Animal Models of Fungal Infection

Critical Systems in Development of New Antifungal Agents

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Disclosures/Disclaimers

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• **Disclaimers:** The views expressed in this talk represent my opinions and do not necessarily represent the views of Weill Cornell Medicine or those of the FDA.
Background

• Animal model systems are a critical component of the process of discovery and development of new antifungal agents for treatment and prevention of invasive fungal diseases (IFDs).

• Models of IFDs in murine, rat, guinea pigs, and rabbits have been developed and studied for development of new systemic antifungal agents.

• We will review the conceptual, scientific, and regulatory framework for utilizing these models, cite specific examples of their application, and discuss their predictability for clinical trials.
Objectives

• Review role of laboratory animal model systems in development of new antifungal agents.

• Assess the predictability of these models for predicting outcome in clinical trials.

• Identify the unmet needs and new directions for markers in preclinical and clinical trials.
What is the Role of Animal Models of IFDs?

- **Development of New Antifungal Agents**
  - Screening murine models
  - PK/PD murine systems
  - Larger animal systems:
    - Rats
    - Guinea pigs
    - Rabbits

- **Pathogenesis**
- **Host Defenses**

- **What are the Characteristics of Predictive In Vivo Models of IFDs**
  - Should reflect the host response relevant to the fungus
  - Should have quantifiable outcome variables
    - survival
    - residual fungal burden:
      - culture
      - PCR
    - biomarkers
    - histology
What are Some of the More Widely Studied Invasive Fungal Diseases?

- **Candida spp.**
  - Neutropenic thigh model
  - Disseminated Candidiasis
    - Acute
    - Subacute
    - Chronic
    - CVC/biofilm studies
  - HCME
  - Oropharyngeal and esophageal candidiasis
  - Cutaneous
  - VVC

- **Aspergillus spp.**
  - IPA
  - CNS disease

- **Mucorales**
  - Pulmonary
  - Disseminated

- **Endemic mycoses**

- **Cryptococcus spp.**
  - CNS infection

- **Hyaline and Dematiaceous moulds**

- **Exserohilum rostratum**
  - CNS phaeohyphomycosis
Murine Neutropenic Thigh Model: Understanding PK/PD Properties of Antifungal Agents

- *Candida* spp.
- Origins in bacterial PK/PD studies
- Provides early guidance toward development of dosing and PK/PD parameters
- Identifies dosage parameters for further exploration

Lepak and Andes, Cold Spring Harbor Press
Mucocutaneous Candidiasis

- Oropharyngeal and Esophageal Candidiasis

- Vulvovaginal Candidiasis

- Cutaneous Candidiasis: *Candida auris*

Walsh TJ et al: J Clin Microbiol. 38:2369-2373;

Ghannoum et al. AAC 2020
Disseminated Candidiasis: Different Patterns of Disease

- **Acute**
  - high inoculum
  - rapidly fatal
  - Reflects hemodynamically unstable patients

- **Subacute**
  - candidemia with deep tissue infection
  - hemodynamically stable
  - most commonly studied

- **Chronic**
  - reflects host patterns of hepatosplenic candidiasis

- **CVC/biofilm treatment studies**

  **Antifungal Agents**
  - DAmB
  - LAmB
  - ABLC
  - Fluconazole
  - Voriconazole
  - Isavuconazole
  - Caspofungin
  - Micafungin
  - Anidulafungin
Hematogenous *Candida* Meningoencephalitis (HCME) and Endophthalmitis
Magnetic Resonance Scanning of Experimental Hematogenous *Candida* Meningoencephalitis: Imaging Focal Disruption of the Blood Brain Barrier

- Delayed imaging post Gd57 infusion reveals significantly greater numbers of lesions as a reflection of focal disruption of the BBB
- Implications for diagnosis and monitoring: increase sensitivity
What is the Effect of Disrupted BBB on Penetration of Echinocandins into Brain Tissue in Experimental HCME?

