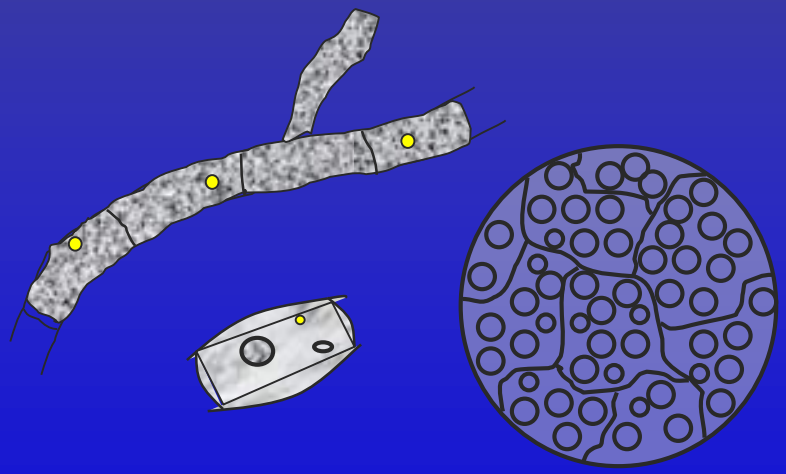
Fighting Coccidioidomycosis
Prevention: Vaccine
Treatment: Nikkomycin Z



Valley Fever Vaccine Project

A brief history…

- Epidemic of early 90's spawned renewed interest in a vaccine
- Bakersfield locals created a group to organize project & secure financing
- With funds in hand, "exploratory phase" was initiated and research begun in '98
- VFVP formally began in 2000 with 5 yrs of funding for 5 research institutions

The Valley Fever Vaccine Project

University of Arizona
- John Galgiani
- Lisa Shubitz

UT San Antonio
- Rebecca Cox
- Mitch Magee (ASU)

Med. College of Ohio
- Garry Cole
- Chiung Yu

UC Davis
- Demo Pappagianis
- Susan Johnson

UC San Diego
- Theo Kirkland

UC San Francisco
- George Rutherford
- Richard Hector

CSU Bakersfield

Supporting Organizations

- California HealthCare Foundation
- State of California (Ashburn, Parra)
- CDC (Thomas)
- Rotary's Valley Fever Project of the Americas, Valley Fever Research Foundation, & many local contributors
- Kern County
- NIH for sequencing of *C. posadasii*

Major Accomplishments

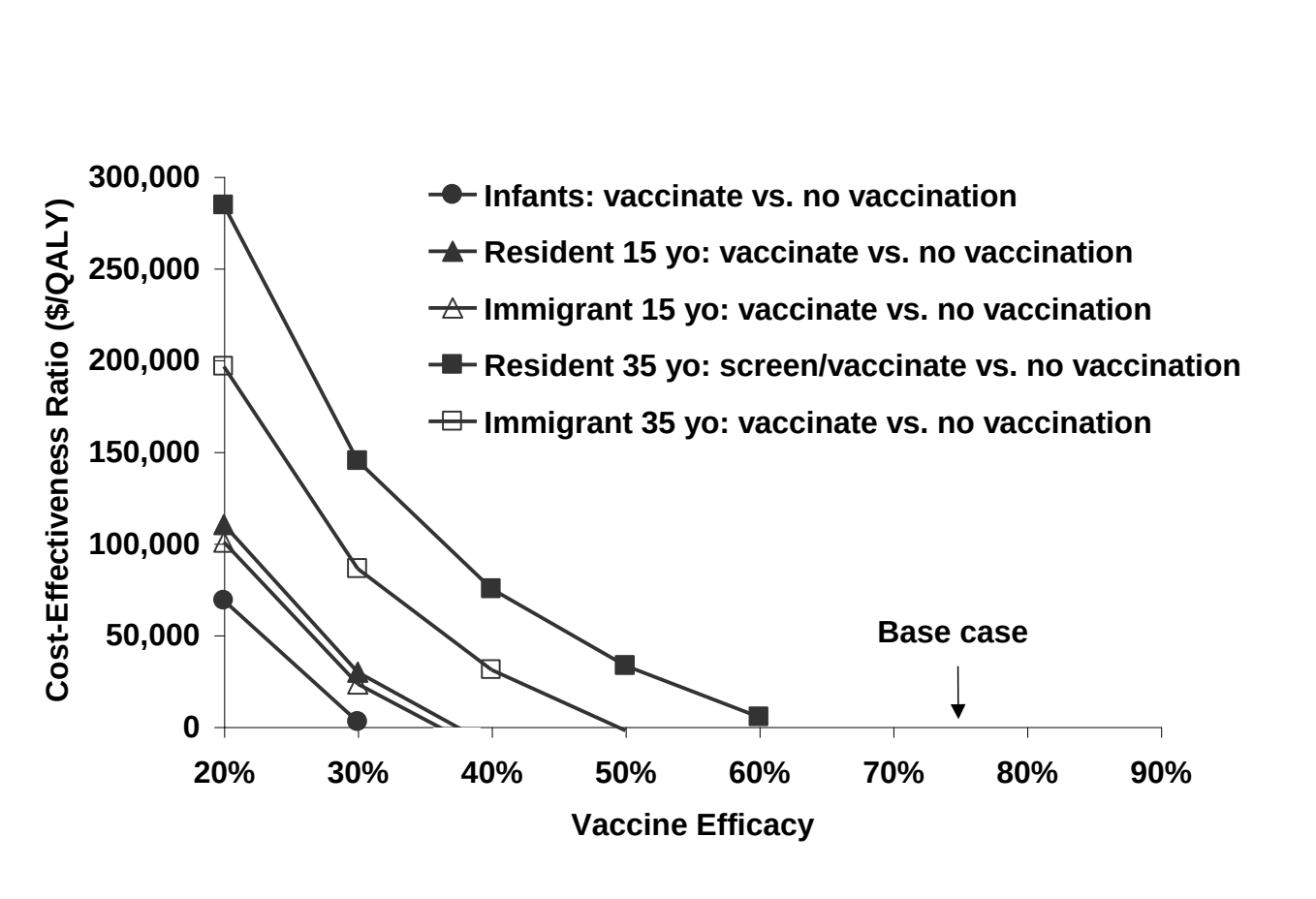
- Dozens of antigens identified, cloned
- Attenuated mutant strains created
- Genome of *C. posadasii* sequenced
- Expression systems & manufacturing evaluated for fusion protein
- Primate trial to evaluate fusion protein
- 10 patents filed; 4 patents issued
- Canine incidence/prevalence trial
- Coccidioidin Phase 1 & 2 trials

Valley Fever Vaccine Project

Possible Value Of A Vaccine

- Prevent ~3,500 disseminated cases/yr
- Prevent ~10-30,000 primary illnesses/yr
  - Public Health risk
    - Residents of California, Arizona, Nevada, Utah
    - Tourists to the Southwest
    - Relocated employees
    - Immunocompromised individuals
  - Military risk
    - Desert training bases within Southwest
    - Biodefense

Cost-effectiveness of a vaccine



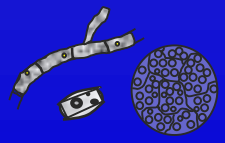
* Barnato et al. Cost-effectiveness of a potential vaccine for coccidioidomycosis. Emerg Infect Dis 2001; 7:797-806.

