The Novel Fungal Cyp51 Inhibitor VT-1129 Demonstrates Potent *In vivo* Activity Against Cryptococcal Meningitis with an Improved Formulation

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BACKGROUND & OBJECTIVE

- Cryptococcosis is a significant cause of morbidity and mortality in immunocompromised patients. Cryptococcal meningitis is the most common manifestation of disseminated disease. This is a significant, life-threatening invasive fungal infection in HIV/AIDS patients.
- In developing countries, intravenous medications are often unavailable for the induction phase of therapy for cryptococcal meningitis. Even with appropriate therapy, morbidity and mortality in such countries remains unacceptably high.
- The investigational agent VT-1129 is a novel fungal Cyp51 inhibitor with potent *in vitro* and *in vivo* activity against *Cryptococcus* spp. (2010 ICAAC, Posters F1-850 and 853). Previous in vivo studies showed highly significant 2-3 log reductions of fungal burden and up to 90% survival with 10 and 20 mg/kg twice daily oral doses. These two doses (using a rudimentary formulation of the parent molecule) produced mean plasma trough levels of 1.1 and 3.5 μg/ml, respectively.
- A new solid-state formulation has been developed to test if lower doses could be as efficacious and if equivalent doses could have even greater efficacy in a murine model of cryptococcus meningitis.

MATERIALS & METHODS

***Cryptococcus neoformans* Isolate**

- *C. neoformans* clinical isolate USC 1597 was used, and was placed into brain heart infusion broth overnight in a shaking incubator. The cells were then collected and washed three times in sterile saline.
- Inocula concentrations were determined using a hemocytometer and confirmed by plating serial dilutions and colony counts.

Murine Model of Cryptococcal Meningitis

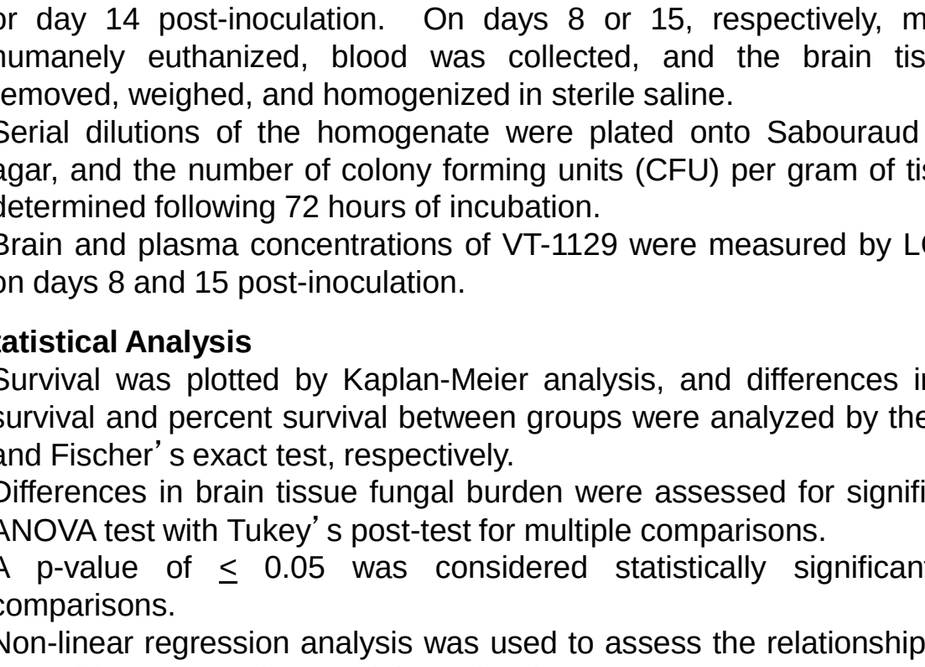
- Immunocompetent ICR mice were anesthetized and inoculated intracranially through a midline puncture in the cranial vault (Nguyen et al. *Antimicrob Agents Chemother* 1997; 41: 1120-3).
- Antifungal therapy was initiated 24 hours after inoculation and consisted of the following groups:
  1. VT-1129 0.1 to 20 mg/kg/day by oral gavage
  2. Fluconazole (FLU) 10 or 20 mg/kg twice daily by oral gavage
  3. Placebo Control
- Survival Arm - antifungal therapy was continued through day 10 post-inoculation, and animals were followed off therapy until day 30.
- Fungal Burden Arm - antifungal therapy was continued either through day 7 or day 14 post-inoculation. On days 8 or 15, respectively, mice were humanely euthanized, blood was collected, and the brain tissue was removed, weighed, and homogenized in sterile saline.
- Serial dilutions of the homogenate were plated onto Sabouraud dextrose agar, and the number of colony forming units (CFU) per gram of tissue was determined following 72 hours of incubation.
- Brain and plasma concentrations of VT-1129 were measured by LC/MS/MS on days 8 and 15 post-inoculation.

