How to build the right model for efficacy demonstration

Cristina Cassetti, PhD.
Senior Scientific Advisor, CEPI

EMA workshop- Amsterdam, Nov 24-25, 2025
“Non-clinical data for regulatory decision-making on the efficacy of medical countermeasures “

Outline

Define the key disease characteristics in humans that we are trying to prevent and model in animals

Define challenge route, dose, and strain

Define the right species to recapitulate human disease

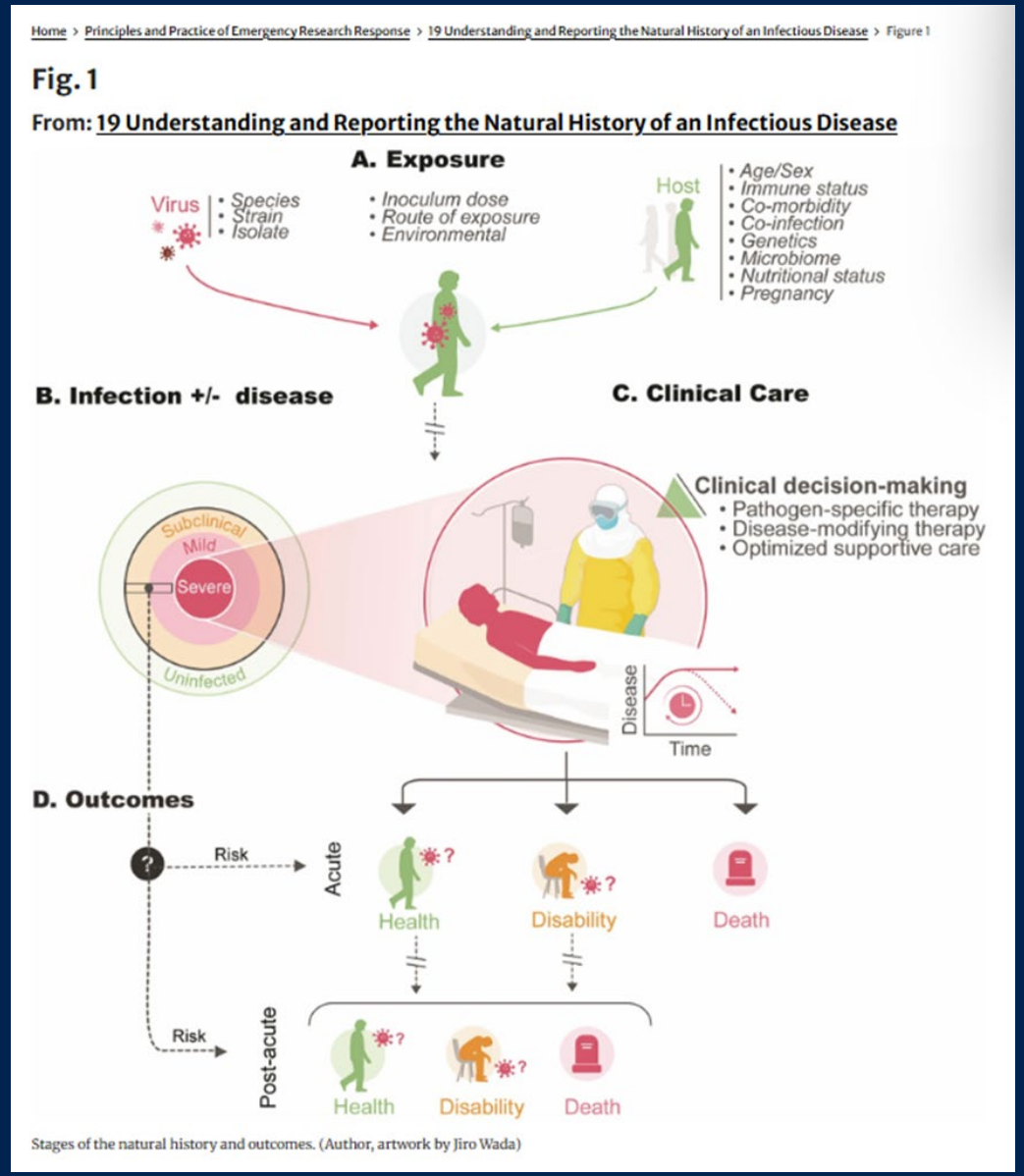
Standardize animal model for reproducibility

Define measurable endpoints in animals that can help elucidate correlates of protection

Conduct standardized animal testing though CROs

Natural History

- The “course a disease takes in the absence of intervention in individuals with the disease, from the disease’s onset until either the disease’s resolution or the individual’s death” (US FDA/CDER 2019)



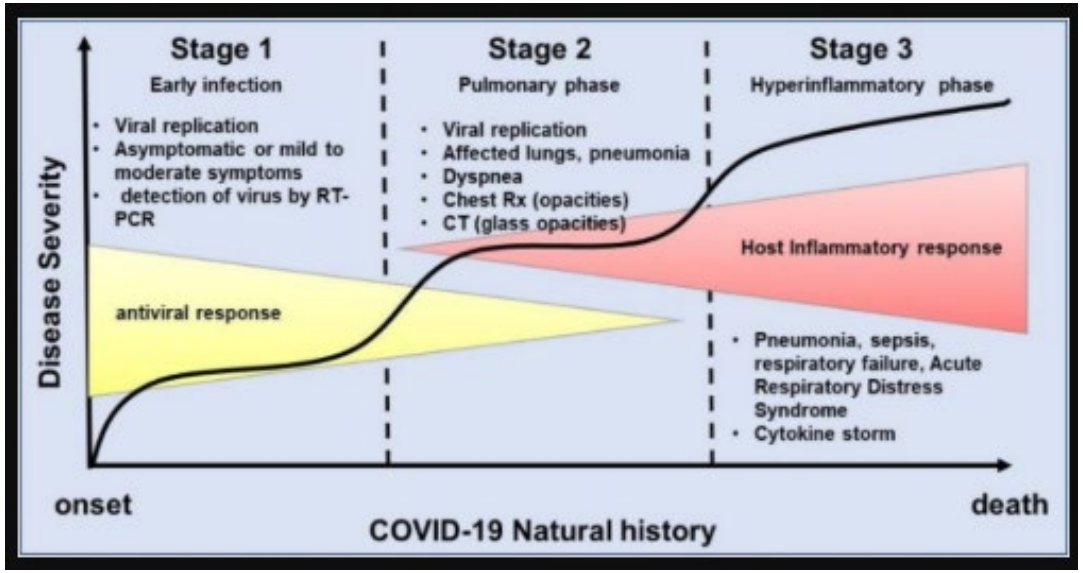
Principles and Practice of Emergency Research Response, 2024

Importance of understanding natural history of IDs

Optimizing Patient Care and Care Guidelines

Rational Design and Implementation of Clinical Trials (who, when, what, how)

Development of in vitro assays and animal models for evaluation of countermeasures



https://www.sciencedirect.com/science/article/pii/S0753332220306867?via%3Dihub

Define the right animal species

What animal species can be infected with the pathogen with minimal adaptation?

