Title

Prophylaxis with Isavuconazole or Posaconazole Protects Immunosuppressed Mice from Pulmonary Mucormycosis

Journal

Antimicrobial Agents and Chemotherapy, 61(5)

ISSN

0066-4804

Authors

Gebremariam, Teclegiorgis
Alkhazraji, Sondus
Baldin, Clara
et al.

Publication Date

2017-05-01

DOI

10.1128/aac.02589-16

Peer reviewed

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EXPERIMENTAL THERAPEUTICS

Prophylaxis with Isavuconazole or Posaconazole Protects Immunosuppressed Mice from Pulmonary Mucormycosis

Teclegiorgis Gebremariam,[a] Sondus Alkhazraji,[a] Clara Baldin,[a] Laura Kovanda,[b] Nathan P. Wiederhold,[c] Ashraf S. Ibrahim[a,d]

Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor-University of California Los Angeles (UCLA) Medical Center and St. John's Cardiovascular Research Center, Torrance, California, USA[a]; Astellas Pharma Global Development, Inc., Northbrook, Illinois, USA[b]; University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA[c]; David Geffen School of Medicine at UCLA, Los Angeles, California, USA[d]

ABSTRACT We assessed prophylactic or continuous therapy of isavuconazole, posaconazole, or voriconazole in treating pulmonary murine mucormycosis. In the prophylaxis studies, only isavuconazole treatment resulted in significantly improved survival and lowered tissue fungal burden of immunosuppressed mice infected with *Rhizopus delemar*. In the continuous treatment studies, isavuconazole and posaconazole, but not voriconazole, equally prolonged survival time and lowered tissue fungal burden compared to placebo-treated mice. These results support the use of isavuconazole and posaconazole in prophylaxis treatment.

KEYWORDS *Rhizopus delemar*, isavuconazole, posaconazole, prophylaxis, mucormycosis, murine, voriconazole

Mucormycosis is a life-threatening infection that occurs predominantly in immunocompromised patients (e.g., diabetic ketoacidosis [DKA] or neutropenia) and is caused by the ubiquitous Mucorales, among which *Rhizopus* is the predominant cause of infection (1–3). Despite disfiguring surgical debridement and adjunctive antifungal therapy, the overall mortality of mucormycosis remains approximately 50% and can approach 100% in hematogenously disseminated disease and central nervous system (CNS) disease as well as in patients with prolonged neutropenia (2, 4–8). Clearly, new strategies to prevent and treat mucormycosis are urgently needed.

In the past 2 decades, mucormycosis has emerged as an important invasive fungal infection in hematologic malignancies, bone marrow transplant (BMT) and solid-organ transplant (SOT) (9, 10). These high-risk patients are often receiving prophylaxis with the azole voriconazole (VOR) or posaconazole (POS). Although VOR is used as a first-line therapy against aspergillosis, it is ineffective against Mucorales. Indeed, outbreaks of mucormycosis cases in patients who received VOR prophylaxis are frequently described (6, 11–14). In contrast, POS has activity against Mucorales *in vitro*, but breakthrough mucormycosis infections (especially with *Rhizopus* species) among patients who received POS prophylaxis have also been reported (15, 16).

Isavuconazole (ISA) has recently been approved by the U.S. Food and Drug Administration (FDA) and the European Commission for treating invasive mucormycosis. We have demonstrated the efficacy of ISA in treating mice infected with *Rhizopus delemar* (17). Hence, we wanted to assess the prophylactic activity of ISA compared to POS and VOR in protecting neutropenic mice from pulmonary mucormycosis.

Animal studies described here were approved by the IACUC of the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, according to the NIH

Received 6 December 2016 **Returned for modification** 29 December 2016 **Accepted** 25 February 2017

Accepted manuscript posted online 6 March 2017

Citation Gebremariam T, Alkhazraji S, Baldin C, Kovanda L, Wiederhold NP, Ibrahim AS. 2017. Prophylaxis with isavuconazole or posaconazole protects immunosuppressed mice from pulmonary mucormycosis. Antimicrob Agents Chemother 61:e02589-16. https://doi.org/10.1128/AAC.02589-16.

