RFA NIH-NIAID-DMID-03-09:
New Animal Models for:
Part B Invasive Aspergillosis
(August 12, 2002)

NIH-NIAID-N01-AI-30041
Invasive Aspergillosis
Animal Models (IAAM)

I think….therefore IAAM.  DMD Sept ‘03
Need for Reference Standard(s)

- Historical perspective: Critical needs in antifungal susceptibility
  - Method reproducible
  - Provides reference standard for comparison of other methods
  - Validated for clinical correlation
- Highest priority for *Aspergillus* and new diagnostics
  - Standardized models aimed at identifying new targets for diagnosis & monitoring of disease progression
SPECIFIC AIMS

• Establish and standardize animal models of IA
  ▪ Mouse Model
  ▪ Guinea pig Model
  ▪ Rabbit Model

• Develop molecular tools to provide standardized procedures for genetic manipulation of *Aspergillus* strains.
  ▪ Molecular Toolbox
  ▪ Pathogenesis Toolbox

• Determine gene expression of both whole cell and individual target genes of *Aspergillus* in experimental infection

• Post-genomic data to develop novel diagnostic and immunologic approaches in the management of IA

• Dissemination of knowledge and skills to qualified scientists and laboratories
  ▪ Training
  ▪ Website
  ▪ Annual workshops
### Animal Models: Key Features

- **Mice & larger animals: rabbit/guinea pigs**
  - Pulmonary (aerosol) challenge

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
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<tbody>
<tr>
<td>Neutropenia/non-neutropenic; other immunosuppression</td>
<td>Continuous blood sampling for surrogate markers</td>
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<tr>
<td>Differentiate: exposure/colonization/infection</td>
<td>Quantify tissue burden (2 methods)</td>
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<tr>
<td>Standardized</td>
<td>Local/disseminated infection</td>
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<tr>
<td>Telemetry/IR fever curves</td>
<td>Genomic approach to molecular diagnosis</td>
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<td></td>
<td>Survival duration allowing for disease progression (4-7 d)</td>
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<td>Assess growth dynamics of fungi</td>
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<td></td>
<td>A. fumigatus (AF293); suitable for others</td>
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<td></td>
<td>Gene profiling</td>
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</table>
Animal Model Design

• Recapitulates human disease
• Cost
• Reproducibility
• Ease of use
• Amenable to studies including:
  ▪ Evaluation of novel diagnostics
  ▪ Evaluation of host response
  ▪ Evaluation of organism virulence factors on diagnosis through molecular manipulations
  ▪ In vivo expression analysis
  ▪ Evaluation of effect of therapy on diagnosis
Madison Aerosol Challenge/Inhalational Chamber

The Reference Standard

Precision delivery to:
- 90 mice
- 18 guinea pigs
- 4 rabbits
Developmental Status Report

• Completion of Phase I—continuation of phase 2 (end of year 4)
  ▪ Deliverables: Standard operating procedures
    - Molecular techniques
    - Animal models
    - www.sacmm.org/iaam.html
  ▪ How to identify “key” questions/new diagnostics
    - Interactions with Steering Committee, Scientific Community & Commercial Partners
  ▪ Procedures for receiving requests and establishing priorities
  ▪ Development of new diagnostics
    - New targets
    - New platforms
    (Provide tissues/samples; testing)
### IAAM: Standard Operating Procedures

#### Invasive Aspergillosis Animal Models

<table>
<thead>
<tr>
<th>Title</th>
<th>File for download</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Operating Procedure for Preparation of Aspergillus fumigatus Test Strains for Inhalational Pulmonary Aspergillosis Animal Studies</td>
<td><a href="#">PDF File</a></td>
</tr>
<tr>
<td>Standard Operating Procedure for Murine Inhalational Pulmonary Aspergillosis</td>
<td><a href="#">PDF File</a></td>
</tr>
<tr>
<td>Standard Operating Procedure for Guinea Pig Inhalational Pulmonary Aspergillosis</td>
<td><a href="#">PDF File</a></td>
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<tr>
<td>Standard Operating Procedure for Animal Tissue Homogenization</td>
<td><a href="#">PDF File</a></td>
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<tr>
<td>Standard Operating Procedure for Processing Animal Tissue Samples for PCR, Gel Electrophoresis, and Storage</td>
<td><a href="#">PDF File</a></td>
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<tr>
<td>Standard Operating Procedure for Aspergillus sp. DNA Extraction for Quantitative Real-time Polymerase Chain Reaction</td>
<td><a href="#">PDF File</a></td>
</tr>
<tr>
<td>Standard Operating Procedure for the Determination of Tissue Fungal Burden Utilizing Quantitative Real-time Polymerase Reaction (qPCR)</td>
<td><a href="#">PDF File</a></td>
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</tbody>
</table>