Does the infection results in clinical manifestations similar to humans?

Are the outcomes in the model measurable?

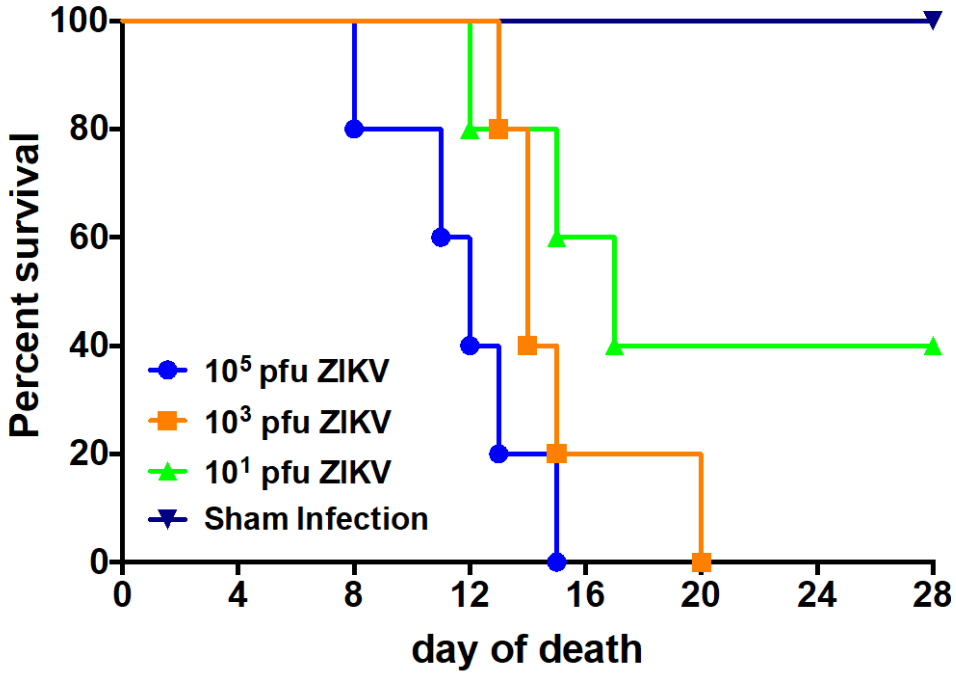
Common limitations of animal models

- Require adaptation of pathogens
- Require partially immune-compromised animals
- Do not have appropriate receptors or receptor distribution
- Induce different immune responses to humans
  - Different kinetics of innate and adaptive responses
  - Immune repertoire and TLR distribution
  - Differ in mucosal and systemic immune response
- Require non-physiological challenge doses and routes
- Cost, availability, reagents

Non-human primate models are usually the best animal models to replicate human disease

- **For efficacy demonstration, animal model data should be used in conjunction with other datasets to best inform regulatory decision-making**
- **It's the totality of the data that should be considered**

Zika Animal models: AG129 mice



Justin Julander, Utah State University

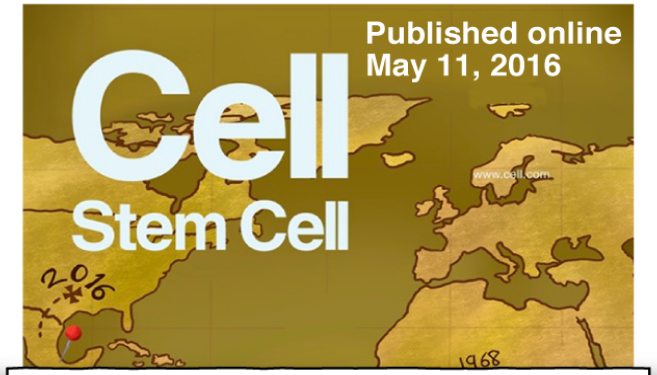
Zika Virus Causes Microcephaly and Other Fetal Abnormalities in Mice

Zika Virus Infection during Pregnancy in Mice Causes Placental Damage and Fetal Demise

JJ Miner, MS Diamond et al.

The Brazilian Zika Virus Strain Causes Birth Defects in Experimental Models

FR Cugola, PC Beltrão-Braga et al.



Zika Virus Disrupts Neural Progenitor Development and Leads to Microcephaly in Mice

C Li, Z Xu et al.

Animal models can help elucidate disease in humans

LETTER

doi:10.1038/nature20556

Zika virus infection damages the testes in mice

Jennifer Govero[1]*, Prabagaran Esakky[2]*, Suzanne M. Scheaffer[2], Estefania Fernandez[3], Andrea Drury[2], Derek J. Platt[4], Matthew J. Gorman[3], Justin M. Richner[1], Elizabeth A. Caine[1], Vanessa Salazar[1], Kelle H. Moley[2,5] & Michael S. Diamond[1,3,4,6]

Article

Cell

Ma et al., 2016

Zika Virus Causes Testis Damage and Leads to Male Infertility in Mice

COMMENT · Volume 17, Issue 11, P1107-1109, November 2017

Download Full Issue

Zika virus infection in semen: effect on human reproduction

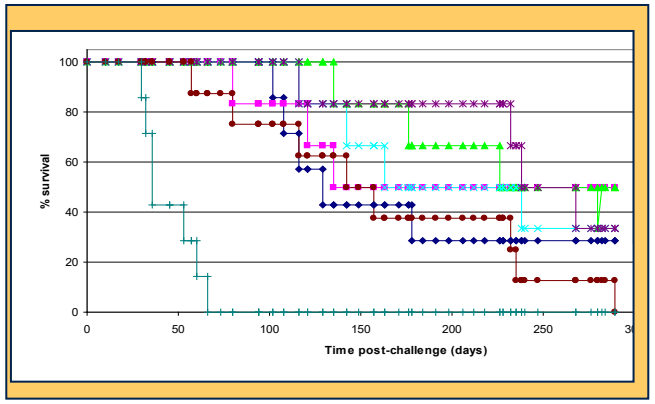
Luisa Barzon · Enrico Lavezzo · Giorgio Palù

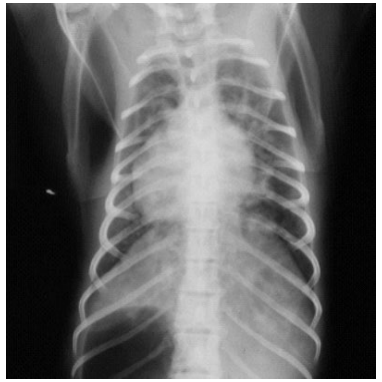
THE LANCET Infectious Diseases

Define measurable endpoints that relate to the desired type of vaccine “protection”

Protection against:

- Death
- Clinical disease
- Congenital disease
- Transmission





Endpoints

- Survival
- Viremia
- Viral load in tissues/organs
- Pathological assessment
  - Lesions in organs
  - X-rays
  - Oxygen levels in lungs
- Clinical assessment
  - Weight loss
  - Temperature

Define route, strain and challenge dose

- Whenever possible use physiologically relevant challenge routes to induce measurable disease
  - E.g. IN and/or aerosol for respiratory infections
  - Highly artificial challenge routes (e.g IP or IC) not very useful to predict efficacy
- Ideally use clinical isolates (minimally passaged and whose sequence has been confirmed and standardized) as challenge strains
- Conduct dose-down studies to discover lowest possible dose to induce physiologically relevant disease
  - Large challenge doses are unnatural and can overcome immunity of vaccines that under normal, real-life situations would be protective

Public Health England

Influenza in Ferrets: How Realistic is the Model?