Address correspondence to Ashraf S. Ibrahim, ibrahim@labiomed.org.

guidelines for animal housing and care (18). Male CD-1 mice (20 to 25 g from Envigo, Indianapolis, IN, USA) were used in this study. They were given irradiated feed and sterile water containing 50 mg/liter Baytril (enrofloxacin: Bayer, Leverkusen, Germany) *ad libitum* on day −3 and then switched to daily ceftazidime (5 mg/mouse) subcutaneous (s.c.) treatment starting day 0 through day +13, relative to infection with *R. delemar* 99-880 (a brain isolate obtained from the Fungus Testing Laboratory at the University of Texas Health Science Center at San Antonio). Neutropenia was induced by cyclophosphamide (200 mg/kg of body weight, administered intraperitoneally [i.p.]) and cortisone acetate (500 mg/kg, s.c.) on days −2, +3, and +8 relative to infection. The treatment regimens resulted in ~16 days of leukopenia with the total white blood cell count dropping from ~130,000/cm^3 to almost no leukocytes detected as determined by using a Unopette system (Becton-Dickinson and Co.). Whenever mice were treated with VOR (Pfizer Pharmaceuticals), they were given 50% grapefruit juice in lieu of drinking water to inhibit metabolism of the drug by cytochrome P450 enzymes (19).

Pharmacokinetic (PK) studies were conducted using ISA (prodrug isavuconazonium sulfate from Astellas USA), POS (oral suspension from Merck & Co., Inc.), and VOR at 110, 30, and 40 mg/kg, respectively. The doses for ISA and POS were chosen because of prior demonstration of activity in murine pulmonary mucormycosis (20, 21). Drugs were given orally three times a day (t.i.d.) for ISA and POS and once daily for VOR for 4 consecutive days. Four hours following the last dose of each drug, the mice were sacrificed, and total blood was collected by cardiac puncture. An analytical method that uses ultraperformance liquid chromatography and single-quad mass spectrometry (UPLC/MS) for the determination of itraconazole and hydroxylitraconazole was adapted and validated for the measurement of ISA and POS (20, 22), while high-performance liquid chromatography (HPLC) was used to measure VOR levels. All three drugs demonstrated serum drug levels in excess of MIC values (defined as the lowest drug concentration that causes 100% growth inhibition) against *R. delemar* 99-880 (MIC values of 0.5, 0.78, and 8.0 μg/ml for ISA, POS, and VOR, respectively [17, 23]) (Fig. 1A).

Next, the efficacy of each of the azoles given as prophylaxis or continuous treatment at the doses investigated in the PK studies was determined. For prophylaxis, treatment started on day −2 and continued until day 0, when the mice were infected 4 h after the last treatment. For continuous therapy, treatment started on day −2 and ended on day +2 relative to infection. Immunosuppressed mice were infected intratracheally (23) with a targeted inoculum of 2.5×10^5 spores of *R. delemar* 99-880. The primary endpoint for efficacy was the time to moribundity of infected mice, while tissue fungal burden in lungs and brains harvested from mice sacrificed on day +3 served as the secondary endpoint. Tissue fungal burden was determined by quantitative PCR (qPCR) (24).

In the prophylaxis studies, only ISA prolonged survival time of mice with pulmonary mucormycosis, albeit this protection was modest with 21-day survival of ~20% for ISA versus 0% for placebo treatment and a median survival time of 15 versus 9 days for ISA and placebo treatment, respectively (Fig. 1B). The improved survival in ISA-treated mice was accompanied by an ~0.5-log-unit reduction in the lung, but not the brain, fungal burden compared to placebo-treated mice (Fig. 1C). With the continuous therapy, both ISA and POS resulted in 50% long-term survival of mice infected with *R. delemar*, whereas VOR did not improve survival versus placebo (Fig. 2A). Protection elicited by ISA or POS was further corroborated by ~1-log-unit reduction in lung and brain fungal burden versus placebo-treated mice (Fig. 2B).

