Newer Combination Therapies

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Combination Therapy Rationale

- Widened spectrum and potency
- More rapid or fungicidal antifungal effect
- Additive or synergistic effects
- Reduce risk of emerging resistance
- Increased penetration / transport

- Inhibit different stages of same pathway
- Simultaneous inhibition of different fungal targets

Voriconazole + Caspofungin

- Neutropenic guinea pig model, IV inoculation
  - Mortality (0/12 animals) and mean times of survival (8 days post infection) SAME in EACH of these arms:
    - Voriconazole 5 mg/kg/d
    - Caspofungin (1 mg/kg/d) + Voriconazole
    - Caspofungin (2.5 mg/kg/d) + Voriconazole
    - Better than AmB (1.25 mg/kg/d) or caspofungin monotherapy (1 or 2 mg/kg/d)
  - Semiquantitative cultures for fungal burden (CFU/g tissue) with combination better than ***untreated controls*** only ($p < 0.0025$)

Kirkpatrick WR, et al. *Antimicrob Agents Chemother* 2002;46:2564-8

Voriconazole + Caspofungin

- Neutropenic guinea pig model, IV inoculation
- Purposefully designed to minimize the therapeutic effects of monotherapies to enhance any combination benefit
  - Voriconazole 1 mg/kg BID (NOT 5 mg/kg/d)
  - Stronger immunosuppression
  - Two higher challenge doses (10^4 CFU/g and 10^3 CFU/g)
  - qPCR to analyze fungal burden
    - Voriconazole 1 mg/kg BID
    - Caspofungin 1 mg/kg/d
    - Voriconazole + Caspofungin

MacCallum DM, et al. *Antimicrob Agents Chemother* 2005;49:3697-3701.

Voriconazole + Caspofungin

- Median Survival Duration
  - Higher inoculum: Caspofungin (p=0.002) and Combination (p=0.0004) over untreated controls only
  - Lower inoculum: caspofungin (p=0.001), voriconazole (p=0.014), and the combination (p<0.001) over untreated controls
    - VCZ + Caspo ALSO greater than Caspo (p=0.048) ***only*** in the 10^3 conidia group
    - No survival differences between voriconazole and combination

- *Kidney A. fumigatus* qPCR fungal burden
  - VCZ + Caspo lower than Caspo (p<0.001) ***only*** in the 10^3 conidia group
  - Again, no difference in voriconazole vs. combination

MacCallum DM, et al. *Antimicrob Agents Chemother* 2005;49:3697-3701.

Ravuconazole + Micafungin

- Neutropenic rabbit model, intratracheally inoculated
  - Micafungin (1 mg/kg/d) (n=8)
  - Ravuconazole (2.5 mg/kg/d) (n=8)
  - Micafungin + Ravuconazole (n=12)

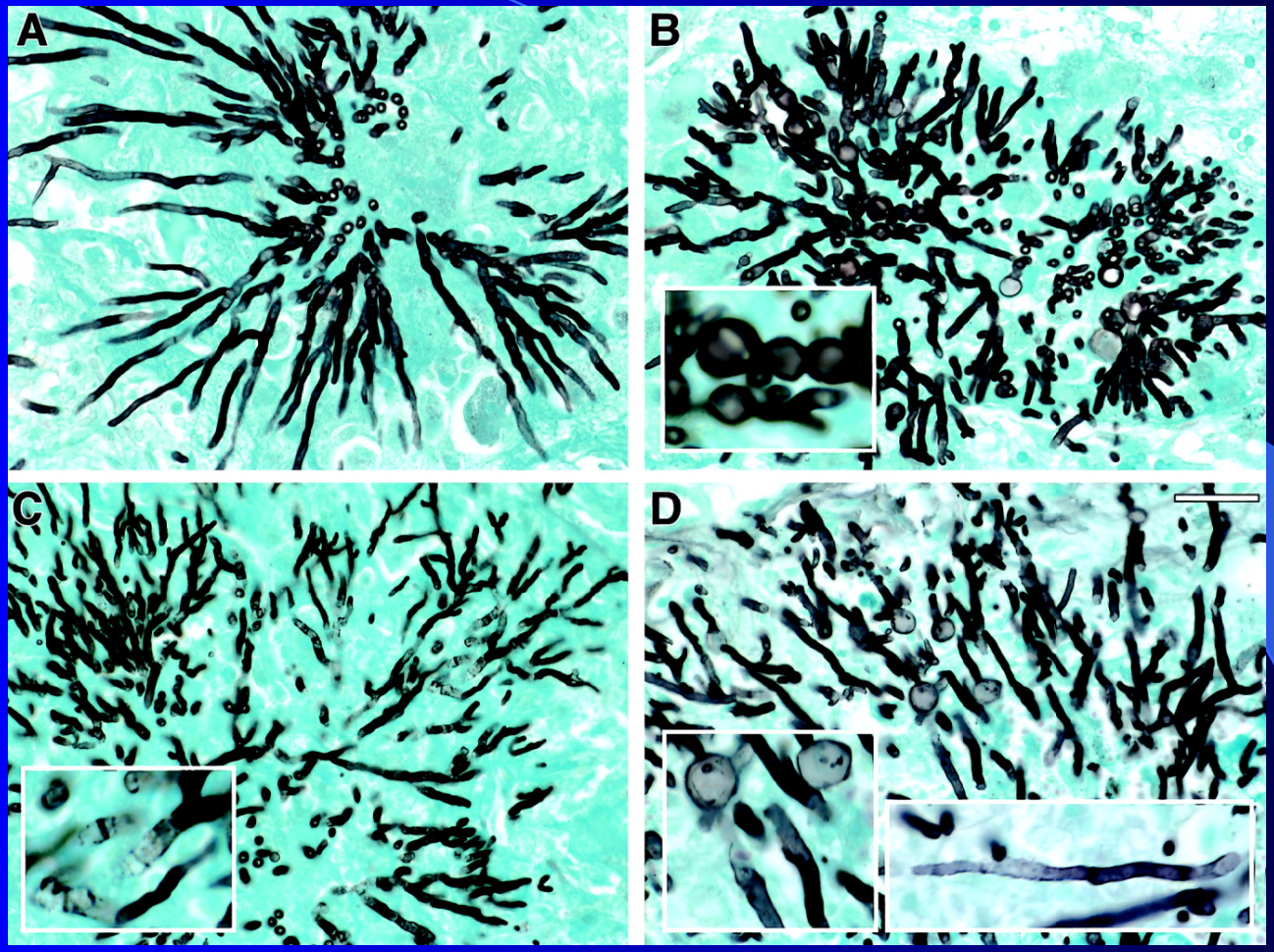
- Survival
  - Micafungin monotherapy 0% (0/8)
  - Ravuconazole monotherapy 25% (2/8)
  - Micafungin + Ravuconazole 75% (9/12) ($p<0.001$)

Petraitis V, et al. *J Infect Dis* 2003;187:1834-43

Ravuconazole + Micafungin Hyphal Damage

Untreated Control

Micafungin



Ravuconazole

Ravuconazole + Micafungin

Petraitis V, et al. *J Infect Dis* 2003;187:1834-43

1966-2001 Review of Combination Therapy: 6,281 Cases

Studies	Syn	Add	Indiff	Antag
In vitro (n=28)	36%	24%	28%	11%
In vivo (n=18)	14%	20%	51%	14%

- AmB + Itraconazole generally indifferent interactions *in vitro*, *in vivo*, and clinically

- 249 cases met combination Rx inclusion criteria
- Most common combinations:
  - AmB + Flucytosine (49%)
  - AmB + Itraconazole (16%)
  - AmB + Rifampin (11%)

- Overall 63% of **clinical cases** reported improvement

Steinbach WJ, et al. *Clin Infect Dis* 2003;37 (suppl 3): S188-224

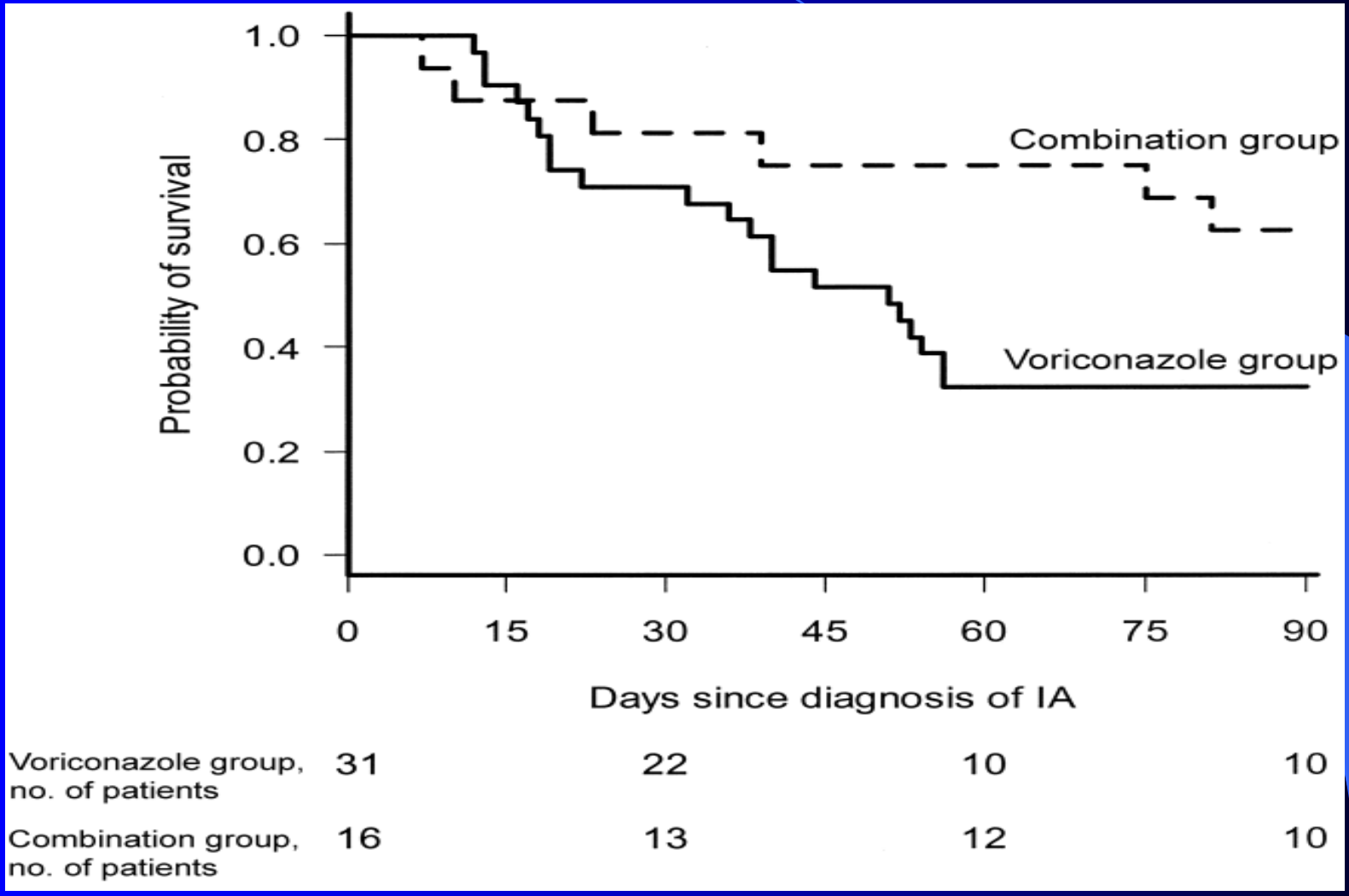
Voriconazole + Caspofungin Salvage Therapy

- 47 patients with proven/probable pulmonary IA from 1997-2001
  - Begin with AmB (≥ 1 mg/kg/d) and then change to voriconazole (6 mg/kg, then 4 mg/kg BID) for salvage therapy (toxicity or progressive disease after ≥ 7 days of AmB therapy)
  - In Feb 2001, practice changed so that caspofungin administered with voriconazole as salvage therapy
- Received either voriconazole (n=31) or voriconazole + caspofungin (n=16) as *salvage therapy*
- Most received salvage therapy due to clinical failure, not intolerance
- Outcomes evaluated relative to both the day of diagnosis and the start of salvage therapy because salvage initiated at different times

Marr KA, et al. *Clin Infect Dis* 2004;39:797-802.

