Biodefense Vaccines, the Animal Rule and Collaboration

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The Filovirus Animal Nonclinical Group (FANG)

• Co-leads
  – Nicole Kilgore, JVAP/CBMS/DoD
  – Ed Nuzum, DMID/NIAID/NIH/HHS
FANG Purpose and Mission

• Purpose: “….support development of Filovirus MCM….focus on Product Development (PD) tools and issues relevant to FDA approval of Filovirus MCM.”

• Mission: “…develop strategies to address broadly applicable and interagency PD issues relevant to licensure…..develop consensus recommendations to facilitate standardization of reagents, methods and procedures across multiple agencies…”
FANG Goals

• Goals:
  – “…enhance communication, coordinate PD efforts and align PD, regulatory and scientific resources…”
  – “…form a cohesive interagency group composed of staff at the “operational” level and SMEs to facilitate decision making and implementation.”
  – “…develop standardized protocols for reagents, assays, models, assessment and measurement of critical endpoints, product manufacturing and characterization.”
  – “…minimize redundancy across agencies…”
  – “…develop unified message to guide product sponsors…”
FANG Participants

- DoD: CBMS-JVAP, TMT, DTRA-JSTO, USAMMDA, USAMRIID
- HHS:
  - FDA: CDER, CBER, OC
  - CDC
  - NIAID intramural and extramural
  - BARDA
- DHS: NBACC
- BSL4 Labs
FANG Accomplishments

• IM challenge dose rationale (White Paper) nearly completed
• Gaps identified
  – Rationale/justification for aerosol challenge dose
  – What is a realistic human aerosol dose?
  – LD99 (not LD50) needed after characterized challenge material is available
  – Reproducible, characterized challenge material for well-characterized challenge studies
FANG Accomplishments (cont.)

• One public workshop completed
• Charter completed
• Monthly meetings and consolidated document “eRoom”
• Subgroups for assays, models, challenge material and human data established
• Challenge strain criteria established
• Challenge strains and sources identified
• Challenge strain characterization criteria in progress
• Standardized and portable plaque assay
• Literature reviews of human and animal data to justify species selection for challenge studies
Backup Slides
Strain Selection Criteria & Selection of Seed Materials

- FDA (CBER and CDER) provided scientific input and suggestions regarding important considerations for Filovirus Challenge Virus Stock for MCMs
- FDA input was important element in development of Challenge Stock selection criteria

1. **Source**: Isolated from outbreaks with a high incidence of mortality and from clinical isolates with known lethal outcome

2. **Background**: Passage history well documented, derived from an isolate that is as close to clinical isolate as possible, low passage number through a well characterized cell line; amplified at a low MOI to minimize Defective Interfering (DI) particles

3. **Panel**: Panel of challenge stocks should be developed that represent the full range of filoviruses

4. **Characterization**: Full genomic sequence, evaluate the seed stock for particle:infectivity ratios; quality control testing (i.e., sterility, mycoplasma, endotoxin, adventitious virus)
Strain Selection Criteria & Selection of Seed Materials

- Survey to help determine key selection criteria

### Survey Results

- **Human Lethal**
- **Stain is or can be well characterized**
- **Low passage number**
- **Whole genome sequence**
- **Pure culture (endotox/mycoplasma)**
- **Historical use for filovirus vaccine testing**
- **Not passaged in an animal**
- **NHP Lethal**
- **Clinical History**
- **High sequence homology to direct human sample**
- **Availability**
- **NHP infection matches human disease**
- **Prototypical isolate from outbreak**
- **Passed at low MOI**
- **Same etiologic agent expected to be encountered**
- **Passed in primate cells**
- **Strain can be shipped internationally**
- **Not plaque picked**
- **Particle to pfu ratio known and within the expected range**
- **High titer growth**
- **Sequence variability within the stock is evaluated preferably at...**
- **Not an outlier**
Strain Selection Criteria & Selection of Seed Materials

- Frequency, Stain is or can be well characterized, 8, 175
- Frequency, Human Lethal, 9, 229
- Frequency, Historical use for filovirus vaccine testing, 6, 99.5
- Frequency, NHP Lethal, 5, 65
- Frequency, Whole genome sequence, 6, 105
- Frequency, Low passage number, 7, 114
- Frequency, Pure culture (endotox/mycoplasma), 5, 102
- Frequency, Not passaged in an animal, 4, 65
- Frequency, Availability, 3, 65
- Frequency, Clinical History, 4, 57.5
- Frequency, High sequence homology to direct human sample, 5, 57
Panel of Challenge Stocks Selected

• Zaire Ebolavirus (ZEBOV)
  • Kikwit ’95 (wild type) for large animal models (NHP)
  • Mayinga ‘76 (mouse/guinea pig adapted) for small animal models

• Marburg virus (MARV)
  • Angola (wild type and guinea pig adapted)

• Sudan Ebolavirus (SEBOV)
  • All available Boneface materials passed in guinea pigs; only higher passage (p4-p6) starting material available; some 100+ nucleotide differences between Boneface and Gulu found with deep sequencing
  • NHP study showed similar virulence/lethality of low passage Gulu and Yambio strains

• Gulu selected
  – From a lethal case and low passage available
  – Passed in NHP cells
  – From a larger outbreak than Yambio
Challenge Strain Characterization

- **Potency**
  - PFU (BSL4)
  - TCID50 (BSL4)

- **Content**
  - Quantitative RT PCR
  - EM: Particle Count, Size Distribution
  - BCA
  - Gene copies to PFU ratio
  - Particle to PFU Ratio
  - DI assessment with low MOI clone

- **Identity**
  - RT-PCR
  - Deep sequencing
  - Negative staining EM
  - Viro Chip? Microarrays?