Previously we have established that the protective activity of ISA is similar to that of liposomal amphotericin B in treating immunosuppressed mice with pulmonary mucormycosis caused by *Rhizopus* or *Mucor* species (17, 20). The efficacious animal data combined with a recently conducted single-arm open-label clinical trial of ISA in the treatment of mucormycosis (25) and case-control analysis resulted in the FDA and European Commission approving ISA for treating invasive mucormycosis. In the present study, we wanted to compare the activity of ISA to currently used azoles with respect to protection against mucormycosis in the prophylaxis setting. At the doses and

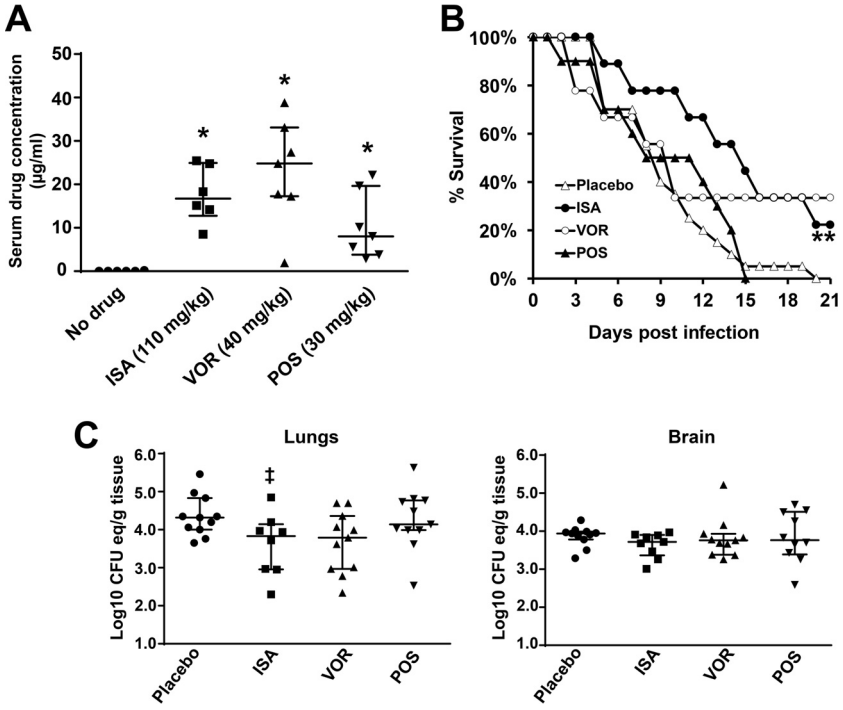

FIG 1 ISA prophylaxis protects immunosuppressed mice from *R. delemar* pulmonary infection. (A) Serum azole concentration of mice (six or seven mice in each group) treated with ISA or POS three times daily or VOR once daily for 4 days at the specified doses. Sera were collected 4 h after the last dose. *, $P < 0.05$ compared to the value for the placebo-treated group (no drug) by the Wilcoxon rank sum test. (B) Survival of mice (20 mice for the placebo group and 9 or 10 mice for all other groups) treated prophylactically (day −2 until day 0 prior to infection) with ISA, POS, or VOR and then infected with *R. delemar* (inhaled inoculum of 4.1×10^3 per mouse). **, $P = 0.01$ compared to the value for the placebo-treated group by the log rank test. (C) Fungal burdens in lungs and brains (9 to 11 mice per arm) harvested on day +3 postinfection. Data are expressed as median ± interquartile range. ‡, $P = 0.03$ compared to the value for the placebo-treated group by the Wilcoxon rank sum test.

treatment regimen tested, we established that ISA, POS, and VOR had serum drug levels that exceeded their MICs against *R. delemar*. At these doses, ISA prophylaxis was superior to POS prophylaxis in protecting mice from pulmonary mucormycosis, while both drugs were equally efficacious in continuous therapy. The difference in efficacy between ISA and POS when administered prophylactically could be due to the possible altered drug levels in the target organs of lungs and brains. For example, it is possible that higher ISA exposure may have been achieved in the lungs and brains after 3 days of dosing compared to that of POS. This assumption is supported by the equal efficacy of ISA and POS when administered in continuous therapy, which lasted for 5 days of treatment.

As expected, the activities of ISA and POS administered as continuous therapy exceeded their respective activities when given as prophylaxis, since the effective treatment continued after infection. Continuous therapy is more likely to be clinically relevant, since it represents treatment of a breakthrough infection while patients are on prophylaxis.