Voriconazole + Caspofungin Salvage Therapy

Kaplan-Meier probability of overall survival at 3-months after day of diagnosis
P = .048, calculated from the likelihood ratio test using Cox regression



Marr KA, et al. *Clin Infect Dis* 2004;39:797-802.

Primary Combination Therapy

- Retrospective single center cohort review of consecutive patients with IA and an underlying hematologic malignancy (Jan 98 – July 03)
- Proven (n=17) / Probable (n=17) / Possible (n=11) by EORTC/MSG
- Data presented below for Proven / Probable cases only

	ALL (n=34)	Combo (n=10)	Mono (n=24)	P value
12 wk Survival	53%	50%	54%	0.82
Median Survival (d)	110	102	115	---
CR/PR	41%	50%	37.5%	0.5
Stable	5.9%	0%	8.3%	--
Failure	53%	50%	54%	0.86

- No differences in survival between primary therapy with mono vs. combo

Munoz LS, et al. ICAAC 2004, Abstract M-1024

Novel Combination Approach Needed

Patient Responses to Calcineurin Inhibitors Already Suggest a Targeting Role for Invasive Aspergillosis

- Use of Cyclosporine (CsA) vs. conventional immunosuppression led to a 54% decrease in invasive aspergillosis in 126 heart transplant recipients

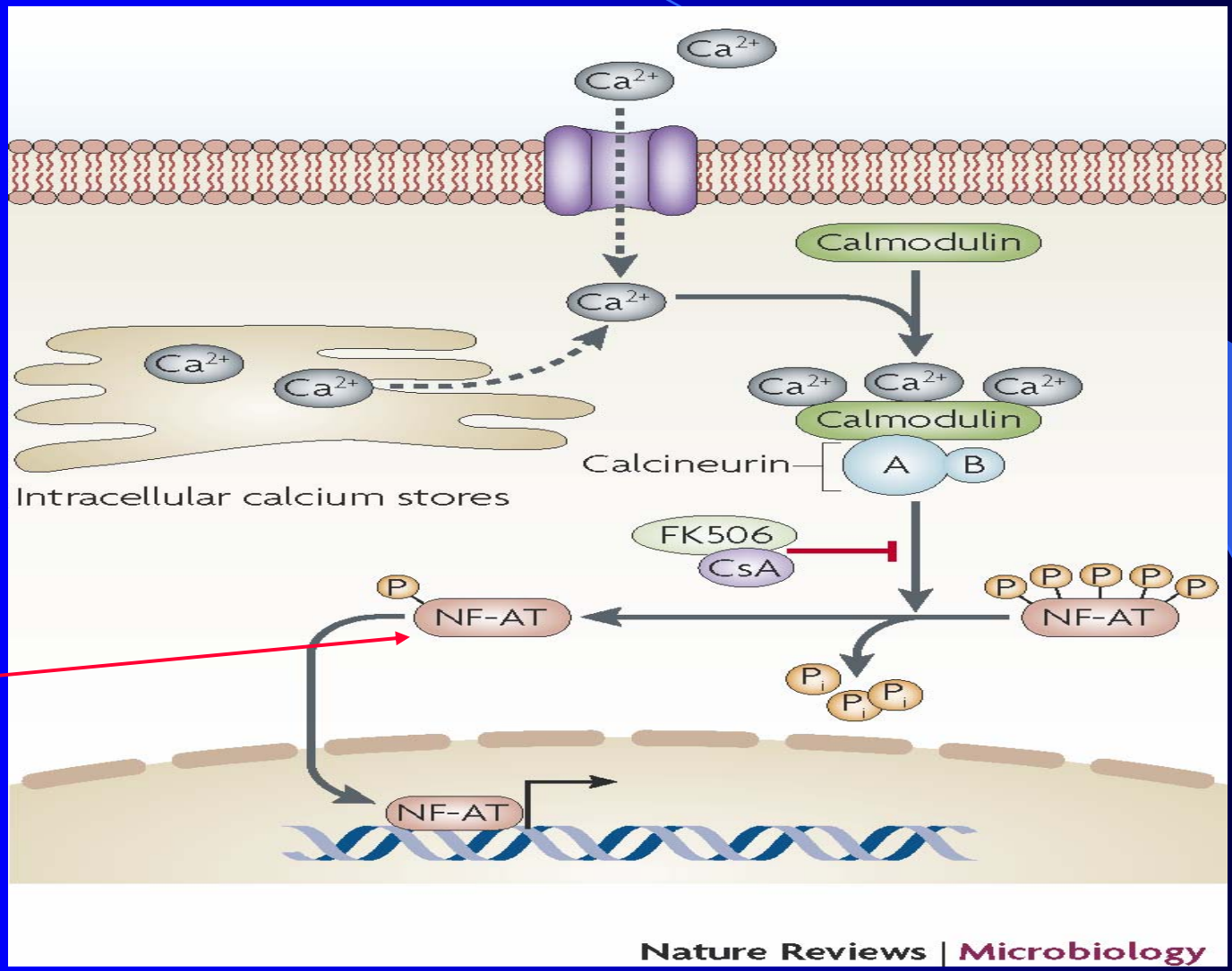
  Hoflin JM, et al. *Ann Intern Med* 1987; 106:209-216

- Liver transplant recipients with invasive aspergillosis who received FK506 showed a significantly lower rate of severe (30% vs. 62%) and brain infection (0% vs. 46%)

  Singh N, et al. *Clin Infect Dis* 2003; 36:46-52

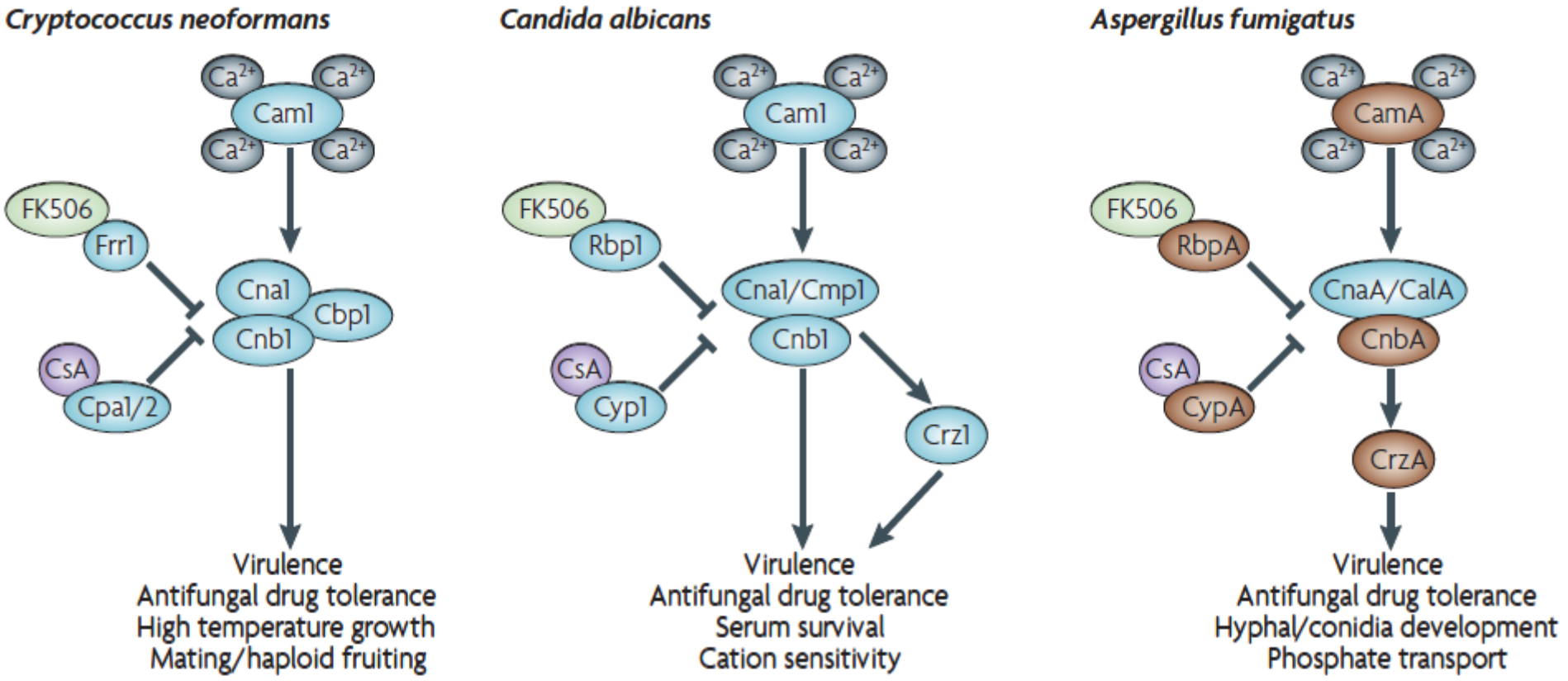
Calcineurin Activation and Inhibition

Fungal *crzA*



Steinbach WJ, *et al*. *Nature Reviews Microbiol 2007;5:418-430.*

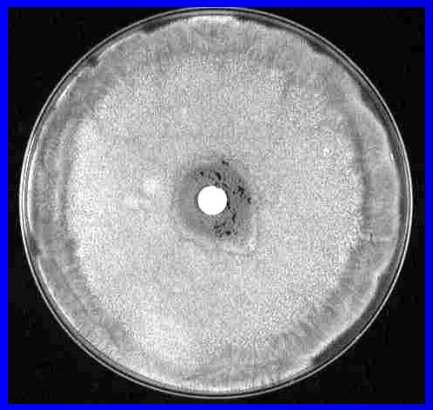
Calcineurin in Pathogenic Fungi



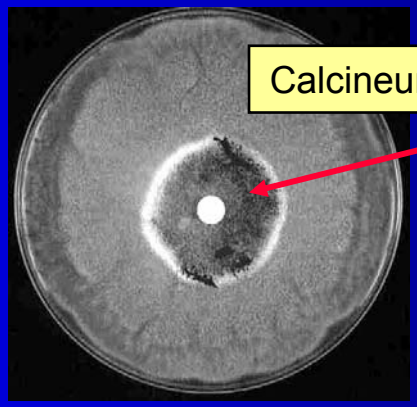
Steinbach WJ, *et al*. *Nature Reviews Microbiol 2007;5:418-430.*