Natural infection -dose ≈ 1?? – 3.0 pfu

Ferret model – dose = 10^6 pfu

Human disease kinetics: incubation period 2-7 days for H1N1.

Would lower dose effect disease kinetics and pathogenesis?

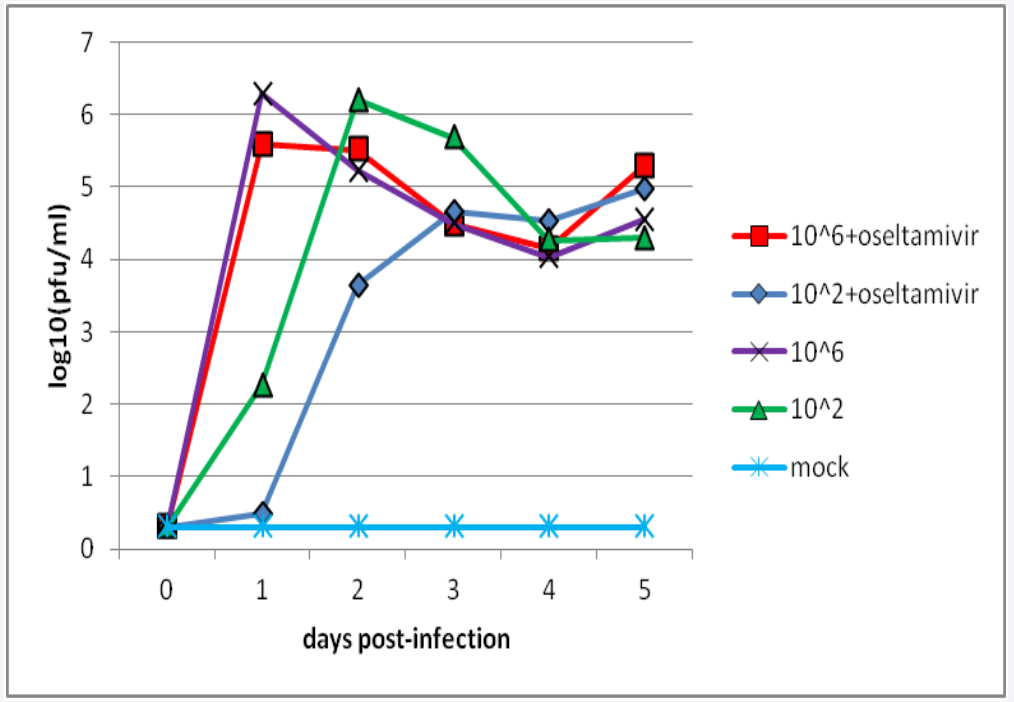
Increased sensitivity for evaluation of vaccines and therapeutics

Slide credit:
Miles Carrol, Oxford U.

Influenza Ferret Model: Development of Vaccines & Therapeutics

Public Health England

Natural infection - dose ≈ 1 – 3.0 pfu Ferret model – dose = 10^6 pfu



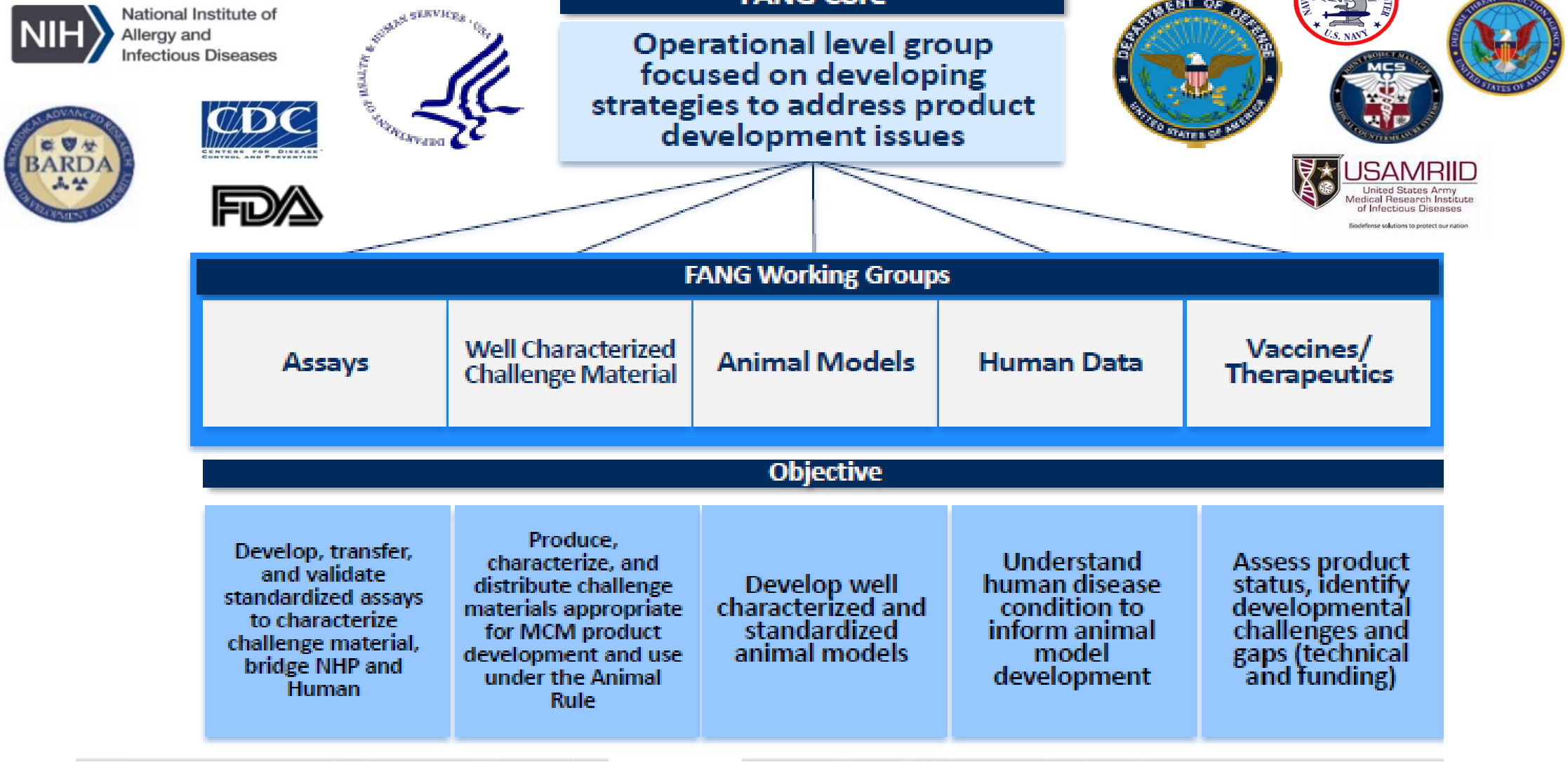
- 10 000 fold challenge reduction: Improved sensitivity
- Assessment of new correlates of protection

Slide credit:
Miles Carrol, Oxford U.

