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

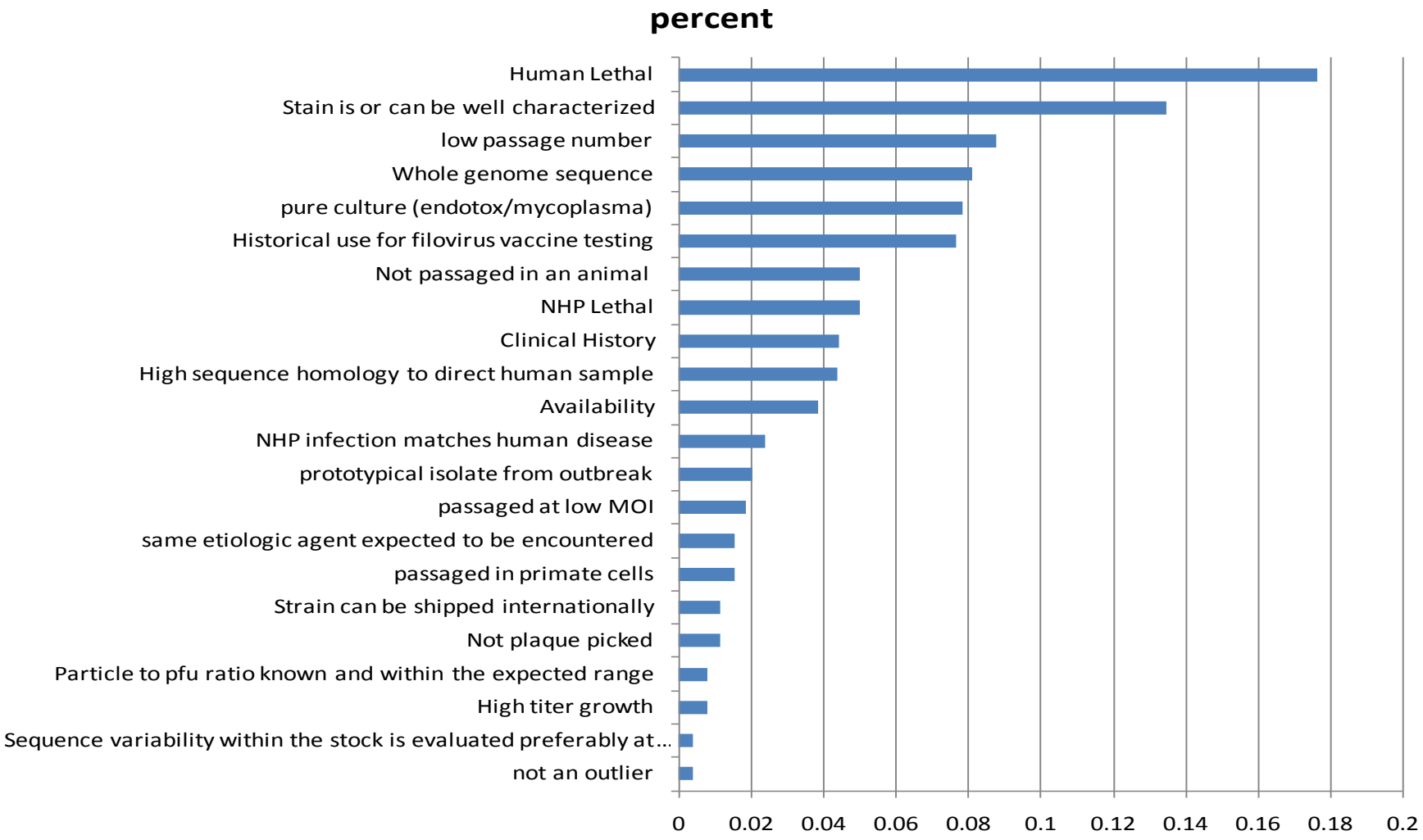
2. Background: Passage history well documented, derived from an isolate that is as Close to clinical isolate as possible, low passage number through a well characterized cell line; amplified at a low MOI to minimize Defective Interferring (DI) particles

