Efficacy of APX2039, A Novel Gwt1 Inhibitor, in a Rabbit Model of Cryptococcal Meningitis

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Funding Sources and Conflicts

• Project funding: NIH 5R01AI144091-02
• Dr. John Perfect, MD
  • Research grants and consulting for Astellas, Pfizer, Merck, Appili, Amplyx, Matinas, Scynexis, and Minnetronix
• Mr. Charles Giamberardino
  • No conflicts
Cryptococcus Overview

- **Cryptococcus**: opportunistic fungal infection that disproportionality impacts women and children.
- Significant morbidity and mortality, frequently disseminated, impacts CNS and leads to cryptococcal meningitis (CM)
- Added to FDA’s Neglected Tropical Disease list in August 2018
- **HIV primary cause of CM**, accounting for 95% of cases in low and middle-income countries
  - **Global annual incidence ~223,100 cases**, of which Sub-Saharan Africa accounted for 73%
  - **Untreated, CM mortality rate ~100%**
    - 1 year mortality >70% in low income countries, ~181,000/yr
- HIV-infected patients at risk when CD4 counts below 100 cells/µL
  - ~4.3 million adults living with HIV with a CD4 count below 100 cells/µL
Current Cryptococcal Meningitis (CM) Treatment

**In resource rich areas a lipid Amphotericin B formulation, 3 – 4 mg/kg/day is also used during Induction.**

**Results of the ACTA study also indicate 1 week of Amphotericin B + Flucytosine is superior to 2 weeks, in resource limited regions during Induction.**

- **Oral Flucytosine**
  - 100 mg/kg/day
  - Induction
- **IV Amphotericin B**
  - 0.7 - 1.0 mg/kg/day
  - Induction
- **Oral Fluconazole**
  - (or other Azole)
  - 400 - 800 mg/day
  - Consolidation
- **Oral Fluconazole**
  - 200 mg/day
  - Maintenance
Challenges with the Current Regimen: Urgent need for a safe, efficacious new oral therapy

Flucytosine
- Remains unlicensed in most African and Asian countries
- 4 doses per day
- Can lead to bone marrow toxicities (cytopenia)

Amphotericin B
- High risk of renal toxicity
- Other formulations have fewer side effects, but are costly ($1000/day) and not feasible in developing countries

Substitution with high-dose Fluconazole
- Clinical studies in Uganda and Malawi report 10-week mortality rates of 58-60% for patients on FLU monotherapy compared to 20% with standard of care regimen

TOXICITIES

ADMINISTRATIVE & COST BARRIERS

POOR EFFICACY
Gwt1 inhibitors: a novel class of antifungal agents

Fosmanogepix (FMGX)

APX001 (Prodrug)

Manogepix (MGX)

APX001A (Active Moiety)

APX2039 (Active Moiety)
Targeting Gwt1

• Gwt1 is essential for trafficking and anchoring mannoprotein to the outer cell wall
• Mannoproteins are required for:
  • cell wall integrity
  • adhesion
  • pathogenicity
  • evading the host immune system
• Pleiotropic effects that result
  • deficiencies in hyphal formation
  • changes in morphology
  • decreased adhesion
  • reduction in cell wall-linked mannoproteins
  • reduction biofilm formation
  • exposure of the glucan layer, and ER stress
• Manogepix and APX2039 do not inhibit PIGW, the closest human homolog
Gwt1 Inhibitors have strong *in vitro* activity against multiple fungal pathogens

APX2039 is 32-fold more potent than APX001A (MGX) vs *Cryptococcus*, but is less active vs *C. albicans* and *A. fumigatus*

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (mcg/mL)</th>
<th>MIC or MEC (mcg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. neoformans</em> (H99)</td>
<td><em>C. gattii</em> (WM276)</td>
</tr>
<tr>
<td>APX001A</td>
<td>0.25</td>
<td>0.125</td>
</tr>
<tr>
<td>APX2039</td>
<td>0.008</td>
<td>0.004</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Caspofungin</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Shaw, et al, AAC 2018
Mouse Delayed Oral Therapy Cryptococcosis Model

Inoculation, IV, 5 x 10^4 cells

Start Therapy

Euthanasia

APX2039, 60 mg/kg, QD + ABT, 50 mg/kg, QD

Days Post Infection
APX2039 Had Superior Efficacy in Mouse Cryptococcosis Model

Log$_{10}$ CFU/g Mouse Tissue - Surviving mice only

- Delayed model (24 h); 7 days of therapy – CFU determined on Day 9 from surviving mice
  - Flu (PO) 150 mg/kg (800 mg humanized dose); AmB (IV) 3 mg/kg; APX2039 (PO, QD) with 50 mg/kg ABT

### Table: Statistical Significance

<table>
<thead>
<tr>
<th></th>
<th>Brain</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2039</td>
<td>&lt;0.0001</td>
<td>0.9935</td>
</tr>
<tr>
<td>2039+FLU</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>AmB</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Flu</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AmB</td>
<td>0.033</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