Calcineurin Inhibitors (FK506 and CsA) are **Superior** to Existing FDA-Approved Antifungal

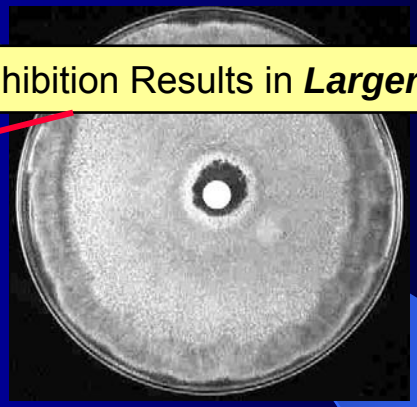
- Drug placed on Disk and Antifungal Activity is Measured by Zone of Clearance of *A. fumigatus* Background Growth



Caspofungin 10 ug



FK506 10 ug

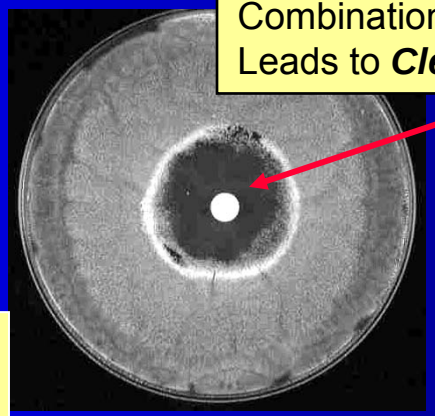


Cyclosporine (CsA) 10 ug

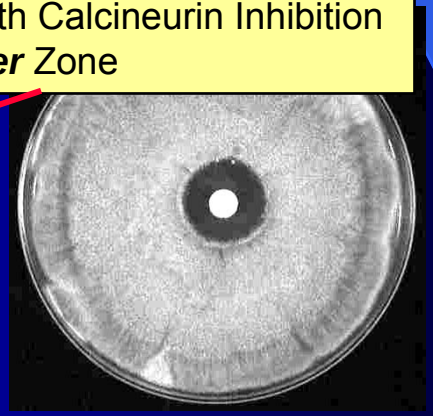
Calcineurin Inhibition Results in ***Larger*** Zone

Caspofungin

FDA-Approved antifungal which inhibits cell wall β1,3-glucan synthesis



Caspofungin + FK506



Caspofungin + Cyclosporine (CsA)

Combination with Calcineurin Inhibition Leads to ***Clearer*** Zone

Calcineurin Inhibitors

FK506 and CsA create ***larger*** and ***clearer*** zones of antifungal activity against background *A. fumigatus*

Steinbach WJ, et al. *Antimicrob Agents Chemother* 2004; 48:1664-9.

Deleting Calcineurin Pathway Genes Leads to Unique Hyphal Defects

Calcineurin pathway genes already deleted to yield unique hyphal defects

Gene	*Gene Name*	*Location*	*Gene Function*	*Mutant Status*
cnaA	**Calcineurin A**	**Afu5g09360**	**Calcineurin catalytic subunit**	***DELETED***
crzA	**Calcineurin-related zinc finger**	**Afu1g06900**	**Calcineurin transcription factor**	***DELETED***
cbpA	**Calcineurin binding protein**	**Afu2g12060**	**Calcineurin binding protein**	***DELETED***
pmrA	**Secretory Ca^{2+}-ATPase**	**Afu2g05860**	**Golgi Ca^{2+}-ATPase pump**	***DELETED***
pmcA	**Vacuolar Ca^{2+}-ATPase**	**Afu7g01030**	**Vacuolar Ca^{2+}-ATPase pump**	***DELETED***

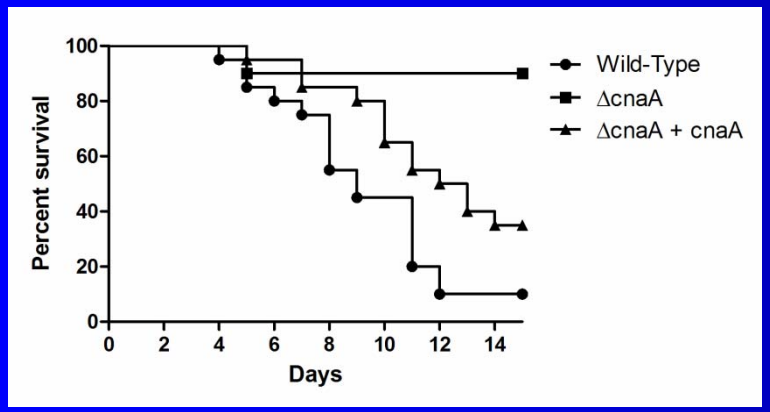
Steinbach WJ, et al. *Eukaryot Cell* 2006;5:1091-103.

Cramer RA Jr, et al. *Eukaryot Cell* 2008;7:1085-97.

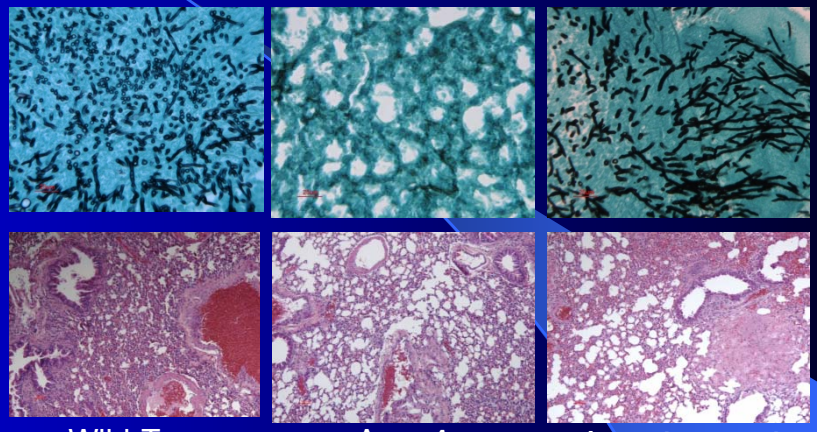
Pinchai N, et al. *Eukaryot Cell* 2009;8:511-19.

Pinchai N et al. *Eukaryot Cell* 2010. *In Press*

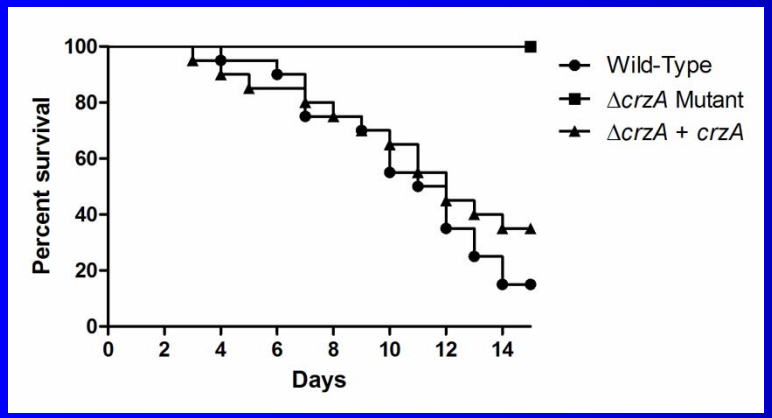
Infections with *A. fumigatus* Lacking *cnaA* or *crzA* Show Limited Disease in Mouse Models



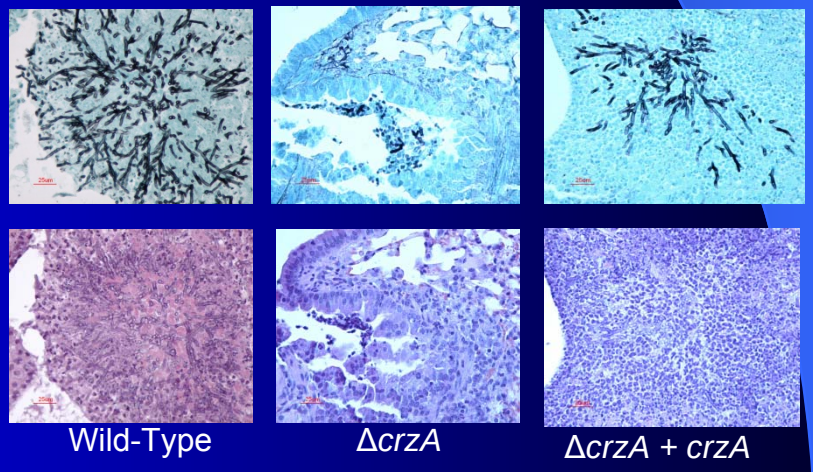
P < 0.001



Steinbach WJ, et al. *Eukaryot Cell* 2006;5:1091-103.