- Echinocandins are an important therapeutic advance against invasive candidiasis
- Whether the large cyclic hexapeptide with low CSF penetration could be used to treat CNS infections was not known
Micafungin is Effective in Experimental HCME

Dose-Effect relationship

\[
\text{Effect (Log}_{10}\text{CFU/g)} = 3.20 - \frac{2.79 \cdot (\text{dose})^{1.12}}{2.15^{1.12} + (\text{dose})^{1.12}}
\]

\[\text{r}^2 = 0.613\]

AUC/MIC ratio-Effect relationship

\[
\text{Effect (Log}_{10}\text{CFU/g)} = 3.20 - \frac{2.81 \cdot (\text{AUC/MIC})^{1.12}}{2499^{1.12} + (\text{AUC/MIC})^{1.12}}
\]

\[\text{r}^2 = 0.614\]
Neonatal Studies

• The studies in HCME established the foundation for PK dose escalation cohort studies in infants and children
• Randomized trial of an echinocandin versus D-AmB in infants.

• Pediatr Infect Dis J. 2013 Nov;32(11):e419-25
Persistently Neutropenic Rabbit Model of Acute Invasive Pulmonary Aspergillosis (IPA)

- The persistently neutropenic rabbit model of invasive pulmonary aspergillosis (IPA) has been a highly predictive system in identifying new antifungal agents for treatment and prevention of this frequently lethal infection.
Persistently Neutropenic Rabbit Model of Invasive Pulmonary Aspergillosis (IPA)

- Central silastic venous catheter permits atraumatic venous access
- Ara-C induction of profound and persistent neutropenia
- Further immunomodulation with CsA and methylprednisolone, where applicable
- Intensive supportive care with at least twice daily monitoring, and 24/7 on-call schedule
What are the Basic Methodologies and Characteristics of the Animal Model?

- Direct endotracheal inoculation of a carefully quantified inoculation under general anesthesia
- Colonization of the tracheobronchial tree
- As immune suppression progresses, colonization progresses to nodular and segmental pneumonia
- Initiation of therapy 24 hours: trigger to treat justification
- Duration of study 12-14 days
What has been the Impact of Markers of Therapeutic Response in the Persistently Neutropenic Rabbit Model of IPA?

- Since its initial development, the persistently neutropenic rabbit model of IPA using a panel of markers of therapeutic response and safety has established a strong preclinical foundation for:
  - dosages
  - drug disposition, pharmacokinetics
  - safety
  - tolerability
  - efficacy

- Clinical trials
  - deoxycholate amphotericin B
  - liposomal amphotericin B
  - amphotericin B lipid complex
  - amphotericin B colloidal dispersion
  - caspofungin
  - micafungin
  - anidulafungin
  - voriconazole
  - posaconazole
  - isavuconazole
  - Ibrexafungerp
Efficacy of Unilamellar Liposomal Amphotericin B in Treatment of Pulmonary Aspergillosis in Persistently Granulocytopenic Rabbits: The Potential Role of Bronchoalveolar D-mannitol and Serum Galactomannan as Markers of Infection

• Humanized dosing
• liposomal amphotericin B, 5 mg/kg/day IV,
• compared with high-dose conventional deoxycholate amphotericin B, 1 mg/kg/day IV
• more effective

• Outcome markers
• increasing survival,
• reducing the number of viable organisms,
• decreasing tissue injury due to *Aspergillus* organisms
• preventing nephrotoxicity
In a double-blind trial, patients with proven or probable invasive aspergillosis or other mold infection were randomized to receive liposomal amphotericin B at either 3 or 10 mg/kg per day for 14 days, followed by 3 mg/kg per day.

Overall survival rate at 12 weeks AmBisome at 3 mg/kg/d was 72%

Overall response rate at 12 weeks was 50%
Pharmacodynamics of Amphotericin B Deoxycholate, Amphotericin B Lipid Complex, and Liposomal Amphotericin B against *Aspergillus fumigatus*