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**Best viewed at 1024x768 resolution.**

[www.sacmm.org/iaam.html](http://www.sacmm.org/iaam.html)
Key Questions

• Qualified investigator
  - Researcher with interest in *Aspergillus*
  - Trained to safely perform requested research
  - Qualifications: Principal investigators; Trainees (Post-doctoral fellows, students); Industry researchers

• Key Questions
  - Gene/gene product as diagnostic target
  - Evaluation of surrogate marker(s)
  - Effects of therapy on disease progression/gene expression
  - Role of virulence determinants in diagnosis
  - Others
Key Questions

• Prioritization of key questions
  ▪ High likelihood of commercialized diagnostic product
  ▪ Data to support development of diagnostic product
  ▪ Pilot studies to test theoretical diagnostic target
    - NIH funded research
    - Preliminary data to support NIH application with favorable priority score on review
    - Pilot studies to evaluate investigator initiated concept
    - Industry sponsored research
### Key Questions

<table>
<thead>
<tr>
<th>Researchers</th>
<th>Project Details</th>
<th>Institutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>W. Steinbach</td>
<td>Calcineurin pathway in IA</td>
<td>Duke</td>
</tr>
<tr>
<td>R. Cramer</td>
<td>Role of gliP in gliotoxin synthesis</td>
<td>Duke</td>
</tr>
<tr>
<td>B. Miller</td>
<td><em>Aspergillus</em> virulence determinants</td>
<td>U Idaho</td>
</tr>
<tr>
<td>N. Wiederhold</td>
<td>Genome-wide expression to echinocandins for <em>Af</em></td>
<td>UTHSCSA</td>
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<tr>
<td>C. Clancy</td>
<td><em>In vivo</em> gene expression of <em>Af</em></td>
<td>U Florida</td>
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<tr>
<td>S. Harris</td>
<td>Polarized Hyphal Growth in <em>Af</em></td>
<td>U Nebraska</td>
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<tr>
<td>R. Akins/J. Sobel</td>
<td>Microfluidic device for rapid pathogenic fungal diagnosis</td>
<td>U Michigan</td>
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<tr>
<td>A. Zaas</td>
<td>Genetic determinants of <em>Af</em> susceptibility</td>
<td>Duke</td>
</tr>
<tr>
<td>R. Calderone</td>
<td>Germination in <em>Af</em></td>
<td>Georgetown</td>
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<tr>
<td>M. Momany</td>
<td>Rho GTPases in polar growth of <em>Af</em></td>
<td>U Georgia</td>
</tr>
<tr>
<td>B. Segal</td>
<td>Development of <em>Aspergillus</em> vaccine</td>
<td>SUNY/Buffalo</td>
</tr>
<tr>
<td>D. Perlin</td>
<td>New Diagnostics for <em>Af</em></td>
<td>New Jersey</td>
</tr>
<tr>
<td>C. Selitrennikoff</td>
<td>Prophylactic and therapeutic <em>Aspergillus</em> vaccines</td>
<td>MycoLogics, Inc, Aurora, CO</td>
</tr>
<tr>
<td>C. Douglas</td>
<td>QPCR for diagnostics of A. fumigatus</td>
<td>Merck and Co., Inc, Rahway, NJ</td>
</tr>
<tr>
<td>J. Loeffler</td>
<td>QPCR for diagnostics of A. fumigatus</td>
<td>University of Wuerzburg, Germany</td>
</tr>
<tr>
<td>G. Ramage</td>
<td>Real-Time PCR assay to detect A. fumigatus</td>
<td>Glasgow Caledonian University, Glasgow, Scotland</td>
</tr>
</tbody>
</table>