- **Safety**
  - Deep sequencing
  - Sterility (BSL4)
  - Endotoxin (BSL4)
  - DNA??
<table>
<thead>
<tr>
<th>Virus</th>
<th>Strain and stock available</th>
<th>Origin</th>
<th>Source</th>
<th>Pass</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zaire ebolavirus</td>
<td>Mayinga 808012</td>
<td>1976 Zaire Outbreak, human blood</td>
<td>Patient F 23yo, nurse, nosocomial infection (onset Oct 13 1976, hospitalized Oct 15, died Oct 21); blood collected day 4 (Oct 17) of the disease</td>
<td>Vero E6+1</td>
<td>Complete sequence in Genbank AY142960 is from USAMRIID. Virus sequenced is likely E6+2 or E6+3</td>
</tr>
<tr>
<td>Zaire ebolavirus</td>
<td>9510621 807223</td>
<td>1995 Kikwit</td>
<td>Patient Clara 65yo (Onset Apr 29, Hosp May 1, Died May 5) sample collected May 4</td>
<td>Vero E6+1</td>
<td>Complete sequence in Genbank AY354458 is from USAMRIID. Virus sequenced is likely E6+2 or E6+3</td>
</tr>
<tr>
<td>Sudan ebolavirus</td>
<td>Boneface 801625</td>
<td>1975 Sudan Outbreak, human blood</td>
<td>patient (F 10-12yo, fatal); Maridi ward; Onset Oct 24, 1976; Hosp Nov 5; Death Nov 11; serum collected 3 days before death</td>
<td>GP3, Vero3</td>
<td>Complete sequence from 806467 GP3,Vero3,3xpp,E6+3</td>
</tr>
<tr>
<td>Sudan ebolavirus</td>
<td>Maleo</td>
<td>1979 Sudan Outbreak, human blood</td>
<td>Yambio/Nzara district. Patient Angelina Maleo 801671</td>
<td>Complete sequence from 808029 Vero+2,E6+1</td>
<td>Complete sequence (Genbank NC_006432) from 808894 E6+2</td>
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<tr>
<td>Sudan ebolavirus</td>
<td>200407831 810500</td>
<td>2004, Sudan Yambio, human blood</td>
<td>patient (Esterina Apparato, F 60yo, mother of a fatal case) onset 6-4-2004, blood collected 6-15-2004, pos (IgM, antigen, RT-PCR). died 6-26-2004,</td>
<td>VeroE6+1</td>
<td>Complete sequence from original clinical material and E6+1 (Genbank EU338380)</td>
</tr>
<tr>
<td>Bundibugyo ebolavirus</td>
<td>200706291 811250</td>
<td>2006 Uganda outbreak, human blood</td>
<td>BUN-038 patient M52yo (Onset Nov 3, 2007; Hospitalized Nov 10; Died Nov 26; from Butalya parish, Kikyo subcounty); blood collected Nov 14</td>
<td>Vero E6+1</td>
<td>Complete sequence from E6+1 Genbank FJ217161</td>
</tr>
<tr>
<td>Ivory Coast ebolavirus</td>
<td>807212</td>
<td>1994 case from Tai Forest, hospitalized in Switzerland</td>
<td>patient F34yo (Onset Nov 24, Hospitalized Nov 26. evacuated to Switzerland Dec 1, Recovered, discharged Dec 8), Blood collected Nov 27</td>
<td>Vero E6+4</td>
<td>Complete sequence from E6+6 (454+primer walking) Genbank FJ217162</td>
</tr>
<tr>
<td>Marburg virus</td>
<td>200501379 Angola 810820</td>
<td>2005, Angola, human blood</td>
<td>Patient F 8 mo, (Onset Feb 24, Hosp Mar 1, Death Mar 14); blood collected Mar 13</td>
<td>Vero E6+1</td>
<td>Complete sequence from clinical sample and E6+1</td>
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<tr>
<td>Marburg virus</td>
<td>Ravn 811103</td>
<td>1987 Kenya Case, human blood. RIID P986</td>
<td>Patient M 15yo, (Onset 8/10/1987, hosp 8/13 Mombasa, 8/19/87 transferred to Nairobi, died 8/21/87). Blood collected day 9 post-onset</td>
<td>RIID P986, Vero E6+2</td>
<td>Complete sequence from 810040 (? , SW13+1, E6+4)</td>
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