In contrast, VOR was not efficacious in protecting mice from mucormycosis despite the fact that serum VOR levels were threefold higher than the MIC of 8 μg/ml. Prophylaxis of BMT patients with VOR has been widely linked to breakthrough infections with mucormycosis (26, 27), and it is acceptable that VOR does not possess activity against Mucorales. In fact, it has been shown that treatment of *Rhizopus* with VOR prior to infecting mice or *Drosophila* increases the virulence of this mold (28). In summary, given their broad-spectrum activities, our findings support the use of ISA and POS in breakthrough infections in patients who are at high risk of contracting mucormycosis.

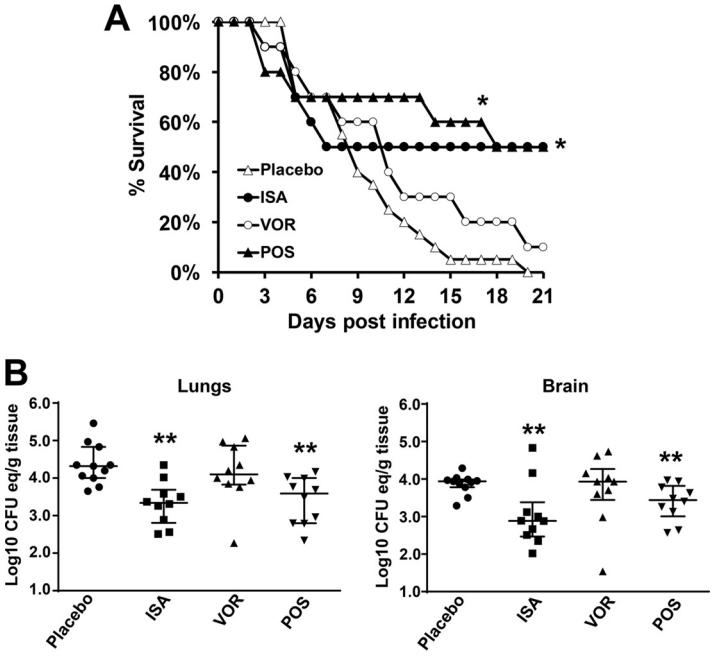

FIG 2 ISA or POS continuous therapy prolongs survival time and reduces tissue fungal burden of immunosuppressed mice with pulmonary mucormycosis due to *R. delemar*. (A) Survival of mice (20 mice in the placebo group and 10 mice for all other groups) treated continuously (day −2 through day +2) with ISA, POS, or VOR. Mice were infected on day 0 with *R. delemar* (inhaled inoculum of 4.1×10^3 per mouse). Values for ISA- or POS-treated mice that are significantly different ($P < 0.05$) from the value for mice treated with placebo by the log rank test are indicated by an asterisk. (B) Fungal burdens of lungs and brains (9 to 11 mice per arm) were harvested on day +3 postinfection. Data are expressed as median ± interquartile range. **, $P < 0.05$ compared to the value for the placebo-treated group by the Wilcoxon rank sum test.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant R01 AI063503 and research and educational grants from Astellas Pharma US to A.S.I. Research described in the manuscript was conducted in part at the research facilities of the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center.

