Filovirus Animal Models

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Goals

- Summarize available filovirus animal models
- Compare model characteristics and translational value
- Highlight regulatory relevance for supporting licensure pathway for filovirus countermeasures

Regulatory Context

Use of Filovirus Animal Models to Support Licensure of Medical Counter Measures (MCM)

- Human efficacy trials impractical and/or infeasible for filoviruses unless outbreaks become large or frequent enough to conduct efficacy trials
  - Human challenge studies are not ethical
- Alternate US regulatory pathways accommodate for these constraints
  - For example, the FDA Animal Rule (21 CFR 601.90) allows approval based on efficacy data generated in nonclinical studies
  - Requires well-characterized model(s) that faithfully recapitulate human filovirus disease and are predictive of human response (natural history studies)
  - Immunobridging or pharmacokinetic bridging from animals to humans is essential to predict clinical efficacy and dose justification of the MCM

Key Requirements for Filovirus Models

No Single model is Perfect – Each Will Have Advantages and Limitations

Natural Susceptibility

Effective models use species naturally susceptible to filovirus infection, avoiding artificial manipulation to ensure realistic disease outcomes.

Mirroring Human Pathology

Models must closely replicate human disease pathology to allow accurate study of disease progression and intervention effects.

Human-Relevant Dosing and Protection

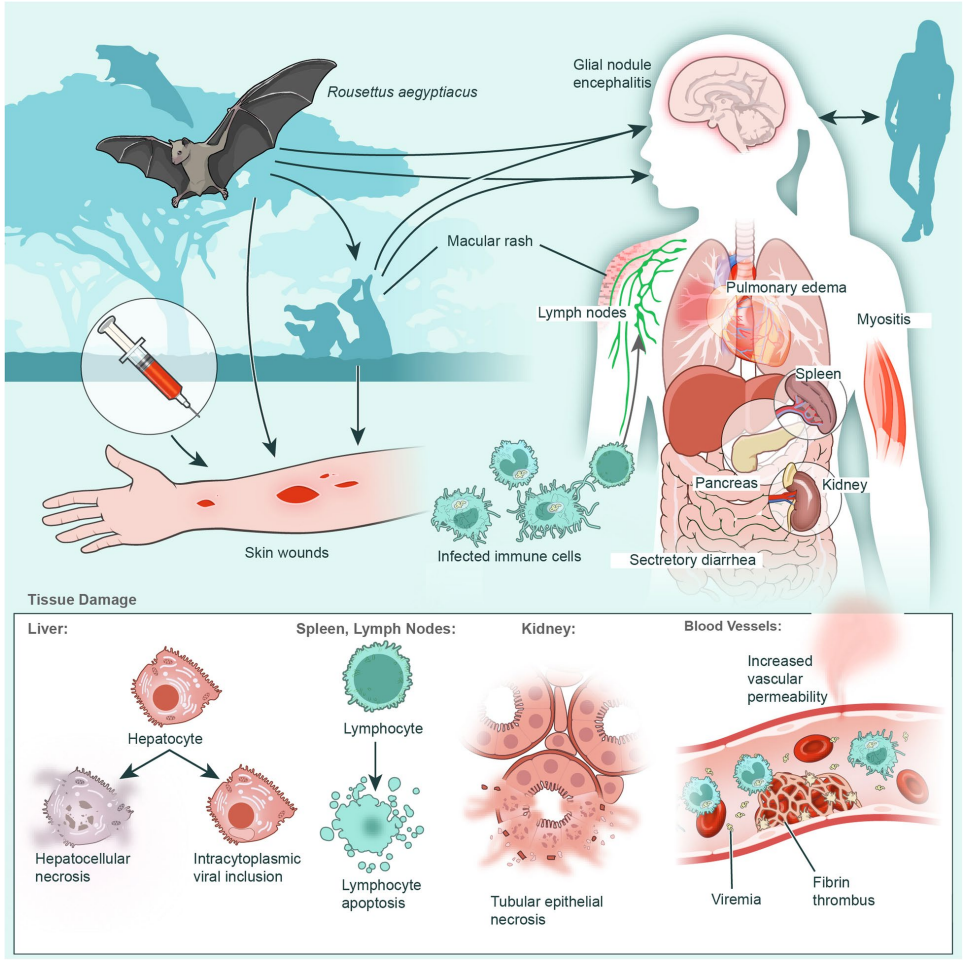
Models should enable identification of doses or protection correlates that are relevant for humans, guiding therapeutic development.

Role of NHPs and Small Animals

Nonhuman primates best fulfill model criteria, but advanced small animal models increasingly support early research and regulatory requirements.

Filovirus Pathogenesis in Humans*

Marburg Virus Transmission and Virus Spread in the Human Body



From Shifflett, K., Marzi, A. Marburg virus pathogenesis – differences and similarities in humans and animal models. *Virol J* **16,** 165 (2019).

