Gwt1 inhibitor, APX2104, prolongs survival against Invasive Aspergillosis in neutropenic mice.

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INTRODUCTION

- *Aspergillus fumigatus* is the leading agent of invasive aspergillosis (IA)—a lethal pulmonary infection among immunocompromised peoples worldwide.
- Resistant strains to current therapies, including triazoles, echinocandin, and polyene treatments, are a growing concern globally.
- New mechanistic inhibitors need to be investigated.
- Gwt1 is a protein that facilitates anchoring cell-wall proteins via glycosylphosphatidylinositol (GPI)- anchoring mechanism.
- Gwt1 inhibitor, APX001, has shown promising pre-clinical results.

OBJECTIVES

- Investigate an APX001 analogue against *A. fumigatus.*

METHODS

- ***In vitro*:**
  - Minimum Effective Concentrations (MECs) were set up with 2 fold serial dilutions.
  - Azole susceptible (CEA10) and azole resistant (F16216) strains were inoculated and cultured in RMPI and their respective antifungal.
  - Images were taken under microscope.
- ***In vivo:***
  - Toxicity studies conducted using 60 mg/kg and 78 mg/kg dosing of APX2104 in immunocompetent mice.
  - Neutropenia induced by injection of 150 mg/kg cyclophosphamide and 250 mg/kg dose of cortisone acetate.
  - Neutropenic mice infected with *A. fumgiatus* CEA10 via an inhalation chamber, dosed with 60 mg/kg APX2104 for 7 days.
  - 1-Aminobenzotriazole (ABT) was used as a Cytochrome P450 suicide inhibitor to facilitate metabolism of APX2104 at a dose of 50 mg/kg orally.
  - Histological slides obtained from mouse tissues at Day +3 of infection.

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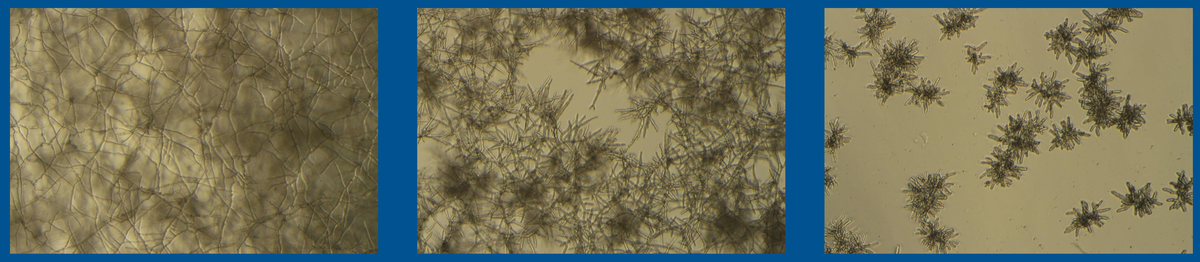
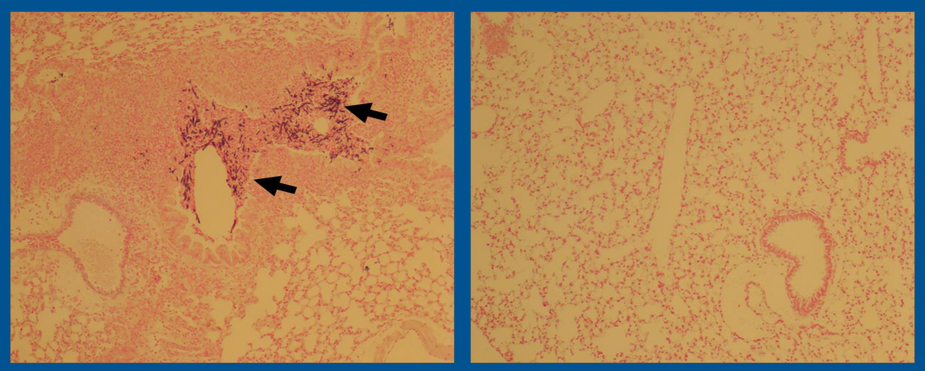
APX2104 pro-drug (µg/mL)

$3.1*10^{-2}$ $6.2*10^{-2}$ 2



APX2104 demonstrates fungistatic inhibition *in vitro* and prolongs survival *in vivo.*



ABT only ABT + APX2104

Histological slide of lungs infected with *A. fumigatus*

RESULTS

Table 1: Minimum Inhibitory/Effective Concentration

[Drug] µg/mL	VCZ	POS	APX2041	AmB
Ku80	0.25	0.25	0.125	1
CEA10	0.25	0.25	0.125	1
F16216	>2	2	0.0625	1

Table 1: CLSI Standards were followed. Drugs were diluted serially by two, and 1000 spores of each strain was carefully added to each well in triplicate. Samples were cultured at 37 °C. Minimum Inhibitory Concentrations (MIC) were attained for Voriconazole (VCZ), Posaconazole (POS), and Amphotericin B (AmB). These values were called when no signs of fungal growth were seen. Minimum Effective Concentration (MEC) was attained only for APX2041, the pro-drug for APX2104. Values were assigned due to inhibition of growth was seen due to the drug. As noted, APX2041 maintains its MEC value across various strains of *A. fumigatus*, including azole resistant strain F16216.

Figure 1: Toxicity Trail of APX2104 in Neutropenic Murine Model

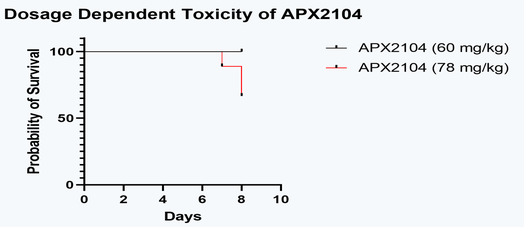


Figure 1: Toxicity studies were conducted in thirteen immunocompetent CD1 male mice at an average weight of 15 g, four mice in 60 mg/kg group and nine mice in 78 mg/kg. Dosages were calculated based on average weight and delivered intraperitoneally. Based on the results, 60 mg/kg was set as the concentration for APX2104 and used as the non-toxic dose in further challenge studies.

Figure 2: Efficacy of APX2104, versus Posaconazole, in Neutropenic Invasive Aspergillosis Murine Model

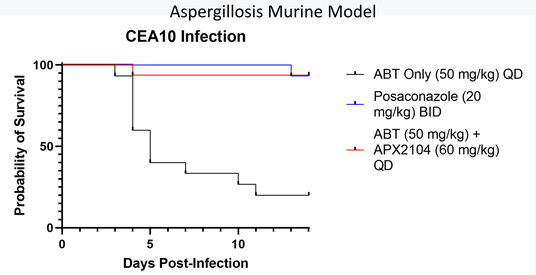


Figure 2: 45 CD1 male mice were separated into three groups of 15 each. Mice were immunosuppressed using cyclophosphamide and triamcinolone. Mice were inoculated with 40 µL $1x10^8$ spores/mL of *A. fumigatus* CEA10. ABT was administered orally, at 50 mg/kg, thirty minutes prior to administration of APX2104, which was delivered intraperitoneally. Posaconazole was dosed orally twice a day. Posaconazole and APX2104 had equal mortality rate at 7%, while ABT-only exhibited a mortality of 80% after 14 days post-infection.

Future Directions

- ***In vitro***
  - Explore synergy between APX2041 and various first line antifungals against CEA10 and F16216
- ***In vivo***
  - Conduct studies comparing IA challenged neutropenic mice with APX2104 and Posaconazole against F16216 strain.