Development of authentic models leads to effective candidate down selection

Standardize the animal model for reproducibility

The Filovirus Animal Non-clinical Working Group
(FANG)
Ebola NHP Model
Standardization Effort (2011-2019)



Slide credit:
Kim Taylor, NIAID

Development of Well Characterized Challenge Material (WCCM)

Strain Selection

Regulatory perspective
USG 2012 data call
CDC Seed Stocks

Key selection criteria

Production & Distribution

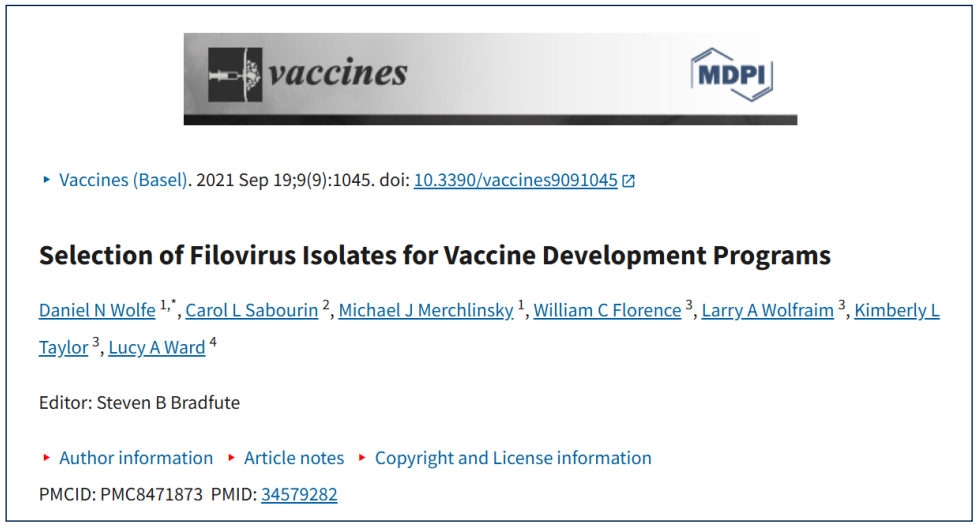
Standardized growth protocol
Master & Working Viral Banks

Stocks Available in BEI Resources

Release, Characterization, Stability Assays

Standardized assays, i.e.
Plaque and TCID50
NGS
7U vs 8U

Certificate of Analysis



vaccines MDPI

Vaccines (Basel). 2021 Sep 19;9(9):1045. doi: 10.3390/vaccines9091045

Selection of Filovirus Isolates for Vaccine Development Programs

Daniel N Wolfe [1,*], Carol L Sabourin [2], Michael J Merchlinsky [1], William C Florence [3], Larry A Wolfraim [3], Kimberly L Taylor [3], Lucy A Ward [4]

Editor: Steven B Bradfute

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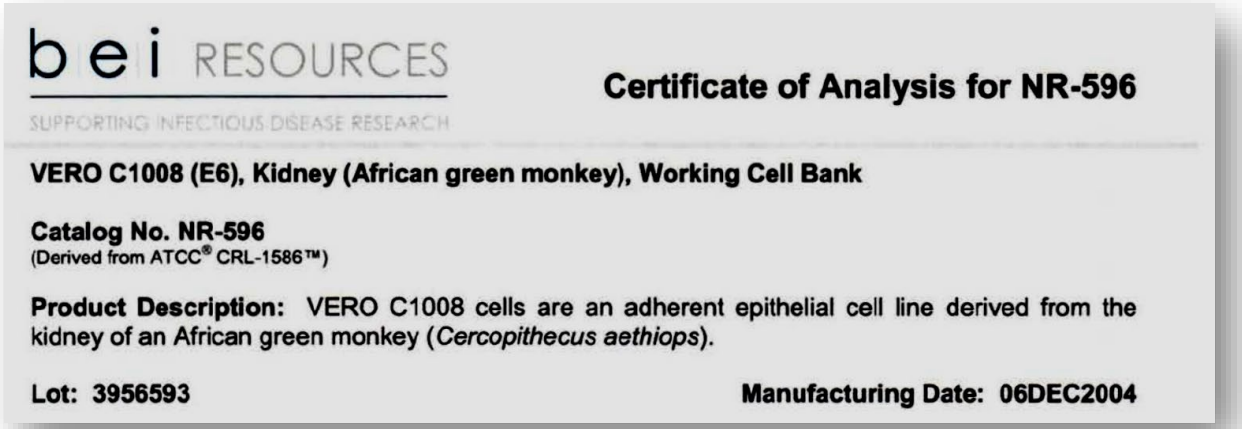
PMCID: PMC8471873 PMID: 34579282



bei RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for NR-596

VERO C1008 (E6), Kidney (African green monkey), Working Cell Bank

Catalog No. NR-596
(Derived from ATCC® CRL-1586™)

Product Description: VERO C1008 cells are an adherent epithelial cell line derived from the kidney of an African green monkey (*Cercopithecus aethiops*).

