Animal models in preclinical trials of aspergillosis

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Why Perform Animal Models of Aspergillosis?

Studies on therapy

Monotherapy & Combination therapy:

AmB preps, azoles, echinocandins, etc.

Compare efficacies at same time!

Results similar among models and to clinical

Immunotherapy:

Various cytokines (interferon-γ, TNF-α, GM-CSF, etc.), other immunomodulators, Ablation of specific cytokines

The Ideal Animal Model

- No "warm fuzzy test tubes"!

  Should mimic clinical disease
- Should be standardized, reproducible & affordable!
- Parameters of survival and infectious burden controllable: define the model first!
- Wait until infection is established!
- Should reflect drug efficacy, toxicity and drug interactions similar to observed clinically

Animal Models – Control of Variables

Animals – preclinical trial	Humans – clinical trial
Syngeneic & many strains --	No way
Age & sex match --	Sort of
Control inocula & route --	No way
Choice of inoculum strain --	No way
Control disease severity --	Sort of, but really no way
Control exp. duration –	Yes – if they show up
Survival & CFU organs --	Survival – yes, CFU - no
Route & duration of dosing --	Yes – to some degree
Control immunosuppression --	Yes – to some degree
Large #'s done at same time --	Limited # pts. & time
Statistical analyses --	Yes – more difficult
Trials are prospective --	Some are - some are not

Animal Models of Aspergillosis

Primary model systems:

- Murine – systemic, pulmonary, CNS, ocular
- Rabbit – systemic, pulmonary, ocular
- Guinea pig – systemic, pulmonary
- Rat - systemic, pulmonary
- Bird – pulmonary

Normal or immunosuppressed - depends on model

Animal Models of Aspergillus

A. fumigatus is the most frequently used

Can test different *Aspergillus* species causing clinically relevant disease.

Clinically:

A. terreus increase frequency & AMB resistant

Models in rabbits and mice:

resistant to AMBs – like clinical

Saper, ICZ and POS effective

Hanson, et al. 1995 JMVM; Dannaoui , et al. 2000 JMM;
Steinbach, et al. 2004 AAC; Walsh, et al. 2003, JID

Models of Aspergillosis

- Need to be cautious interpreting results!

What looks good in one model may not look good in another model!

Models of Aspergillosis

- Systemic model
  - synergy between Mica and Nik Z
- Pulmonary model
  - NO synergy between Mica and Nik Z

Different models using same drugs may not give same result

Capilla Luque, et al, ’03. AAC. Clemons et al. 2006. Med Mycol

Models of Aspergillosis

Use models for drug efficacy comparisons not likely to be done in a clinical trial

For example – is one lipid formulation of AmB superior for treatment of aspergillosis?

Summary - All Lipid AmB comparison Systemic Aspergillosis

1. All formulations prolonged survival,
2. AmBi nearly equivalent to dAmB; ABCD and ABLC were less effective or equivalent depending on the severity
3. No survivors cured in both kidneys and brain
4. Each formulation efficacious, esp. in kidneys.
5. No lipid-formulation consistently superior to Fungizone even though higher doses given
6. All were equivalent to each other