Valley Fever Vaccine Project

Vaccine Candidates

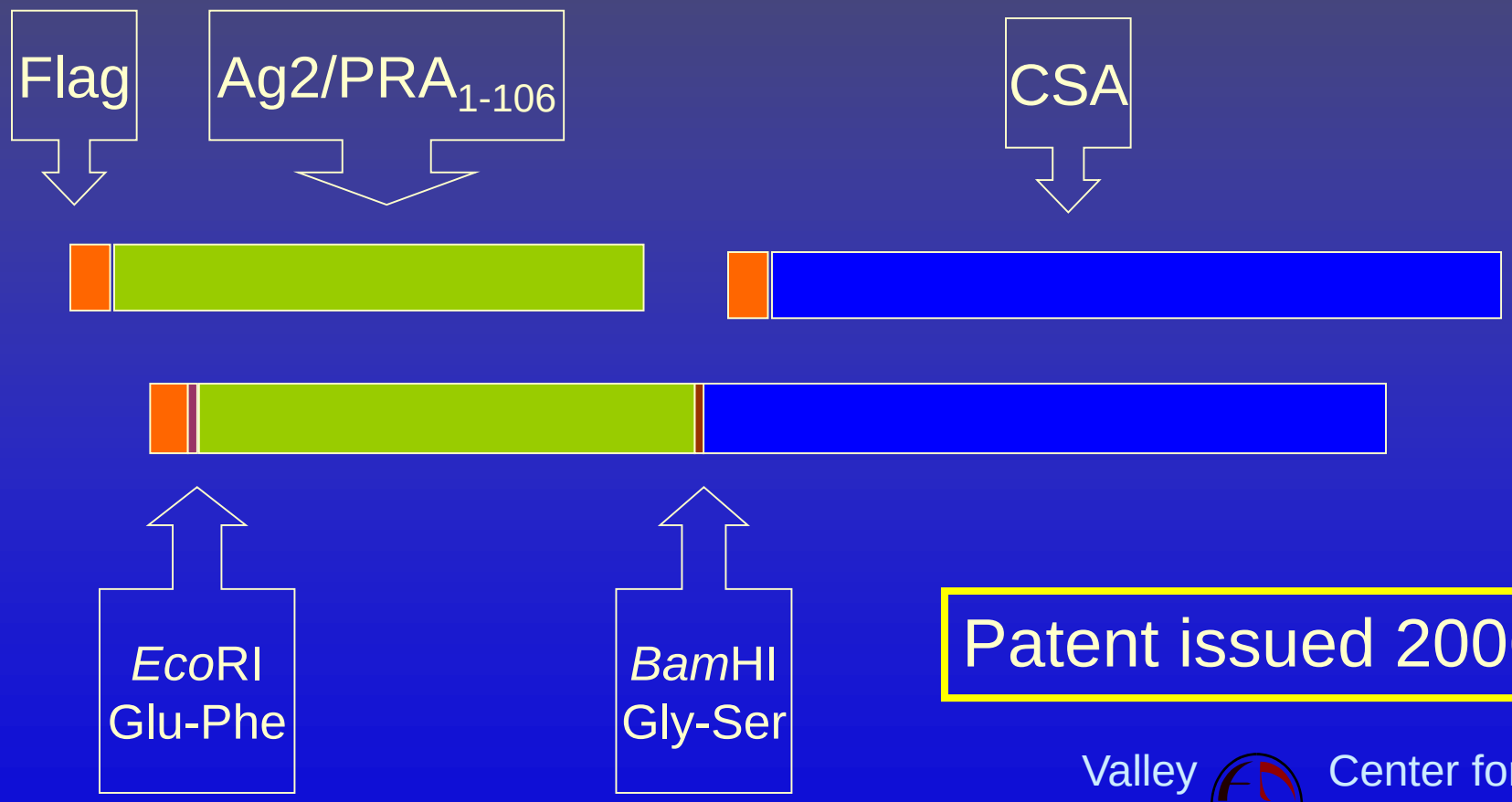
- Recombinant proteins:
  - Ag2/PRA106+Csa
  - ELI1

