posaconazole 0.25, terbinafine 0.06, and voriconazole 0.25. The patient underwent multiple surgical debridements and was treated over time with various antifungal agents (ambrobicin B, miconafungin, terbinafine, voriconazole, posaconazole), adjunct cytokines (IFN-γ, GM-CSF), and hyperbaric oxygen. However, the infection progressed into the right middle cranial fossa and meningitis and the patient died 1 year after presentation. The autopsy revealed a de novo splice-site mutation in STAT3 (c.1140 3C>G). cDNA sequencing showed nonsense-mediated decay of the affected allele. No mutations in CARD9 or NADPH oxidase subunits were found; a DHR test was normal. The patient had normal blood myeloid cell subsets. Serum IgE level was elevated at 833 IU/mL based on CLSI method and in the patient’s memory CD4+ T cells and CD11c+ myeloid cells had reduced pSTAT3 levels compared with control cells. Cellular analysis of SOCS3, a STAT3-dependent downstream target, is underway to evaluate for functional STAT3 haploinsufficiency.

Conclusion. A novel de novo STAT3 splice site mutation results in impaired pSTAT3, and is associated with elevated IgE, eosinophilic esophagitis, and sino-oral aspergillosis without other common features of Job’s syndrome.

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273. C5ar1-dependent phagocyte effector functions protect against systemic candidiasis

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Background. Systemic candidiasis, the most common nosocomial human fungal infection, leads to mortality of >40% despite antifungal therapy. Myeloid phagocytes, not lymphocytes, are critical for protection; yet, the molecular basis of phagocyte-mediated immune protection remains elusive. Since patients with inherited C5 deficiency and those treated with the anti-C5 humanized monoclonal antibody eculizumab have been reported to occasionally develop candidiasis, we examined the role of C5a signaling in anti-Candida host defense.

Methods. Wild-type (WT) and C5ar1 knockout (KO) mice were infected with C. albicans and were euthanized on days 1 and 3 post-infection for fungal burden, histological, mRNA, ELISA and FACS analyses. Bone marrow (BM) radiation chimeras, GFP-C5ar1 reporter and conditional C5ar1 KO mice were used to examine the role of myeloid C5ar1 expression in protection. A Candida strain that simultaneously reports phagocytosis and fungal viability in vivo was generated to probe phagocyte effector functions.

Results. C5ar1 and C5ar2 transcripts and their ligand C5a were induced post-infection in WT mouse kidney. C5ar1 KO mice showed dramatically increased susceptibility as compared with WT mice (100% mortality vs. 40%; P < 0.0001). Significantly greater fungal burden and tissue injury were observed in C5ar1 KO, but not in C5ar2 KO kidneys. BM radiation chimera experiments showed that hematopoietic, not stromal, C5ar1 expression promotes protection. Using GFP-C5ar1 reporter mice, we found that C5a and C5aR1 expression were upregulated in macrophages and neutrophils in the infected kidney, but not in granulocytes and macrophage-derived macrophages. The macrophage cell-type specific contribution is under investigation using S100a8a-Cre/GFP-C5ar1fl/fl and Cx3cr1-Cre-GFP-C5ar1fl/fl mice as are the molecular mechanisms that promote C5ar1-dependent effector function.

Conclusion. C5ar1 is required for host survival during systemic candidiasis via regulating the antifungal effector function of phagocytes.

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274. VT-1598 Inhibits the in vitro Growth of Mucosal Candida Isolates and Protects Against Oropharyngeal Candidiasis in IL-17 Deficient Mice

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Background. Patients with chronic mucocutaneous candidiasis (CMC) often develop drug-resistant fungal infections, making treatment difficult due to lack of oral antifungal drug options. VT-1598 is a novel broad-spectrum fungal CYP51 inhibitor designed for selective toxicity for the fungal target versus human CYP enzymes to circumvent classic azole side effects like drug–drug interactions. We report the efficacy of VT-1598 in the treatment of oral Candida infection (including by azole-resistant strains).

Methods. The in vitro MIC values of 28 Candida species isolated from patients with CMC due to AIRE mutations were tested against VT-1598 and fluconazole (FLC), using CLSI broth microdilution M27-S4. Plasma VT-1598 levels were measured using LC-MS/MS with electrospray ionization. Tongue fungal load was determined in IL-17 deficient Act1-/- mice following sublingual C. albicans infection and once-daily oral treatment for 4 days with 25 mg/kg FLC or 3.2, 8, and 20 mg/kg VT-1598 starting 18 hours post-infection.