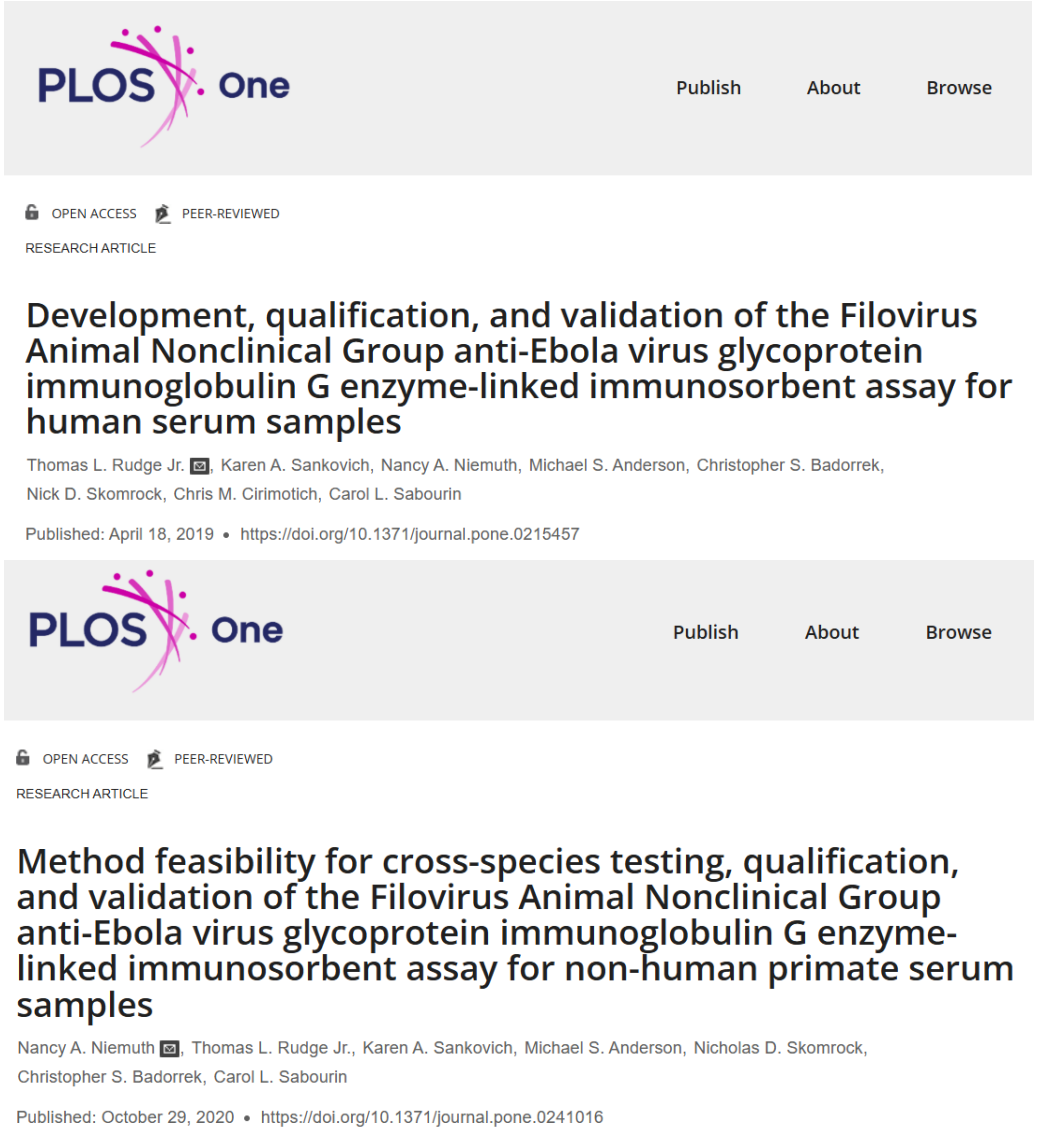
Lot: 3956593 **Manufacturing Date: 06DEC2004**

Slide credit: Kim Taylor, NIAID

Immunogenicity Assay Standardization

- Develop, standardize, qualify, and validate a single immune assay
- Human anti-EBOV glycoprotein (GP) IgG Enzyme-Linked Immunosorbent assay (ELISA)
- DOD funded Battelle
- Collaboration with USG partners (NIAID & BARDA) and product developers
- DOD submitted a Master File to the FDA

Slide credit:
Kim Taylor, NIAID



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RESEARCH ARTICLE

Development, qualification, and validation of the Filovirus Animal Nonclinical Group anti-Ebola virus glycoprotein immunoglobulin G enzyme-linked immunosorbent assay for human serum samples

Thomas L. Rudge Jr., Karen A. Sankovich, Nancy A. Niemuth, Michael S. Anderson, Christopher S. Badorrek, Nick D. Skomrock, Chris M. Cirimotich, Carol L. Sabourin

Published: April 18, 2019 • https://doi.org/10.1371/journal.pone.0215457

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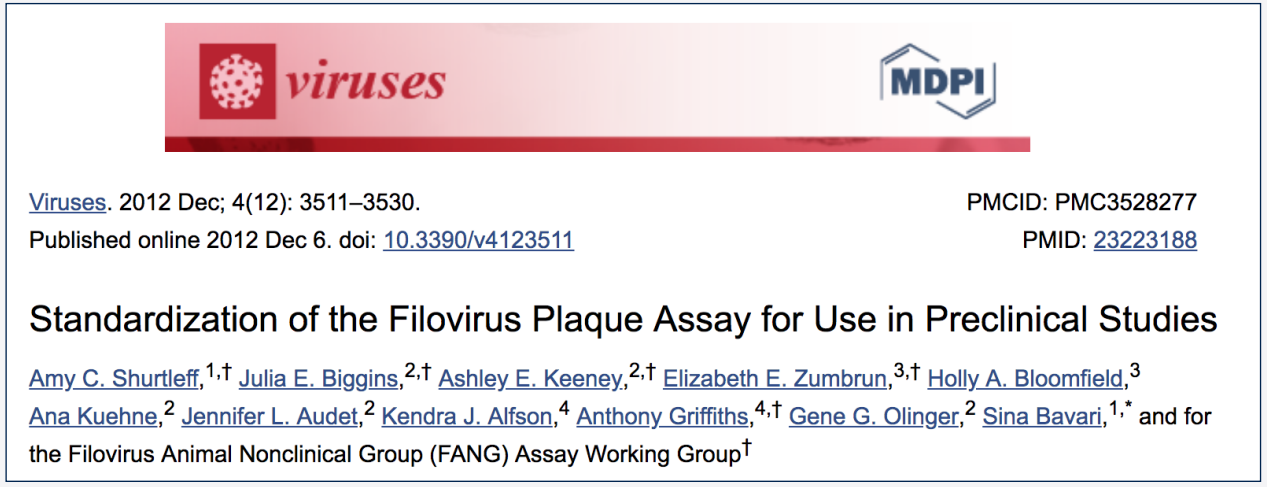
RESEARCH ARTICLE

Method feasibility for cross-species testing, qualification, and validation of the Filovirus Animal Nonclinical Group anti-Ebola virus glycoprotein immunoglobulin G enzyme-linked immunosorbent assay for non-human primate serum samples

Nancy A. Niemuth, Thomas L. Rudge Jr., Karen A. Sankovich, Michael S. Anderson, Nicholas D. Skomrock, Christopher S. Badorrek, Carol L. Sabourin