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

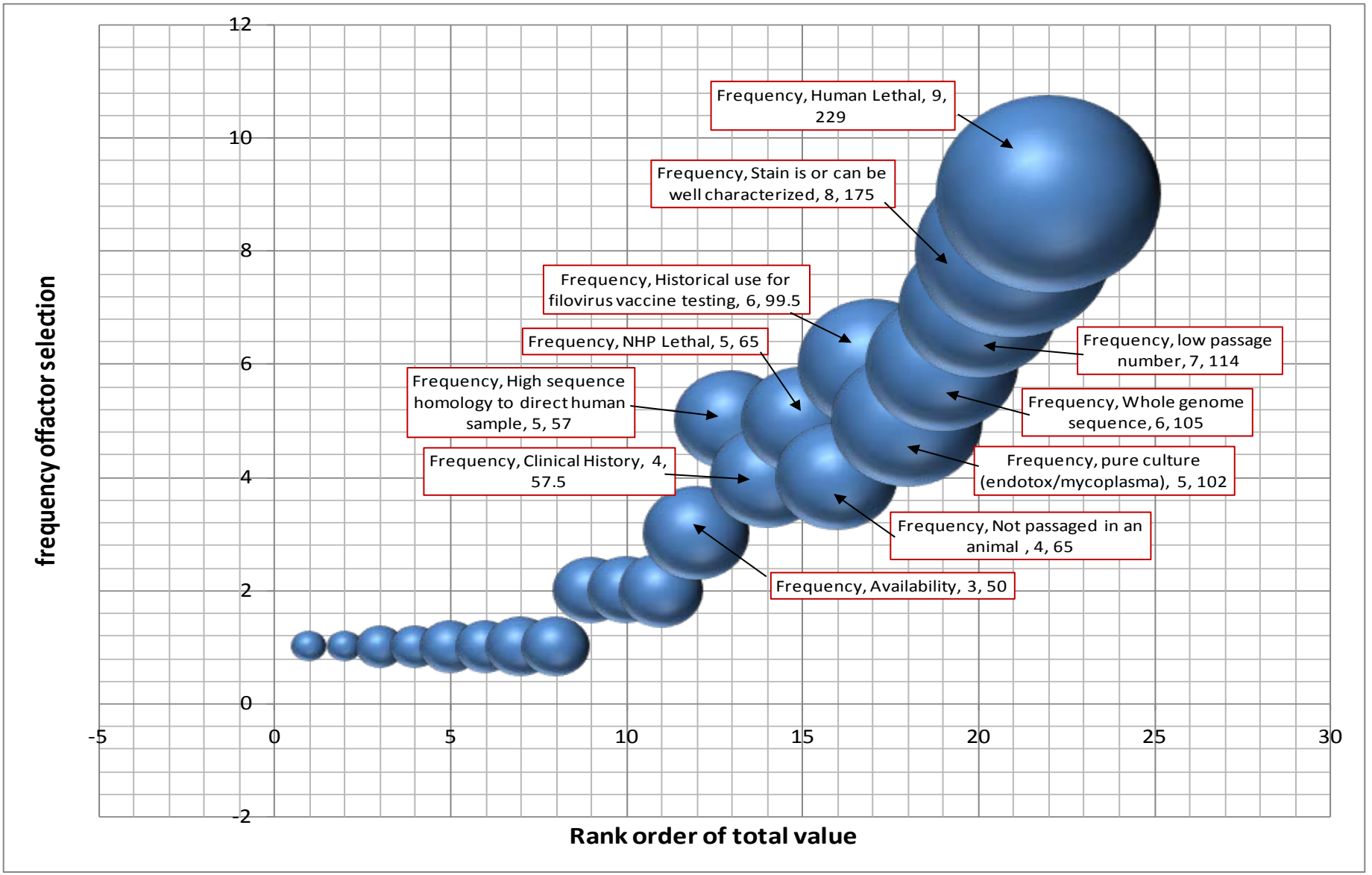
4. Characterization: Full genomic sequence, evaluate the seed stock for particle:infectivity ratios; quality control testing (i.e., sterility, mycoplasma, endotoxin, adventitious virus)

Strain Selection Criteria & Selection of Seed Materials

- Survey to help determine key selection criteria



Strain Selection Criteria & Selection of Seed Materials



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??

Virus	Strain and stock available	Origin	Source	Pass	Sequence
Zaire ebolavirus	Mayinga 808012	1976 Zaire Outbreak, human blood	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	Vero E6+1	Complete sequence in Genbank AY142960 is from USAMRIID. Virus sequenced is likely E6+2 or E6+3
Zaire ebolavirus	9510621 807223	1995 Kikwit	Patient Clara 65yo (Onset Apr 29, Hosp May 1, Died May 5) sample collected May 4	Vero E6+1	Complete sequence in Genbank AY354458 is from USAMRIID. Virus sequenced is likely E6+2 or E6+3
Sudan ebolavirus	Boneface 801625	1975 Sudan Outbreak, human blood	patient (F 10-12yo, fatal); Maridi ward; Onset Oct 24, 1976; Hosp Nov 5; Death Nov 11; serum collected 3 days before death	GP3, Vero3	Complete sequence from 806467 GP3,Vero3,3xpp,E6+3
Sudan ebolavirus	Maleo	1979 Sudan Outbreak, human blood	Yambio/Nzara district. Patient Angelina Maleo 801671		Complete sequence from 808029 Vero+2,E6+1
Sudan ebolavirus	200011676 808892	2000, Uganda Gulu Outbreak, human blood	patient Obol M 35yo, onset Oct 10, Hosp Oct 12, Death Oct 16. (collected postmortem)	VeroE6+1	Complete sequence (Genbank NC_006432) from 808894 E6+2

Virus	Strain and stock available	Origin	Source	Passage	Sequence
Sudan ebolavirus	200407831 810500	2004, Sudan Yambio, human blood	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,	VeroE6+1	Complete sequence from original clinical material and E6+1 (Genbank EU338380)
Bundibugyo ebolavirus	200706291 811250	2006 Uganda outbreak, human blood	BUN-038 patient M52yo (Onset Nov 3,2007; Hospitalized Nov 10; Died Nov 26; from Butalya parish, Kikyo subcounty); blood collected Nov 14	Vero E6+1	Complete sequence from E6+1 Genbank FJ217161
Ivory Coast ebolavirus	807212	1994 case from Tai Forest, hospitalized in Switzerland	patient F34yo (Onset Nov 24, Hospitalized Nov 26. evacuated to Switzerland Dec 1, Recovered, discharged Dec 8), Blood collected Nov 27	Vero E6+4	Complete sequence from E6+6 (454+primer walking) Genbank FJ217162
Marburg virus	200501379 Angola 810820	2005, Angola, human blood	Patient F 8 mo, (Onset Feb 24, Hosp Mar 1, Death Mar 14); blood collected Mar 13	Vero E6+1	Complete sequence from clinical sample and E6+1
Marburg virus	Ravn 811103	1987 Kenya Case, human blood. RIID P986	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	RIID P986, Vero E6+2	Complete sequence from 810040 (?,SW13+1, E6+4)