New Pharmacological Targets for Fungal Infections

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Disclosures

Grant Support

- Astellas
- bioMérieux
- F2G
- Merck
- Pfizer
- Viamet

Advisory Boards

- Astellas
- Merck
- Toyama
- Viamet

In Kind Support

- Associates of Cape Cod
Targets of Antifungal Drugs

- Cell well
  - *Echinocandins*

- Cell membrane
  - *Polyenes*
  - *Azoles*

- Nucleic acid synthesis
  - *Flucytosine*
Targeting Ergosterol

• Directly binding to ergosterol within fungal cell membrane
  – *Amphotericin B, nystatin*

• Inhibition of enzymes in ergosterol biosynthetic pathway
  – *Azoles & terbinafine*
Amphotericin B
*(Mechanism of Action & Toxicity)*

- Binds ergosterol 1:1 complex
- Other side = hydrophilic pore
  - Progressive ion & small molecule loss
  - Drop in pH
  - Death/Lysis
- Poor binding to cholesterol in human cell membranes
  - Not 100% selective

**Nephrotoxicity**

*Infusion related reactions*
Azoles & Ergosterol Biosynthesis

Lanosterol

14α-demethylase (a CYP450-like enzyme)

Ergosterol

Triazoles bind to heme-containing pocket of enzyme & prevent demethylation of lanosterol (metallo-enzyme inhibitors)

- Impair membrane fluidity
- Accumulation of toxic sterols
- Growth arrest
Azoles & Drug-Drug Interactions

• Selectivity for fungal CYP enzymes *not* exclusive
  – $IC_{50}$ against *Candida* CYP = $10^{-9}$M
  – $IC_{50}$ against human CYP450 = $10^{-6}$M

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Fluconazole</th>
<th>Voriconazole</th>
<th>Posaconazole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhibitor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP 2C19</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>CYP 2C9</td>
<td>++</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>CYP 3A4</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Substrate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP 2C19</td>
<td>–</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>CYP 2C9</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>CYP 3A4</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

*Increased affinity to fungal CYP enzymes results in reduced specificity*
Echinocandin Mechanism of Action

- Cyclic lipopeptide antibiotics that interfere with fungal cell wall synthesis by inhibition of β-(1,3) D-glucan synthase
  
  ➢ Loss of cell wall glucan results in osmotic fragility

New Agents of Available Classes

- **Isavuconazole** *(Basilea, Astellas)*
  - Broad-spectrum triazole
  - Oral & IV formulations
    - Prodrug (BAL-8557) cleaved to active component (BAL-4815) by plasma esterases
    - Long $t_{1/2}$
    - Phase 3 studies

- **ASP9726** *(Astellas)*
  - Echinocandin
  - IV formulation
  - Preclinical studies
What’s old is new...
Novel Cyp51 Inhibitors VT-1129 & VT-1161

Viamet Pharmaceuticals, Inc.

• Investigational fungal Cyp51 inhibitors

• MOA similar to azoles
  – *Without imidazole/triazole structure*

• Highly selective for fungal Cyp51 enzyme vs. human Cyp450 enzymes (more so than the azoles)
  – *$K_d$ against fungal Cyp51 $\leq 39$ nM*
  – *Failed to inhibit human Cyp51 at 50 $\mu$M*

MTP = metallophilic
X, Y = preferably halogen
Ar = aryl moiety

Hoekstra et al. *ICAAC* 2010.
VT-1129 & VT-1161 In vitro Potency

Potent in vitro activity against *Cryptococcus* & *Candida* species

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>VT-1129</th>
<th>VT-1161</th>
<th>FLU</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC50</td>
<td>0.015</td>
<td>0.03</td>
<td>2</td>
<td>NT</td>
</tr>
<tr>
<td>MIC90</td>
<td>0.06</td>
<td>0.125</td>
<td>8</td>
<td>NT</td>
</tr>
<tr>
<td>GM MIC</td>
<td>0.03</td>
<td>0.04</td>
<td>2.32</td>
<td>NT</td>
</tr>
<tr>
<td><em>Cryptococcus gattii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC50</td>
<td>0.06</td>
<td>0.06</td>
<td>2</td>
<td>NT</td>
</tr>
<tr>
<td>MIC90</td>
<td>0.125</td>
<td>0.25</td>
<td>4</td>
<td>NT</td>
</tr>
<tr>
<td>GM MIC</td>
<td>0.05</td>
<td>0.08</td>
<td>2.28</td>
<td>NT</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC50</td>
<td>0.03</td>
<td>0.03</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>MIC90</td>
<td>0.03</td>
<td>0.03</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>GM MIC</td>
<td>0.03</td>
<td>0.03</td>
<td>0.15</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Cryptococcal Meningitis - VT-1129