Published: October 29, 2020 • https://doi.org/10.1371/journal.pone.0241016

Assay standardization and strain characterization data was shared through publications



viruses

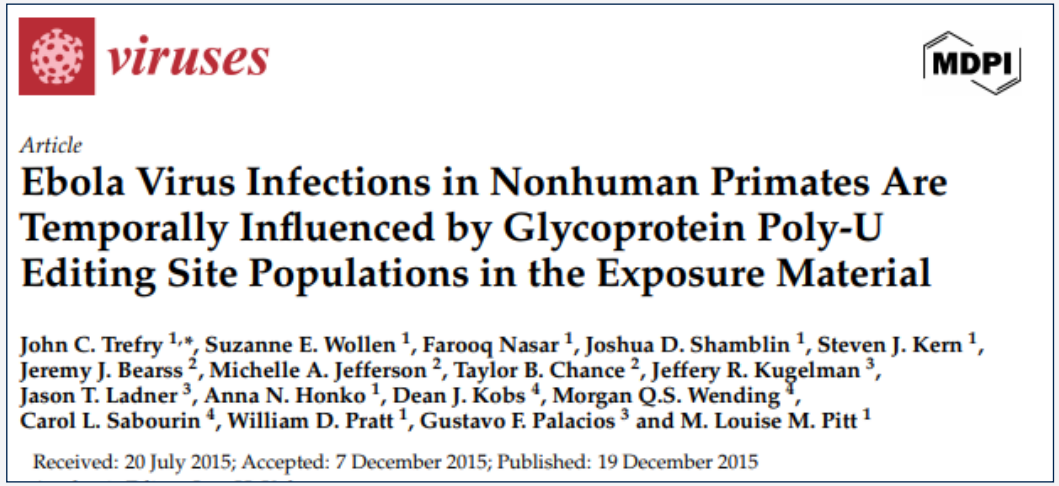
MDPI

Viruses. 2012 Dec; 4(12): 3511–3530.
Published online 2012 Dec 6. doi: 10.3390/v4123511

PMCID: PMC3528277
PMID: 23223188

Standardization of the Filovirus Plaque Assay for Use in Preclinical Studies

Amy C. Shurtleff,[1,†] Julia E. Biggins,[2,†] Ashley E. Keeney,[2,†] Elizabeth E. Zumbrun,[3,†] Holly A. Bloomfield,[3] Ana Kuehne,[2] Jennifer L. Audet,[2] Kendra J. Alfson,[4] Anthony Griffiths,[4,†] Gene G. Olinger,[2] Sina Bavari,[1,*] and for the Filovirus Animal Nonclinical Group (FANG) Assay Working Group[†]



viruses

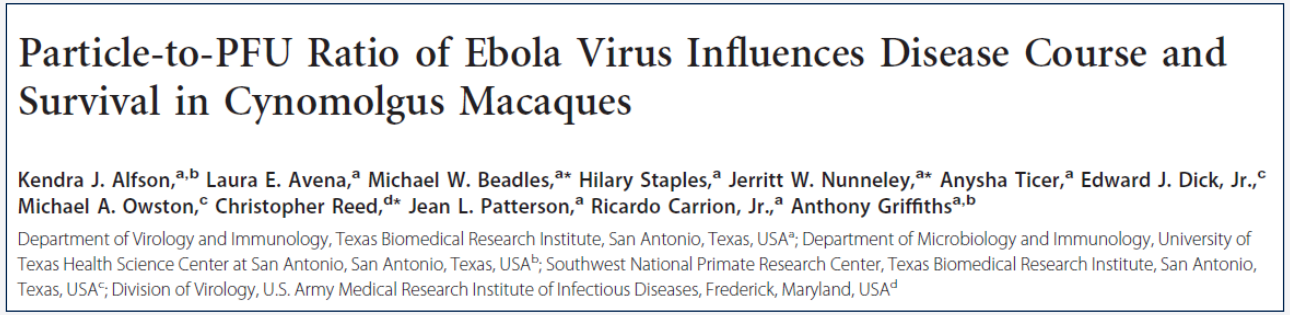
MDPI

Article

Ebola Virus Infections in Nonhuman Primates Are Temporally Influenced by Glycoprotein Poly-U Editing Site Populations in the Exposure Material

John C. Trefry [1,*], Suzanne E. Wollen [1], Farooq Nasar [1], Joshua D. Shamblin [1], Steven J. Kern [1], Jeremy J. Bearss [2], Michelle A. Jefferson [2], Taylor B. Chance [2], Jeffery R. Kugelman [3], Jason T. Ladner [3], Anna N. Honko [1], Dean J. Kobs [4], Morgan Q.S. Wending [4], Carol L. Sabourin [4], William D. Pratt [1], Gustavo F. Palacios [3] and M. Louise M. Pitt [1]

Received: 20 July 2015; Accepted: 7 December 2015; Published: 19 December 2015



Particle-to-PFU Ratio of Ebola Virus Influences Disease Course and Survival in Cynomolgus Macaques

Kendra J. Alfson,[a,b] Laura E. Avena,[a] Michael W. Beadles,[a*] Hilary Staples,[a] Jerritt W. Nunneley,[a*] Anysha Ticer,[a] Edward J. Dick, Jr.,[c] Michael A. Owston,[c] Christopher Reed,[d*] Jean L. Patterson,[a] Ricardo Carrion, Jr.,[a] Anthony Griffiths[a,b]

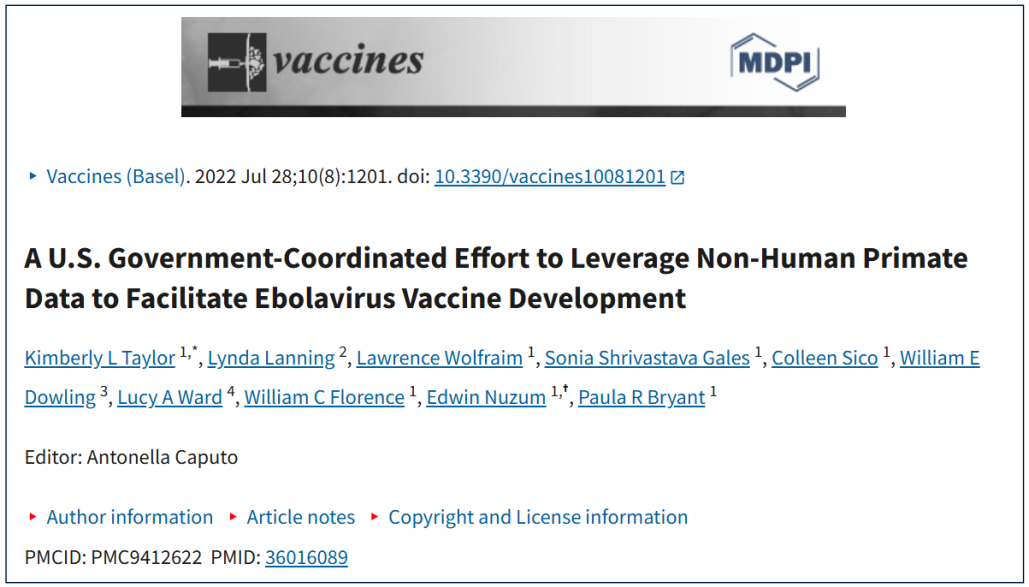
Department of Virology and Immunology, Texas Biomedical Research Institute, San Antonio, Texas, USA[a]; Department of Microbiology and Immunology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA[b]; Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, Texas, USA[c]; Division of Virology, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Maryland, USA[d]

Slide credit:
Kim Taylor, NIAID

The FANG leveraged existing NHP control data from vaccine studies to support IND submissions