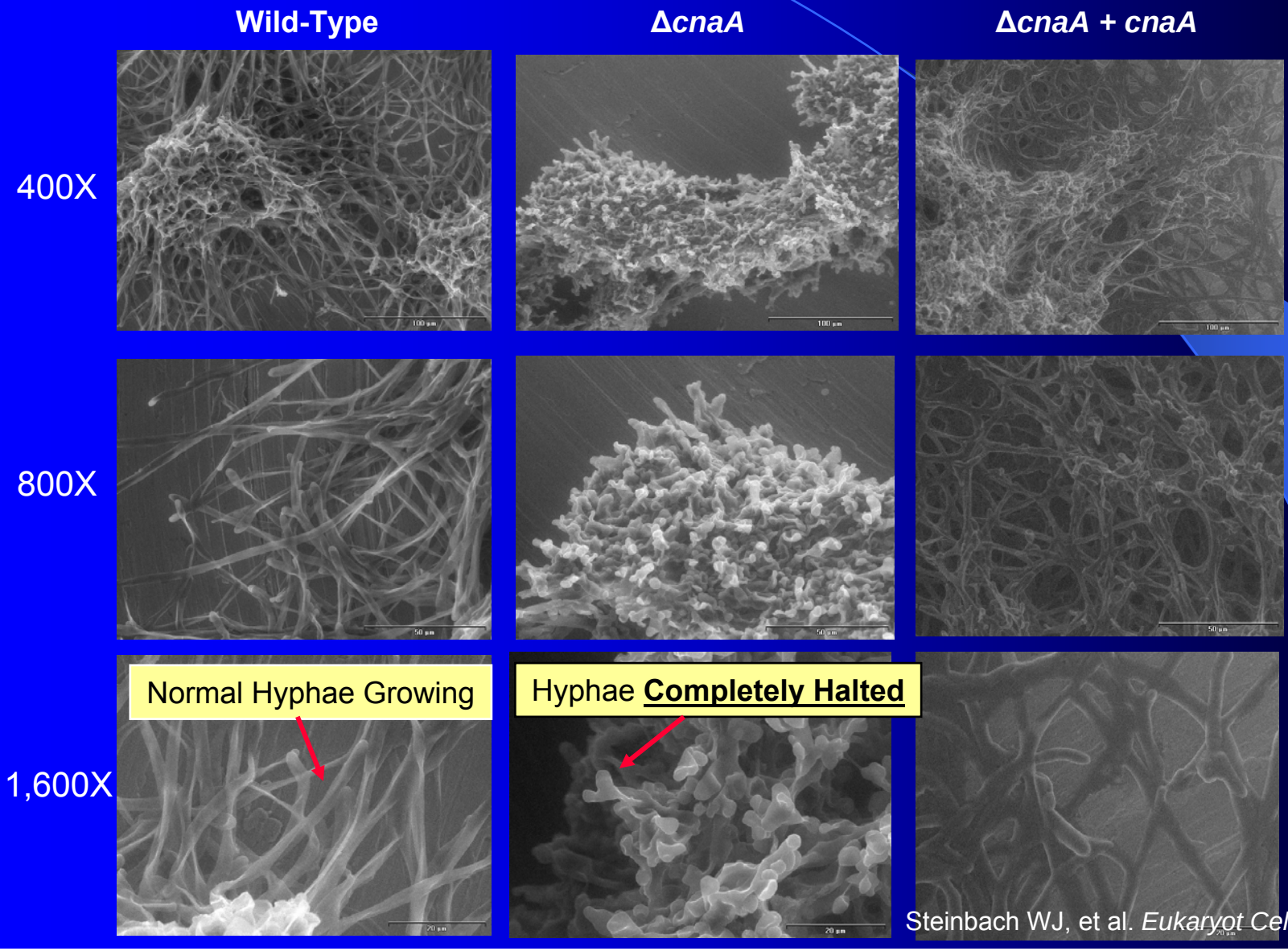


P < 0.001



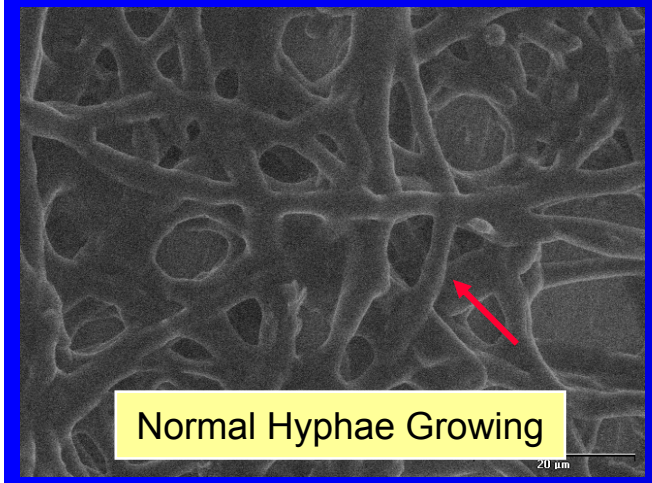
Cramer RA Jr, et al. *Eukaryot Cell* 2008;7:1085-97.

Hyphal Growth is Completely Halted in Δ*cnaA*

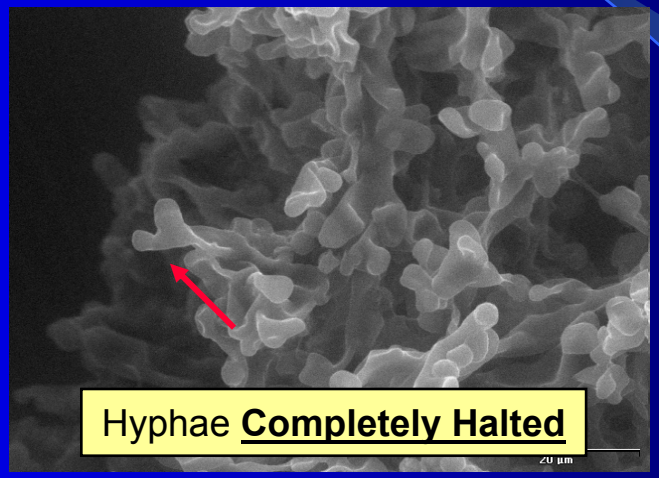


Steinbach WJ, et al. *Eukaryot Cell* 2006;5:1091-103.

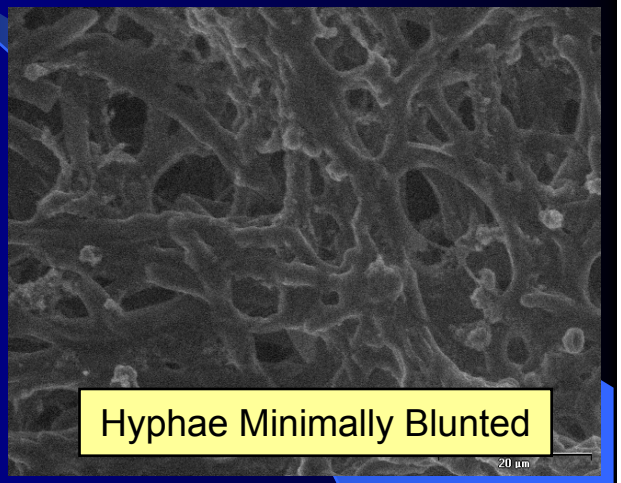
Inhibiting Calcineurin is More Effective than FDA-Approved Antifungal



Wild-Type (WT)



Δ*cnaA* or
WT + FK506 (20 ng/ml)



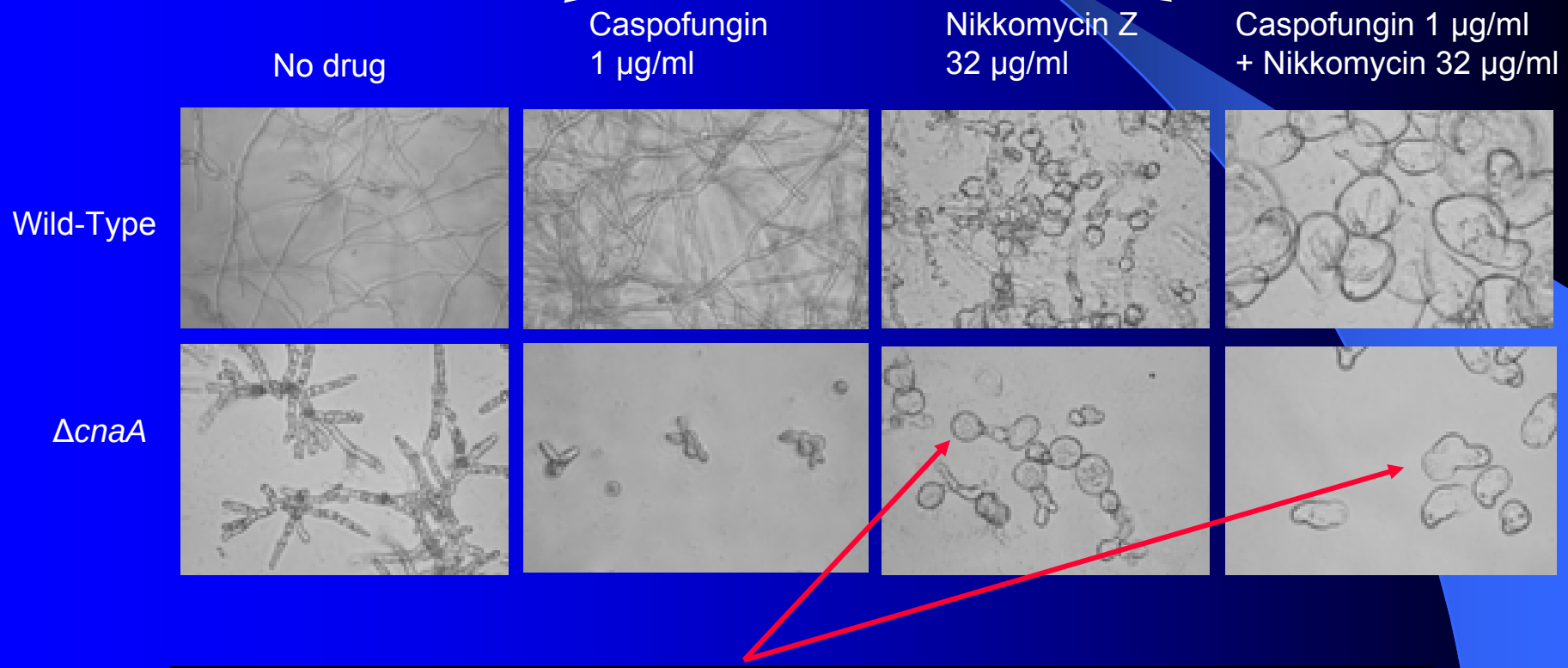
WT + Caspofungin 1 μg/ml

Calcineurin inhibition **> 50 X more effective** in inhibiting hyphal growth and invasion than the FDA-approved and clinically used antifungal Caspofungin

Adding Calcineurin Inhibition Improves Killing

Caspofungin
Antifungal which inhibits cell wall β1,3-glucan synthesis

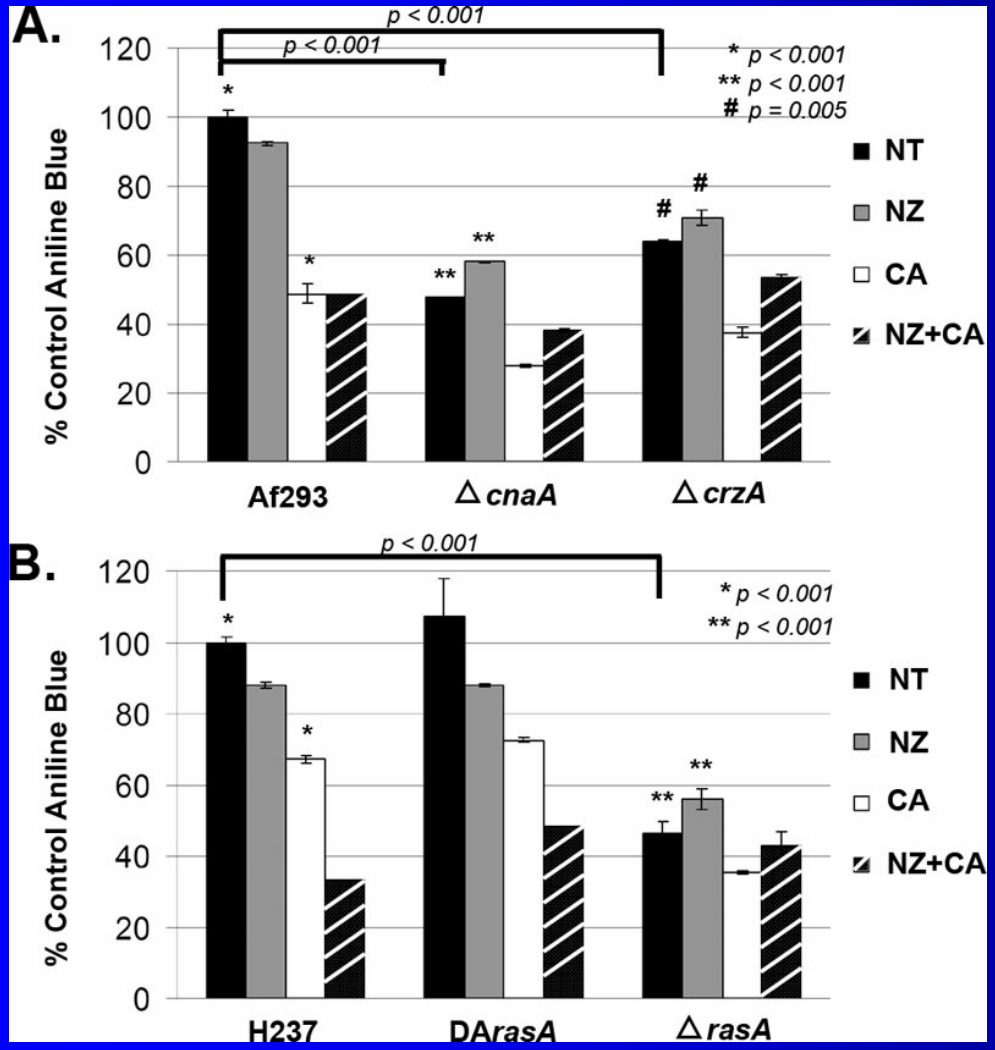
Nikkomycin Z
Antifungal which inhibits cell wall chitin synthesis



Hyphae are appear only as cell blebs when treatment is TRIPLE combination with calcineurin inhibition

Steinbach WJ, et al. *Antimicrob Agents Chemother* 2007;51:2979-2981.