- We studied the pharmacokinetics (PK) and pharmacodynamics (PD) of amphotericin B deoxycholate (DAMB), amphotericin B lipid complex (ABLC), and liposomal amphotericin B (LAMB) by using the neutropenic-rabbit model of IPA.
- Near-maximal antifungal activity was evident with DAMB at 1 mg/kg/day and ABLC and LAMB at 3 to 5 mg/kg/day.
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- All formulations of amphotericin B induce a dose-dependent reduction in markers of lung injury and circulating fungus-related biomarkers.
- A clinical dosage of liposomal amphotericin B of 3 mg/kg/day is predicted to cause complete suppression of galactomannan and (1,3)-beta-D-glucan levels in the majority of patients.
Antifungal Activity and Pharmacokinetics of Posaconazole (SCH 56592) in Treatment and Prevention of Experimental Invasive Pulmonary Aspergillosis – Correlation with Galactomannan Antigenemia

- Antifungal therapy:
  - posaconazole at 2, 6, and 20 mg/kg of body weight per os
  - itraconazole (ITC) at 2, 6, and 20 mg/kg per os
  - Deoxycholate amphotericin B (AMB) at 1 mg/kg IV
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![Graphs showing concentration profiles of POC and ITC](image)
Treatment of Invasive Aspergillosis with Posaconazole in Patients Who Are Refractory to or Intolerant of Conventional Therapy: An Externally Controlled Trial

- Efficacy and safety of posaconazole oral suspension (800 mg/day in divided doses) as monotherapy
- open-label, multicenter study in patients with invasive aspergillosis

Global response by salvage therapy

- The overall success rate (DRC-assessed global response at the end of treatment)
- 42% for posaconazole recipients
- 26% for control subjects
- (odds ratio, 4.06; 95% confidence interval, 1.50–11.04; \( P \) p .006).

<table>
<thead>
<tr>
<th>Therapy</th>
<th>No. of subjects</th>
<th>No. (%) of responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>Posaconazole group</td>
<td>107</td>
<td>45 (42)</td>
</tr>
<tr>
<td>Control group: all therapies</td>
<td>86</td>
<td>22 (26)</td>
</tr>
<tr>
<td>Amphotericin B(^a)</td>
<td>80</td>
<td>16 (20)</td>
</tr>
<tr>
<td>Itraconazole(^b)</td>
<td>49</td>
<td>14 (29)</td>
</tr>
<tr>
<td>Amphotericin B and itraconazole</td>
<td>45</td>
<td>10 (22)</td>
</tr>
<tr>
<td>Other therapies(^c)</td>
<td>36</td>
<td>11 (31)</td>
</tr>
</tbody>
</table>
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![Survival distribution function graph]
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<table>
<thead>
<tr>
<th>Quartile</th>
<th>No. of subjects(^a)</th>
<th>Plasma C(_{\text{max}}) Mean ng/mL</th>
<th>CV, %</th>
<th>Plasma C(_{\text{avg}}) Mean ng/mL</th>
<th>CV, %</th>
<th>No. (%) of responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>142</td>
<td>51</td>
<td>134</td>
<td>45</td>
<td>4 (24)</td>
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<tr>
<td>2</td>
<td>17</td>
<td>467</td>
<td>27</td>
<td>411</td>
<td>21</td>
<td>9 (53)</td>
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<tr>
<td>3</td>
<td>17</td>
<td>852</td>
<td>15</td>
<td>719</td>
<td>12</td>
<td>9 (53)</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>1480</td>
<td>16</td>
<td>1250</td>
<td>28</td>
<td>12 (75)</td>
</tr>
</tbody>
</table>

**NOTE.** C\(_{\text{avg}}\), average plasma concentration; C\(_{\text{max}}\), maximum plasma concentration; CV, coefficient of variation.

\(^a\) Data were available for 67 patients with available plasma concentrations of posaconazole.
In patients undergoing chemotherapy for acute myelogenous leukemia or the myelodysplastic syndrome, posaconazole prevented invasive fungal infections more effectively than did either fluconazole or itraconazole and improved overall survival.
Combination Antifungal Therapy

- Three major preclinical studies in the persistently neutropenic rabbit model using the broad range of therapeutic response biomarkers laid the preclinical foundation for the combination therapy with a mould active triazole and an echinocandin for primary treatment of IPA.

