What are the Clinical Implications of Antifungal Resistance: Aspergillus/Moulds & Clinical Trials

Thomas F. Patterson, MD, FACP, FIDSA
Professor of Medicine
Vice-Chair for Faculty Development
Chief, Division of Infectious Diseases
Director, San Antonio Center for Medical Mycology

UT Health San Antonio, Texas, USA

Presenter Disclosures
Thomas F Patterson MD

The following relationships with commercial interests related to this presentation existed during the past 24 months:

<table>
<thead>
<tr>
<th>Company Name:</th>
<th>Nature of Relationship: Consultant, Speakers Bureau, Sponsored Research, Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grant support</td>
<td>None</td>
</tr>
<tr>
<td>Consultant</td>
<td>Astellas, Basilea, Gilead, Merck, Pfizer, Scynexis, Toyama</td>
</tr>
<tr>
<td>Speakers' bureau</td>
<td>None</td>
</tr>
<tr>
<td>Major Stock holder</td>
<td>None</td>
</tr>
<tr>
<td>Other material support</td>
<td>NIH/NIAID (Amplyx, Cidara, F2G, Matinas, Mirati, Scynexis, Toyama, Viamet, Vical, Valley Fever Solutions)</td>
</tr>
</tbody>
</table>
Clinical Implications of Antifungal Resistance: Key Questions?

- Extent of problem
  - Local/geographic variability?
  - Clinical outcomes?
- Role for susceptibility
  - Recommendations for testing?
  - Implications for management?
- Management options
  - Efficacy of new agents?
  - Strategies for optimizing outcomes?

Resistance in Aspergillus – How big of a problem?

- Itraconazole R - first described in 1997
- Specific mutations in CYP51A (azole target)
  - Global emergence of point mutations with TR in promoter region (TR34/L98H & TR46/Y121F/T289A) — environmental
  - Specific “hot-spots”:
    - G54, L98, G138, M220, G448
- Overexpression of CYP51B/efflux pump
- “Pan” azole resistant isolates—cavitary disease, also in azole naïve patients
- UTHSCSA: Azole A. fumigatus resistance (MICs) UTHSCSA
  - Itraconazole (≥ 4): 2.58%
  - Voriconazole (≥ 4): 3.46%
  - Posaconazole (≥ 1): 4.06%


Slide adapted from GR Thompson. Thanks GR!
New reports (especially with TR/mutations): Italy, Tanzania, Sweden, Kuwait, Poland, Japan, France, China, Germany, Australia, Colombia, & US!


Mechanisms of Azole Resistance in Aspergillus fumigatus in the United States

- Clinical A. fumigatus isolates with elevated azole MICs screened.
- 26 non-duplicate A. fumigatus isolates evaluated.
- Point mutations associated with azole resistance present in 20/26.
- Two TR34/L98H and two TR46/Y121F/T289A mutations were also identified, which have not been previously found in U.S. isolates.
- Two isolates with elevated azole MICs did not contain Cyp51 mutations.

<table>
<thead>
<tr>
<th>Mutation (State)</th>
<th>Year</th>
<th>ITR</th>
<th>POS</th>
<th>VOR</th>
<th>ISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR34/L98H (Pennsylvania)</td>
<td>2010</td>
<td>&gt;16</td>
<td>2</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>TR34/L98H (Pennsylvania)</td>
<td>2014</td>
<td>&gt;16</td>
<td>1</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>TR46/Y121F/T289A (Arizona)</td>
<td>2008</td>
<td>4</td>
<td>1</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>TR46/Y121F/T289A (Reference lab)</td>
<td>2012</td>
<td>4</td>
<td>1</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
</tbody>
</table>

Azole Resistance in Aspergillus fumigatus in the US

- Passive screen by CDC of A. fumigatus isolates from US
  - 2011-2013: 1026 isolates screened for itra resistance: no isolates identified with TR34 or TR46 mutations
  - 2015-2017: 1356 isolates screened; 20 (1.4%) with increased MICs, 5 with TR34/L98H mutations

- Detection of TR34/TR46 from soil
  - Peanut farm debris screened for resistant A. fumigatus
  - Extensive use of tebuconazole and propiconazole (previously suggested to be associated with development of TR34/TR46)
  - No Aspergillus detected from 26 samples from current crop; all 6 samples from compost pile positive for Aspergillus
  - 200 isolates collected: 38 itra resistant; 20 with TR34/L98H mutations
  - Microsatellite typing: clonality of 73% of TR34/L98H vs no clonality in susceptible strains.