Clemons and Stevens. AAC, 2004

Aspergillus Animal Models

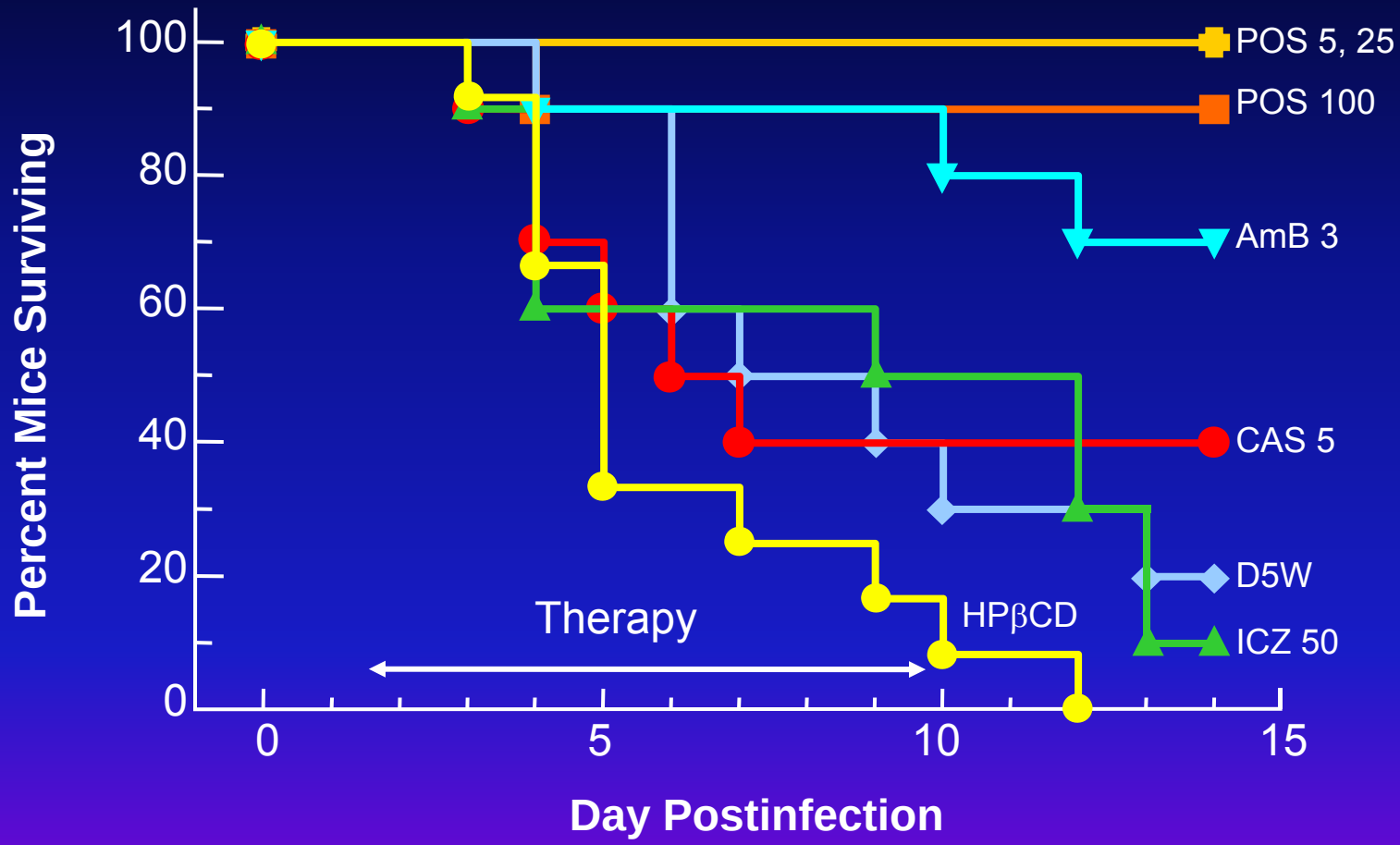
Use models to look at new indications not possible in a clinical trial

Test efficacy of new and “old” drugs

- Alone – monotherapy
- Combination

Example – CNS aspergillosis

CNS Aspergillosis Monotherapy Studies



Imai, et al. 2004, AAC

Monotherapy against CNS Aspergillosis

Amphotericin B

- dAmB, iv partially effective
- AmBisome or ABLC iv effective not curative

Azoles

- ICZ and VCZ PO partially effective
- POS very effective

Echinocandins

- Mica or CAS equally effective

NONE CURATIVE!

Aspergillus Animal Models

Use to examine combination therapies
Use to try regimens to achieve cure

CNS aspergillosis

Clinically, CNS disease a common site of dissemination. Poor response to treatments and high mortality.

CNS Model Objectives

- Examine combinations of antifungal drugs (AmBi, ABLC, dAmB, MICA, VCZ, ICZ, CAS) or escalating dosages

Combinations against CNS aspergillosis

- AmBi + echinocandins –improved survival, but no significant enhancement
- AmBi + VCZ – *significant enhancement*
- Sequential AmBi + VCZ ca. as effective as combination
- dAmB + CAS – indifferent
- Only combination regimens reduced CFU
- Only AmBi +VCZ caused significant reduction in brain and kidney
- No total cures were obtained

Imai, et al. 2005, JAC; Clemons, et al. 2005 AAC

Combinations and CNS aspergillosis

- ABLC at 4 mg/kg = to higher dosages
  - more not better
- ABLC + echinocandins – improved survival, but ns.
  - (lower doses of combination equal to higher doses!)
- ABLC + ICZ – indifferent
- ABLC + VCZ – *significant enhancement*
- AmB + ICZ – slight enhancement?
- AmB + CAS – indifferent

Similar to results with AmBisome (Clemons et al. AAC, 2005)