### Graph:
- Dextrose Vehicle
- ABT + DMSO Tween vehicle
- APX2039 60 mg/kg +ABT
- APX2039 + FCN 60 mg/kg +ABT
- Amphotericin b
- FCN
Rabbit Cryptococcal Meningitis Study Design: More Challenging and Greater Clinical Relevance

Therapy
- Oral APX2039, 50 mg/kg, BID
- IV Amphotericin B, 1 mg/kg, QD
- Oral Fluconazole, 80 mg/kg, QD

Start Therapy
- CSF Tap
- PK Blood
- CSF Tap
- CSF Tap
- Euthanasia

Corticosteroids

Days Post Infection

-1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Oral APX2039 Rapidly Reduces Fungal Burden in CSF Better than AMB and FLC

![Graph showing the reduction of CFU/mL (Log10) over days post infection. The graph compares Control, Fluconazole, Amphotericin B, and APX2039 (50 mg/kg, BID). Error bars indicate 95% CI, T Distribution.](image)
Oral APX2039 has a Rate of Reduction of $\sim 0.4 \text{ log/day}$ in the Rabbit Model

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Average Total Change</th>
<th>Average Change Per Day</th>
<th>SD, Change Per Day</th>
<th>95% CI, T, Change Per Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14</td>
<td>-0.10</td>
<td>0.01</td>
<td>0.21</td>
<td>-0.11 - 0.14</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>5</td>
<td>-2.28</td>
<td>-0.16</td>
<td>0.04</td>
<td>-0.21 - -0.11</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>5</td>
<td>-3.92</td>
<td>-0.28</td>
<td>0.05</td>
<td>-0.34 - -0.22</td>
</tr>
<tr>
<td>APX2039 (50 mg/kg, BID)</td>
<td>8</td>
<td>-4.60</td>
<td>-0.35</td>
<td>0.06</td>
<td>-0.4 - -0.29</td>
</tr>
</tbody>
</table>
Oral APX2039 Reduces Fungal Burden in Brain Tissue Better than AMB and FLC

Kruskal-Wallis, p = 1.2e-05

Bars represent mean, p values obtained using Wilcoxon
APX2039 Plasma PK (5 rabbits)
Pharmacokinetics of APX2039 in Rabbits

<table>
<thead>
<tr>
<th>Mean</th>
<th>APX2039* Geomean</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (mcg/ml)</td>
<td>3.7</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>2</td>
</tr>
<tr>
<td>$AUC_{\text{last}}$ (mcg*h/ml)</td>
<td>26.3</td>
</tr>
<tr>
<td>$T_{\frac{1}{2}}$ (h)</td>
<td>3.1</td>
</tr>
<tr>
<td>$AUC_{\text{inf}}$ (mcg*h/ml)</td>
<td><strong>29.4</strong></td>
</tr>
</tbody>
</table>

*AUC, $C_{\text{max}}$, $T_{\text{max}}$: Geometric mean and coefficient of variation

0-12 hr, 50 mg/kg dose, 5 rabbits
APX2039 CSF Levels Remain >10 fold above the MIC of 0.008 mcg/mL
APX2039 CSF Levels Remain >10 fold above the MIC of 0.008 mcg/mL
CSF Levels of APX2039 are ~ 7% of Plasma Levels

<table>
<thead>
<tr>
<th>APX2039 CSF Concentration/APX2039 Plasma Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit ID</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>8148</td>
</tr>
<tr>
<td>8162</td>
</tr>
<tr>
<td>8170</td>
</tr>
<tr>
<td>8186</td>
</tr>
<tr>
<td>8194</td>
</tr>
<tr>
<td>Mean</td>
</tr>
</tbody>
</table>
Concentration of APX2039 in Rabbit Brain Tissues after Oral Dosing 50 mg/kg, BID

MIC C. neoformans H99 = 0.008 mcg/ml
MIC_{90} = 0.03 mcg/ml
Summary

• APX2039 is a novel oral antifungal agent that targets Gwt1, an early step in GPI anchor biosynthesis

• APX2039 has very low MICs vs Cryptococcus

• **APX2039 showed a ~4.5 log reduction in brain and CSF in the rabbit model of CM** likely due to its penetration into the CNS as well as its potent fungicidal activity, similar to what was seen in the murine model of cryptococcosis
  • This reduction was **significantly better than AMB or FLC**

• In humans, a rapid reduction of fungal burden in CSF is associated with better outcome
  • APX2039 demonstrated rapid sterilization of CSF by Day 10 which was not achieved by AMB, or FLC by Day 14, or any other drug previously evaluated in this model

• These findings may have important clinical ramifications for an improved treatment option for CM with a novel oral monotherapy
Team Members

- **Perfect Lab – Rabbit Experiments**
  - Dr. Jennifer Tenor, PhD
  - Dr. Dena Toffeletti, PhD
  - Ms. Julia Palmucci
  - Ms. Kim Boua
  - Ms. Choiselle Marius
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  - Ms. Nancy Myers

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  - Dr. Arthur Mosely, PhD

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  - Dr. Kyha Williams, DVM
  - Mr. Jeff Lee
  - Ms. Gabriella Dancourt

- **Amplyx**
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  - Dr. Quinlyn Soltow, PhD