Characteristics of Nine Patients from Whom A. fumigatus Resistant to Multiple Triazoles Was Cultured

Table 1. Characteristics of Nine Patients from Whom A. fumigatus Resistant to Multiple Triazoles Was Cultured.

<table>
<thead>
<tr>
<th>Sex</th>
<th>Year of Age</th>
<th>Underlying Disease</th>
<th>Date of Isolation</th>
<th>Site of Isolation</th>
<th>Disease Classification*</th>
<th>Previous Azole Exposure*</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male 1</td>
<td>53</td>
<td>X-linked chronic granulomatous disease</td>
<td>April 4, 2002</td>
<td>Sputum</td>
<td>Breakthrough invasive pulmonary aspergillosis, proven</td>
<td>None</td>
<td>Voriconazole (high dose)</td>
<td>Survived</td>
</tr>
<tr>
<td>Male 2</td>
<td>56</td>
<td>Pulmonary fibrosis</td>
<td>June 26, 2005</td>
<td>Sputum</td>
<td>Invasive pulmonary aspergillosis, possible</td>
<td>Voriconazole</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>Male 3</td>
<td>60</td>
<td>Chronic granulomatous disease</td>
<td>Nov 1, 2004</td>
<td>Lung aspirate</td>
<td>Breakthrough invasive pulmonary aspergillosis, probable</td>
<td>Voriconazole</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>Male 4</td>
<td>62</td>
<td>Acute myeloid leukemia</td>
<td>Feb 14, 2006</td>
<td>Bronchoalveolar lavage fluid</td>
<td>Disseminated invasive pulmonary aspergillosis, probable</td>
<td>Voriconazole</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>Female 5</td>
<td>62</td>
<td>Chronic obstructive pulmonary disease</td>
<td>Apr 5, 2006</td>
<td>Bronchoalveolar lavage fluid</td>
<td>Invasive pulmonary aspergillosis, possible</td>
<td>Voriconazole</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>Male 6</td>
<td>62</td>
<td>Chronic granulomatous disease</td>
<td>Apr 15, 2006</td>
<td>Bone</td>
<td>Breakthrough aspergillosis osteomyelitis, proven</td>
<td>Voriconazole, caspofungin, and posaconazole</td>
<td>Survived</td>
<td></td>
</tr>
<tr>
<td>Male 7</td>
<td>55</td>
<td>Acute myeloid leukemia and allergic hemato poetic stem cell transplantation</td>
<td>May 11, 2006</td>
<td>Bone</td>
<td>Breakthrough aspergillosis osteomyelitis, proven</td>
<td>Voriconazole, caspofungin, and posaconazole</td>
<td>Survived</td>
<td></td>
</tr>
</tbody>
</table>

* Diseases were classified according to consensus criteria defined by the European Organisation for Research and Treatment of Cancer and the National Institute of Allergy and Infectious Diseases Mycoses Study Group.


- Detection of resistant A. fumigatus withitra screen in 82/1792 (4.6%) isolates
- Confirmation of TR34/L98H clone in 74 (92%)
- Overall prevalence of 5.3%