Clemons et al. JAC, 2006

Combination Therapy Pulmonary Aspergillosis

In steroid-suppressed DBA/2 pulmonary model

-AmBi + VCZ, CAS, or Mica
-dAmB + Mica
-Mica + NikZ
-ICZ + Mica

None better than monotherapy

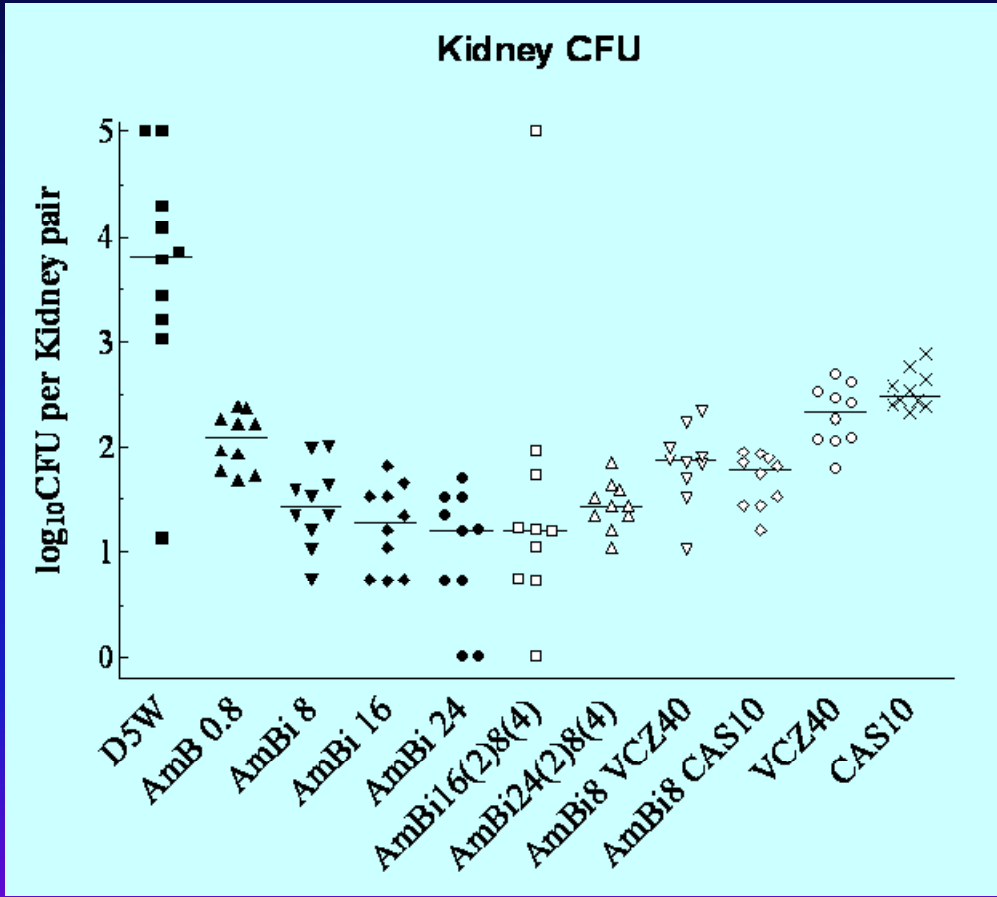
Aspergillus Animal Models

Cure, “The Holy Grail” of clinical medicine

Can you achieve cure
Using high doses?
Using combination therapy?

Is more better?
At what cost to tissues?

