Approaches to Developing and Refining Animal Models for Use in Assessing the Efficacy of Medical Countermeasures According to the FDA Animal Rule

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Track A – Animal Models
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Outline

• Why is the “Animal Rule” necessary?
• What is the “Animal Rule”?
• Animal Model Development Under the Animal Rule
  – Anthrax
  – Smallpox
  – Botulinum
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What are the Biological Threats?

Traditional Threats
- Priority Pathogens-Category A
  - Anthrax
  - Plague
  - Ebola
- Priority Pathogens-Category B
  - Glanders
  - Q Fever
- Priority Pathogens-Category C
  - Yellow fever
  - Rabies

Advanced Threats
- Add, delete, or mutate genes to engineer pathogens that are more resistant, transmissible, virulent
- Create viruses de novo
  (synthetic organisms - e.g., polio, 1918 influenza)

Emerging Threats
- “Natural” and emerging diseases:
  - Pandemic flu
  - SARS
  - Drug-resistant TB
  - Malaria
  - Cholera
  - MRSA

Enhanced Threats
- “Bioprospecting”: Finding particularly virulent strains in nature
- Cultivating particularly virulent strains of pathogens in the laboratory

Emerging Threats
- Multi-drug and vaccine resistant pathogens
Diseases with antibiotics, therapeutics, vaccines that require testing under the Animal Rule

- Anthrax
- Smallpox (monkeypox; rabbitpox)
- Botulinum toxin
- Plague
- Tularemia
- Meliodosis
- Glanders
- Brucellosis
- Filoviruses

BSL-3 or BSL-4
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The Animal Rule

- The Animal Rule *amended* the regulations to allow for approval of a new drug or biologic product for which safety has been established in humans and efficacy has been demonstrated in adequate and well-controlled animal studies believed to be predictive of the desired benefit in humans.

- Licensing of CBRN medical products when normal clinical trails are not possible requires alternative approaches to demonstrate *efficacy*.

- The Animal Rule was published in the Federal Register with an effective date of July 1, 2002.
  
  Subpart I – Approval of New Drugs When Human Efficacy Studies Are Not Ethical or Feasible (21 CFR Parts 314.600-650)

  Subpart H – Approval of Biological Products When Human Efficacy Studies Are Not Ethical or Feasible (21 CFR Parts 601.90-95)
Animal Rule: Tenets

- Reasonably well-understood pathophysiological mechanism of the toxicity of the substance (agent) and its prevention/reduction by the test product
  - "The effect can be demonstrated in a single animal species if there is a sufficiently well-characterized animal model for predicting the response in humans; no where in the Rule is “Two Animals” stated"

- Effect is demonstrated in an animal species expected to react with a response predictive of human

- Animal study outcome is clearly related to the desired benefit in human
  - Reduced morbidity/mortality

- Data on pharmacokinetics/dynamics of the product in animals and humans allows selection of an effective dose in humans
Potential misunderstandings

• The Rule does not apply if product approval can be based on standards described elsewhere in FDA's regulations – normal human trials can be done

• Safety must still be demonstrated in human subjects enrolled in Phase I, II & III clinical trials

• The Rule is not an Accelerated or Fast-Track approval and is not a short-cut to approval, in fact, it may take longer
Two products are approved under the Animal Rule

• Pyridostigmine bromide, indicated for prophylaxis against the lethal effects of soman nerve agent poisoning, was approved in 2003.

• Hydroxocobalamin, indicated for the treatment of known or suspected cyanide poisoning, was approved in 2006.