- Also predicted the outcome of the clinical trial for voriconazole plus anidulafungin for treatment of IPA.
Combination Therapy in Treatment of Experimental Pulmonary Aspergillosis

*In Vitro and In Vivo* Correlations of the Concentration- and Dose-Dependent Interactions between Anidulafungin and Voriconazole by Bliss Independence Drug Interaction Analysis
Combination Therapy in Treatment of Experimental Pulmonary Aspergillosis

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*In Vitro* and *In Vivo* Correlations of the Concentration- and Dose-Dependent Interactions between Anidulafungin and Voriconazole by Bliss Independence Drug Interaction Analysis
Combination Antifungal Therapy for Invasive Aspergillosis: A Randomized Trial

- Mortality rates at 6 weeks were 19.3% (26 of 135) for combination therapy and 27.5% (39 of 142) for monotherapy (difference, −8.2 percentage points [95% CI, −19.0 to 1.5]; \( P = 0.087 \)).

- Multivariable regression analysis suggested that maximum galactomannan value, Karnofsky score, and baseline platelet count had prognostic significance.

- Post hoc analysis: 6-week mortality was lower in combination therapy than monotherapy (15.7% [17 of 108] vs. 27.3% [30 of 110]; difference, −11.5 percentage points [CI, −22.7 to −0.4]; \( P = 0.037 \)).
APX001 Pharmacokinetic/Pharmacodynamic Target Determination against *Aspergillus fumigatus* in an *In Vivo* Model of Invasive Pulmonary Aspergillosis

Zhao et al AAC 2019
Efficacy of the Investigational Echinocandin ASP9726 in a Guinea Pig Model of Invasive Pulmonary Aspergillosis

Survival in guinea pigs with invasive pulmonary aspergillosis treated with the placebo control, voriconazole (VRC; 10 mg/kg twice daily by oral gavage), caspofungin (CAS; 3 mg/kg/day by intraperitoneal injection), and ASP9726 (2.5, 5, and 10 mg/kg/day by subcutaneous injection).
Efficacy of the Investigational Echinocandin ASP9726 in a Guinea Pig Model of Invasive Pulmonary Aspergillosis

Pulmonary fungal burden and serum surrogate marker concentrations in guinea pigs with invasive pulmonary aspergillosis treated with the placebo control, voriconazole (VRC; 10 mg/kg twice daily by oral gavage), caspofungin (CAS; 3 mg/kg/day by intraperitoneal injection), and ASP9726 (2.5, 5, and 10 mg/kg/day by subcutaneous injection).
AmB Lipid Formulations: Murine Pulmonary Mucormycosis

Formulation of AMB changes rate and extent of lung drug distribution in murine pulmonary mucormycosis:

72 h, EC50=MFC

Lewis RE et al. AAC 2010
Diabetic Murine Model of Disseminated Mucormycosis

L-AmB: Dose-dependent Efficacy *in vivo*

Ibrahim et al. AAC 03
LAMB vs. Isavuconazole – Efficacy in vivo

Cyclophosphamide/cortisone immunocompromised murine model of *M. circinelloides* pulmonary mucormycosis

LAMB was dosed at 15 mg/kg/d

Gebremariam et al. JAC 17
Zebra fish as a Model Host for Invasive Fungal Infections

*J. Fungi* 2018, 4(4), 136;  
https://doi.org/10.3390/jof4040136
Non-Vertebrate Animal Model Systems

**Caenorhabditis elegans**
- ✓ Slow & fast killing assays
- ✓ Hermaphroditic lifestyle
- ✗ Uptake by feeding
- ✗ 37 °C

**Drosophila melanogaster**
- ✓ Different sites for infection
- ✓ Biofilm formation
- ✗ 37 °C

**Galleria mellonella larvae**
- ✓ Direct injections
- ✓ Melanization
- ✓ Toxicity testing
- ✓ 37 °C

doi:10.1002/1873-3468.1245
Future Directions

• Implementation of biomarkers from preclinical data into clinical endpoint response criteria
• Development of new models of emerging pathogens
• Systematic integration of data from several models in predicting outcome
Conclusions

• The decision to move from laboratory to clinical trials should be predicated upon a portfolio of complementary and mutually validating preclinical laboratory animal models.

• Meticulous preclinical investigation of a candidate antifungal compound in a robust series of predictive laboratory animal models will optimize study design, de-risk clinical trials, and ensure tangible benefit to our patients.
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