Statistical Analysis

- Survival was plotted by Kaplan-Meier analysis, and differences in median survival and percent survival between groups were analyzed by the log rank and Fischer's exact test, respectively.
- Differences in brain tissue fungal burden were assessed for significance by ANOVA test with Tukey's post-test for multiple comparisons.
- A p-value of ≤ 0.05 was considered statistically significant for all comparisons.
- Non-linear regression analysis was used to assess the relationship between VT-1129 concentrations and tissue burden.

RESULTS

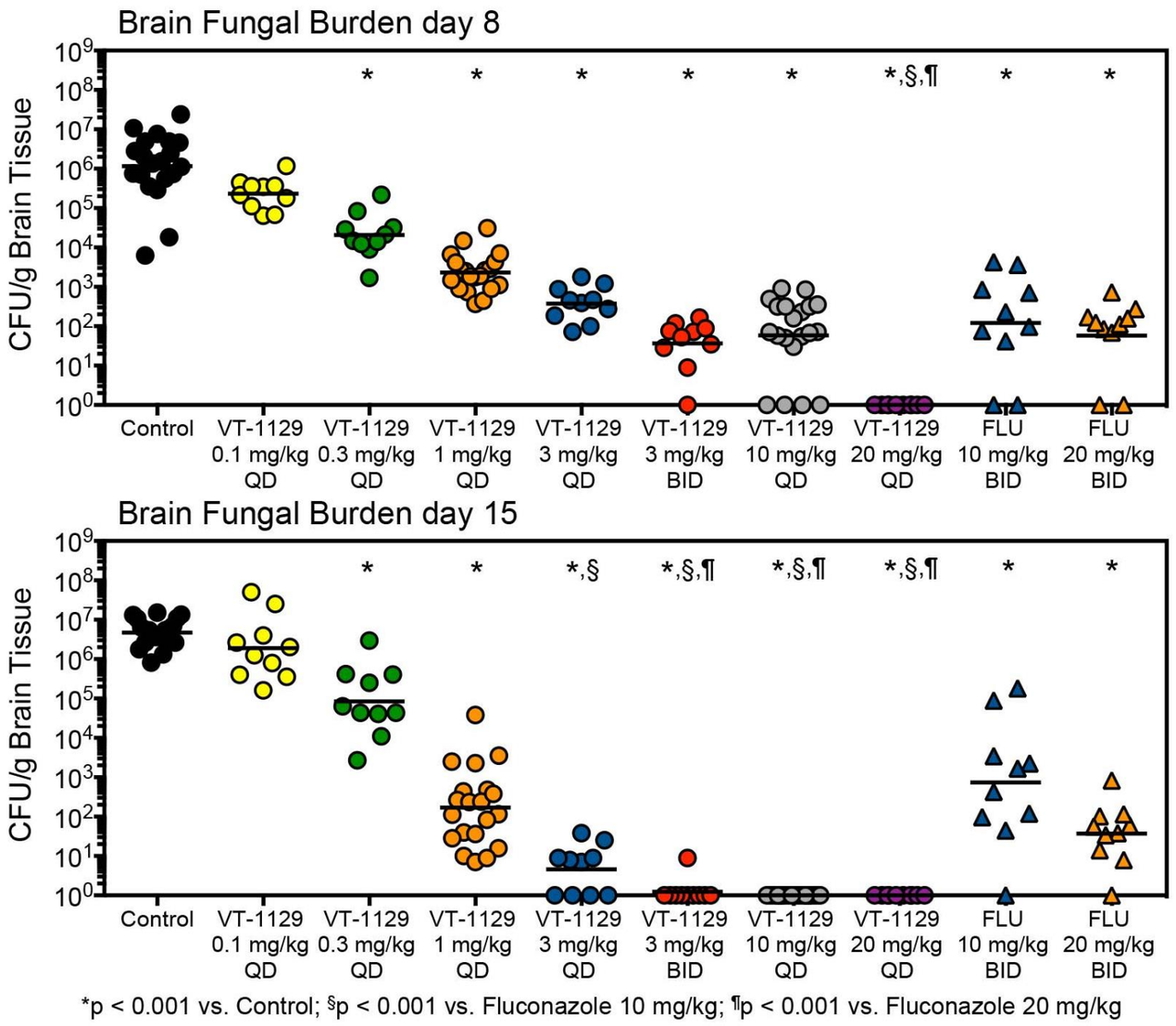
Figure 1. Survival data for A.) VT1129 and B.) fluconazole to 30 days post-inoculation in mice infected intracranially with *C. neoformans*.



