	16	0.00375	0.00375
10176	16	0.0075	0.0075
10187	16	0.00375	0.015
10191	16	0.1	0.1
10194	16	0.025	0.025
10203	16	0.025	0.025
10213	16	0.1	0.25
10217	16	0.1	0.25
10224	16	0.1	0.1
10227	16	0.1	0.25
10247	16	0.025	0.25
10090	32	0.05	0.05
10117	32	0.25	0.25
10192	32	0.015	0.25
10214	32	0.5	>1
10225	32	0.05	0.1
10242	32	0.1	0.25
10168	32	0.05	0.05
10168	32	0.05	0.05
10054	64	0.05	0.05
10124	64	1.0	>1
10137	64	0.05	0.25
10218	64	0.075	0.025
10218	64	0.25	0.25
10233	64	0.015	0.015
KN99α Ref	2	0.025	0.05

Conclusions

- VT-1129 has robust activity against *Cryptococcus* isolates with elevated fluconazole MICs and may be a viable option in persons infected with such strains.
- A Phase 1 study of VT-1129 in healthy volunteers is scheduled to begin by the end of 2015. Phase 2 trials in persons with cryptococcal meningitis are targeted to begin by the end of 2016.