Invasive Aspergillosis Animal Models (IAAM)
<table>
<thead>
<tr>
<th>Name</th>
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</tr>
</thead>
<tbody>
<tr>
<td>C. Cray</td>
<td>Murine model of Pulmonary invasive Aspergillosis</td>
<td>University of Miami Miller School of Medicine, Miami FL</td>
</tr>
<tr>
<td>D. Sheppard</td>
<td>GM diagnostics in A. fumigatus</td>
<td>McGill University, Montreal Canada</td>
</tr>
<tr>
<td>B. Wickes</td>
<td>PCR diagnostics in A. fumigatus</td>
<td>UTHSCSA</td>
</tr>
<tr>
<td>S. Filler / D. Sheppard</td>
<td>Host response to invasive aspergillosis</td>
<td>UCLA - Harbor / McGill University</td>
</tr>
<tr>
<td>S. Baker</td>
<td>Proteomics approach to A. fumigatus detection</td>
<td>Pacific Northwest National laboratory, Richland, WA</td>
</tr>
<tr>
<td>R. Cramer</td>
<td>Metabolomics approach to A. fumigatus detection</td>
<td>Duke</td>
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<tr>
<td>N. Wiederhold</td>
<td>Chitin assay development for pulmonary aspergillosis</td>
<td>UTHSCSA</td>
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<tr>
<td>N. Wiederhold</td>
<td>effect of paradoxical effect on diagnosis of IPA during echinocandin therapy</td>
<td>UTHSCSA</td>
</tr>
<tr>
<td>A. Vallor</td>
<td>utility of serum vs whole blood for assessment of fungal burden in IPA</td>
<td>UTHSCSA</td>
</tr>
<tr>
<td>S. Filler / D. Sheppard</td>
<td>Effect of different aspergillus isolates on experimental murine IPA</td>
<td>UCLA - Harbor / McGill University</td>
</tr>
<tr>
<td>M. Del Poeta</td>
<td>Detection of anti-glucosylceramide antibody in an Invasive Aspergillosis</td>
<td>Medical Univ. Of S. Carolina, Charleston, SC</td>
</tr>
<tr>
<td>Russ Lewis</td>
<td>Animal models for diagnosis and treatment (Use of SOPs)</td>
<td>MD Anderson, Houston TX</td>
</tr>
<tr>
<td>Terry Sweeney</td>
<td>ABIP in an inhalational model of aspergillosis (Use of SOPs)</td>
<td>Nektar Therapeutics, San Carlos, CA</td>
</tr>
</tbody>
</table>
IAAM - INVASIVE ASPERGILLOSIDIC ANIMAL MODELS:
Fourth Annual Meeting
The University of Texas Health Science Center at San Antonio
Academics and Administration Building Room 114
7703 Floyd Curl Drive
San Antonio, TX 78284-3900
Telephone (210) 567-4527
Thursday, October 18, 2007
8:00 AM - 1:00 PM

Welcome & Introductions
• Dennis Dixon / Rony Duncan / Tom Patterson
  Welcome / Meeting Expectations 8:00 - 8:15 AM
• Tom Patterson
  Presentation: Contract Review & Key Questions 8:15 - 8:30 AM

Session 1  MODEL UPDATES
• Scott Filler / Laura Najjar
  Presentation: Murine Models 8:30 - 8:50 AM
• Rick Kirkpatrick / Peter Warn
  Presentation: Guinea Pig and Rat Models 8:50 - 9:00 AM

Session 2  SURROGATE MARKERS AND GENE EXPRESSION
• Ana Vallor / Nathan Wiederhold
  Presentation: PCR / Beta-D-glucan 9:00 - 9:20 AM
• Don Sheppard
  Presentation: In vivo Galactomannan Release
  Gene Expression 9:20 - 9:40 AM

Session 3  MOLECULAR TOOLBOX
• Brian Wickes / David Denning
  Presentation: Aspergillus Strain Identification 9:40 - 10:00 AM
• Panel Discussion and audience interaction 10:00 - 10:25 AM

Break 10:25 - 10:50 AM

Session 4  KEYNOTE LECTURE: CLINICAL NEEDS
• John Wingard  University of Florida Shands Cancer Center

Session 5  NEW DIAGNOSTIC PLATFORMS AND TARGETS
• Margo Moore, Simon Fraser University
  Presentation: Siderophores for IA Diagnosis 11:15 - 11:30 AM
• Annette Fothergill / Wieslaw Farmaga, University of Massachusetts
  Presentation: Proteomics in IA Diagnostics 11:30 - 11:45 AM
• Steven B. Kleboecker, Pacor
  Presentation: Real time PCR of A. fumigatus 11:45 - 12:00 PM
• Marta Feldmesser, Albert Einstein College of Medicine
  Presentation: Diagnostic Tests for A. fumigatus 12:00 - 12:15 PM
• Tom Walsh, National Institutes of Health/National Cancer Institute
  Presentation: Host/Pathogen Proteomics of Experimental IPA 12:15 - 12:30 PM
• Panel Discussion and audience interaction 12:30 - 12:35 PM

Conclusions: Future Directions
• Tom Patterson 12:35 - 1:00 PM

Departures – Lunch 1:00 PM
Future Directions

• Deliverables/Model refinement
  ▪ SOPs online
  ▪ Alternative Af strains
  ▪ Role of host responses
  ▪ Distinction of colonization vs disease
  ▪ Impact of sample types, collection, storage

• New target identification
  ▪ Gene product(s) in disease
  ▪ Host gene responses
  ▪ Novel approaches

• Diagnostic development
  ▪ Industry partners
  ▪ Pre-clinical support for diagnostics
  ▪ Community awareness/interaction
3rd ADVANCES AGAINST ASPERGILLOSIS
January 16-19, 2008
Miami Beach Resort & Spa
Miami, Florida
USA
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