• Many more under development
Drug development process

Animal Rule Nonclinical Efficacy Studies

Drug Discovery → Preclinical → Phase 1 → Phase 2 → Phase 3 → FDA Review And Approval

Number of Compounds:
- 5,000 -10,000
- 250
- 5

One FDA Approved Drug

Number of Subjects:
- 20-100
- 100-500
- 1,000-5,000

3-6 years → 6-7 years → 0.5-2 years

IND Submitted → NDA Submitted

Phase 4: Post-marketing surveillance
The Biological Agent

- Etiologic agent same as that causing disease in humans
  - Surrogates can be acceptable (e.g. Monkeypox)
- Pathogenic determinants
  - How does the agent cause the pathology
    - Toxin production of a bacteria
    - Target and disrupt a target organ
  - This should be the same in both humans and the animal species
- Route of exposure should be same as the threat to humans
  - Aerosol challenge is often the route of exposure
- Quantification of exposure
  - Well characterized agent
  - Reliable and reproducible challenge dose
  - Show scalable relationship between dose and outcome (especially in humans)
The Host

• Host susceptibility and response
  – Animal species chosen should be susceptible to the agent – seems obvious but requires consideration of the dose compared to the human dose

• Natural history of the disease
  – Pathophysiologically should be comparable to humans
  – Time course of disease
  – Manifestations of disease (signs, symptoms)
  – Pathology
  – Outcome (death, recovery)

• Endpoints
  – Ultimate key to success is the study endpoints
Study endpoints in addition to mortality

- Clinical observations/body weight
- Telemetry – body temperature and activity
- Hematology/clinical chemistry
- Bacteremia/Viral titers
- Cytokine levels, Toxin levels
- Immune response
- Pathology
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Natural history study of inhalational anthrax in Cynomolgus macaques

- **LD$_{50}$** – 61,800 cfu
- **Gross lesions**
  - Splenomegaly, lymph node enlargement, hemorrhage particularly involving the meninges and lungs
- **Endpoint** - death due to toxemia

Development of a monotherapy model in Cynomolgus macaques

- Characterize the disease progression observed in cynomolgus macaques following exposure to *B. anthracis* via the inhalational route of exposure

- Develop and utilize critical assays for support of future efficacy studies in the cynomolgus macaque

- Evaluate in a product neutral fashion the therapeutic efficacy of adjunct therapies

*Henning et al., Development of an Inhalational Bacillus anthracis Exposure Therapeutic Model in Cynomolgus Macaques. Clinical and Vaccine Immunology, accepted.*
Design for the efficacy study of a anti-PA monoclonal antibody

- Objective: Assess the disease progression in untreated animals (challenged and unchallenged) to define appropriate indicators of illness and preliminarily assess the efficacy an anti-PA mAb
  - 30 Cynomolgus macaques were randomized into three groups
    - 24 monkeys challenged with 432 (+/-156) B. anthracis LD\textsubscript{50} equivalents (LD50 = 61,800 cfu)
      - 12 monkeys treated at ECL positive with 10mg/kg anti-PA mAb
      - 12 monkeys untreated
    - 6 monkeys unchallenged

<table>
<thead>
<tr>
<th>Group ID</th>
<th>Monkeys per Group</th>
<th>mAb Dose</th>
<th>Treatment Point Post-Challenge</th>
<th>Dose Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
<td>10 mg/kg</td>
<td>Individual times will be based on serum PA levels (ECL Positive)</td>
<td>IV (bolus injection)</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>3</td>
<td>6 (unchallenged)</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Study design is complex

• Blood Collections
  – Day -7,
  – Hours 24, 30, 36, 42, 48, 54, 60, 66, 72 post median challenge time
  – Days 5, 8, 14, and 28 post challenge
    - Bacteremia culture, qPCR, CBC (differential), and C-reactive protein (CRP)
    - ECL, PA-ELISA

• Clinical Monitoring
  – Telemetry - Hourly Body Temperature and Activity
  – Outward Clinical Signs - Every six hours

• Pathology
  – Gross necropsy and Histopathology

• Treatment mAb administered on an individual basis (IV bolus) at first positive ECL result
Clinical profiles

Unchallenged Control Animals
- WBC – Diurnal
- N/L Ratio – Unchanged
- Bacteremia – Negative
- PA – Negative
- Temperature - Disrupted