- Live, attenuated mutants:
  - Δcts2/Δard1/ Δcts3 strain
  - Δchs5 strain



Valley Fever Vaccine Project

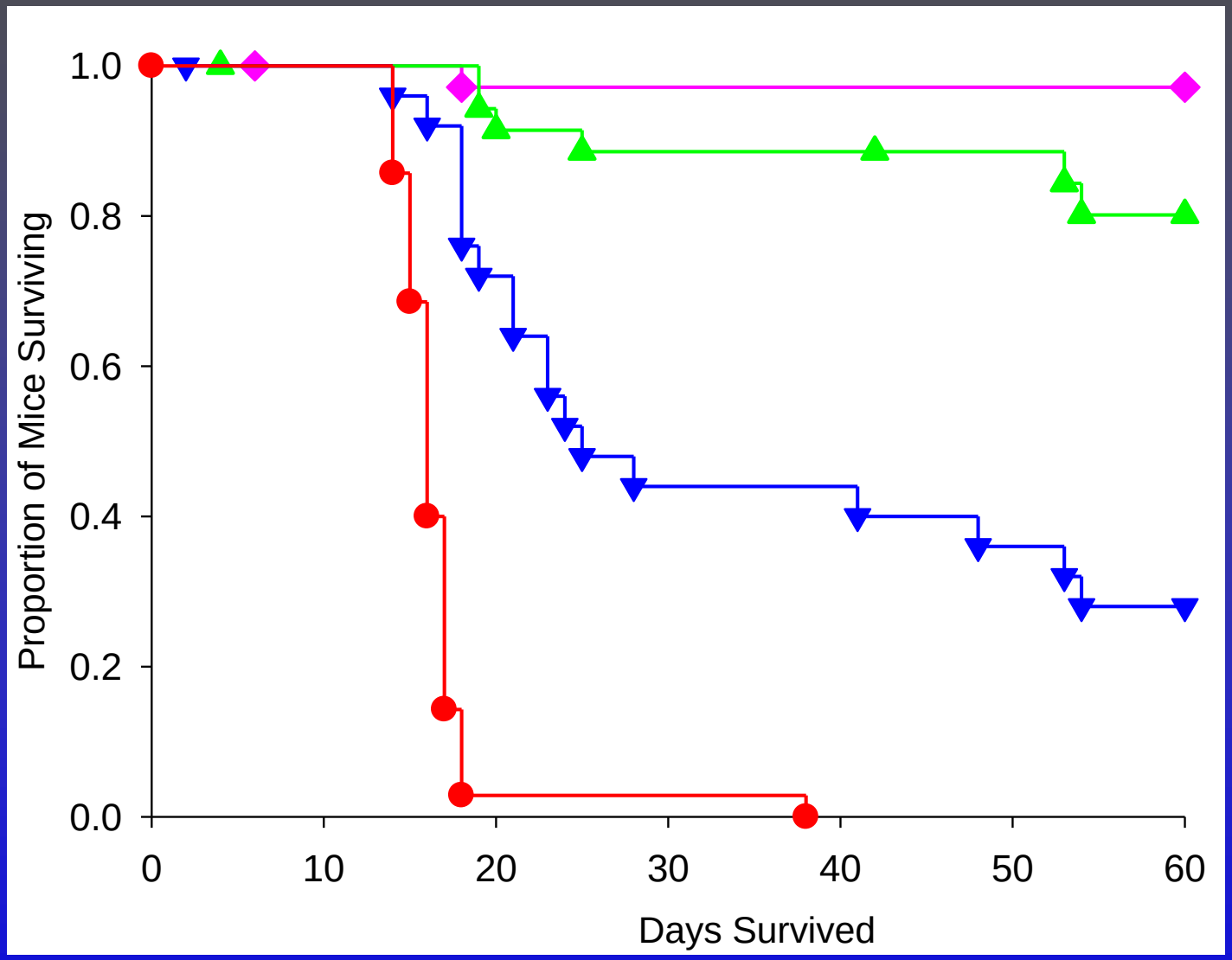
Ag2/PRA106-CSA Chimeric Ag



Patent issued 2006

Valley Fever Center for Excellence

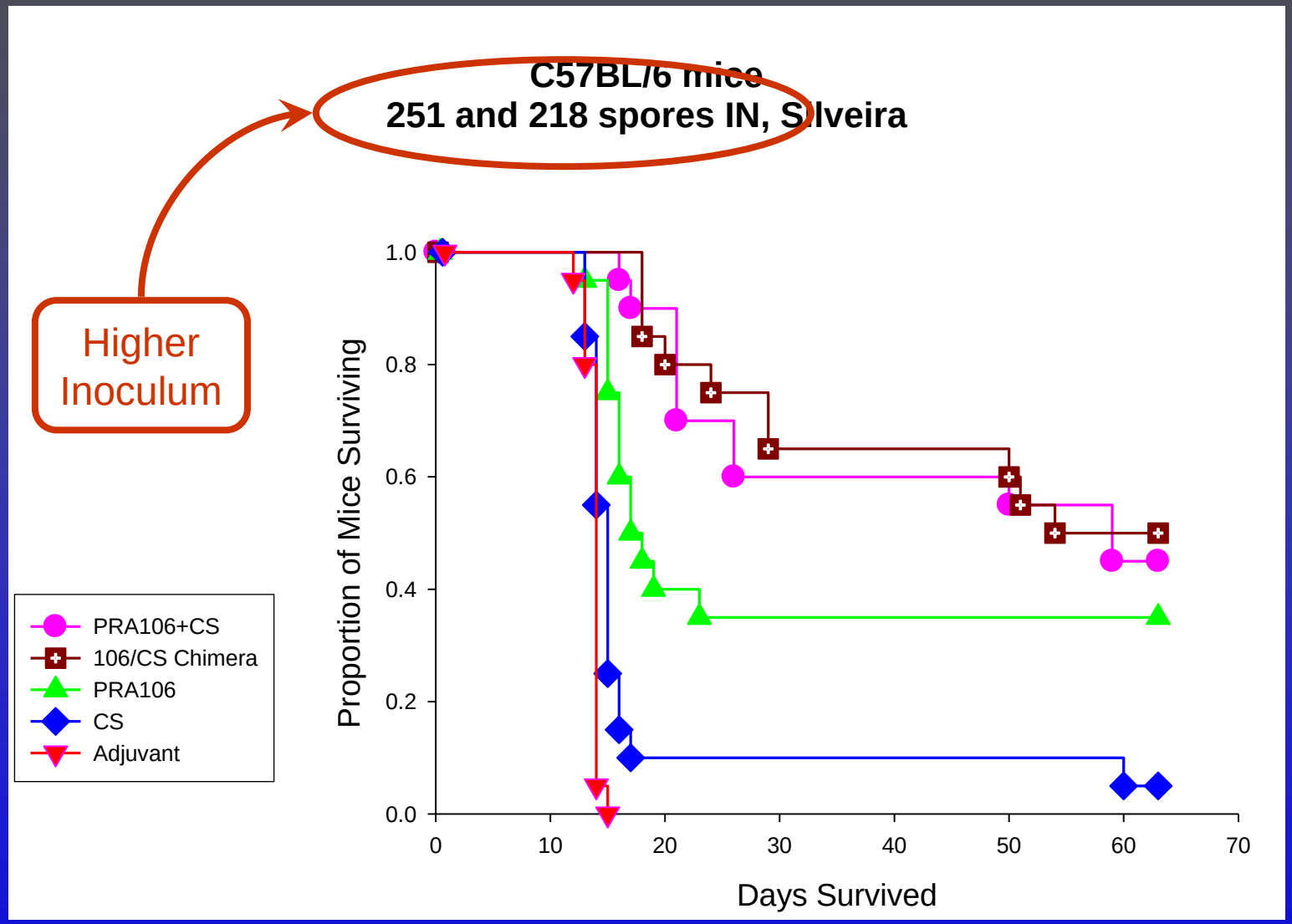
Mouse Survival Studies with Fusion Protein*