Results. Among 28 Candida isolates tested (22 C. albicans, three C. glabrata, and one each of C. utilis, C. dubliniensis, and C. krusei), 10 (36%) were not susceptible to FLC, based on CLSI breakpoints (1 mg/L vs. 4 mg/L, respectively). Oral administration of VT-1598 led to mean drug levels in mouse plasma (2.6, 3.0, and 11 mg/ml at the low, mid, and high doses, respectively) that were higher than the MIC values. In vivo, VT-1598 was significantly more effective, compared with FLC, against susceptible and -resistant C. albicans, and led to elimination of fungal growth even at the lowest tested dose (3.2 mg/kg). After a 10-day washout period from the last dose, mice treated with VT-1598 did not have mucosal fungal growth, while mice treated with FLC had tongue fungal loads similar to vehicle control.

Conclusion. VT-1598 shows in vitro activity against mucosally derived Candida, including FLC-resistant strains. In vivo, VT-1598 achieves high plasma concentrations allowing effective C. albicans killing. Future work will examine the potential for VT-1598 as a novel agent for the treatment of CMC.

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275. Replicative Aging in Candida auris Has an Effect on Antifungal Resistance

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Background. Candida auris can colonize patients for a prolonged time causing life-threatening systemic infections. Replicative aging is a result of asymmetric division known as replicative aging. The objective of this study was to evaluate C. auris replicative aging and assess its effect on antifungal resistance.

Methods. We used an F. grallii strain harboring a GFP-C5ar1 reporter and conditional C5ar1 KO mice as are the molecular mechanisms that promote C5ar1-dependent effector function.

Conclusion. C5ar1 is required for host survival during systemic candidiasis via regulating the antifungal effector function of phagocytes.

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276. Mechanisms of Fluconosine Resistance in Cryptococcus gattii May Be Independent of the FCY2-FCY1-FUR1 Pathway

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Background. Cryptococcus is an opportunistic fungal infection caused by both *Cryptococcus neoformans* and its sibling species, *Cryptococcus gattii*. Flucytosine (5FC) is one of the most widely used antifungals against *Cryptococcus spp.*, yet very few studies have looked at the molecular mechanisms responsible for 5FC resistance in this pathogen.

Methods. Eleven *Cryptococcus gattii* clinical isolates were selected based on differential 5FC susceptibility. All isolates underwent whole-genome sequencing and genomic differences in key genes involved in flucytosine metabolism were examined. Heterologous expression of FTC1 and spot sensitivity assays were performed to examine regions of interest based on genomic differences.

Results. Susceptibility assays and sequencing analysis revealed an association between a point mutation in cytosine deaminase (FTC1) and 5FC resistance in two *C. gattii* clinical isolates, B0322 and J55. This mutation results in the replacement of arginine for histidine at position 29 and occurs within an unconserved stretch of amino acids. Heterologous expression of FTC1 and spot sensitivity assays demonstrated that the point mutation did not have any effect on FTC1 activity and was not responsible for 5FC resistance. Comparative sequence analysis further showed that no amino acid changes were observed in either cytosine permeases (FCY2-4) or uracil phosphoribosyltransferase (UPRTase, encoded by FUR1) among 5FC-resistant and 5FC susceptible *C. gattii* isolates.

Conclusion. Together, our work suggests that the mediator(s) of 5FC resistance in J55 is likely found either downstream of FUR1 or on disparate regulatory regions of interest based on genomic differences.

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277. Fungal Mechanobiology: High Shear Forces Increase *Rhizopus Virulence* Alexander Tatara, PhD1; Nathaniel Albert, MS2; and Dimitrios P. Kontoyiannis, MD, PhD1.*1* FIDSA, 1*1* FIDSA, 1*1* Medical College of Wisconsin, Milwaukee, Wisconsin, Milwaukee, Wisconsin, 1*1* The University of Texas MD Anderson Cancer Center, Houston, Texas, 1*1* The University of Texas MD Anderson Cancer Center, Houston, Texas, 1*1* Stanford University School of Medicine, Stanford, California, 1*1* Stanford University School of Medicine, Stanford, California, 1*1* INRS Institute Armand-Frappier, Laval, QC, Canada, 1*1* INRS Institute Armand Frappier, Laval, QC, Canada

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**Background.** It has been observed in both civilian and military populations that high-energy events, such as tornadoes and blast injuries, have been associated with mucormycosis in otherwise immunocompetent patients. However, the effects of high shear force directly on fungal biology have not been explored. In order to elucidate the relationship between fungal mechanobiology and virulence, *R. oryzae* was exposed to high shear stress. Subsequent changes in virulence were measured in a validated fly model of mucormycosis. Finally, spores were simultaneously exposed to high shear forces and calcofluorin inhibitors to determine whether this classical stress pathway was involved in changes in virulence in response to shear force.