- Effective as monotherapy & in combination with amphotericin B against cryptococcal meningitis
  - Murine model with intracranial inoculation
  - Improvements in survival & significant reductions in fungal burden (>4 log reduction CFU/g when combined with AMB)

VT-1129 & VT-1161 In vitro Potency (Azole Resistance)

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>VT-1129</th>
<th>VT-1161</th>
<th>FLU</th>
<th>VOR</th>
<th>CAS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Azole SDD &amp; High Level Resistance Isolates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC Range</td>
<td>≤ 0.03 – 0.5</td>
<td>≤ 0.03 – 0.5</td>
<td>32 - &gt;64</td>
<td>0.06 - &gt;16</td>
<td>0.25 – 2</td>
</tr>
<tr>
<td>GM MIC</td>
<td>0.12</td>
<td>0.11</td>
<td>58.7</td>
<td>1.84</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Defined Azole Mechanism of Resistance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC Range</td>
<td>≤ 0.03 – 0.5</td>
<td>≤ 0.06 – 0.25</td>
<td>32 - &gt;64</td>
<td>≤ 0.25 – 0.5</td>
<td>≤ 0.25 – 0.5</td>
</tr>
<tr>
<td>GM MIC</td>
<td>0.12</td>
<td>0.12</td>
<td>50.8</td>
<td>0.40</td>
<td>0.31</td>
</tr>
</tbody>
</table>

- *Candida albicans* clinical isolates from patients with OPC or candidemia
- Mechanisms of azole resistance included point mutations in *ERG11* and overexpression of *CDR1, CDR2, & MDR1*

Fothergill et al. ICAAC 2011.
Echinocandin and azole resistant *Candida albicans*

- Improved survival and reductions in kidney fungal burden vs. caspofungin and fluconazole

Najvar et al. *ISHAM* 2012.
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

In vitro & in vivo activity against Fusarium

## T-2307 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 (40)</td>
<td>0.00025 – 0.0039</td>
<td>0.0005</td>
<td>0.002</td>
</tr>
<tr>
<td>C. glabrata (25)</td>
<td>0.0039 - 0.0078</td>
<td>0.0039</td>
<td>0.0078</td>
</tr>
<tr>
<td>C. guilliermondii (17)</td>
<td>0.001 – 0.0039</td>
<td>0.002</td>
<td>0.0039</td>
</tr>
<tr>
<td>C. krusei (16)</td>
<td>0.0005 – 0.002</td>
<td>0.001</td>
<td>0.002</td>
</tr>
<tr>
<td>C. parapsilosis (20)</td>
<td>0.00025 – 0.002</td>
<td>0.0005</td>
<td>0.0005</td>
</tr>
<tr>
<td>C. tropicalis (20)</td>
<td>0.00025 – 0.002</td>
<td>0.0005</td>
<td>0.0005</td>
</tr>
<tr>
<td>C. neoformans (20)</td>
<td>0.0078 – 0.0625</td>
<td>0.0156</td>
<td>0.0313</td>
</tr>
<tr>
<td>A. fumigatus (20)</td>
<td>0.125 – 4</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

Maintained activity against azole susceptible dose-dependent & resistant *C. albicans* isolates (MIC range 0.0005 – 0.001 μg/mL)

T-2307 In vivo Efficacy

In vivo efficacy demonstrated in murine models of invasive fungal infections

<table>
<thead>
<tr>
<th>Invasive Fungal Infection</th>
<th>Invasive Candidiasis (C. albicans)</th>
<th>Systemic Cryptococcosis (C. neoformans)</th>
<th>Systemic Aspergillosis (A. fumigatus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ED50 (mg/kg)</td>
<td>0.00755</td>
<td>0.117</td>
<td>0.391</td>
</tr>
</tbody>
</table>

Novel targets...
Glycosylphosphatidylinositol (GPI) Biosynthesis

Microbial attachment to host cell surfaces

Colonization & replication on host mucosal or endothelial surfaces

Penetration across mucosal barriers / systemic dissemination

Some fungal ligands responsible for adhesion derived from GPI-anchored proteins

*Candida albicans* possess ~115 GPI-anchored proteins
• Als protein family members

GPI Biosynthesis Inhibition - E1210
(Eisai)

GWT1 gene encodes Gwt1p
- an inositol acyltransferase in early GPI biosynthesis pathway

