M-433

The University of Arizona
John Galgiani
520-626-4968
spherule@email.arizona.edu

VT-1161, a Novel Fungal CYP51 Inhibitor, Improved Survival in Murine Models of Coccidioidomycosis

LF Shubitz,[1] HT Trinh,[1] **JN Galgiani**,[1] ML Lewis,[1] EP Garvey,[2] WJ Hoekstra,[2] WR Moore,[2] RJ Schotzinger[2]
[1]The University of Arizona, Tucson, AZ; [2]Viamet Pharmaceuticals, Durham, NC, USA

Abstract

Background: VT-1161 (VT), a novel CYP51 inhibitor with low toxicity and a long half-life, is currently in Phase IIa studies. The MIC_{50} against *Coccidioides* spp. is 1 µg/ml.

Methods: Swiss-Webster mice received lethal doses of *Coccidioides posadasii*. Mice infected intranasally (i.n.) with 500 spores were treated from 120 h post-infection (p.i.) for 7 d with VT 10 mpk (VT10) or 50 mpk (VT50), 50 mpk of fluconazole (FLU), or placebo (P), and sacrificed 1 d following the end of treatment (n=8) or 14 d later (n=8). Other mice were infected intracerebrally (i.c.) with 70-90 spores, and were treated from 48 h p.i. for 7 d with VT 25 mpk (VT25), VT50, FLU, or P and sacrificed 2 d after treatment. In an i.c. survival study, mice were treated for 14 d with VT25, FLU, or P and sacrificed up to 28 d later. Fungal burdens of lungs (LFB) or of brains/spinal cords were compared by ANOVA for significance of differences.

Results: In i.n. mice, all antifungal treatments reduced LFB compared to P (P≤0.03) and none died; all P mice died by d 13. LFB of treated mice were similar to each other 1 d after treatment. However, two weeks after treatment, VT50 mice had significantly lower LFB than VT10 and FLU mice (P<0.001 for both). In mice with CNS infection treated for 7 days, all P mice died before scheduled sacrifice. Brain and spinal cord fungal burdens were decreased in VT mice compared to P or FLU mice (P≤0.007). VT plasma concentrations were 26 and 52 µg/ml in the VT25 and VT50 mice, respectively, 2 d after last dose. In the CNS survival study, all VT25 or FLU mice survived the treatment period, while P mice died between days 7 and 9. Of 13 FLU mice, 10 died within 7 d of treatment. VT mice remained healthy until the middle of the third week when some developed transient CNS signs and/or lost weight, and two were euthanized in week 4. VT mice lived longer than FLU mice or P mice (P<0.001), and had lower brain and spinal cord fungal burdens. Four weeks following treatment, mean plasma concentration of VT was 1.5 µg/ml.

Conclusions: VT-1161 has a long half- life and significantly reduced lung, brain and spinal cord fungal burden and dissemination to other organs compared to P or FLU, as well as extending survival, in murine models of coccidioidomycosis. VT-1161 merits further exploration as a treatment for this potentially deadly disease.

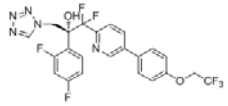
Background

Coccidioidomycosis

- Respiratory and systemic fungal infection endemic to the southwestern United States and Mexico.
- Infection ranges from asymptomatic to fatal. Coccidioidal meningitis is a severe and life-threatening consequence of infection (1).
- Coccidioides is a possible bioterrorism weapon (NIAID Cat. C agent) (2).

VT-1161

- Highly selective for fungal CYP51 versus human CYPs (3).
- Crosses blood-brain barrier.
- Long half life (>24 hrs) in all species examined.
- MIC_{50} *C. posadasii*, strain Silveira, is 1 µg/ml and MIC_{100} is 4 µg/ml.
- In Phase 2a studies for superficial and mucosal fungal infections.



