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
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
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

Vaccine Candidates

- Recombinant proteins:
  Ag2/PRA106+Csa
  ELI1

- Live, attenuated mutants:
  \(\Delta cts2/\Delta ard1/ \Delta cts3\) strain
  \(\Delta chs5\) strain
Ag2/PRA106-CSA Chimeric Ag

Patent issued 2006
Mouse Survival Studies with Fusion Protein*

*Intranasal infection with 80 arthroconidia
Shubitz et al., Vaccine 24:5904, 2006
Mouse Survival Study with Higher Inoculum

C57BL/6 mice
251 and 218 spores IN, Silveira

Days Survived
0 10 20 30 40 50 60 70
Proportion of Mice Surviving
0.0
0.2
0.4
0.6
0.8
1.0
PRA106+CS
106/CS Chimera
PRA106
CS
Adjuvant

Gehans Wilcoxon: Both > Ag2/PRA\textsubscript{1-106} > 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 $\Delta$cts2/$\Delta$ard1/$\Delta$cts3
Attenuated Mutant
Morphology of Δcts2/Δard1/Δcts3 triple mutant in vitro vs in vivo

Parental strain: C735

Cultures in Converse medium

Δcts2/Δard1/Δcts3

Infected murine lung tissue

Δcts2/Δard1/Δcts3

Sterile spherules

Δcts2/Δard1/Δcts3
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

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. Strains</th>
<th>Geometric Mean MIC&lt;sub&gt;100&lt;/sub&gt; (µg/ml)</th>
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<tbody>
<tr>
<td>Coccidioides immitis</td>
<td>1</td>
<td>0.0625</td>
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<tr>
<td>Blastomyces dermatitidis</td>
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<td>0.25</td>
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<tr>
<td>Histoplasma capsulatum</td>
<td>9</td>
<td>2.47</td>
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<tr>
<td>Sporothrix schenkii</td>
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<td>0.407</td>
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<tr>
<td>Candida albicans</td>
<td>59</td>
<td>5.56</td>
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<tr>
<td>Candida parapsilosis</td>
<td>10</td>
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<tr>
<td>Candida rugosa</td>
<td>1</td>
<td>7.8</td>
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<tr>
<td>Candida tropicalis</td>
<td>7</td>
<td>&gt;500</td>
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<tr>
<td>Candida krusei</td>
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<td>445</td>
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<tr>
<td>Candida lusitaniae</td>
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<tr>
<td>Cryptococcus neoformans</td>
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<tr>
<td>Torulopsis glabrata</td>
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<td>&gt;500</td>
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<tr>
<td>Aspergillus flavus</td>
<td>2</td>
<td>500</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>2</td>
<td>500</td>
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</tbody>
</table>
Control vs. NZ-treated
SURVIVAL EXPERIMENT WITH COCCIDIOIDOMYCOSIS

ANIMALS INFECTED with $9 \times 10^3$ CFU I.N.

- Control
- Nik Z 5 mg/kg
- Nik Z 20 mg/kg
- Nik Z 50 mg/kg

Hector et al. AAC, 1990
# SHORT-TERM ORGAN LOADS (Lung) WITH COCCIDIOIDES IMMITIS

<table>
<thead>
<tr>
<th>GROUP</th>
<th>DOSE (mg/kg)</th>
<th>MEAN LOG CFU ± S. E. M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>-</td>
<td>6.35 ± .06</td>
</tr>
<tr>
<td>FLUCONAZOLE</td>
<td>2.5</td>
<td>3.77 ± 1.12</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>2.62 ± .82</td>
</tr>
<tr>
<td>NIK Z</td>
<td>50</td>
<td>0.37 ± .37</td>
</tr>
</tbody>
</table>
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)