*Intranasal infection with 80 arthroconidia

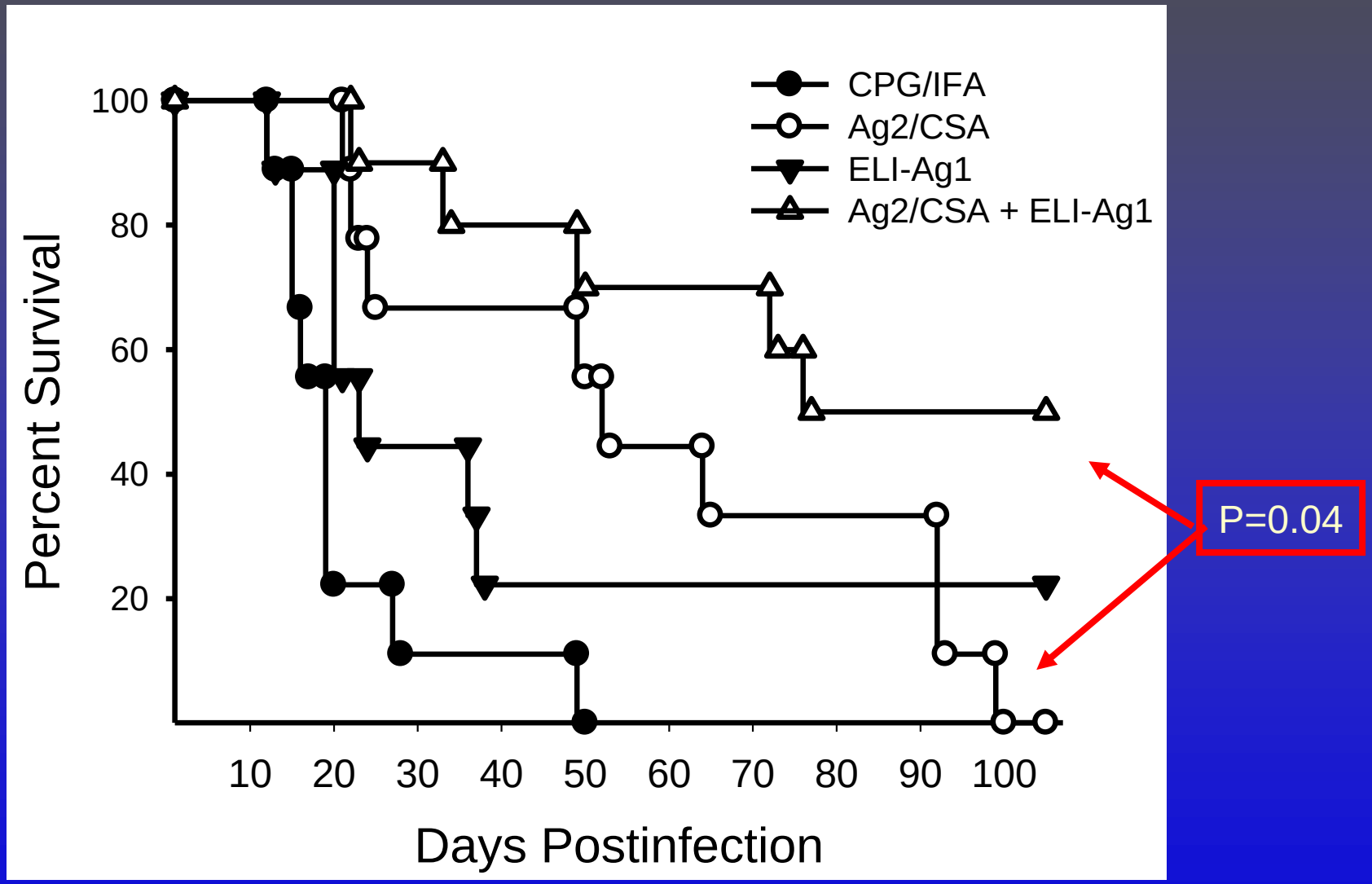
Shubitz et al., Vaccine 24:5904, 2006

Mouse Survival Study with Higher Inoculum



Gehans Wilcoxon: Both > Ag2/PRA$_{1\text{-}106}$ > CSA > Adjuvant

Adding ELI-1 to Ag2/PRA+CSA



Magee et al, 2006

Fusion Vaccine Issues

- Efficacy: may prevent dissemination, but clearly does not prevent infection
  - Recombinant vaccines have not worked in diseases like TB
  - No data on duration of immunity
- Adjuvants: few CMI-promoting adjuvants
  - MPL is not available to us
  - Alternatives are few & have safety issues
- Manufacturing issues
  - Expression is poor; aggregation is a problem

Flagellin as an Adjuvant Substitute

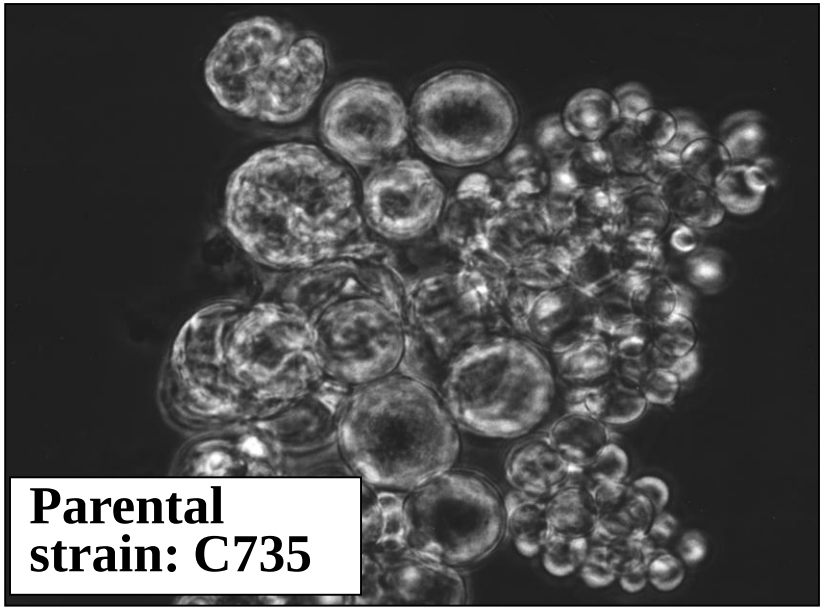
- Flagellin binds to TLR 5 to stimulate DC activation and antigen presentation.
- Flagellin + antigen fusion proteins have been shown in mouse models to stimulate cell-mediated immunity
- This technology is being commercialized by Vaxinnate, Inc.; clinical trials underway
- Flagellin + Ag2/PRA+Csa experiment in progress in Galgiani lab