Clinical Characteristics of Patients with Azole-Resistant A. fumigatus

<table>
<thead>
<tr>
<th>Patient age, y</th>
<th>Underlying disease</th>
<th>Disease</th>
<th>No. positive cultures</th>
<th>Resistance mechanism</th>
<th>VCZ MIC, mg/L</th>
<th>Prior azole treatment (duration)</th>
<th>Outcome at 12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>66/M</td>
<td>Lung carcinoma</td>
<td>Proven pulmonary aspergillosis</td>
<td>1 TR/L98H</td>
<td>None</td>
<td>VCZ</td>
<td>Died</td>
<td></td>
</tr>
<tr>
<td>59/M</td>
<td>Hematologic malignancy, allo-SCT, GVHD</td>
<td>Proven pulmonary aspergillosis</td>
<td>4 TR/L98H</td>
<td>8</td>
<td>VCZ (&gt;1 mo)</td>
<td>VCZ</td>
<td>Died</td>
</tr>
<tr>
<td>54/M</td>
<td>Acute myeloid leukemia, relapse, allo-HSCT</td>
<td>Proven pulmonary aspergillosis</td>
<td>1 TR/L98H</td>
<td>8</td>
<td>ITZ (2-4 wk)</td>
<td>VCZ</td>
<td>Died</td>
</tr>
<tr>
<td>50/M</td>
<td>Non-Hodgkin lymphoma, allo-SCT, GVHD, lung cavities</td>
<td>Probable pulmonary aspergillosis</td>
<td>2 TR/L98H</td>
<td>16</td>
<td>VCZ (&gt;1 mo)</td>
<td>VCZ</td>
<td>Died</td>
</tr>
<tr>
<td>36/F</td>
<td>Breast carcinoma with metastasis</td>
<td>Probable pulmonary aspergillosis</td>
<td>1 TR/L98H</td>
<td>1</td>
<td>None</td>
<td>VCZ</td>
<td>Died</td>
</tr>
<tr>
<td>13/F</td>
<td>Non-Hodgkin lymphoma</td>
<td>Proven pulmonary and CNS aspergillosis</td>
<td>1 TR/L98H</td>
<td>16</td>
<td>None</td>
<td>VCZ, CAS, AMB</td>
<td>Died</td>
</tr>
<tr>
<td>58/M</td>
<td>Liver transplantation for hepatic failure after methotrexate treatment for arthritis</td>
<td>Proven pulmonary and CNS aspergillosis</td>
<td>5 TR/L98H</td>
<td>2</td>
<td>None</td>
<td>AMB, CAS</td>
<td>Died</td>
</tr>
<tr>
<td>69/M</td>
<td>Acute myeloid leukemia, allo-SCT, GVHD</td>
<td>Proven pulmonary and CNS aspergillosis</td>
<td>3 TR/L98H</td>
<td>4 FCO (1-2 wk)</td>
<td>VCZ, CAS, Survived</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VCZ: voriconazole; TR: itraconazole; CAS: caspofungin; AMB: amphotericin B; FCO: fluconazole; POS: posaconazole.

Invasive Aspergillosis in Patients Admitted to ICU with Severe Influenza

- Retrospective cohort study in Belgium/Netherlands 2009-2016
- IA dx in 83 (19%) of 432 patients with influenza; median 3 days after admission
  - Immunocompromised: 32% (38 of 117)
  - Non-immunocompromised: 14% (45 of 315)
  - Controls: 5% (16 of 315)
- IA in 20% with Influenza A (H1N1); 16% Influenza B
- Resistant *A. fumigatus* 4/17 (23%)
- 90 d mortality: 51% with IA vs 28% no IA
- Risk factors: influenza (OR 5.19), higher APACHE score, male, corticosteroids

✓ Importance of consideration for IA with severe influenza and potential for azole resistance

IDSA Susceptibility Testing Recommendations: Challenges of GRADE

IDSA 2016:
24. Routine antifungal susceptibility testing of isolates recovered during initial infection is not recommended. Antifungal susceptibility testing of Aspergillus isolates using a reference method is reserved for patients suspected to have an azole resistant isolate, who are unresponsive to antifungal agents, or for epidemiological purposes (Strong Recommendation; Moderate Quality Evidence).