**Methods.** 10 or 100 spores/ml of *R. oryzae* in 100 ml saline were either: (1) grown in static conditions (CONTROL); (2) subjected to stirring at 1100 RPMs for 30-45 minutes (Tornado Physical Shear Challenge, TPSC); or (3) subjected to TPSC in the presence of the calcofluorin inhibitor tacrolimus (TPSCS + TAC). Wild-type flies were subsequently infected via dorsal thorax inoculation and monitored for survival over 7 days (n = 26 per group; performed in triplicate).

**Results.** Flies inoculated with *R. oryzae* exposed to high shear stress experienced significantly greater mortality compared with spores grown under static conditions (P < 0.001). Co-culture of spores grown under TPSC with tacrolimus (1 mg/ml) resulted in increased fly survival (P < 0.001). In fact, there was no significant difference between flies inoculated with spores subjected to high shear and TAC and spores grown under static conditions (P = 0.934).

**Conclusion.** Fungal exposure to high shear stress increases virulence. As calcineurin exposure to high shear force directly on fungal biology have not been explored. In order to elucidate the relationship between fungal mechanobiology and virulence, *R. oryzae* was exposed to high shear stress. Subsequent changes in virulence were measured in a validated fly model of mucormycosis. Finally, spores were simultaneously exposed to high shear forces and calcofluorin inhibitors to determine whether this classical stress pathway was involved in changes in virulence in response to shear force.

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278. Optimization of the CRISPR/Cas9 System to Manipulate Gene Function in *Rhizopus delemar* Clara Baldis, PhD1; Sameh Soliman, PhD2; Heewon Jeon, BS3; Christopher Skory, PhD; John Edwards, MD4; and Ashraf Ibrahim, PhD1,4.*1* Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California, 1*1* Department of Medicinal Chemistry, College of Pharmacy, University of Sharjah, Sharjah, United Arab Emirates, 1*1* Agricultural Research Services, USDA, Peoria, Illinois, 1*1* David Geffen School of Medicine at UCLA, Los Angeles, California

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**Background.** The genus *Rhizopus* is the main cause of mucormycosis, a life-threatening infection that affects predominantly hosts with an impaired immune system. However, patients with severe trauma and burns, without prior immune deficiency, are also at increased risk of developing mucormycosis. Despite aggressive treatment that includes disfiguring surgery and antifungal therapy, mortality rates range from 56-100%. Gene editing with CRISPR/Cas9 is critical for identifying fungal targets to develop more effective therapies. However, Rhizopus genetics are challenging because of lack of dominant selection markers, low efficiency of transformation, and rarity of chromosomal integration. Here we attempted to adapt the CRISPR/Cas9 technology to disrupt genes in *R. delemar*.

**Methods.** We used the Gibson cloning strategy to assemble all necessary elements of the CRISPR/Cas9 system in *R. delemar* plasmid using the pyrF as a selection marker. The targeted gene for disruption was a toxin-encoding gene with similarity to ricin. This disruption cassette was transformed using biolistic delivery system into *R. delemar* pyrF strain (M16). Recombination events were studied by Southern blot analysis. The presence of the toxin gene expression was confirmed a partial deletion of the ricin gene, in the region where the guide RNA was designed. Moreover, gene disruption was confirmed by agaration of ricin expression in comparison to reference strains (wild type or mutant with the CRISPR/Cas9 plasmid void of ricin gene sequence).

**Results.** Five stable transformants were obtained with the CRISPR/Cas9 construct. Southern blot analysis and gene expression were confirmed by qRT-PCR. Furthermore, damage to alveolar epithelial cells (A549) and nasal epithelial cells (CCL30) was studied with Cr-release assay.

**Conclusion.** We have successfully adapted the CRISPR/Cas9 system to disrupt the ricin-like gene in *R. delemar*. This tool will enable us to better understand the pathogenesis of mucormycosis, designing novel and more successful strategies to manage this lethal fungal infection.

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