Challenged Control Animals
- WBC – Elevated
- N/L Ratio – Increase
- Bacteremia – Positive
- PA – Positive
- Temperature - Increased
Mortality – significantly different between the treated and untreated

<table>
<thead>
<tr>
<th>Group</th>
<th># Survived/ # Total</th>
<th>Survival Percent</th>
<th>Mean Time to Death (hr)</th>
<th>Fisher's Exact Test (p-value)</th>
<th>Log Rank Test (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9/12</td>
<td>75%</td>
<td>90.5</td>
<td>0.0061*</td>
<td>0.0127*</td>
</tr>
<tr>
<td>2</td>
<td>2/12</td>
<td>17%</td>
<td>133.21</td>
<td>0.0061*</td>
<td>0.0127*</td>
</tr>
<tr>
<td>3</td>
<td>6/6</td>
<td>100%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

p-value = 0.0061
NHP monotherapy model: Conclusions

- Cynomolgus macaques challenged with *Bacillus anthracis* via the inhalational exposure route exhibit clinical and physiological abnormalities following challenge.

- Specific diagnostic techniques including ECL, PA-ELISA, qPCR, and Bacteremia cultures can be used to confirm infection.

- The clinical findings (abnormal CBC, body temperature disruption, decreased activity) observed in Cynomolgus macaques post-exposure appear to be delayed relative to confirmation of infection.

- Treatment of animals confirmed to be infected with a mAb against PA is efficacious in preventing death.
Progression of studies for vaccine development

• Pharmacokinetic/Toxicity and Immunogenicity
  – Dose regimen/schedule
  – Dose ranging/Dose response

• Efficacy
  – Dose ranging/Dose response
  – Regimen and schedule

• Correlate Refinement Studies
  – Passive transfer of human antibody
  – Time to protection
  – Duration of protection
  – Breakthrough
Endpoints of an anthrax vaccine study

• Survival / Lethality
• Non-lethal pathology or clinical observations
  – Pneumonia
  – Fever
  – Hematology (white blood cell shift reflecting infection)
  – Clinical Chemistries (i.e., Liver Function Tests)
• Bacteremia
• Immunomodulation assessments
  – Antibody levels and function
  – Cellular immune response
US Licensed Anthrax Vaccine

• Anthrax Vaccine Adsorbed, USP
  – ‘BioThrax’
  – Aluminum hydroxide adsorbed
  – Sterile, cell-free filtrate made from microaerophilic cultures of avirulent, non-encapsulated *B. anthracis* V770-NP1-R
  – Primary immunogen is PA
  – Manufactured
    - Michigan Dept of Health until 1998
    - Emergent BioSolutions

• Adjuvant and additives
  – Adjuvant 1.2 mg/mL aluminum (Al(OH)₃, 0.85% NaCl)
  – Preservatives: 25 µg/mL benzethonium chloride and 100 µg/mL formaldehyde
CDC Anthrax Vaccine Research Program

- Vaccinate NHPs with dilutions of the Anthrax Vaccine Adsorbed at week 0 and boosted at 4 and 26 weeks
  - Blood collections during the vaccination schedule to assess the immunological response and correlation with survival at 12, 30, and 52 months
  - Anthrax aerosol challenges >150 NHP
  - Humoral and cellular immune response at >20 time points (10 assays)

- Parallel human clinical trial schedule and route

- Analysis of correlates of protection
Dose-dependent antibody levels in response to AVA correlated with human clinical trial

- Antibody levels were induced after vaccination
- Peak levels occurred 2-4 weeks post the 3rd vaccination at week 28
- Post week 30, antibody levels gradually decreased to low but detectable levels
- NHPs receiving three doses of AVA were protected for 52 months from an aerosol challenge

Quinn et al., Clin Vaccine Immunol 2012 Aug 29. [Epub ahead of print]
Next generation anthrax Recombinant Protective Antigen vaccine in multiple species