Loss of Ras or Calcineurin Signaling Decreases Baseline β-Glucan

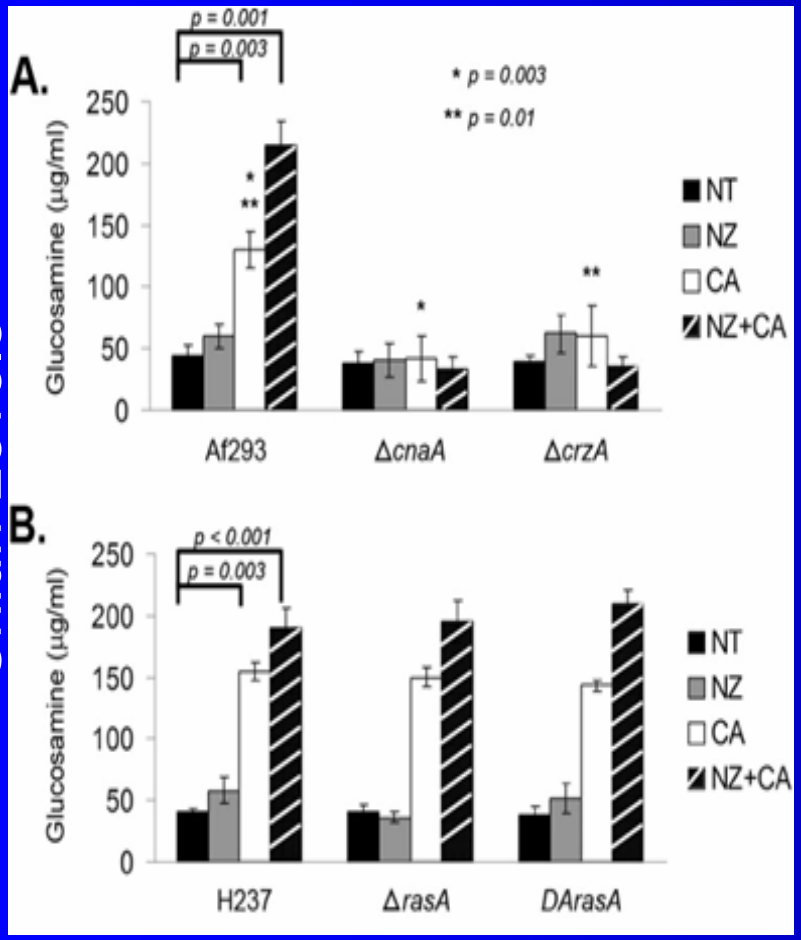


B-Glucan Levels

- Caspofungin treatment further decreases β-glucan
- Nikkomycin Z treatment increases β-glucan in Δ*cnaA*, Δ*crzA*, Δ*rasA*

Fortwendel JR, *et al*. *Antimicrob Agents Chemother* 2009;53:476-482.

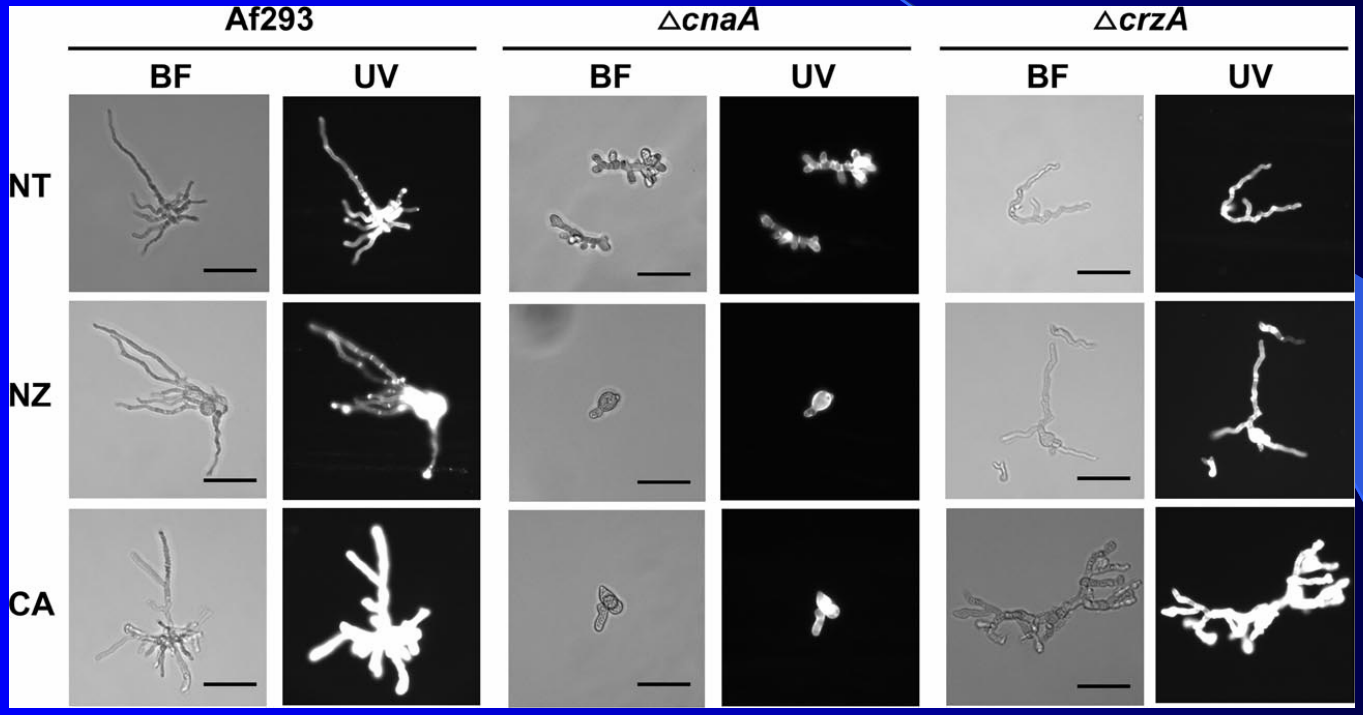
Compensatory Regulation of Chitin and β-Glucan Synthesis



- Caspofungin treatment of Δ*cnaA* or Δ*crzA* did NOT increase chitin content like seen in WT, but treatment of Δ*rasA* increased chitin
- Micafungin or Anidulafungin treatment of Δ*cnaA* was unable to increase chitin to WT level
- **Suggests that the calcineurin pathway regulates some aspect of compensatory chitin response to glucan inhibition**
- Calcineurin and Ras act in parallel

Fortwendel JR, *et al*. *Antimicrob Agents Chemother* 2009;53:476-482.

Calcofluor Staining Confirmation of Absence of Chitin Increase



The absence of compensatory increase in chitin following Caspofungin treatment of calcineurin pathway mutants

Fortwendel JR, *et al*. *Antimicrob Agents Chemother* 2009;53:476-482.

Paradoxical Echinocandin Effect Against *Aspergillus* spp.

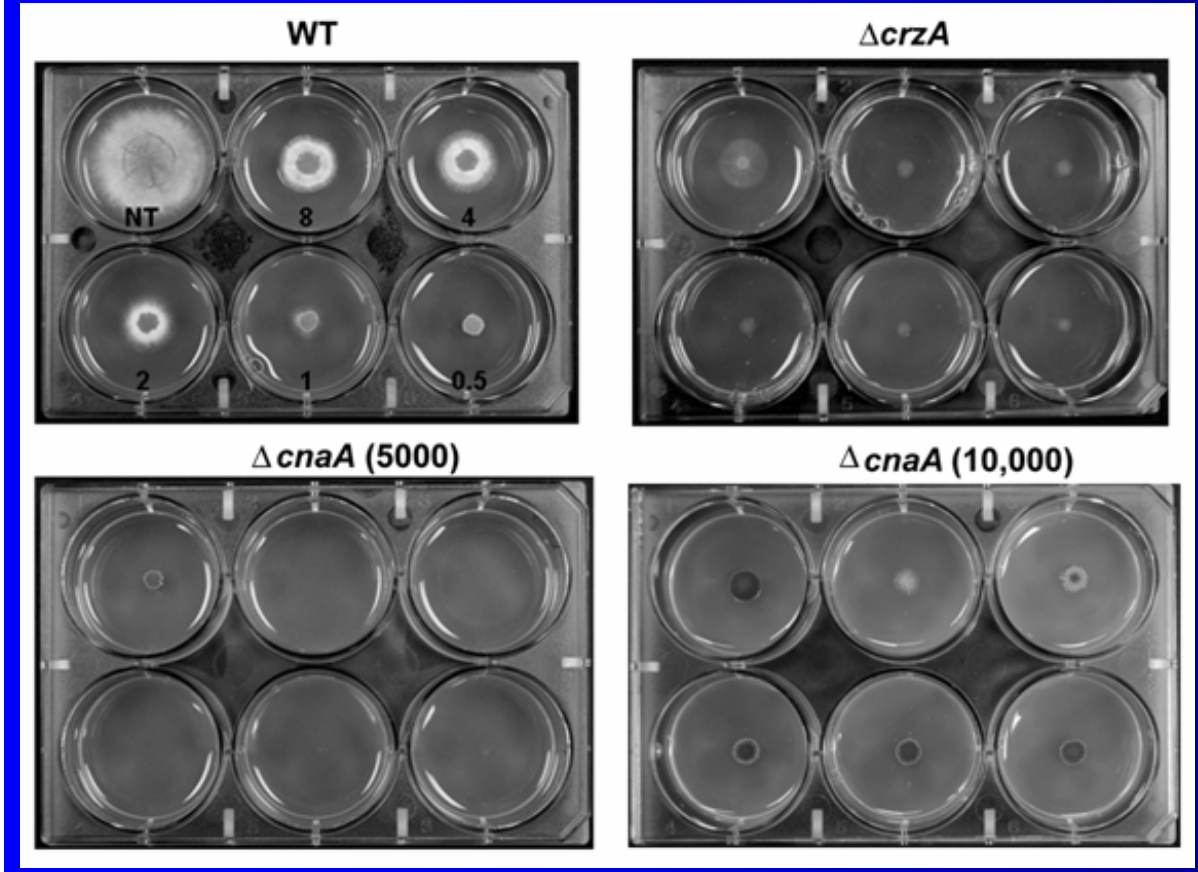
- XTT metabolic assay along with microscopic MEC
- Reduction in metabolic activity in *Aspergillus* treated with caspofungin
  - Reduction more ($p<0.01$) for *A. flavus* (25% of control)
  - Reduction less in *A. fumigatus* (42% of control) and *A. terreus* (53% of control)

- **Paradoxical** increase in metabolic activity at caspofungin concentrations > MEC
  - *A. fumigatus* (5/9 strains) and *A. terreus* (6/12 strains)

- *A. fumigatus* = Metabolic activity at 8 µg/ml increased by 1.82 compared to MEC ($p<0.0001$)
- *A. terreus* = Metabolic activity increased by 1.47 ($p<0.0001$)
- A. flavus = Metabolic activity increased by only 1.15 (p=NS)

Antachopoulos C, et al. Antimicrob Agents Chemother 2007;51:881-887.