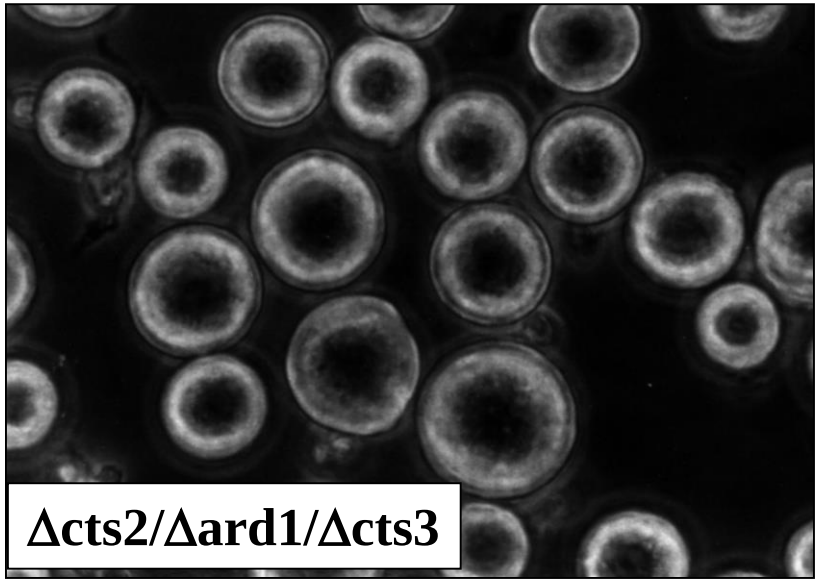
C. posadasii Δcts2/Δard1/Δcts3 Attenuated Mutant

Valley Fever Vaccine Project

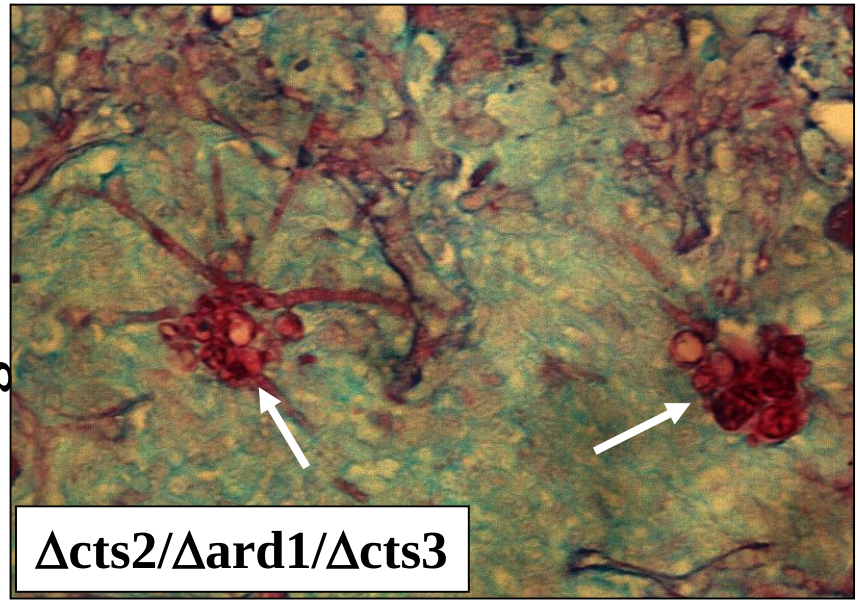
Morphology of Δcts2/Δard1/Δcts3 triple mutant in vitro vs in vivo