Methods

- **Mice:** 8-week old F Swiss-Webster (Harlan Sprague Dawley, Indianapolis).
- **Fungal Strain:** *Coccidioides posadasii*, strain Silveira, arthroconidia (spores) were counted with a hemacytometer and viability was determined by colony-forming units (CFU) on glucose-yeast extract (GYE) plates.(2) Final suspensions in sterile 0.9% saline were adjusted to deliver **500 spores** in 30 µl for the **respiratory** challenge and **90 spores** in 30 µl for the **CNS** infection (4,5).
- **Drug Treatments: VT-1161** was provided as a powder (Viamet Pharmaceuticals, Inc, Durham, NC) and dissolved in 20% Cremaphor EL (Sigma, St. Louis, MO). **Fluconazole** was purchased commercially and concentration adjusted with sterile water. 20% Cremaphor EL was used for **placebo**. **All treatments were administered by oral gavage once (VT-1161, placebo) or twice (fluconazole) daily.**

Methods

Study 1

- Mice (n=16/grp) were infected IN under anesthesia.(6)
- Treatment as follows for 7 days, starting 120 h p.i.:
  Grp 1: VT-1161 10 mpk once daily (**VT10**)
  Grp 2: VT-1161 50 mpk once daily (**VT50**)
  Grp 3: Fluconazole 50 mpk, divided BID (**Flu**)
  Grp 4: Placebo Cremaphor EL once daily (**P**)
- 8 mice were sacrificed 1 d post-treatment (p.t.) (d 12 p.i.) and 8 sacrificed 14 d p.t. (d 26 p.i.); moribund mice were sacrificed as needed.
- Lungs were quantitatively cultured by serial dilution (6); spleens were plated whole to assess dissemination.
- Plasma was collected from VT-1161-treated mice at euthanasia, sterilized, and frozen for analysis.

Study 2

- Mice (n=11/grp) were anesthetized, skulls shaved and prepped, and injected with 30 µl inoculum intracerebrally (IC) with 29G needle.(3)
- Treatment as follows for **7 days,** starting 48 h p.i.:
  Grp 1: VT-1161 25 mpk once daily (**VT25**)
  Grp 2: **VT50** once daily
  Grp 3: **Flu** divided BID
  Grp 4: **P** once daily
- Mice were sacrificed 2 days p.t. and plasma collected as for Study 1.
- Brains and spinal cords were weighed, homogenized, and CFU determined on a per gram of tissue basis; lungs, livers and spleens were plated whole to assess dissemination.

Study 3

- Mice (n=13/grp) were infected as for Study 2. Treatment started 48 h p.i. for **2 weeks** as follows:
  Grp 1: **VT50** once daily
  Grp 2: **Flu** divided BID
  Grp 3: **P** once daily
- Mice were sacrificed 4 weeks p.t. to assess survival. VT-1161-treated mice were bled for plasma as above and brains and spinal cords cultured quantitatively as in Study 2. Lungs, livers and spleens were plated whole to determine dissemination.

***Plasma drug levels*:** VT-1161 concentration was measured by LC-MS from the frozen plasma and quantified by linear regression compared to a standard curve.

Statistical analysis: Fungal burdens (CFU) were log transformed and compared by ANOVA. Survival was compared using Kruskall-Wallis. (Systat 8.0) Differences were significant at P<0.05.

Results

Study 1 – Respiratory infection

- Mice were sacrificed one day and 14 days p.t. All **P** mice died by d 13 (most moribund d 12). Drug-treated groups were healthy throughout treatment. **VT10** mice lost weight (data not shown) and one died 3 d p.t; all other drug-treated mice survived until end of study.
- Antifungal treatment significantly reduced lung fungal burden compared to placebo (P≤0.03, all comparisons).
- Antifungal treatments did not differ from each other 1 d p.t., but at 2 weeks p.t., **VT50** was better than **VT10** (P<0.001) and **Flu** (P<0.001). (Table 1)

Table 1 – 1 day and 2 weeks post-treatment for primary lung infection

	Mean Lung Fungal Burdens (Log10 CFU)			
	VT10	VT50	Flu	P
day 1	5.60	5.24	5.4	7.56
day 14	5.58	3.99	5.32	7.58

Results

Study 1 - Dissemination

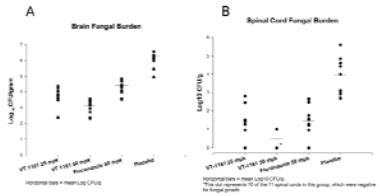
	Splenic Dissemination			
	VT50	VT10	Flu	P
Day 1	0/8	0/8	0/8	8/8
Day 14	1/8	6/7[1]	3/4[2]	7/8