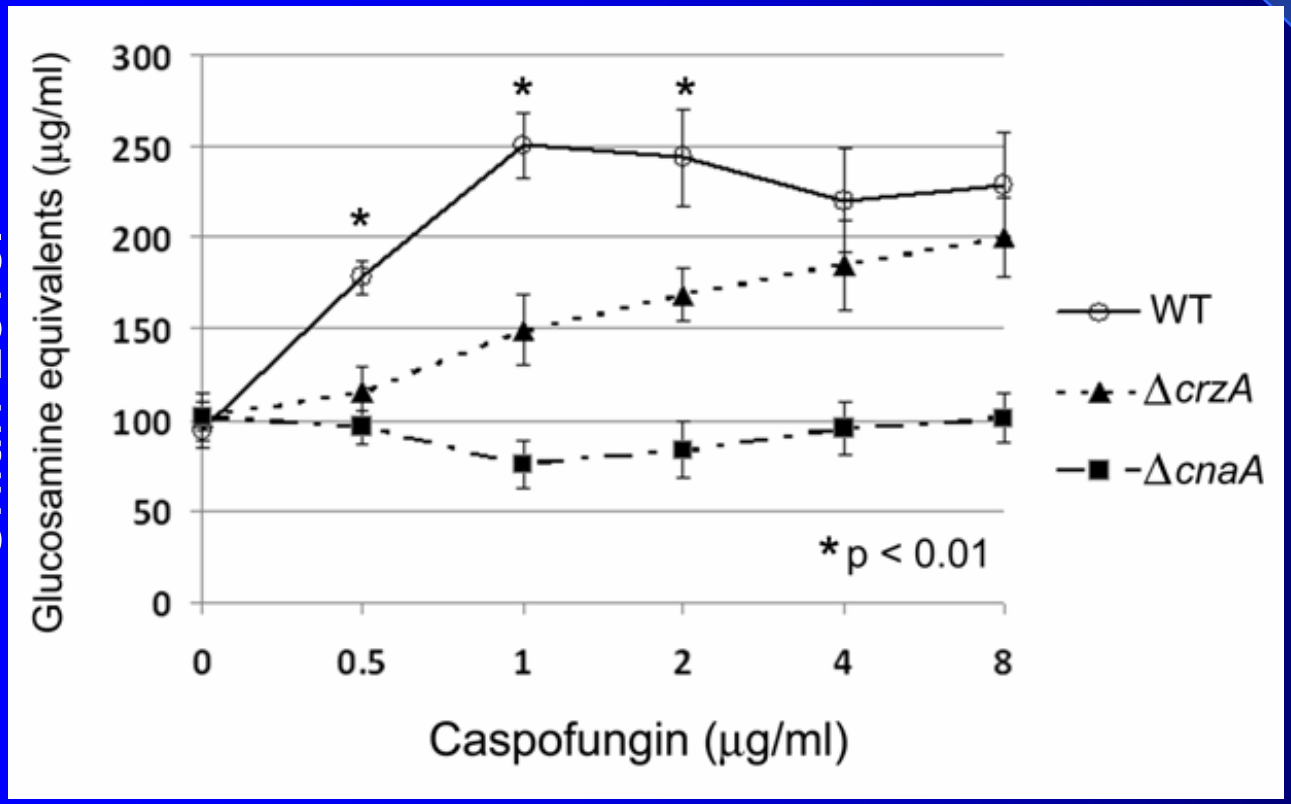
Calcineurin Pathway - Dependent "Paradoxical Effect"



- No paradoxical effect seen in Δ*cnaA* or Δ*crzA*
- Δ*rasA* has paradoxical growth
- Paradoxical effect removed in all strains when treated with FK506
- Echinocandin- and strain- specific

Fortwendel JR, *et al*. *Antimicrob Agents Chemother* 2010, *In Press.*

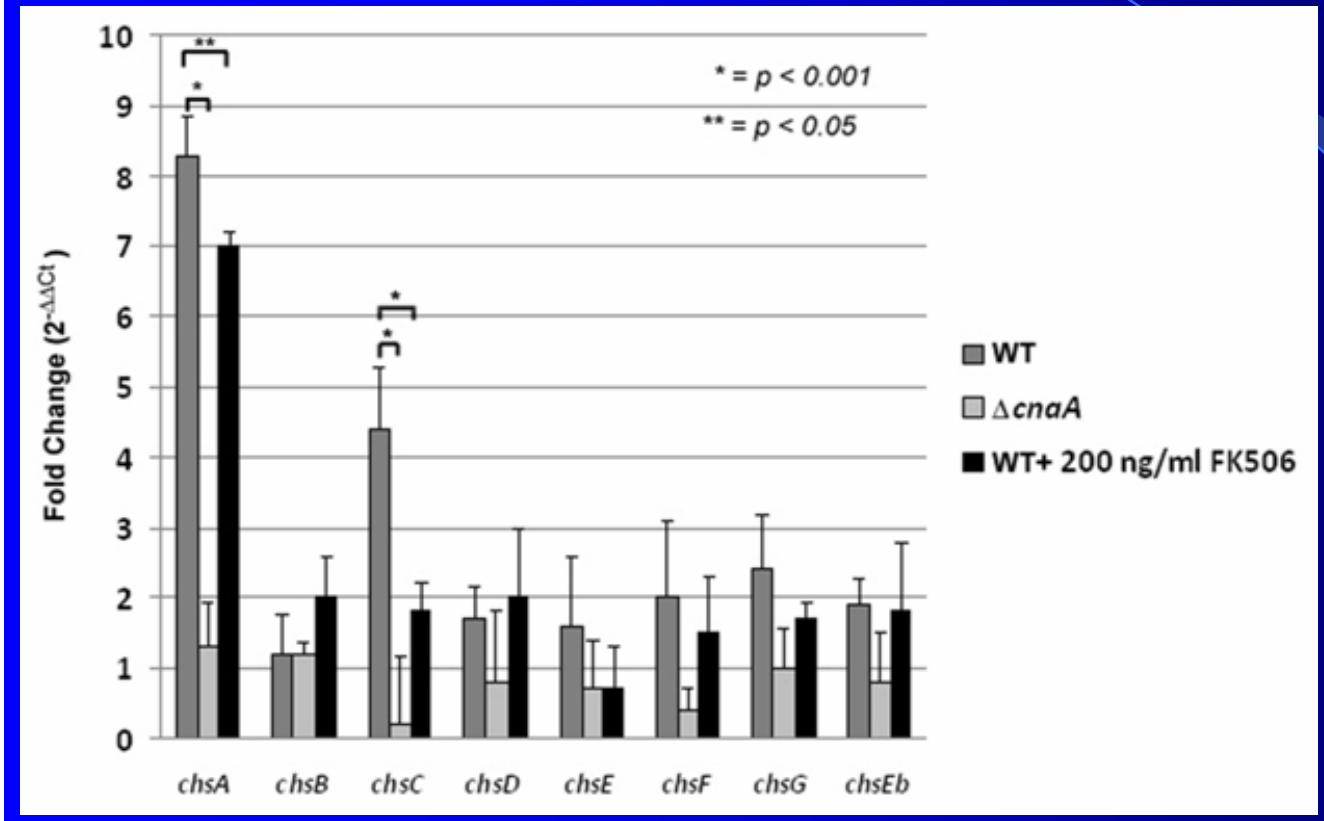
No Paradoxical Chitin Increase in Δ*cnaA*



- Less increase in chitin in Δ*crzA*
- β-glucan content decreased in all strains following caspofungin

Fortwendel JR, *et al*. *Antimicrob Agents Chemother* 2010, *In Press.*

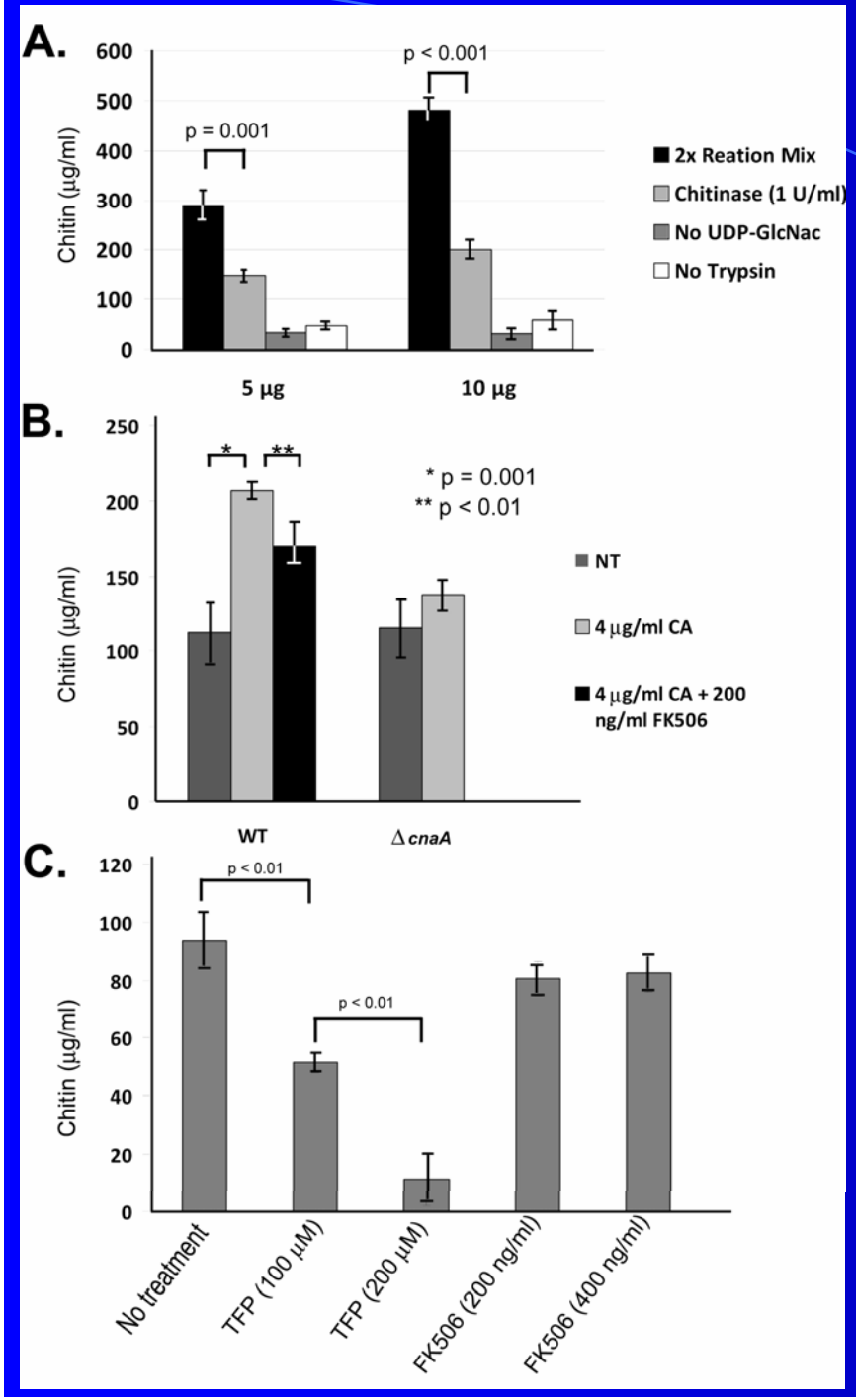
ChsA and ChsC Upregulated During Paradoxical Growth