- Lower reported rates of A. fumigatus resistance in US
- Limited availability of testing in many medical centers
- Local testing often not timely in return
- Limited clinical correlation
- Largely relies on ‘expert opinion’
✓ BUT: Significant potential harms with missed detection of resistance


International Expert Opinion on the Management of Infection Caused by Azole-resistant Aspergillus fumigatus

- Expert panel to provide practical ‘guidance’ not ‘guideline’
  - Establish microbiological diagnosis
  - Identify to species complex
  - In regions / institutions with high (>10%) rates of resistance test all isolates for azole susceptibility
    - Reliability of azole-based agar screens
  - Report results within 72 hrs
  - Ideally test 5 isolates for possible different phenotypes

ESCMID-ECMM-ERS 2018:
Antifungal susceptibility testing should be performed in patients with invasive disease in regions with resistance found in contemporary surveillance programmes (All), especially in patients unresponsive to antifungal therapy or suspected of having azole resistant isolates (AIII).

PCR-Based Detection of A. fumigatus Cyp51A Mutations on BAL Fluid: Validation of the AsperGenius Assay

- AsperGenius multiplex RT-PCR identifies 4 clinical relevant Aspergillus spp. and 4 resistant associated mutations (RAMs)
  - TR34/L98H/T289A/Y121F
- 201 hematology pts
  - BAL GM/culture controls pos 74/88 (80%)
    - Pos GM alone in 32/74 PCR+
  - BAL neg controls: 23/113 (84%)
- Azole failures: 6/8 (75%) with RAMs vs 12/45 (26%) without
- 6 wk mortality: 50% with RAMs vs 18% without


ESCMID-ECMM-ERS Guidelines for Susceptibility Testing and Choice of Therapy

<table>
<thead>
<tr>
<th>Azole MIC testing: choice of azole compounds</th>
<th>Population</th>
<th>Intention</th>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>To determine susceptibility to itraconazole</td>
<td>MIC (EUCAST/CLSI) A  III</td>
<td>In general, a sensitive marker for azole resistance in Aspergillus; test itraconazole and voriconazole as a maximum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>To determine susceptibility to voriconazole</td>
<td>MIC (EUCAST/CLSI) A  III</td>
<td>Resistance/reduced susceptibility to other azole(s) may accompany that of voriconazole; isolated voriconazole resistance described related to TR34 mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>To determine susceptibility to posaconazole</td>
<td>MIC (EUCAST/CLSI) B  III</td>
<td>Posaconazole resistance without itraconazole resistance not reported so far; current EUCAST breakpoint will misclassify approximately 15% susceptible isolates as IRC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>To determine susceptibility to isavuconazole</td>
<td>MIC (EUCAST/CLSI) A  III</td>
<td>MIC often similar to voriconazole, but needs testing separately, if isavuconazole is to be used; lower MIC of isavuconazole as compared to itraconazole and voriconazole for A. flavus and A. udagawae (A. fumigatus complex) (CLSI)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Optimal therapy in documented azole-resistance

<table>
<thead>
<tr>
<th>Population</th>
<th>Intention</th>
<th>Intervention</th>
<th>SoR</th>
<th>QoE</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolate with voriconazole MIC = 2 mg/mL</td>
<td>To cure IA</td>
<td>Voriconazole + echinocandin combination therapy or L-AmB monotherapy for IA (as well as for CPA)</td>
<td>A  III</td>
<td>The probability of voriconazole treatment failure may be higher than in voriconazole MIC &lt;2</td>
<td></td>
</tr>
<tr>
<td>Isolate with voriconazole MIC &gt;2 mg/mL</td>
<td>To cure IA</td>
<td>L-AmB lipid complex</td>
<td>A  IL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Voriconazole &amp; anidulafungin</td>
<td>B  III</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Posaconazole &amp; caspofungin</td>
<td>C  III</td>
<td>Posaconazole not licensed for primary treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Caspofungin or micafungin</td>
<td>C  III</td>
<td>Patients with contra-indications to AmB and other azoles</td>
<td></td>
</tr>
</tbody>
</table>

Study Design for PN069 Invasive Aspergillosis (PN069) Based on 2008 EORTC/MSG Criteria