Achieving cure- systemic *Aspergillus*



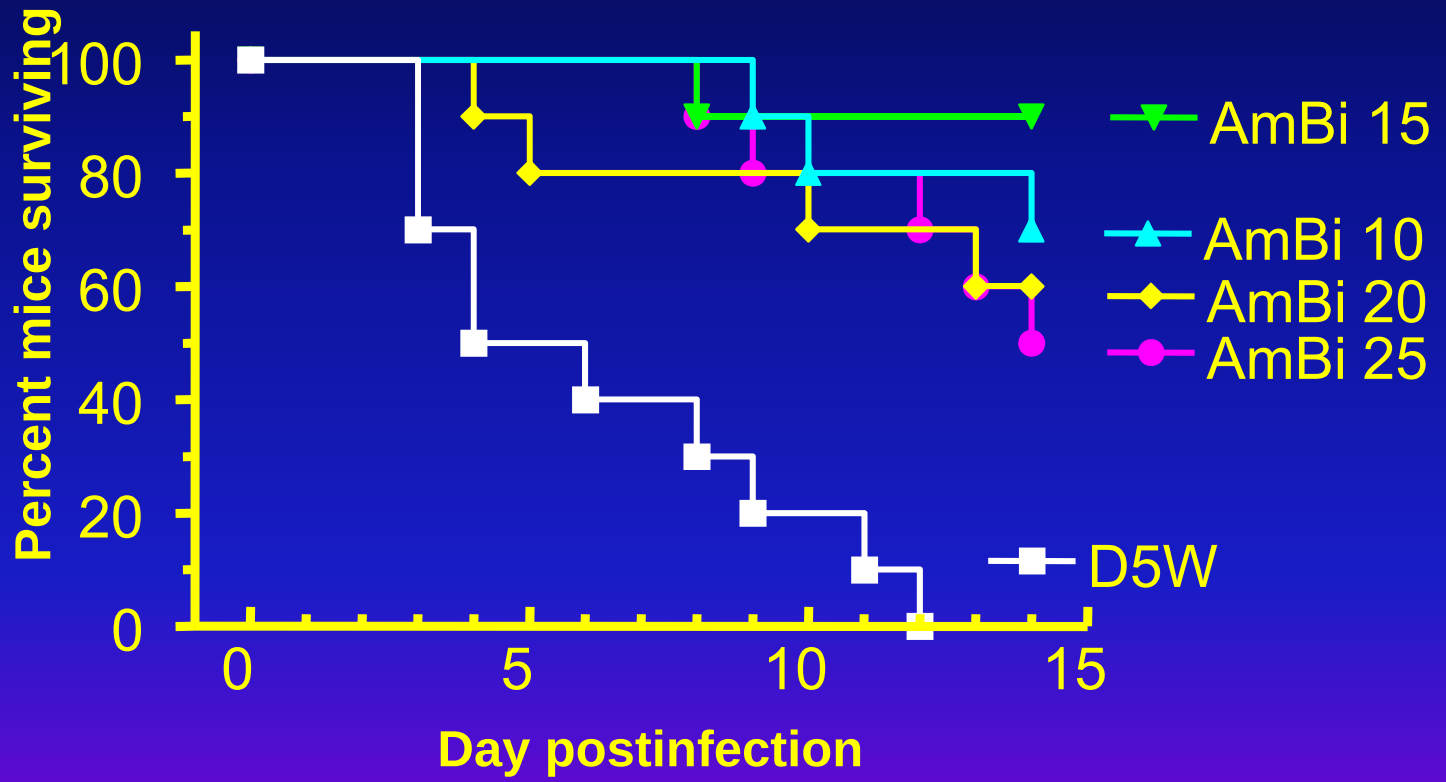
Test AmBi, CAS, VCZ

- Dose-escalation
- Loading doses AmBi
- Combination
  AmBi + CAS or VCZ

No improvement in frequency of CURES!

Clemons, et al. AAC, 2005

Achieving cure for CNS Aspergillosis by dose-escalation



NO CURES & MORE IS NOT BETTER!

Clemons, et al. AAC, 2005

Achieving cure-
Systemic, Pulmonary or CNS *Aspergillus*

- More isn't better and may be bad!
  - Increased dosages did not improve efficacy.
  - Dose-escalation of AmBi or ABLC may be deleterious
  - AmBi loading doses no improvement
- MICA or CAS – flat dose-response – 1 =10 mg/kg
- Suboptimal combo ≅ high dose AmBi or ABLC
- Sequential therapy not effective for cure
- No improvement in cure rate by any regimen

What about other filamentous fungi?

