PREVENTION OF *Pneumocystis Pneumonia* (PCP) BY THE NOVEL ECHINOCANDIN, CD101

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Pneumocystis

- Obligate fungi
- Microscopic (5-8µm)
- Host-dependent
  - Detected in the lungs of most mammals
- Cause lethal pneumonia
  - Immune system debilitation
    - HIV-infected; chemotherapy; corticosteroids; other diseases states
Asci and trophic forms are the predominant life cycle stages.
Trophic forms are microscopically enumerated by staining of their nuclei.

Cysts are microscopically enumerated by staining their cell wall.
**Pneumocystis Pneumonia (PCP) Therapy**

**Moderate to Severe PCP**
- **Corticosteroids w/in 72 h**
- **TMP-SMX**
  - Emerging resistance
  - Severe allergic reactions
  - 20-85% in patients with AIDS
- **Pentamidine (IV)**
  - Wide variety of toxicities
  - Relapse
- **Clindamycin-primaquine**
  - Higher efficacy, fewer side effects

**Mild to Moderate PCP**
- **Dapsone and TMP**
  - Methemoglobinemia and hemolysis
  - Rash and fever
- **Primaquine + clindamycin**
  - Methemoglobinemia and hemolysis
  - Anemia, rash, fever, and diarrhea
- **Atovaquone**
  - Less effective, but fewer side effects
  - Headache, nausea, diarrhea, rash, and transaminase elevations

PCP Prophylaxis

For patients with:

- CD4+ counts < 200 cells/mm³
- History of oral candidiasis
- CD4+ cell percentage of < 14%
- Hx of AIDS-defining illness

- **TMP-SMX**
  - TMP 15–20 mg/kg/d and SMX 75–100 mg/kg/d), PO in 3 divided doses or TMP-SMX DS, 2 tablets TID
- **Dapsone**
  - Plus pyrimethamine + leucovorin
- **Aerosolized pentamidine with Respirgard II nebulizer**
- **Atovaquone**
  - Plus pyrimethamine + leucovorin
- **NOT recommended**: oral clindamycin plus primaquine
CLEAR NEED FOR ALTERNATIVE THERAPIES AND PROPHYLAXIS
ECHINOCANDINS

• New class of lipopeptide antifungal agents available since 2001
• Non-competitive inhibition of (1,3)-β-D-glucan synthesis
• Probably targets glucan synthetase
  – By inhibiting this enzyme, echinocandins prevent > 90% of glucose incorporation into glucan
• Approved for use against other fungal infections
## Efficacy of Echinocandins for Treatment of PCP?

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kamboi et al. <em>CID</em>, 2006</td>
<td>Caspofungin only; both died</td>
<td></td>
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<tr>
<td>Utili et al. <em>Transplantation</em>, 2007</td>
<td>TMP-SMX plus caspofungin as salvage therapy</td>
<td>• Led to a rapid improvement and complete cure of 2/2 patients, no side effects or drug interactions were observed</td>
</tr>
</tbody>
</table>
| Tu et al. *Nephrology*, 2013  | TMP-SMX plus caspofungin, tacrolimus, mycophenolate | • 3 cases in renal transplant pts  
  • Some improvement by imaging; after 35d succumbed to pneumothorax  
  • TMP-SMX, moxifloxacin, caspofungin- discharged with partially normal lung fields  
  • Caspofungin and TMP-SMX; remarkable improvement with discharge |
| Kim et al. *Scand J Infect Dis*, 2013 | Single center review in China                  | • 3/10 received TMP-SMX with caspofungin as salvage/combination died  
  • 1/3 who received caspofungin-only died |


β-1,3-D-glucan and *Pneumocystis*

- The cell wall of *Pneumocystis* cysts/asci has been reported to contain 2 types of glucan, β-1,3-D-glucan and α-1,4 glucan, but little to no chitin
- *Gsc-1* in *Pneumocystis*, was characterized and found to encode a 214kDa integral membrane protein with 12 transmembrane domain, which was preferentially expressed in cysts but not in trophic forms
  - Kottom & Limper, JBC 2000

Cushion et al. PLoS ONE, 2010
CD101 for Prophylaxis of PCP in the Mouse Model
MOUSE MODEL OF PROPHYLAXIS

Immunosuppression:
Dexamethasone (4µg/L) in acidified drinking water (sulfuric acid; 1ml/L); i.s continued through 6 wk

Infection:
2 x 10^6/50 µl, P. murina Intra-nasally (2d post-i.s.)

Treatment:
10%DMSO/1% Tween/PBS IP/mg/kg

Microscopic enumeration
log transformed, Kruskall-Wallis, Dunn’s post test for multiple comparisons (GraphPadPrismv.6).

<table>
<thead>
<tr>
<th>Grp</th>
<th>Drug</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Neg. control</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>CD101</td>
<td>20 mg/kg 3x/wk</td>
</tr>
<tr>
<td>3</td>
<td>CD101</td>
<td>20 mg/kg 1x/wk</td>
</tr>
<tr>
<td>4</td>
<td>CD101</td>
<td>2 mg/kg 3x/wk</td>
</tr>
<tr>
<td>5</td>
<td>CD101</td>
<td>2 mg/kg 1x/wk</td>
</tr>
<tr>
<td>6</td>
<td>CD101</td>
<td>0.2 mg/kg 3x/wk</td>
</tr>
<tr>
<td>7</td>
<td>CD101</td>
<td>0.2 mg/kg 1x/wk</td>
</tr>
<tr>
<td>8</td>
<td>TMP/SMX</td>
<td>50/250 mg/kg 3x/wk</td>
</tr>
</tbody>
</table>

10 animals in each group
RESULTS

*Denotes statistical significance reduction from C/S group - p value <0.05
RESULTS

- Prophylactic treatment with CD101 resulted in statistically significant reductions in total numbers of *P. murina* (nuclei) at all doses but 0.2 mg/kg/1X/week vs untreated controls.
- Three groups were as efficacious as the TMP-SMX group, with no nuclei observed by microscopic evaluation.
- ALL CD101 treatment groups showed significant reductions in asci levels vs untreated controls.
- There was no difference in efficacy among 5 of the CD101 treatment groups and TMP/SMX, with no observed organisms.
**CONCLUSIONS**

- **Previous studies:** Cysts/asci repopulated the infections upon cessation of echinocandin treatment *Pneumocystis* when administered therapeutically (Cushion, 2010)
  - Mice treated with anidulafungin and lacking detectable cysts could not transmit the infection, suggesting the cyst is the agent for transmission
- Cysts/asci are necessary for transmission
- With this study, we now postulate that cyst/asci formation is needed for a productive infection
- Prophylaxis with CD101, which blocked cyst/asci formation, offers a new means to prevent PCP
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