Efficacy studies in rabbits

Stark et al., 8th Annual ASM Biodefense & Emerging Diseases, Baltimore, MD, February 21-24, 2010
Fay et al., Sci Transl Med. 2012 Sep 12;4(151):151ra126
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Monkeypox virus is a surrogate for Variola major (Smallpox)

- Members of the Poxviridae family
- Incubation period for smallpox is 1 to 12 days before the symptoms of fever, headache and rash appear
- Pathology of monkeypox infection in cynomolgus monkeys is similar to that of smallpox infections in humans (Zaucha et al., Lab Invest 2001;81:1581)
- Secondary parameters of infection
  - Lesion presentation, weight loss, hematological changes, virus shedding in saliva, virus recovery from tissues, and histopathology
Natural history study IN vs. IV challenge for Monkeypox showed differential mortality

- Differential mortality observed in high dose groups of NHPs challenged via the IV and IN routes
- Increased percent body weight declination and severe clinical observations are more prevalent in the IN dose response

Schmidt et al., XVII International Poxvirus and Iridovirus Conference, Grainau, Germany. June 8-12, 2008.
Responses varied by the route of challenge

- IV Responses
  - Greater lesion development
  - Increased clinical assessment scores
  - Increased mortality

Schmidt et al., XVII International Poxvirus and Iridovirus Conference, Grainau, Germany. June 8-12, 2008.
IN and IV challenge routes showed similar temperature and activity

- Similar Telemetry data observed in monkeys developing severe infection in both challenge routes
- Temperature increase, which did not return to baseline levels or a normal diurnal pattern
- Significantly reduced activity levels

Schmidt et al., XVII International Poxvirus and Iridovirus Conference, Grainau, Germany. June 8-12, 2008.
Efficacy of ACAM2000 in NHPs

- An adapted vaccinia virus that was derived from the existing Dryvax® vaccine and grown in cell culture (ACAM2000)
- Monkeys were vaccinated with ACAM2000 and challenged IV at 2 months with a lethal dose of monkeypox virus
- Both vaccines were immunogenic and efficacious and no viremia was identified

Licensing medical countermeasures for Smallpox

- ACAM 2000 was licensed based on non-inferiority in a clinical trial
- Anti-virals are under development
- A rabbitpox intradermal model is currently under development
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Botulinum Neurotoxin

• Botulinum neurotoxin is the most lethal toxin known to man
• Seven toxins (A-G) – different types affect different animals
• Causes the same disease after inhalation, oral ingestion, or injection
• Botulinum vaccine
• Botulinum anti-toxin
Natural history study to determine the LD$_{50}$ of botulinum neurotoxin

- Clinical and physiological parameters to aid in the understanding of disease progression
  - Clinical observations
  - Body weight
  - Clinical hematology
  - Clinical chemistry
  - Telemetric monitoring
  - Circulating neurotoxin levels

Sanford et al., Clin Vaccine Immunol. 2010 September; 17(9): 1293–1304.
Botulinum neurotoxin: physiological parameters in the NHP

Sanford et al., Clin Vaccine Immunol. 2010 September; 17(9): 1293–1304.
A botulinum vaccine showed efficacy in the NHP model

- All vaccinated animals survived challenge and remained asymptomatic during the 30 day post-challenge observation period
- Circulating neurotoxin was detected in the serum of non-vaccinated control animals but not in vaccinated animals
- Vaccine provides excellent protection in rhesus macaques

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Courtesy of DynPort Vaccine Co. LLC (DVC), a CSC company; DoD Contract DAMD 17-98-C-8024
Key points for a successful efficacy study under the Animal Rule

• Challenge material is a “critical reagent” - preparation and characterization of the infectious agent must be standardized, consistent, and reproducible

• Optimized/validated assays to monitor the response and bridge data to humans (non-validated assays may be useful and acceptable)

• Statistical plan in place

• Pivotal studies conducted under the FDA GLP guidelines
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Questions?