- CDRE sequences in each of the Chs promoters
- No upregulation in Δ*cnaA*
- Suggests the molecular role for calcineurin in the chitin compensatory response

Fortwendel JR, *et al*. *Antimicrob Agents Chemother* 2010, *In Press.*

Chitin Synthase Activity Not Increased in Δ*cnaA* "Paradoxical Effect"



- Activity of calmodulin and calcineurin necessary for chitin synthase
- Trifluoperazine (TFP) (calmodulin inhibitor) treatment of microsomal extracts decreased chitin synthase activity

Fortwendel JR, *et al*. *Antimicrob Agents Chemother* 2010, *In Press.*

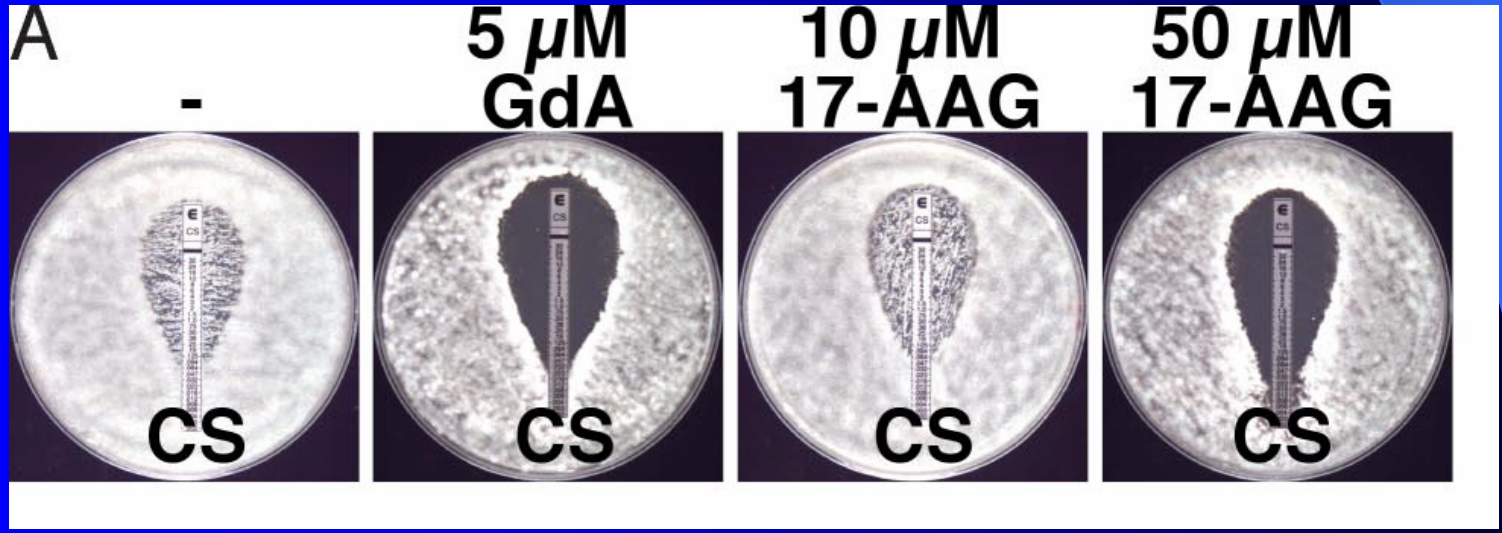
Proposed Paradoxical Growth Control Model

- Calmodulin-mediated activation of CnaA in response to cell wall damage following caspofungin treatment
- Activated CnaA can then dephosphorylate transcription factor CrzA
- This induces transcription of *chsA* and *chsC*, which may be responsible for chitin response
- OR potentially calcineurin post-translational control over chitin synthases (dephosphorylation?)

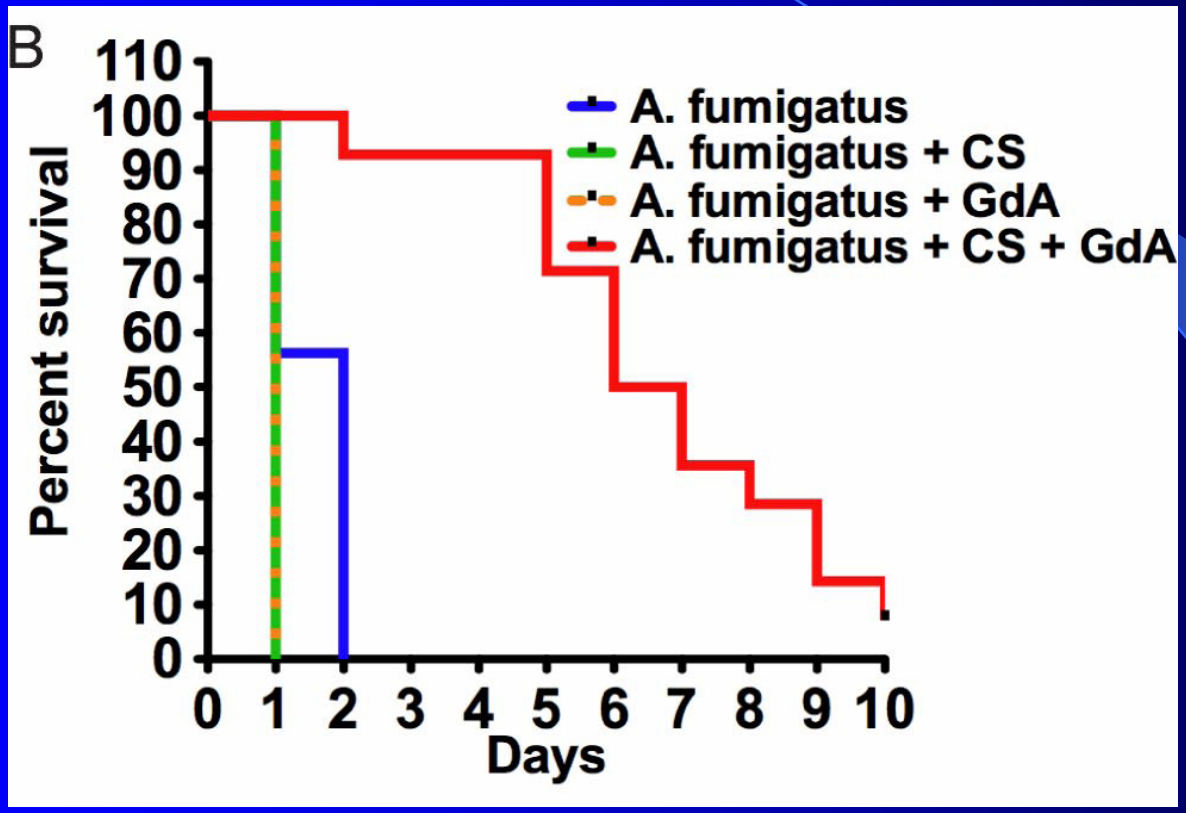
Hsp90 Inhibition reduces Echinocandin Resistance

- *In vitro* geldanamycin reduced *A. fumigatus* and *A. terreus* resistance to caspofungin (little effect with voriconazole)

Cowen LE, et al. *Science* 2005;309:2185-9
Cowen LE, et al. *PNAS* 2009;106:2818-23.



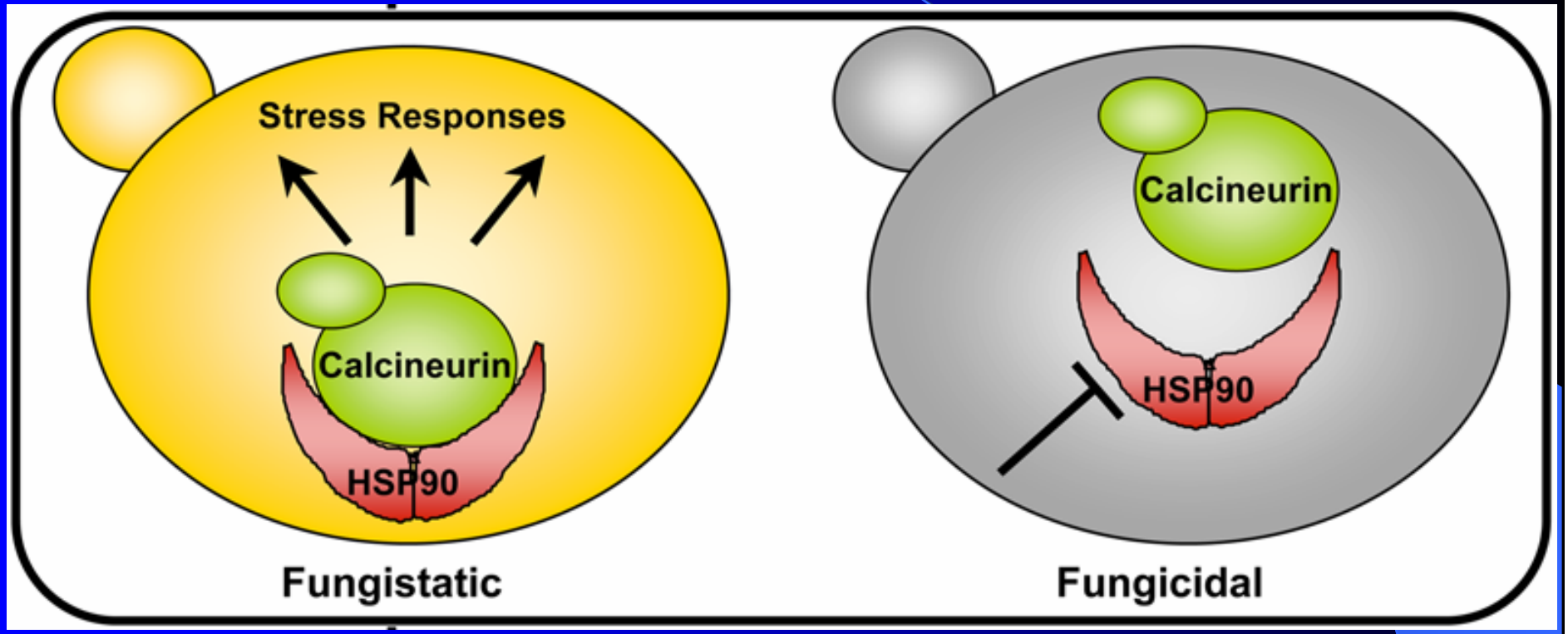
Hsp90 Inhibition + β-Glucan Inhibition



- In *G. mellonella* (wax moth larvae) model, geldanamycin + caspofungin improved survival

Cowen LE, et al. *PNAS* 2009;106:2818-23.

