S3.4a
The repurposing approach identifies pitavastatin (coxzim) making fluconazole fungicidal by inhibiting ergosterol synthesis

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Objectives: Making fluconazole (FLC) fungicidal in combination with adjuvants is a promising strategy to avoid the emergence of FLC resistance and eliminate the persistence and recurrence of fungal infections. To address this question, we combined an in vitro screening of a library of FDA-approved drugs to identify compounds for making FLC fungicidal.

Methods: We performed a high-throughput screen of an FDA-approved compound library (MycoLab, MCB), which contains 2152 drugs, to identify potentially novel FLC synergistic lethal adjuvants using both microdilution and dose matrix titration assays. The abilities of candidate drugs to turn FLC from fungistatic to fungicidal were further investigated by FLC disk diffusion assays carried out by four tested strains with different FLC tolerance levels (SC5314, SN12, cmp1 and Δ(cmp1-aid1), and ADH1p-UPC2 strain). We determined the median lethal dose (LD50) of Candidate compounds by the Up-and-Down procedure (DPI). (ORCID 6:25, 2008) via the intraperitoneal route in adult mice and used cyclosporine A and geldanamycin as control drugs to screen FLC synergistic lethal adjuvants with lower toxicity. Finally, we constructed heterogeneous aleurin mutants for ergosterol synthesis-related genes to identify the mechanism of action of the synergistic lethality of pitavastatin (coxzim) (PIT) and FLC (Fig. 1a).

Results: We found that 200 compounds (≤100 μg/ml) could make FLC (4 μg/ml) fungicidal and further confirmed that 30 compounds tested FLC (4 μg/ml) from fungistatic to fungicidal at a concentration lower than 12.5 μg/ml by broth microdilution assays (Fig. 1b). We further identified 1200 compounds (≤25 μg/ml) can make FLC fungicidal (≤9 μg/ml) using dose matrix titration assays. Among these compounds, PIT can make FLC fungicidal at as low as 0.78 μg/ml (Fig. 1c). In the FLC disk diffusion assay, we identified 8 compounds (≥1 μg/ml) that were superior to or equivalent to the abilities of the control drugs to eliminate the FLC tolerance of four tested strains. It was worth noting that PIT could make FLC fungicidal against all four tested strains (Fig. 1d). The LD50 value of PIT is 103.4 mg/kg and the highest of the tested compounds. Spot assay results showed feeding compound 100 μg/g ergosterol counteracted the antifungal activity of PIT (10 μg/ml) (Fig. 2a), but did not restore the growth defect of Tet-(HMG1)Δ mutant, in which the HMG1 gene expression would be inhibited by tetracycline.
Lactoferrin, a natural source of peptides that potentiate the antifungal activity of amphotericin B

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Objective: It is notoriously difficult to prevent and treat fungal infections, however, the natural world has come up with remedies that are non-toxic, effective, and safe in use. Here we investigate lactoferrin, an iron-binding glycoprotein found in milk, tears, and sweat, for its capacity to inhibit fungi and to synergize with commonly used antifungal drugs, with the aim of determining its mode of action.

Methods: Lactoferrin (LF) was obtained from a commercial supplier and two dairy companies. LF was tested on three species of pathogenic yeast and mold spores for inhibition using CLSM and microwell methods. Synagis was determined with antifungal drugs amphotericin B (AMB), nystatin (NYL), flucytosine (5-FC), and 5-lactoferrin (5-LF). The effect of LF on fungal cells was analyzed using scanning electron microscopy (SEM). The active peptides within LF were then predicted from peptides and in silico digestion, synthesized, and tested for synergy with amphotericin B (AMB).

Results: LF demonstrated antifungal activity against yeast species Cryptococcus, Candida, and Saccharomyces and was much less effective against molds. Good synergy was achieved with AMB but not nystatin or chloramphenicol. While the iron-chelating capacity of LF was important for the antifungal activity it was not involved in synergy. SEM revealed cell damage suggesting an interaction between AMB, LF, and the fungal membrane or cell wall. A 10-residue peptide from the C-lobe of LF was synthesized and tested for activity and synergy. This peptide, dubbed lactoferrin (LFG), was inactive alone but was potently synergistic with AMB, indicating a direct role in augmenting AMB activity. Synthetic membranes loaded with ergosterol but not cholesterol were disrupted by AMB + LFG, demonstrating that activity was fungal-specific and was mediated through ergosterol binding.

Conclusion: LF is a complex molecule that causes fungal inhibition via iron binding and when cleaved by pepstatin can produce active peptides. An AMB is a highly toxic treatment, the use of LFG as a synergist could help increase activity while lowering the effective dose, thereby reducing undesirable side effects. The action of AMB + LFG appears dependent on ergosterol, suggesting substitution will be highly fungal-specific.

S3.4c A pipeline toward the identification of novel antifungal compounds derived from the microbial dark matter

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Background: The current armamentarium of antifungal drugs and the restricted variety in antifungal drug classes combined with the ever-rising threat of resistant fungal pathogens highlighted the urgent need for novel antifungal compounds. Natural