[1]1 died of gavage; [2]3 mice accidentally euthanized d 1 p.t., and1 mouse deemed uninfected based on no fungus found at sacrifice d 14

Study 2 - 1 Week Treatment of CNS Infection

- Mice received 90 spores IC. **P** mice were euthanized with CNS signs on days 7-8 p.i. All antifungal treated mice remained healthy until sacrifice on d 10 p.i.
- **VT25** and **VT50** significantly reduced brain cfu/g compared to **Flu** (P≤0.007) and **P** (P<0.001). (Fig. 1a) Spinal cord data were similar, though 10/11 VT50 mice had no fungal growth. (Fig. 1b)

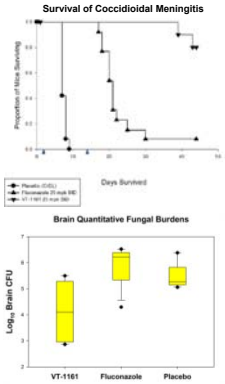
Fig. 1



Study 3 - 2 Week Treatment for CNS Infection

- Mice received 70 spores IC. All **P** mice died between 7-9 days p.i.
- **Flu** mice were healthy during treatment, but began to develop CNS signs and require euthanasia within 48 h of stopping; all but one died prior to scheduled sacrifice. (Fig. 2)
- **VT25** mice remained clinically normal until the third week p.t.; 2 died prior to scheduled sacrifice with weight loss and dehydration, not neurologic signs. (Fig. 2)

Fig. 2



- All **Flu** and **P** mice disseminated to lung, liver and/or spleen; only 5/10 **VT25** mice, including early deaths.
- **VT25** – 5/10 spinal cords neg.
- None of the brains were sterile, though CFU significantly lower in **VT25** than **Flu** or **P** (P≤0.001). (Fig. 3)

Results

Plasma Levels of VT-1161

Table 2 - VT-1161 plasma concentrations (mean ± std deviation) from murine coccidioidomycosis studies

VT-1161 dose	Respiratory Study		CNS Study	
	1 day PT[1]	14 days PT	2 days PT	28 days PT
10 mg/kg	11 ± 2 µg/ml[2]	0.9 ± 0.5µg/ml[2]	-	-
25 mg/kg	-	-	26 ± 8 µg/ml[2]	1.5 ±0.5 µg/ml[3]
50 mg/kg	46 ± 14 µg/ml[2]	23 ± 8 µg/ml[2]	52 ± 24 µg/ml[2]	-

[1]Post-treatment; [2]Dosed once daily for 7 days; [3]Dosed once daily for 14 days

- Plasma levels of VT-1161 accumulated with repeat daily doses.
- Proportional with dose and reproducible between studies.
- Estimated half-lives from these data were 3-14 days depending on dose.

VT-1161 Conclusions

- Significantly reduced fungal burden and prolonged survival in two fatal models of coccidioidomycosis in mice, pulmonary and CNS.
- More efficacious than fluconazole at prolonging survival and suppressing fungal burden in a CNS model of coccidioidomycosis.
- Not fungicidal with any dose or treatment times tested in these models, consistent with the fungistatic mechanism of CYP51 inhibition.
- Long half-life and strong selective binding for fungal cytochrome vs. mammalian have the following potential advantages over azole class antifungals:
  - reduced frequency of dosing
  - improved efficacy
  - fewer side effects
- Merits further exploration and development as a treatment for coccidioidomycosis.

References

1) Xxx
2) Dixon, DM. 2001. Coccidioides immitis as a select agent of bioterrorism. J. Appl. Microbiol. **91**:602-605.
3) Warrilow AGS, Mertel CM, Parker JE, Lamb DC, Garvey EP, Hoekstra WJ, Moore WR, and Schotzinger RJ, Kelly DE, Kelly SL: The clinical antifungal, VT-1161, is a highly potent inhibitor of *Candida albicans*, but not human, CYP51. Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), 2011; F1-1386.

Acknowledgements

This work was funded by NIH NIAID grant number 1-R21-AI101497-01 and by Viamet Pharmaceuticals, Inc. Plasma concentrations of VT-1161 were determined at OpAns, LLC (Durham, NC).