Increased frequency of zygomycosis

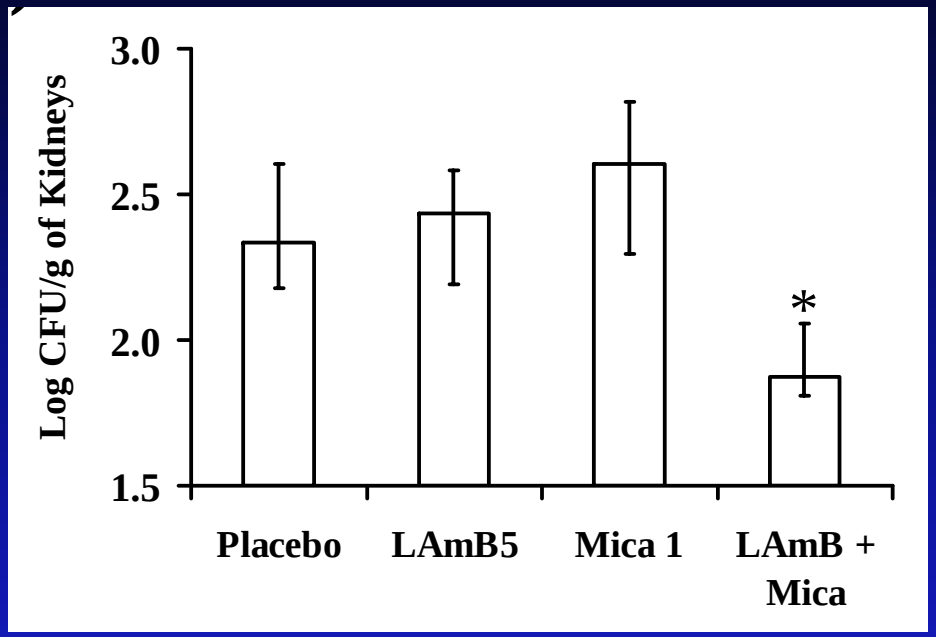
Treatments are poorly effective.

What works?