Filovirus Animal Models

Mice

Guinea Pigs

Hamsters

Ferrets

NHP

Filovirus	Mouse			Guinea pig*	Syrian hamster*	Ferret	NHP
	Immuno-competent*	Immuno-compromised	Humanized				
EBOV	+	+	+	+	+	+	+
MARV	+	+	+	+	+	+*	+
RAVV	+	+	+	+	-	-	+
SUDV	-	+	+	+	-	+	+
TAFV	-	-	+	-	-	+	+
BDBV	-	-	+	-	-	+	+
RESTV	-	-	+	-	-	+	+

* Requires adapted virus; NHP: Nonhuman primate; EBOV: Ebola virus; MARV: Marburg virus; RAVV: Ravn virus; SUDV: Sudan virus; TAFV: Tai Forest virus; BDBV: Bundibugyo; RESTV: Reston virus; +: Available; -: Not available

Adapted from T. W. Geisbert, Galveston National Laboratory, UTMB

Filovirus Pathogenesis

Differences in Marburg Virus Disease Animal Models

	Human	NHP	Outbred Guinea Pig	Inbred Guinea Pig	Immuno-competent Hamster	Immuno-deficient Hamster	Immuno-competent Mouse	Immuno-deficient Mouse	Humanized BLT Mouse
Hemorrhage	✓	✓	✓	✓	✓	✗	✗	✗	✗
Maculo-papular Rash	✓	✓	✗	✗	✓	✗	✗	✗	✗
Thrombo-cytopenia	✓	✓	✓	?	○	○	✓	?	?
Lymphopenia followed by neutrophilia	✓	✓	✓	?	✓	○	✓	✓	?
DIC	✓	✓	✓	?	✓	?	✗	✗	?
Severe Hepatic Damage	✓	✓	✓	✓	✓	✓	✓	✓	?
Severe Renal damage	✓	✓	✓	?	✓	✓	✓	✗	?
Neurological Symptoms	✓	✓	✗	?	✓	✓	✗	✗	?
Immune disregulation	✓	✓	✓	?	✓	✓	✓	?	?
Lymphocyte Apoptosis	✓	✓	✓	✓	✓	✓	✓	?	?

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Nonhuman Primates

Gold Standard Model

- Cynomolgus and rhesus macaques are highly susceptible to wild-type EBOV, SUDV, and MARV
  - <1 pfu can be lethal, indicating extreme susceptibility comparable to humans
  - Susceptible to intramuscular, aerosol or mucosal routes of infection causing extremely high rates of mortality, even with low challenge doses
  - African green monkeys, marmosets, chimpanzees and baboons also tested
- Disease mirrors human filovirus infection
  - Viral tropism
  - Clinical lab findings and dysregulated immune responses
  - Fever, rash, coagulopathy, multi-organ failure and shock
- No virus adaptation required; consistent lethality and pathology
- Gold-standard model for vaccine and therapeutic efficacy
  - “Protection” in NHPs – e.g. survival or significant reduction in viremia/pathology compared to controls – is a strong indicator that an MCM could save human lives during an outbreak

Filovirus NHP Model Comparison

Time to Death, Fever, and Clinical Signs (Courtesy of Texas BioMed)

	EBOV (Kikwit)	SUDV (Gulu)	MARV (Angola)
Design	16 SE; 4 SP-TK; 4 control	10 SE; 8 SP-TK; 2 control	10 SE; 8 SP-TK; 2 control
Mortality	100%	88% (1 survivor)	100%
Time to Death [Days]	7 to 10 (average 8 days)	7 to 13 (average 9 days)	7 to 9 (average 8 days)
Peak Temp (telemetry)	Days 3 to 5	Days 4 to 8	Days 5 to 7
Fever [39°C] (telemetry)	65% on Day 5	86% on Day 3; 100% by Day 5	67% on Day 4; 92% by Day 5
Serum Viremia	Appear Day 3; All Day 5	Appear Day 3; All Day 5	Appear Day 2; All Day 5
Increased ALT, ALP, GGT	Day 5	Day 7	Day 5
Decreased ALB	Day 7	Day 5	Day 5
Coagulopathy (aPTT and/or PT)	Day 6	Day 7	Day 7
Appearance of Anatomical Pathology	Day 3	Day 2	Day 5

SE = scheduled euthanasia; SP-TK = Survival and Physiology Time Kinetics

Rodent Models

Mice, Guinea Pigs and Hamsters

- Wild-type filoviruses don’t cause lethal disease in normal rodents
- Adapted virus strains enable lethal models
- Knockout mice and humanized mice allow wild-type virus use but lack normal immune functions
- Guinea pigs and hamsters show better disease fidelity than mice
- Ideal for early screening and dose optimization, therefore essential for preclinical MCM development
- Help define immune correlates and viral kinetics
- Do not always correlate with outcomes in NHPs
- Likely insufficient for regulatory approval alone