Molecular Chaperone Hsp90 Stabilizes Calcineurin



Hsp90 enables calcineurin-dependent stress responses for survival

Cowen LE. *PLoS Pathogens* 2009;5:1-3.

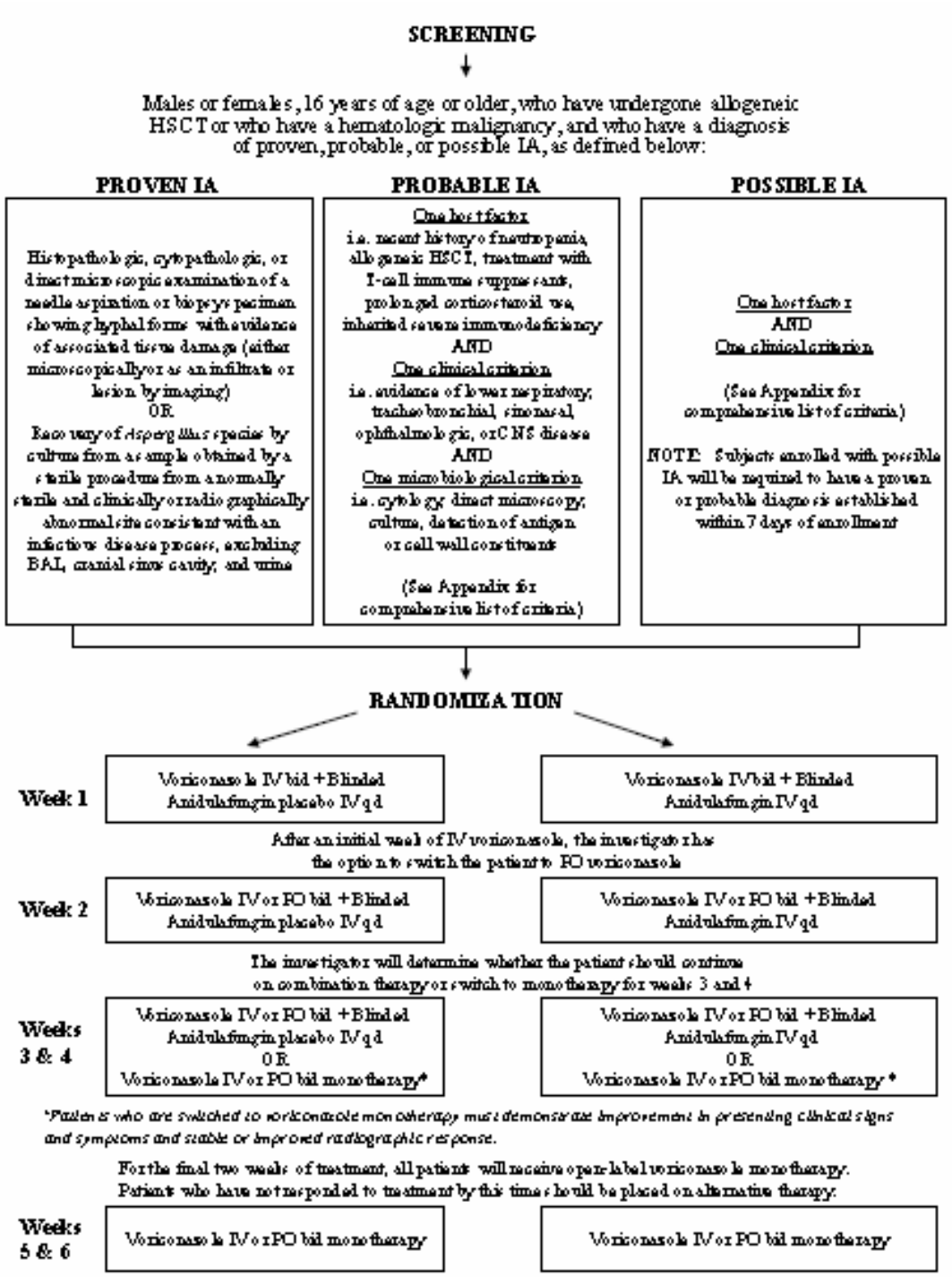
Voriconazole vs. Voriconazole + Anidulafungin for Combination Therapy of IA

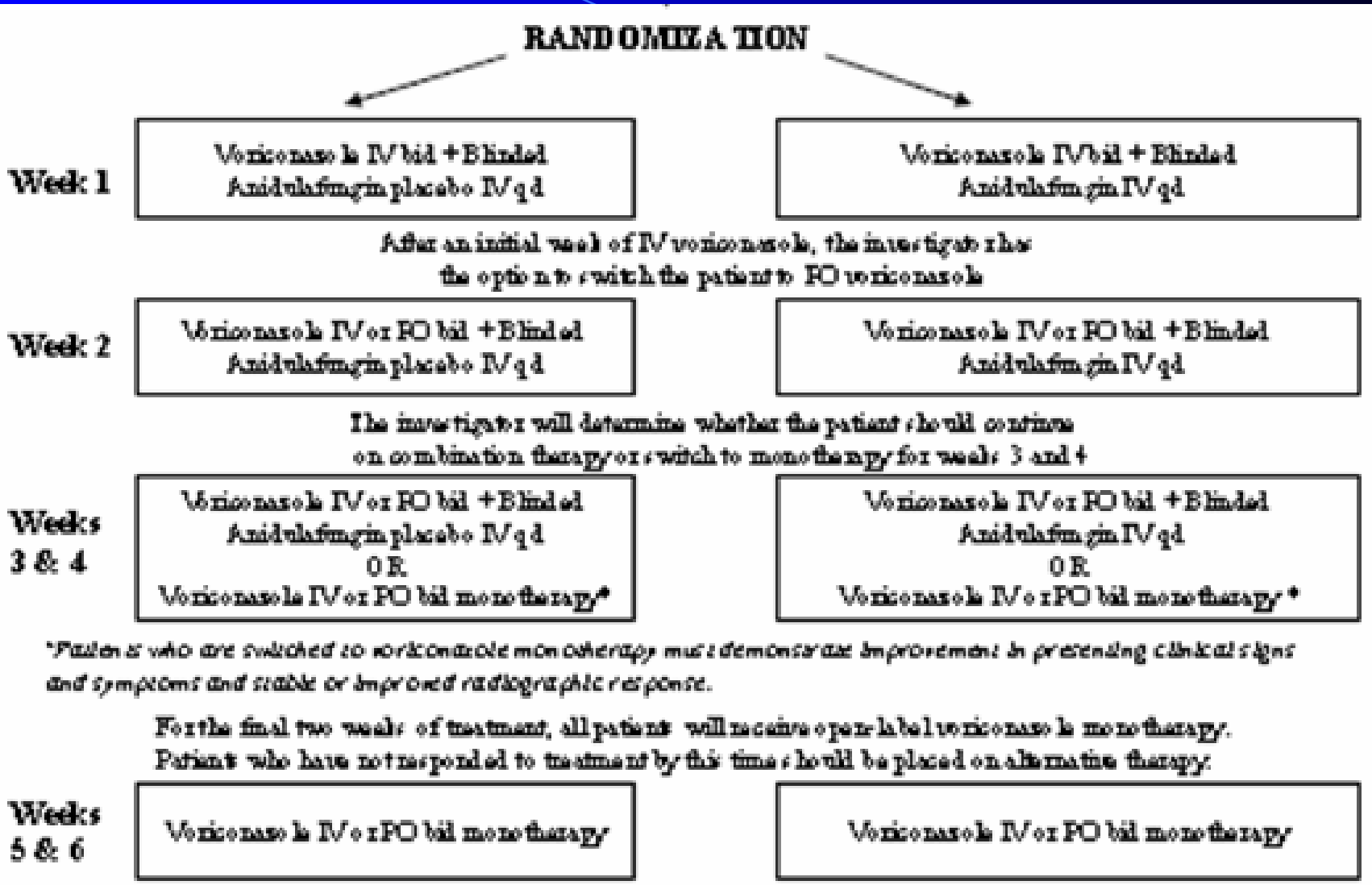
Pfizer – A8851009 / MSG-03

MSG-03/A8851009: Voriconazole & Anidulafungin Combination for Invasive Aspergillosis

- 405 patients needed
  - Superiority of combination therapy in improving survival compared to monotherapy at week 6
- Hematologic malignancies, HCT – to decrease impact of underlying disease on treatment outcomes
- Exclusion based on organ dysfunction
  - Estimated OS generated from Herbrecht study, FHCRC database – 20%

Pfizer A8851009 – MSG 003




RANDOMIZATION
Week 1
Voriconazole IV bid + Blinded Anidulafungin placebo IV qd
Voriconazole IV bid + Blinded Anidulafungin IV qd
After an initial week of IV voriconazole, the investigator has the option to switch the patient to PO voriconazole
Week 2
Voriconazole IV or PO bid + Blinded Anidulafungin placebo IV qd
Voriconazole IV or PO bid + Blinded Anidulafungin IV qd
The investigator will determine whether the patient should continue on combination therapy or switch to monotherapy for weeks 3 and 4
Weeks 3 & 4
Voriconazole IV or PO bid + Blinded Anidulafungin placebo IV qd OR Voriconazole IV or PO bid monotherapy*
Voriconazole IV or PO bid + Blinded Anidulafungin IV qd OR Voriconazole IV or PO bid monotherapy*
*Patients who are switched to voriconazole monotherapy must demonstrate improvement in preexisting clinical signs and symptoms and stable or improved radiographic response.
For the final two weeks of treatment, all patients will receive open-label voriconazole monotherapy. Patients who have not responded to treatment by this time should be placed on alternative therapy.
Weeks 5 & 6
Voriconazole IV or PO bid monotherapy
Voriconazole IV or PO bid monotherapy

Sites

- 28 countries
- 163 sites activated
  - 34 in US
- 63 sites have enrolled a subject
  - 11 in US

Enrollment

- First subject randomized in July 2008
- 295 subjects randomized to date
  - 62% with probable/proven diagnoses
- Study is enrolling *approximately seven months ahead of schedule*
  - Should finish enrolling in Summer of 2010
- DSMB
  - Quarterly safety meetings
  - Second interim efficacy analysis planned for March
- DRC established

Newer Combination Conclusions

- Likely mechanistically different approaches will work best
- Await Triazole + Echinocandin large clinical trial results
- Newer molecular approaches involve cell signaling or stress response attacks