LAmB and echinocandin combination therapy against experimental mucormycosis

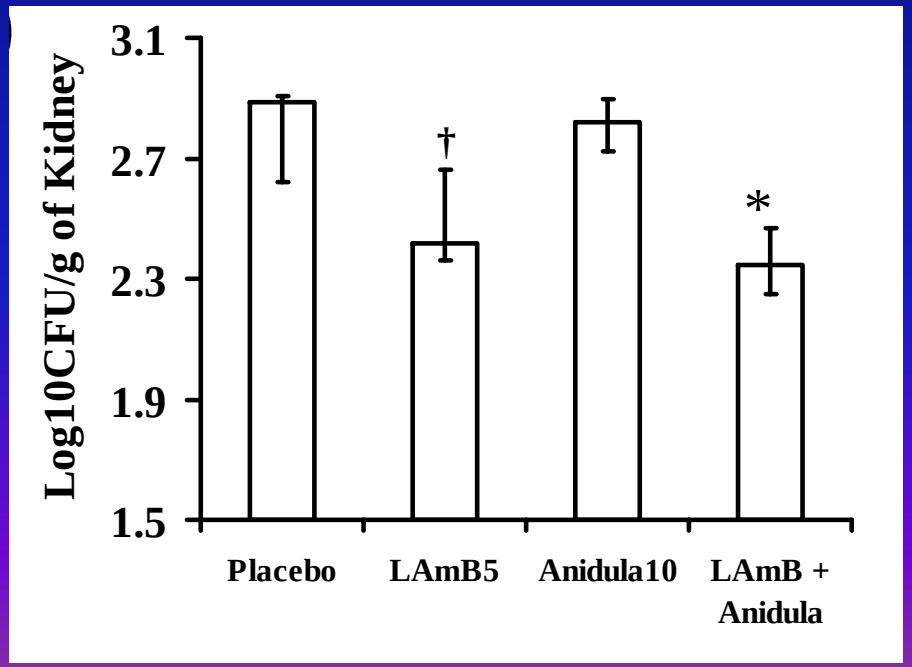
Ibrahim et al. 08 AAC:52:1556-8

LAmB +Mica or LAmB +Anidula: DKA mouse model



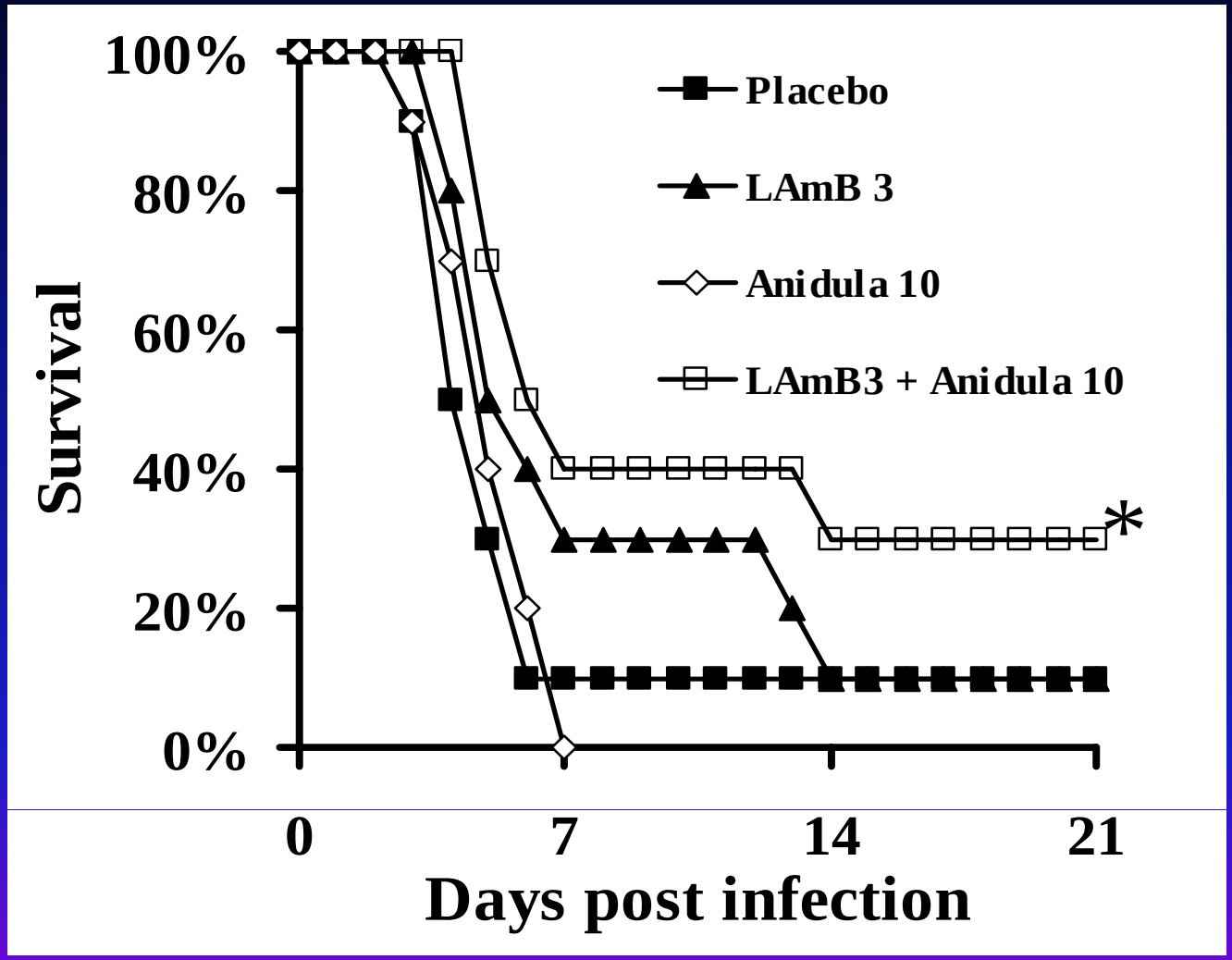
LAmB + Mica
Enhanced survival & CFU

LAmB + Anidula
Enhanced survival
Less for CFU



Ibrahim et al. 08 AAC:52:1556-8

LAmB +Anidula: Neutropenic mouse model



Ibrahim et al. 08 AAC:52:1556-8

What's on the horizon for antifungals ?

Azoles

- Isavuconazole (BAL-4815; Basilea)
  - Prodrug, active against *Candida* and *Aspergillus*
- Albaconazole (UR9825; Uriach)
  - broad spectrum
- Ravuconazole (BMS207147 or ER30346: Eisai)
  - broad spectrum
- ITF2534 (Italfarmaco)
  - broad spectrum, in vivo *Candida, Aspergillus Cryptococcus*

Pasqualotto and Denning, JAC, 2008; Clemons, et al ICAAC, 2008

What's on the horizon for antifungals ?

Polyenes

- Patricin A – (SPA-753)
  - *Candida* & *Cryptococcus in vivo*,
  - *Aspergillus in vitro* *(Rimaroli &* Bruzzese, Chemother, 2000)
- New formulations of amphotericin B

Echinocandins

- Aminocandin (HMR-3270; Novexel)
  - *Candida* and *Aspergillus*

New classes

- *Arylamidine* (T-2307)
  - *Candida, Aspergillus, & Cryptococcus* (Mitsuyama et al AAC 2008)
- Sordarins??

Summary of animal models in preclinical trials aspergillosis

- Results provide a basis for running clinical trials of antifungal therapy for aspergillosis
- Have been the basis for treatment with AmBs, various azoles and echinocandins now approved for therapy
- Provide the rationale for potential clinical trial of various combination therapies
  - (e.g., liposomal AmB + VCZ, or CAS or MICA)
- Will be necessary to bring new drugs to trial

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