• Phase 3, randomized, double-blind study of POS versus VOR in subjects

STUDY UPDATE:
Began enrollment in October, 2013; As of September 2018, enrollment of > 85% of target ~ 600 subjects
Stratification at baseline with ~ 40% classified as high risk
- High risk includes: liver transplant recipients, relapsed leukemia undergoing salvage chemotherapy, and receipt of allogeneic stem cell transplant
Disease classification at baseline:
- ~ 10% proven IA
- ~ 55% probable IA
- ~ 35% possible IA

To evaluate the all-cause mortality at Weeks 6 and 12 in subjects with a diagnosis of possible, probable, or proven IA receiving POS vs. VOR (Intention to Treat [ITT] population/Clinically evaluable dataset)
• To evaluate the safety of POS compared to VOR therapy (ITT)
• Other PK/PD, pharmacogenomic, and susceptibility endpoints.

Slide adapted from Dr. Hetty Waskin. Thanks! Hetty

CD101 (Rezafungin)
A novel echinocandin antifungal (Cidara)

Structural modification yields chemical stability & enhanced biological properties
Permanent charge & highly stable ring structure...
- Prolongs PK: once weekly dosing
- Eliminates toxic degradation products: improved safety & dose range
- Allows high exposures: treats less susceptible pathogens
- Enables multiple formulations: systemic and topical

In vitro activity: C. auris including some echinocandin resistant strains
Aspergillus including azole resistant strains

Phase 2 trial in candidemia vs caspo now complete NCT 02734862
Activity of Rezafungin against *Aspergillus fumigatus* including Resistant Isolates & Cryptic Species

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rezafungin</th>
<th>Caspofungin</th>
<th>Micafungin</th>
<th>Posaconazole</th>
<th>Voriconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>All <em>Aspergillus</em> isolates (N = 78)</td>
<td>0.015 μg/mL</td>
<td>&gt;0.015 μg/mL</td>
<td>&gt;0.015 μg/mL</td>
<td>0.06 μg/mL</td>
<td>0.125 μg/mL</td>
</tr>
</tbody>
</table>

Phase 3, Multicenter, Randomized, Double-Blind Study (ReSPECT) of the Efficacy and Safety of Rezafungin for Injection Versus the Standard Antimicrobial Regimen to Prevent Invasive Fungal Infections in Adults Undergoing Allogeneic Hematopoietic Stem Cell Transplantation.

Rezafungin for Injection will be compared with the current accepted SAR as prophylaxis against IFIs (inclusive of *Pneumocystis* pneumonia [PCP]) in patients undergoing allogeneic hematopoietic stem cell transplant (HSCT). ~450 subjects will be randomly assigned (2:1 ratio) to receive either Rezafungin for Injection or SAR for prevention of IFI caused by yeasts, molds, and *Pneumocystis*.

Glucan Synthase Inhibitors: Ibrexafungerp – SCY-078

(Scynexis)

- Semi-synthetic derivative of natural product
  - New molecular class
- Potent β 1,3 glucan synthesis inhibitor (GSI)
  - Same target as echinocandin antifungals. Blocks synthesis of essential component of cell wall of pathogenic fungi
  - Unique target not in mammalian cells
- Excellent in vitro & in vivo activity against *Candida* (including *C. auris*), *Aspergillus*, *Pneumocystis*
  - Active in vitro against azole & echinocandin resistant strains
- Orally bioavailable
- Extensive tissue distribution: kidney, lungs

- Phase I/II - generally well tolerated with good pharmacokinetics (QD); low drug-drug interactions
- Phase III trial *C. auris* (CARES) NCT03363841
- Phase III trial IFI resistant/unresponsive to therapy (FURI) NCT03059992

Ibrexafungerp *In vitro* Activity against *Aspergillus* spp.