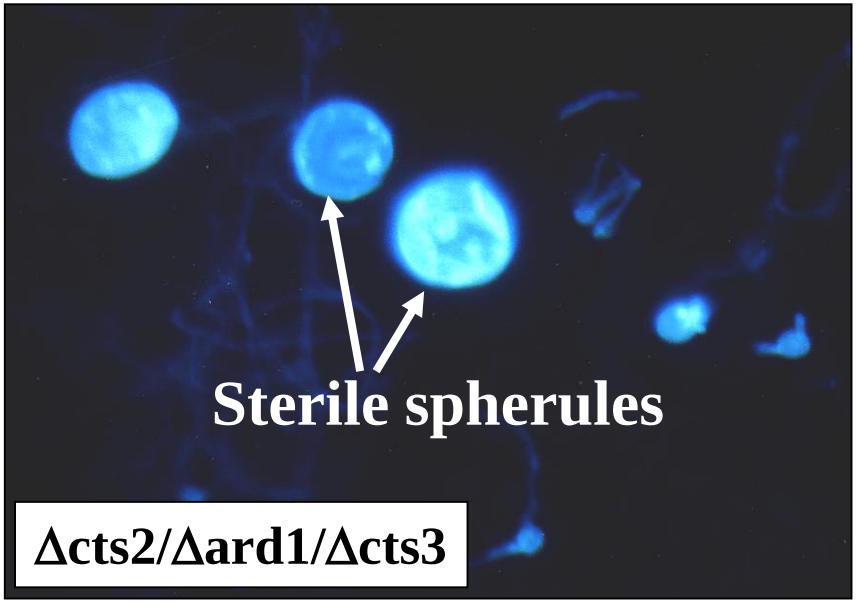
Cultures in Converse medium



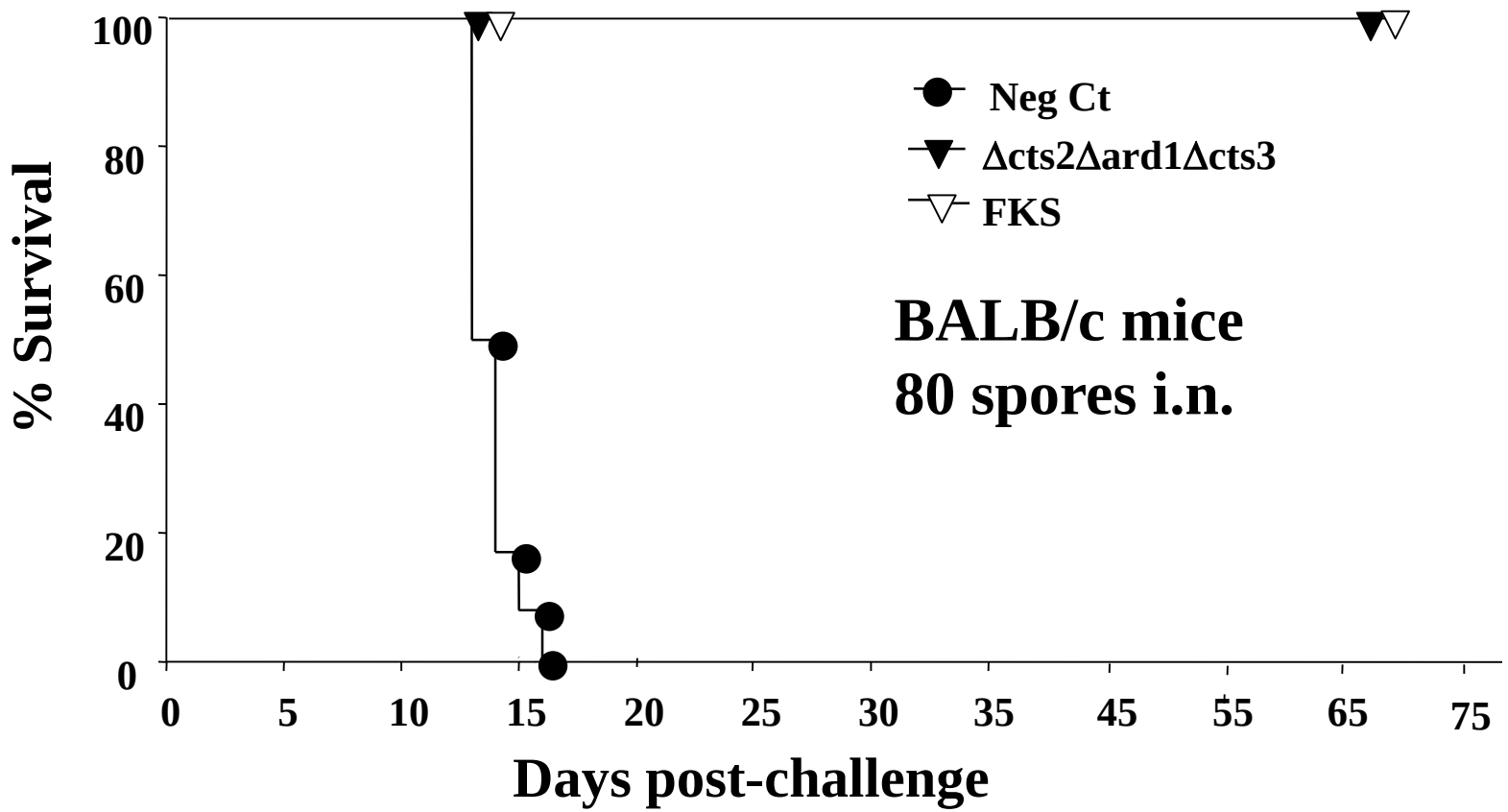


Infected murine lung tissue





Evaluation of survival of BALB/c mice vaccinated with the live Δcts2/Δard1/Δcts3 mutant or the FKS vaccine and i.n.-challenged



Data courtesy Garry Cole, MUO

Attenuated Mutant: Why it's a "no go" for humans

- Safety: possibility of reversion
- Restrictions on use in humans
- Immunogenicity vs. Reactogenicity
- Manufacture of spore-former

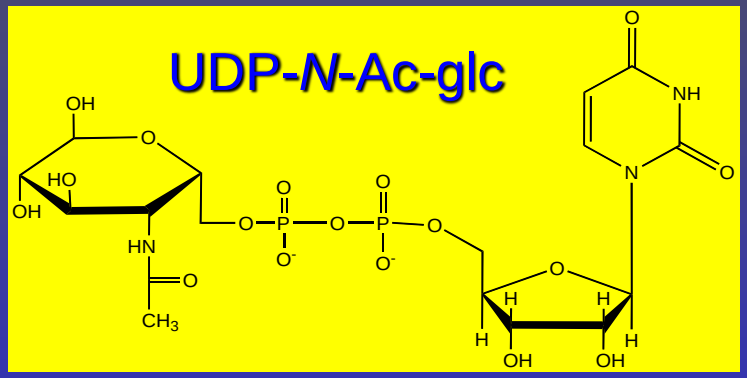
Nikkomycin Z: The perfect drug for coccidioidomycosis?

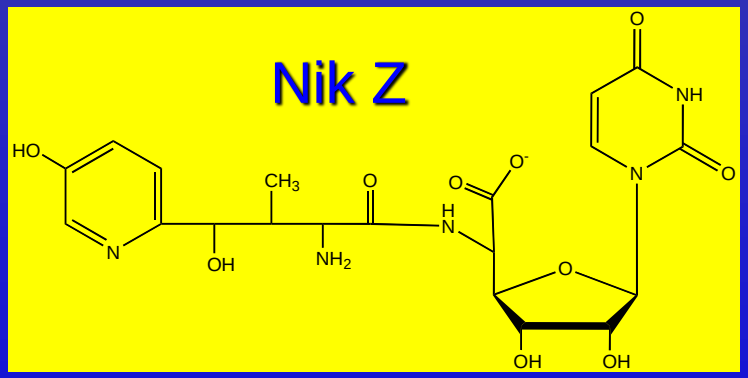
History of Nikkomycin Z

- 1970s: Evaluated by Bayer as agricultural fungicide
- 1980s: Cured mice with cocci (Hector/Pappagianis)
- 1990s: Clinical Development started by Shaman Pharmaceuticals: Phase Ia conducted
- 2001: Purchased at auction by CSUBF
- 2005: Rights transferred to University of Arizona
- 2006: Nik Z designated as orphan drug
- Oct 2007: Patients enrolled in Phase Ib/II @ UA