- No formal natural history studies conducted for Ebolavirus Zaire Kikwit
- Multiple sponsors/ PDs were conducting vaccine immunogenicity and efficacy studies sites
- Stringency/reliability of Model at different
  - BSL-4 sites
  - Challenge dose? (100 vs 1000 PFU)
- Collaborative effort with USG partners and vaccine developers
- Conducted meta-analysis of NHP control data under NIAID PCS
- NIAID sponsor/ submitted Master File to the FDA

Slide credit:
Kim Taylor, NIAID



vaccines MDPI

Vaccines (Basel). 2022 Jul 28;10(8):1201. doi: 10.3390/vaccines10081201

A U.S. Government-Coordinated Effort to Leverage Non-Human Primate Data to Facilitate Ebolavirus Vaccine Development

Kimberly L Taylor [1,*], Lynda Lanning [2], Lawrence Wolfraim [1], Sonia Shrivastava Gales [1], Colleen Sico [1], William E Dowling [3], Lucy A Ward [4], William C Florence [1], Edwin Nuzum [1,†], Paula R Bryant [1]

Editor: Antonella Caputo

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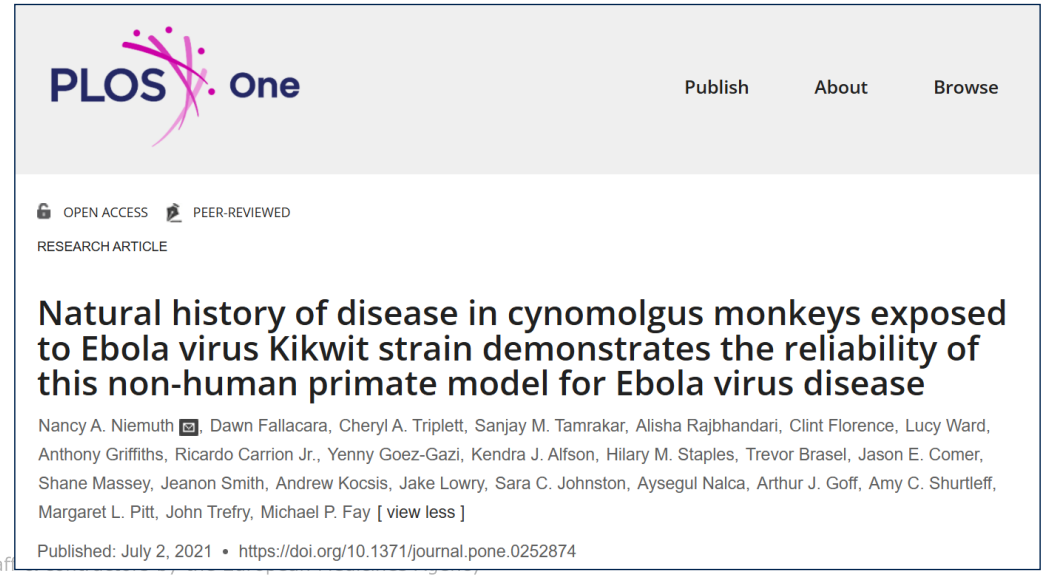
PMCID: PMC9412622 PMID: 36016089



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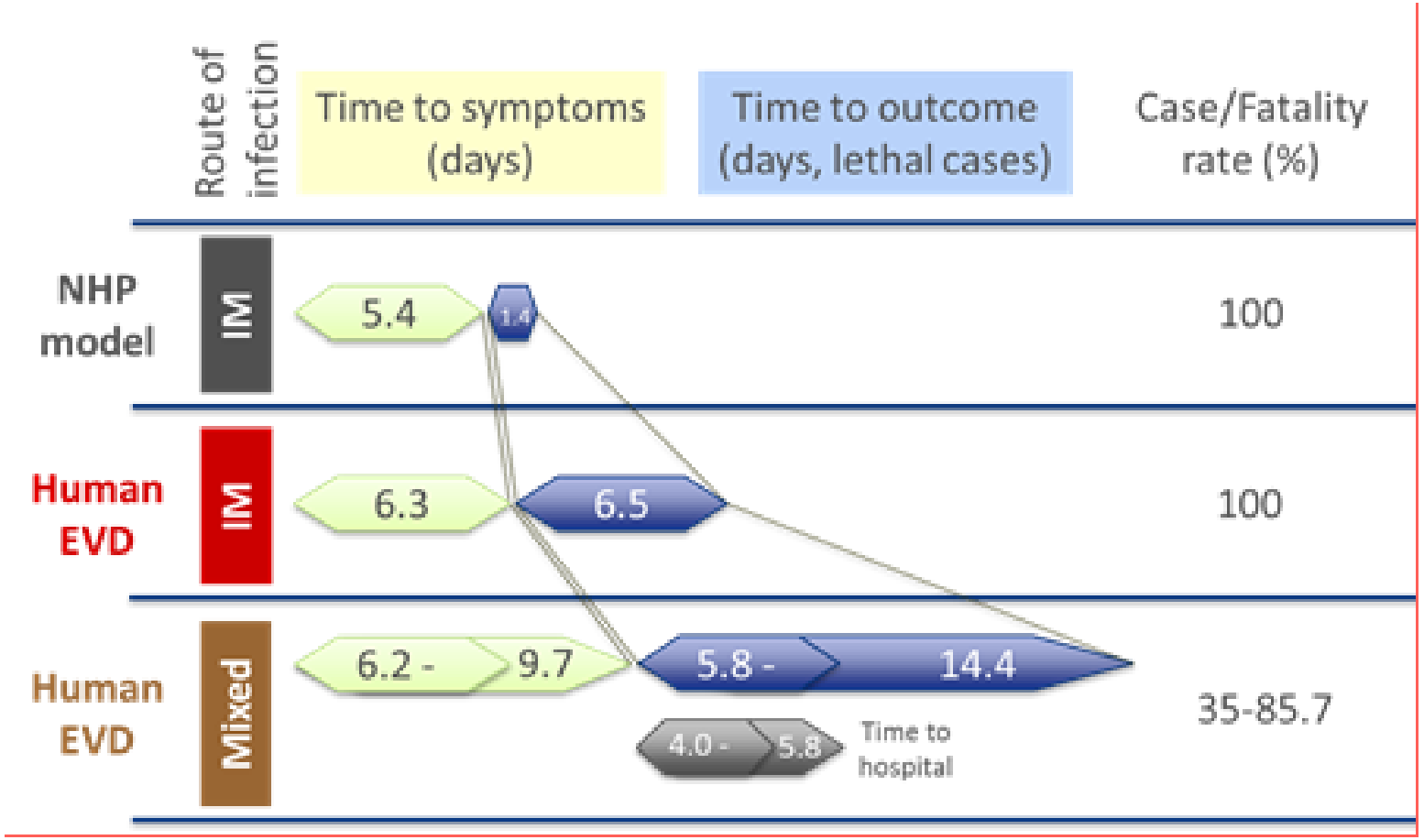
RESEARCH ARTICLE