- **SCYNERGIA- Phase 2 Invasive Pulmonary Aspergillosis (IPA) Study**
  - Objective: To assess the safety and efficacy of combination therapy (oral Ibrexafungerp + voriconazole) versus voriconazole monotherapy.
  - Intended Sample Size: ~60 patients (30 each arm)
    - Arm 1: Voriconazole + Oral SCY-078
    - Arm 2: Voriconazole + Oral Placebo
  - Combination therapy for the entire duration of anti-fungal treatment

<table>
<thead>
<tr>
<th>Other spp. (n=24)</th>
<th>&lt;0.06 – 0.25</th>
<th>&lt;0.06</th>
<th>&lt;0.06</th>
</tr>
</thead>
<tbody>
<tr>
<td>All isolates (n=311)</td>
<td>&lt;0.06 – 4</td>
<td>&lt;0.06</td>
<td>0.125</td>
</tr>
</tbody>
</table>

- IBX in combination with Vori, Isa and Amph B demonstrated synergistic activity against the majority of *A. fumigatus* isolates tested
- Efficacy of combination therapy with IBX + Isa in a rabbit model of IA

Vidmantas P, et. al. Advances Against Aspergillosis 2018

**Orotomide:**

**Olorofim (F901318)**

**Mechanism of Action, Spectrum**

- F901318 is a potent inhibitor of *A. fumigatus* DHODH
  - DHODH (Dihydroorotate dehydrogenase) is a key enzyme involved in pyrimidine biosynthesis
- Mechanism was identified using genetic studies in *Aspergillus nidulans*
  - Confirmed by in vitro enzyme assays
- Humans also have this enzyme
  - But, > 2000-fold difference in IC$_{50}$ between human and fungal enzymes
- Spectrum of activity: highly active against moulds/endemics including resistant *Lomentospora, Scedosporium, Aspergillus, Coccidioides*

F901318 structure
**Aspergillus: In vitro**

- Olorofim MICs are tightly clustered
  - MIC\textsubscript{90} are 0.03-0.06 mg/L (CLSI and EUCAST)
  - MICs are the same for all *Aspergillus* spp. tested
    - >1100 isolates from 35 species including:
      - >1100 isolates from 35 species including:
        - 615 isolates of 4 most common species\(^1\)
        - 220 isolates of 16 cryptic species\(^2\)
- Resistance not seen in surveys to date
- Resistance not induced with serial passage
- Cross-resistance is not seen
  - Same MICs in azole-resistant isolates
  - Same MICs in amphotericin-resistant species

\(^1\) A. fumigatus, flavus, niger, and terreus
\(^2\) A. alliaceus, aureostraeus, calidoustus, carneus, citrinoterreus, fumigatiaffinis, hiratsukae, hortai, insuetus, kevlei, lentulus, ochraceus, pseudofischeri, sclerotiorum, tubingensis, and udagawae

Other fungi fall into 3 buckets

- MICs similar to *Aspergillus*
  - *Scedosporium* spp., including *L. prolificans*
  - A long list of other moulds*
  - All the endemics
- Mixed and method-dependent results
  - *Fusarium* spp.
- Little or no activity
  - Mucorales
  - *Candida* spp., *Cryptococcus neoformans*

*Tested to date: Acremonium persicinum, Acrothialphora fusispora, Rasamsonia spp., Phaeoacremonium spp., Sarcolaudium kilenses, Scopulariopsis brevicaulis, Microascus spp., Sporothrix schenckii, Trichodemus spp., Ramschiorum (Myrmecridium) schulzeri, Paecilomyces spp., Pleurostomophora richardiae, Verruconis gallipavus, Chaetomium spp., and Penicillium spp. (including *P. marneffii*).

Wiederholdt et al. JAC 2107, Biswas et al. manuscript in preparation; Alastuey et al. TIMM 2017; Oliver et al. PNAS 2016; F2G Ltd. data on file
In vivo activity

- In vivo activity (mostly murine, some rabbit) shown for
  - A. fumigatus, flavus, terreus, nidulans, tanneri
  - Scedosporium apiospermum
  - Lomentospora prolificans
  - Pseudallescheria boydii
  - C. immitis (sterilizing activity in the brain!)

- In vivo activity has been shown PO & IV
  - Endpoints: Survival, tissue burden, and GM

- Equal efficacy on azole- and amphotericin B-resistant isolates
  - Azole-resistant (A. fumigatus, A. tanneri, L. prolificans)
  - Amphotericin B-resistant (A. terreus, A. tanneri)

- Open-label salvage study is open, other studies to follow!