*p < 0.05 vs. Control; §p < 0.05 vs. Fluconazole

RESULTS (continued)

Figure 2. Brain tissue fungal burden data on days 8 & 15 post-inoculation.

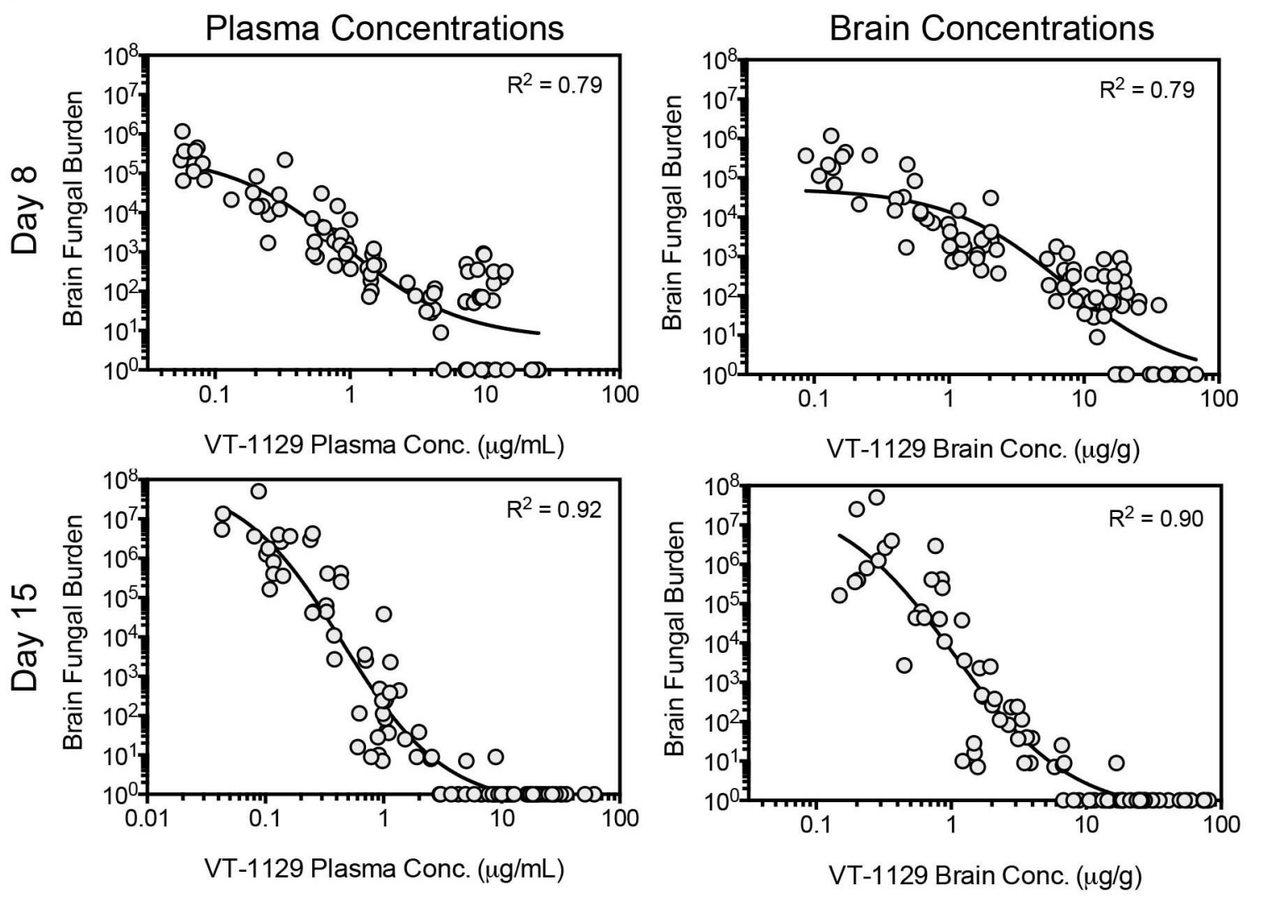


*p < 0.001 vs. Control; §p < 0.001 vs. Fluconazole 10 mg/kg; ¶p < 0.001 vs. Fluconazole 20 mg/kg

Table 1. Mean (SD) brain tissue fungal burden & plasma concentrations (μg/mL) on days 8 & 15.

Group	Control	VT-1129 0.1 mg/kg QD	VT-1129 0.3 mg/kg QD	VT-1129 1 mg/kg QD	VT-1129 3 mg/kg QD	VT-1129 3 mg/kg BID	VT-1129 10 mg/kg QD	VT-1129 20 mg/kg QD
Log CFU/g Day 8	6.06 (0.45)	5.37 (0.39) (Δ -0.69 log CFU/g)	4.31 (0.57) (Δ -1.75 log CFU/g)	3.36 (0.48) (Δ -2.7 log CFU/g)	2.58 (0.45) (Δ -3.48 log CFU/g)	1.56 (0.66) (Δ -4.5 log CFU/g)	1.77 (1.00) (Δ -4.29 log CFU/g)	0.0 (0) (Δ -6.06 log CFU/g)
Plasma Conc. Day 8	---	0.067 (0.010)	0.238 (0.060)	0.751 (0.157)	1.41 (0.183)	4.31 (1.22)	9.35 (2.39)	21.4 (4.38)