1-(4-butylbenzyl)isoquinoline (BIQ)

E1210
- (3-(3-{4[pyridin-2-yloxy)methyl]benzyl}isoxazol-5-yl)pyridin-2-amine

GPI Biosynthesis Inhibition - E1210

- E1210 inhibits inositol acylation in fungi
  - IC$_{50}$ C. albicans 0.27 μM
  - IC$_{50}$ A. fumigatus 0.60 μM
  - IC$_{50}$ Human Gwt1p >100μM

- Reduces expression of Als1p on C. albicans cell surface
  - Not in crude extracts of C. albicans

## 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)

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E1210 – In vivo Efficacy

In vivo efficacy demonstrated in murine models of invasive fungal infections

<table>
<thead>
<tr>
<th>PK parameter</th>
<th>Tmax</th>
<th>Bioavailability</th>
<th>Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/kg PO X1</td>
<td>0.5 hours</td>
<td>57.5%</td>
<td>2.2 hours</td>
</tr>
</tbody>
</table>

Histones & Epigenetic Regulation

- Post-translational modifications regulated at epigenetic level
  - **Histone acetyl transferases**
  - **Histone deacetylases**

Histones & Epigenetic Regulation

• Histone deacetylases remove acetyl groups leading to condensing of chromatin

• Histone deacetylases control important fungal cellular functions
  – *HDA1 & RPD3* involved in high frequency phenotypic switching
  – Adhesion of yeast to epithelial cells
  – Germ tube formation

Histone Deacetylase Inhibitors

• Histone deacetylase inhibitors may enhance susceptibility of fungi to azoles
  – Reduction in azole-dependent upregulation of efflux pumps (CDR1 & CDR2)
  – Reduction in transcription of genes encoding ergosterol biosynthesis pathway (ERG1 & ERG11)

• Reduced transcription (50%) when fluconazole or terbinafine combined with trichostatin A (TSA)
  – Non-specific histone deacetylase inhibitor

MGCD290
(MethylGene)

• Synergistic with azoles against yeast & moulds

<table>
<thead>
<tr>
<th>MGCD290 Combined with...</th>
<th>Percent Synergy</th>
<th>Percent Antagonism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconazole</td>
<td>60% (50/91 isolates)</td>
<td>1.1% (1/91 isolates)</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>51% (46/91 isolates)</td>
<td>1.1% (1/91 isolates)</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>53% (48/91 isolates)</td>
<td>3.3% (3/91 isolates)</td>
</tr>
</tbody>
</table>

• Synergy when combined with fluconazole
  – 87% Candida isolates (all that were fluconazole resistant [≥64 μg/mL] became susceptible [<8 μg/mL])
    • All 5 C. krusei isolates
  – 60% (6/10) Aspergillus isolates

MGCD290
(MethylGene)

• Synergy when combined with posaconazole
  – 60% (18/30) Candida isolates
  – 93.3% (14/15) Zygomycetes (9 Rhizopus & 5 Mucor)

• Synergy when combined with voriconazole
  – 73.3% (11/15) Zygomycetes
    • MIC change >8 μg/mL to <1 μg/mL for 9 isolates
  – 75% (6/8) Fusarium species

Phase 2 VVC study completed

Heat Shock Protein 90

• Essential molecular chaperone
  – *Regulates form and function of signal transducers*
  – *Enables emergence of drug resistance in fungi*
  • Also plays role in maintaining resistance

• Blockage of Hsp90 abolishes resistance
  – Azoles & *Candida*
  – Echinocandins & *Aspergillus*

Hsp90 Inhibitors

- In vitro synergy with fluconazole and caspofungin against *C. albicans* & *A. fumigatus*

- Combination therapy with fluconazole and caspofungin beneficial in vivo
  - *Galleria mellonella* (wax moth larvae) model
    - Improvements in survival
  - Murine model disseminated candidiasis
    - Reductions in tissue fungal burden
  - Rat venous catheter model
    - Eradicating biofilms

Summary

• Several new agents in preclinical and clinical development
  – Next generation agents in established classes
    • Isavuconazole (azole)
    • ASP9726 (echinocandin)
  – New designs for established targets
    • VT-1129 & VT-1161 (ergosterol)
    • T-2307 (pentamidine-like)
  – Novel mechanisms of action
    • E1210 (GPI biosynthesis)
    • MGCD290 (histone deacetylation)
    • Hsp90 inhibitors

• Pharmacokinetics & pharmacodynamics not well defined
  – Formulation & toxicity profile also lacking