### GPI Biosynthesis Inhibition (Amplyx)

**APX001[E1210] In vitro Activity**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Range</th>
<th>MIC50</th>
<th>MIC90</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans (52)</td>
<td>( \leq 0.008 ) – 0.016</td>
<td>( \leq 0.008 )</td>
<td>( \leq 0.008 )</td>
</tr>
<tr>
<td>C. glabrata (44)</td>
<td>( \leq 0.008 ) – 0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>C. tropicalis (23)</td>
<td>( \leq 0.008 ) – 0.03</td>
<td>0.016</td>
<td>0.03</td>
</tr>
<tr>
<td>C. parapsilosis (26)</td>
<td>( \leq 0.008 ) – 0.016</td>
<td>( \leq 0.008 )</td>
<td>0.016</td>
</tr>
<tr>
<td>A. fumigatus (20)</td>
<td>0.03 – 0.13</td>
<td>0.06</td>
<td>0.13</td>
</tr>
<tr>
<td>A. terreus (23)</td>
<td>0.015 – 0.06</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>F. solani (23)</td>
<td>0.03 – 0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>F. oxysporum (15)</td>
<td>0.03 – 0.25</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>S. prolificans (28)</td>
<td>0.03 – 0.25</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>S. apiospermum (28)</td>
<td>0.03 – 0.12</td>
<td>0.06</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*50% inhibition of growth for Candida; MEC endpoint for moulds (static activity)
**Inactive against C. krusei and members of the Order Mucorales
Active against fluconazole-resistant Candida (MIC90 - 0.03 μg/mL)


Slide adapted from John Rex, F2G; Thanks, John!
Clinical Relevance of Antifungal Resistance: Aspergillus/Other Moulds and Clinical Trials

September 27, 2018

GPI Biosynthesis Inhibition (Amplex) APX001[E1210] – In vivo Efficacy

In vivo efficacy demonstrated in murine models of invasive fungal infections

Invasive Candidiasis (C. albicans)

Invasive Pulmonary Aspergillosis (A. flavus)

Disseminated Fusariosis (F. solani)

PK parameter  T<sub>max</sub>  Bioavailability  Half-life

1 mg/kg PO X1  0.5 hours  57.5%  2.2 hours

✓ Efficacy in vitro and in vivo against resistant C. albicans & C. auris
✓ Phase 2 trials for Candida & moulds in development


VL-2397 – Activity against Aspergillus

Individual reference strains tested using standard CLSI methodology

<table>
<thead>
<tr>
<th>Aspergillus species</th>
<th>VL-2397</th>
<th>Voriconazole</th>
<th>Amphotericin</th>
<th>Caspofungin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>&gt;16</td>
<td>1</td>
<td>1</td>
<td>0.12</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>0.06</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>&gt;16</td>
<td>2</td>
<td>0.25</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Panels of reference strains tested using standard CLSI methodology

<table>
<thead>
<tr>
<th>Aspergillus species</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>MIC Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus (49)</td>
<td>0.5</td>
<td>0.06 - 0.5</td>
</tr>
<tr>
<td>Aspergillus fumigatus – azole-resistant (4)</td>
<td>0.06 - 1</td>
<td></td>
</tr>
<tr>
<td>Aspergillus flavus (17)</td>
<td>2 - &gt;16</td>
<td></td>
</tr>
<tr>
<td>Aspergillus terreus (20)</td>
<td>0.25 - 8</td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger (40)</td>
<td>1 - 16</td>
<td></td>
</tr>
</tbody>
</table>

- VL-2397 activity against A. fumigatus similar to voriconazole, amphotericin B
  - Active against azole-resistant A. fumigatus
  - Also active against C. glabrata, F. solani
- Phase 2 Clinical Trial: Open label multicenter randomized trial for Invasive Aspergillosis
- VL-2397 vs Standard Therapy
**Novel Cyp51 Inhibitors VT-1598**  
*(Viamet Pharmaceuticals, Inc.)*