Log CFU/g Day 15	6.67 (0.35)	6.28 (0.80) (Δ -0.39 log CFU/g)	4.93 (0.87) (Δ -1.74 log CFU/g)	2.23 (0.98) (Δ -4.44 log CFU/g)	0.66 (0.61) (Δ -6.61 log CFU/g)	0.09 (0.30) (Δ -6.58 log CFU/g)	0.0 (0) (Δ -6.67 log CFU/g)	0.0 (0) (Δ -6.67 log CFU/g)
Plasma Conc. Day15	---	0.117 (0.166)	0.337 (0.726)	0.951 (0.190)	2.96 (1.11)	8.16 (2.21)	15.5 (5.31)	32.9 (13.2)

Figure 3. Concentration-response curves in plasma and brain tissue on days 8 & 15.



CONCLUSIONS

A new formulation of VT-1129 was efficacious in this murine model of cryptococcal meningitis. Relative to previous studies, lower doses of the new VT-1129 formulation demonstrated similar efficacy and plasma and brain exposures. Efficacy was further improved and higher exposures were achieved with higher doses, and brain CFUs were undetectable at these doses. These data demonstrate the potential utility of VT-1129 to have a marked impact in the treatment of cryptococcal meningitis.

This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. HHSN261200800001E, and the National Center for Advancing Translation Sciences