REFERENCES

1. Chakrabarti A, Chatterjee SS, Das A, Panda N, Shivaprakash MR, Kaur A, Varma SC, Singhi S, Bhansali A, Sakhuja V. 2009. Invasive zygomycosis in India: experience in a tertiary care hospital. Postgrad Med J 85:573–581. https://doi.org/10.1136/pgmj.2008.076463.
2. Marr KA, Carter RA, Crippa F, Wald A, Corey L. 2002. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis 34:909–917. https://doi.org/10.1086/339202.
3. Rammaert B, Lanternier F, Zahar JR, Dannaoui E, Bougnoux ME, Lecuit M, Lortholary O. 2012. Healthcare-associated mucormycosis. Clin Infect Dis 54(Suppl 1):S44–S54. https://doi.org/10.1093/cid/cir867.
4. Gleissner B, Schilling A, Anagnostopolous I, Siehl I, Thiel E. 2004. Improved outcome of zygomycosis in patients with hematological diseases? Leuk Lymphoma 45:1351–1360. https://doi.org/10.1080/10428190310001653691.
5. Ibrahim AS, Edwards JE, Jr, Filler SG, Spellberg S. 2011. Mucormycosis and entomophthoramycosis (zygomycosis), p 265–280. *In* Kauffman CA, Pappas PG, Sobel JD, Dismukes WE (ed), Essentials of clinical mycology, 2nd ed. Springer, New York, NY.
6. Kauffman CA. 2004. Zygomycosis: reemergence of an old pathogen. Clin Infect Dis 39:588–590. https://doi.org/10.1086/422729.
7. Kontoyiannis DP, Wessel VC, Bodey GP, Rolston KV. 2000. Zygomycosis in the 1990s in a tertiary-care cancer center. Clin Infect Dis 30:851–856. https://doi.org/10.1086/313803.
8. Husain S, Alexander BD, Munoz P, Avery RK, Houston S, Pruett T, Jacobs R, Dominguez EA, Tollemar JG, Baumgarten K, Yu CM, Wagener MM, Linden P, Kusne S, Singh N. 2003. Opportunistic mycelial fungal infections in organ transplant recipients: emerging importance of non-Aspergillus mycelial fungi. Clin Infect Dis 37:221–229. https://doi.org/10.1086/375822.
9. Skiada A, Pagano L, Groll A, Zimmerli S, Dupont B, Lagrou K, Lass-Florl C, Bouza E, Klimko N, Gaustad P, Richardson M, Hamal P, Akova M, Meis JF, Rodriguez-Tudela JL, Roilides E, Mitrousia-Ziouva A, Petrikkos G. 2011. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007. Clin Microbiol Infect 17:1859–1867. https://doi.org/10.1111/j.1469-0691.2010.03456.x.
10. Lanternier F, Dannaoui E, Morizot G, Elie C, Garcia-Hermoso D, Huerre M, Bitar D, Dromer F, Lortholary O. 2012. A global analysis of mucormycosis in France: the RetroZygo Study (2005-2007). Clin Infect Dis 54(Suppl 1):S35–S43. https://doi.org/10.1093/cid/cir880.
11. Marty FM, Cosimi LA, Baden LR. 2004. Breakthrough zygomycosis after voriconazole treatment in recipients of hematopoietic stem-cell transplants. N Engl J Med 350:950–952. https://doi.org/10.1056/NEJM200402263500923.
12. Imhof A, Balajee SA, Fredricks DN, Englund JA, Marr KA. 2004. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. Clin Infect Dis 39:743–746. https://doi.org/10.1086/423274.
13. Ide L, Buysschaert I, Demuynck H, De Man R, Verlinde A, De Laere E, Surmont I. 2004. Zygomycosis in neutropenic patients with past Asper-