- Investigational fungal Cyp51 inhibitors (VT-1129, 1161, 1598)
- MOA similar to azoles
- Highly selective for fungal Cyp51 enzyme vs human Cyp450 enzymes (more so than the azoles)
  - $K_d$ against fungal Cyp51 ≤39 nM
  - Failed to inhibit human CYP450 at 50 µM
- VT-1598 Activity against yeasts & moulds
  - *Candida, Cryptococcus*, endemics, & *Aspergillus* including non-fumigatus spp. but less activity against azole-resistant strains

**T-2307**  
*(Toyama)*

- Chemical screen conducted by Toyama Chemical Co.
- Member of a class of aromatic diamidines
  - Similar to pentamidine
  - Mechanism of action not fully understood: appears to target fungal mitochondria
  - In vitro & in vivo activity against broad spectrum yeasts and moulds & activity vs Pneumocystis

---


In vitro activity: Antifungal activity against Candida, Cryptococcus and Aspergillus sp.

<table>
<thead>
<tr>
<th>No. of strain</th>
<th>MIC range (μg/mL)</th>
<th>Drug-resistant strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azole-resistant <em>C. albicans</em> (8)</td>
<td>0.0005–0.001</td>
<td>CAS/VRG: 0.0625–0.125* FLC/ITR: 16–64</td>
</tr>
<tr>
<td>Candidin-resistant <em>C. albicans</em> (18)</td>
<td>≤0.008</td>
<td>NT</td>
</tr>
<tr>
<td>Candidin-resistant <em>C. glabrata</em> (17)</td>
<td>≤0.008–0.015</td>
<td>NT</td>
</tr>
<tr>
<td><em>C. auris</em> (10) #</td>
<td>≤0.008–0.015</td>
<td>0.125–0.5 0.5–64</td>
</tr>
<tr>
<td><em>Crypt. neoformans</em> (20)</td>
<td>0.0078–0.0625</td>
<td>&gt;64*</td>
</tr>
<tr>
<td><em>Crypt. gattii</em> (16)</td>
<td>0.0078–0.0625</td>
<td>&gt;128*</td>
</tr>
<tr>
<td><em>A. fumigatus</em> (20)</td>
<td>0.125–4</td>
<td>0.0313*</td>
</tr>
<tr>
<td><em>A. terreus</em> (27)</td>
<td>0.0156–0.125</td>
<td>0.0313–0.0625*</td>
</tr>
</tbody>
</table>

CAS: caspofungin, FLC: fluconazole, ** MICs of micafungin, *** MIC of voriconazole, **** MIC of itraconazole
# This work utilized NIAID's suite of preclinical services for in vitro assessment.
(Contract No. HHSN272201100018I HHSN27200012 )

T-2307 showed broad and potent antifungal activity of against major pathogenic fungi including *C. auris* and drug-resistant strains.

Mitsuyama et al., Antimicrob Agents Chemother 2008; 52: 1318-1324; Toyama, Data on file

T-2307 In vivo Efficacy

In vivo efficacy demonstrated in murine models of invasive fungal infections

- In vivo efficacy against echinocandin-resistant *C. albicans* and *C. auris* in a murine model

Clinical Implications of Antifungal Resistance:

**Summary**

- **Extent of problem**
  - Widespread detection of resistance in *A. fumigatus* and other moulds
  - Regional/location variation in prevalence
  - High mortality with resistant strains

- **Role for susceptibility**
  - Need for testing in high prevalence settings
  - Importance of timely availability
  - Local laboratory screening methods
  - Potential role for molecular testing
  - Implications for management

- **Management options**
  - New compounds with activity against resistant *Aspergillus* and other moulds beginning clinical trials!
  - Strategies needed for optimizing outcomes for resistant isolates
Thank you!

Special thanks!
Drs. Nathan Wiederhold & GR Thompson
Industry Colleagues:
Tom Chen, Paul Daruwala, Ed Garvey, Mike Hodges, Junichi Mitsuyama, John Rex, Haran Schlamm, Hetty Waskin