Ferret Model

Breakthrough for Ebolaviruses

- Susceptible to wild-type EBOV, SUDV, BDBV, and TAFV — no virus adaptation needed
- Recapitulates key human disease features: fever, coagulopathy, organ failure
- Uniformly lethal disease with realistic time-to-death (6–9 days)
- Not susceptible to wild type Marburg virus—limited to ebolaviruses
- Useful for vaccine and therapeutic efficacy testing
- Enables transmission and pathogenesis studies not feasible in NHPs
- Emerging model for Ebolaviruses

Comparison of Animal Models of MVD

Other Considerations For Selection of Animal Models

	NHP	Outbred Guinea Pig	Inbred Guinea Pig	Immuno-competent Hamster	Immuno-deficient Hamster	Immuno-competent Mouse	Immuno-deficient Mouse	Humanized BLT Mouse
WT or Adapted Virus	WT	Adapted	Adapted	Adapted	WT	Adapted	WT	WT
Immuno-competent Host	Yes	Yes	Yes, but not robust	Yes	No	Yes	No	Yes, but at the cost of anemia
Ethical Limitations	High	Low	Low	Low	Low	Low	Low	High
Cost	$$$$	$$	$$	$$	$$	$	$$	$$$
Animal Husbandry	Extensive	Moderate	Moderate	Simple	Simple	Simple	Moderate	Moderate
Availability of Commercial Reagents and Assays	Good	Poor	Poor	Poor	Poor	Excellent	Excellent	Excellent

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Future of Filovirus Animal Models

Potentials for Replacement and Improvements

- Consider refinement: Alternate routes, doses or strains?
- New models: Survivor models? Different species? Use of supportive care, therapies or sub-optimal care therapies?
- Replacement of animal models: Organ on a chip or organdies
- Continue characterization and use of current models
  - Focus on well documented, well controlled studies
  - Assess with new tools and revisit through new lenses

There are a number of a variables that we can change in our models; however, after 40-50 years of animal model development and testing:

- Should we continue to adapt or change them?
- Is it worth changing these when disease is a spectrum in humans and no model will ever be sufficient to model all aspects of clinical disease.

Filovirus Animal Model Summary

Relevance to Support of Licensure of MCM

Model	Virus Adaptation	Disease Fidelity	Regulatory Role	Conclusions
NHPs	None	High	Primary	• NHPs remain central to filovirus MCM development • Provide critical evidence and closest human analog
Mice and Guinea Pigs	Required For Immuno-competent Models	Low to Moderate	Supportive	• Rodent models support early-stage screening • Provide scalability and mechanistic insights with important caveats
Ferrets	None (Ebolaviruses)	High	Emerging	• Ferrets offer scalable, realistic disease modeling • A recent promising addition that combines best traits of small and large animal models • Not useful for wild type Marburg virus

Summary & Outlook

- Filovirus MCM candidates are vetted through multiple models of rigorous animal testing
  - Provides regulatory confidence based on multi-model, evidence-based approach
  - Definitive ‘gold standard’ should continue to be NHPs for pivotal efficacy studies wherever possible
- Path forward:
  - Potential to refine or replace current models is low
  - Vaccines: NHP efficacy + human immunogenicity = licensure
  - Therapeutics: NHP efficacy + PK bridging = licensure?

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Multiple Variables to Consider When Developing and Selecting Animal Models

Virus species (and/or strain or isolate)	Route of infection of animals	Starting dose or LD50 determination (generally between 1,000-10,000 pfu)

• Plaque-purified, passage number, etc.
• How should stocks be characterized
  • Stability of virus
• Animal species / strain
  • Small animal vs large; old world vs new world NHP; cyno vs rhesus; Indian vs. Chinese, etc.
	• intramuscular, subcutaneous, aerosol, intravenous, intraperitoneal, oral	• What is more important: a lethal model or reflection of human disease

FDA Animal Rule Requirements

Animal Rule Requirements	Study Design
Understand the disease	• Lethal dose for 50% of animals (LD_{50}) • Natural history (pathophysiology)
Demonstrate efficacy in more than one species which react as humans would with similar endpoint (e.g., survival)	• Test article administration combined with an active challenge • Evaluate immune response • Establish assay to be used as correlate • Breakthrough of protection
Select effective human dosage	• Establish vaccination regimen that induces immune response similar to that observed in the clinic. • Demonstrate efficacy in challenge study • Evaluate dosage in clinical trials

Hallmarks of Filovirus Disease

NHP Disease Signs and Symptoms are Similar But Not Identical to Humans

	Humans	NHP Models
Fever	✓	✓
Lethargy /Reduced response	✓	✓
Serum Viremia	✓	✓
Hemorrhage	✓	✓
Rash	✓	✓
Thrombocytopenia	✓	✓
Atypical white blood cell counts	✓	✓
Lymphocyte apoptosis	✓	✓
Coagulopathy	✓	✓
Evidence of Hepatic Damage	✓	✓
Evidence of Renal Damage	✓	✓
Neurologic Symptoms	✓	✓
Immune dysregulation	✓	✓
Gastrointestinal symptoms	✓	-
Long-term sequelae	✓	✓

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