gillus infection: a role for posaconazole? Clin Microbiol Infect 10:862–863. https://doi.org/10.1111/j.1469-0691.2004.00945.x.
14. Vigouroux S, Morin O, Moreau P, Mechinaud F, Morineau N, Mahe B, Chevallier P, Guillaume T, Dubruille V, Harousseau JL, Milpied N. 2005. Zygomycosis after prolonged use of voriconazole in immunocompromised patients with hematologic disease: attention required. Clin Infect Dis 40:e35–e37. https://doi.org/10.1086/427752.
15. Schlemmer F, Lagrange-Xelot M, Lacroix C, de La Tour R, Socie G, Molina JM. 2008. Breakthrough *Rhizopus* infection on posaconazole prophylaxis following allogeneic stem cell transplantation. Bone Marrow Transplant 42:551–552. https://doi.org/10.1038/bmt.2008.199.
16. Lerolle N, Raffoux E, Socie G, Touratier S, Sauvageon H, Porcher R, Bretagne S, Bergeron A, Azoulay E, Molina JM, Lafaurie M. 2014. Breakthrough invasive fungal disease in patients receiving posaconazole primary prophylaxis: a 4-year study. Clin Microbiol Infect 20:O952–O959. https://doi.org/10.1111/1469-0691.12688.
17. Luo G, Gebremariam T, Lee H, Edwards JE, Jr, Kovanda L, Ibrahim AS. 2014. Isavuconazole therapy protects immunosuppressed mice from mucormycosis. Antimicrob Agents Chemother 58:2450–2453. https://doi.org/10.1128/AAC.02301-13.
18. National Institutes of Health. 2002. Public Health Service policy on humane care and use of laboratory animals. Office of Laboratory Animal Welfare, National Institutes of Health, Bethesda, MD.
19. Sugar AM, Liu XP. 2000. Effect of grapefruit juice on serum voriconazole concentrations in the mouse. Med Mycol 38:209–212. https://doi.org/10.1080/mmy.38.3.209.212.
20. Gebremariam T, Wiederhold NP, Alqarihi A, Uppuluri P, Azie N, Edwards JE, Jr, Ibrahim AS. 24 October 2016. Monotherapy or combination therapy of isavuconazole and micafungin for treating murine mucormycosis. J Antimicrob Chemother https://doi.org/10.1093/jac/dkw433.
21. Lewis RE, Albert ND, Kontoyiannis DP. 2014. Comparative pharmacodynamics of posaconazole in neutropenic murine models of invasive pulmonary aspergillosis and mucormycosis. Antimicrob Agents Chemother 58:6767–6772. https://doi.org/10.1128/AAC.03569-14.
22. Wiederhold NP, Pennick GJ, Dorsey SA, Furmaga W, Lewis JS, II, Patterson TF, Sutton DA, Fothergill AW. 2014. A reference laboratory experience of clinically achievable voriconazole, posaconazole, and itraconazole concentrations within the bloodstream and cerebral spinal fluid. Antimicrob Agents Chemother 58:424–431. https://doi.org/10.1128/AAC.01558-13.
23. Luo G, Gebremariam T, Lee H, French SW, Wiederhold NP, Patterson TF, Filler SG, Ibrahim AS. 2013. Efficacy of liposomal amphotericin B and posaconazole in intratracheal models of murine mucormycosis. Antimicrob Agents Chemother 57:3340–3347. https://doi.org/10.1128/AAC.00313-13.
24. Ibrahim AS, Bowman JC, Avanessian V, Brown K, Spellberg B, Edwards JE, Jr, Douglas CM. 2005. Caspofungin inhibits Rhizopus oryzae 1,3-β-D-glucan synthase, lowers burden in brain measured by quantitative PCR, and improves survival at a low but not a high dose during murine disseminated zygomycosis. Antimicrob Agents Chemother 49:721–727. https://doi.org/10.1128/AAC.49.2.721-727.2005.
25. Marty FM, Ostrosky-Zeichner L, Cornely OA, Mullane KM, Perfect JR, Thompson GR, III, Alangaden GJ, Brown JM, Fredricks DN, Heinz WJ, Herbrecht R, Klimko N, Klyasova G, Maertens JA, Melinkeri SR, Oren I, Pappas PG, Racil Z, Rahav G, Santos R, Schwartz S, Vehreschild JJ, Young JA, Chetchotisakd P, Jaruratanasirikul S, Kanj SS, Engelhardt M, Kaufhold A, Ito M, Lee M, Sasse C, Maher RM, Zeiher B, Vehreschild MJ, VITAL and FungiScope Mucormycosis Investigators. 2016. Isavuconazole treatment for mucormycosis: a single-arm open-label trial and case-control analysis. Lancet Infect Dis 16:828–837. https://doi.org/10.1016/S1473-3099(16)00071-2.
26. Siwek GT, Dodgson KJ, de Magalhaes-Silverman M, Bartelt LA, Kilborn SB, Hoth PL, Diekema DJ, Pfaller MA. 2004. Invasive zygomycosis in hematopoietic stem cell transplant recipients receiving voriconazole prophylaxis. Clin Infect Dis 39:584–587. https://doi.org/10.1086/422723.
27. Kontoyiannis DP, Lionakis MS, Lewis RE, Chamilos G, Healy M, Perego C, Safdar A, Kantarjian H, Champlin R, Walsh TJ, Raad II. 2005. Zygomycosis in a tertiary-care cancer center in the era of Aspergillus-active antifungal therapy: a case-control observational study of 27 recent cases. J Infect Dis 191:1350–1360. https://doi.org/10.1086/428780.
28. Lamaris GA, Ben-Ami R, Lewis RE, Chamilos G, Samonis G, Kontoyiannis DP. 2009. Increased virulence of Zygomycetes organisms following exposure to voriconazole: a study involving fly and murine models of zygomycosis. J Infect Dis 199:1399–1406. https://doi.org/10.1086/597615.