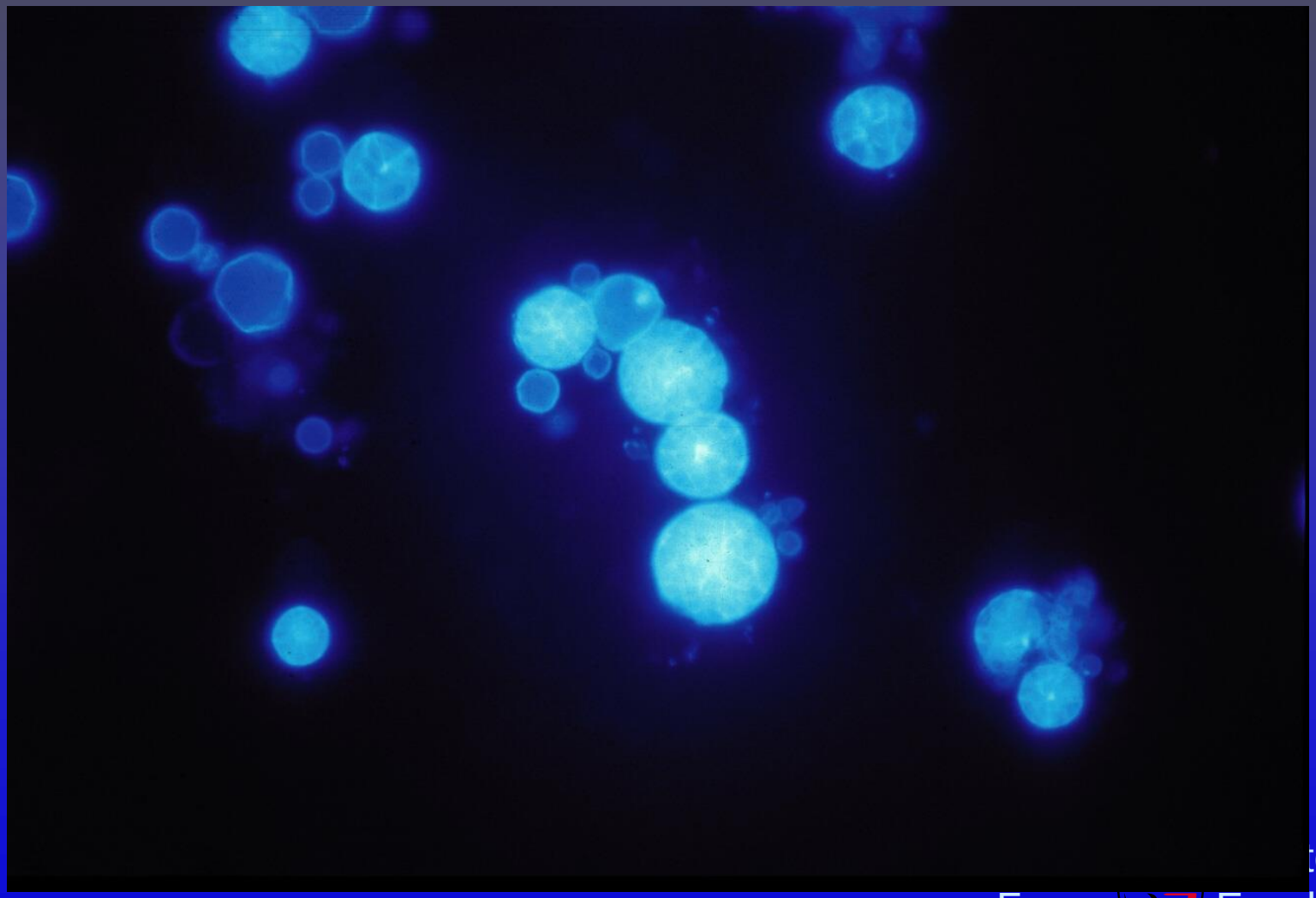
Nikkomycins Inhibit Chitin Synthase

- UDP-*N*-acetylglucosamine is a precursor to chitin
- Nikkomycin Z is a competitive inhibitor of chitin synthase

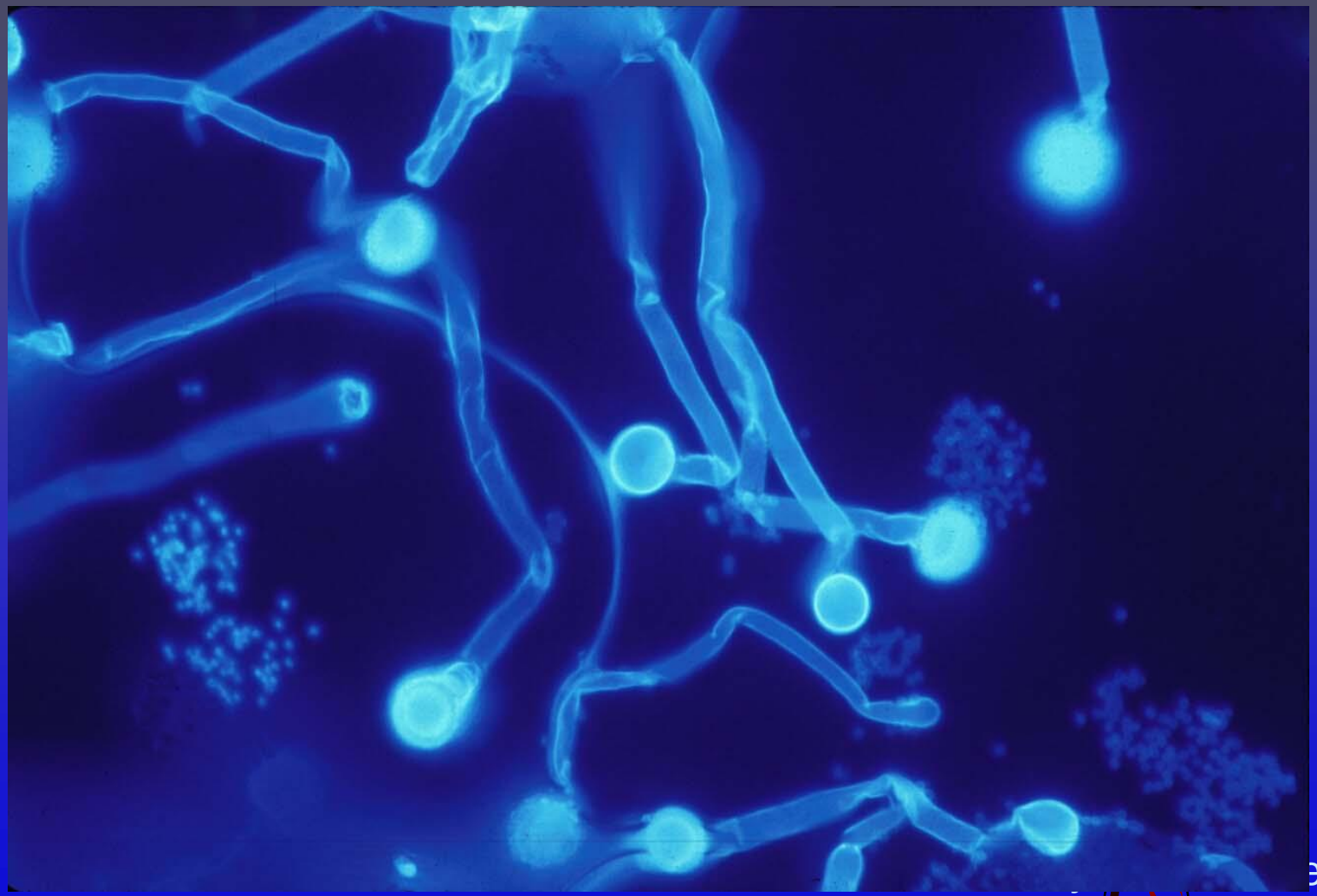




Mature *C. immitis* stained with calcofluor white



A. fumigatus stained with calcofluor white

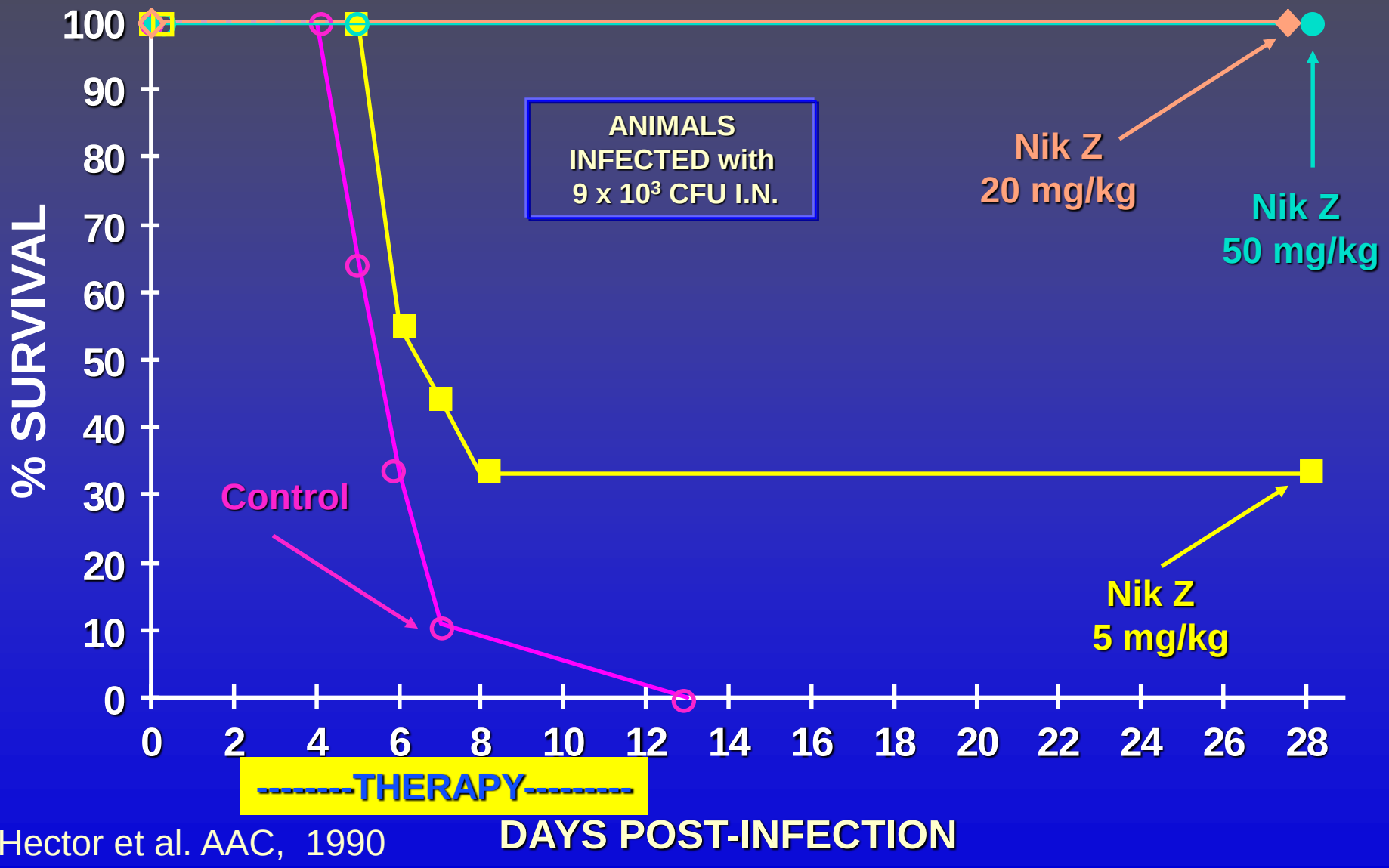


SUSCEPTIBILITY OF DIVERSE FUNGI TO NIKKOMYCIN Z

Organism	No. Strains	Geometric Mean MIC_{100} (μg/ml)
Coccidioides immitis	1	0.0625
Blastomyces dermatitidis	10	0.25
Histoplasma capsulatum	9	2.47
Sporothrix schenkii	10	0.407
Candida albicans	59	5.56
Candida parapsilosis	10	4.29
Candida rugosa	1	7.8
Candida tropicalis	7	>500
Candida krusei	5	445
Candida lusitaniae	1	>500
Cryptococcus neoformans	30	144
Torulopsis glabrata	21	>500
Aspergillus flavus	2	500
Aspergillus fumigatus	2	500

Control vs. NZ-treated

SURVIVAL EXPERIMENT WITH COCCIDIOIDOMYCOSIS



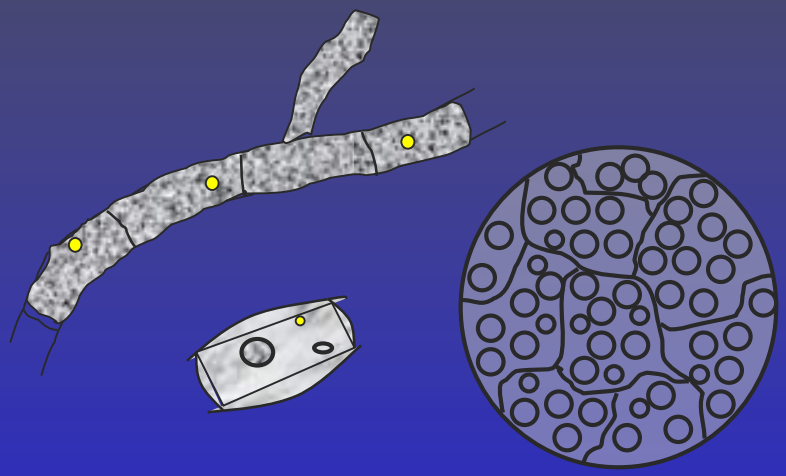
Hector et al. AAC, 1990

SHORT-TERM ORGAN LOADS (Lung) WITH COCCIDIOIDES IMMITIS

GROUP	DOSE (mg/kg)	MEAN LOG CFU ± S. E. M.
CONTROL	-	6.35 ± .06
FLUCONAZOLE	2.5	3.77 ± 1.12
“	25	2.62 ± .82
NIK Z	50	0.37 ± .37

UA: Phase Ib/II Design

- Enrollment: 60 seropositive subjects with uncomplicated cocci pneumonia
- Rising multiple doses: 100-2250 mg x 14 days vs. placebo
- End of treatment response based on signs & symptoms vs. pre-dose
  - Subjective questionnaire for 12 symptoms
  - Lab: ESR, C-reactive protein, procalcitonin, lung lesion volume (CT)



Valley Fever Vaccine Project