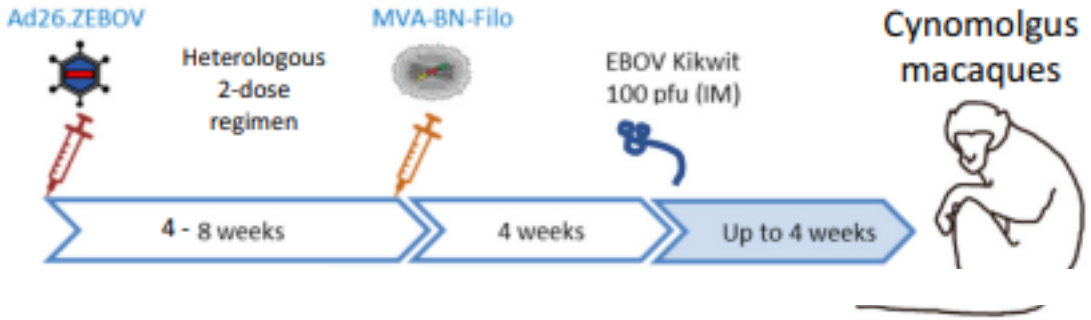
Natural history of disease in cynomolgus monkeys exposed to Ebola virus Kikwit strain demonstrates the reliability of this non-human primate model for Ebola virus disease

Nancy A. Niemuth, Dawn Fallacara, Cheryl A. Triplett, Sanjay M. Tamrakar, Alisha Rajbhandari, Clint Florence, Lucy Ward, Anthony Griffiths, Ricardo Carrion Jr., Yenny Goez-Gazi, Kendra J. Alfson, Hilary M. Staples, Trevor Brasel, Jason E. Comer, Shane Massey, Jeanon Smith, Andrew Kocsis, Jake Lowry, Sara C. Johnston, Aysegul Nalca, Arthur J. Goff, Amy C. Shurtleff, Margaret L. Pitt, John Trefry, Michael P. Fay [view less]

Published: July 2, 2021 • https://doi.org/10.1371/journal.pone.0252874

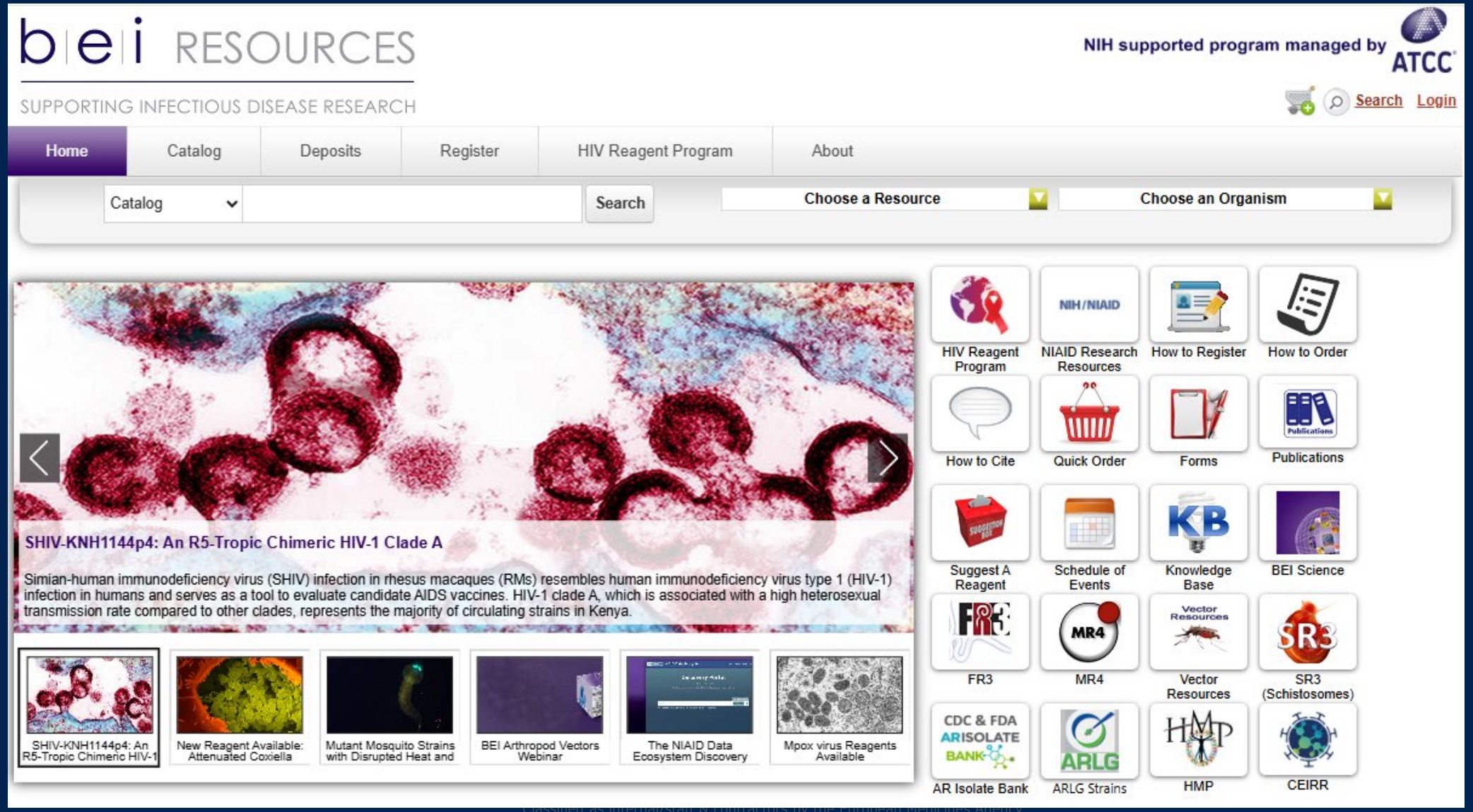
Nonhuman primate to human immunobridging to infer the protective effect of an Ebola virus vaccine candidate





[Nonhuman primate to human immunobridging to infer the protective effect of an Ebola virus vaccine candidate (nature.com)]

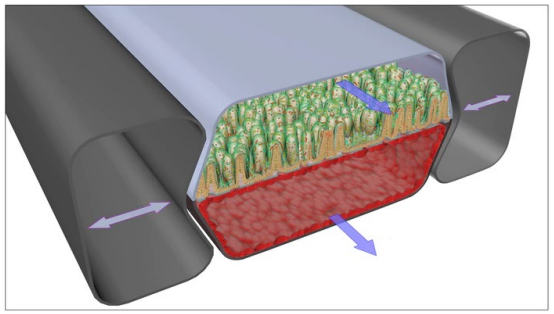
Reagents Repositories



CROs to conduct standardized animal testing- NIAID Pre-Clinical Models of Infectious Diseases

- Small animals – e.g., mouse, rat, hamster, ferret, rabbit
- NHPs – rhesus, cynos, AGM, pig-tailed
- Non-traditional & animal replacement (organ on a chip)
- "Animal Rule"
- Model Development & Reagent Production
- Pivotal animal models
  - GLP capability
- FDA interactions

https://www.niaid.nih.gov/research/pre-clinical-models-infectious-disease

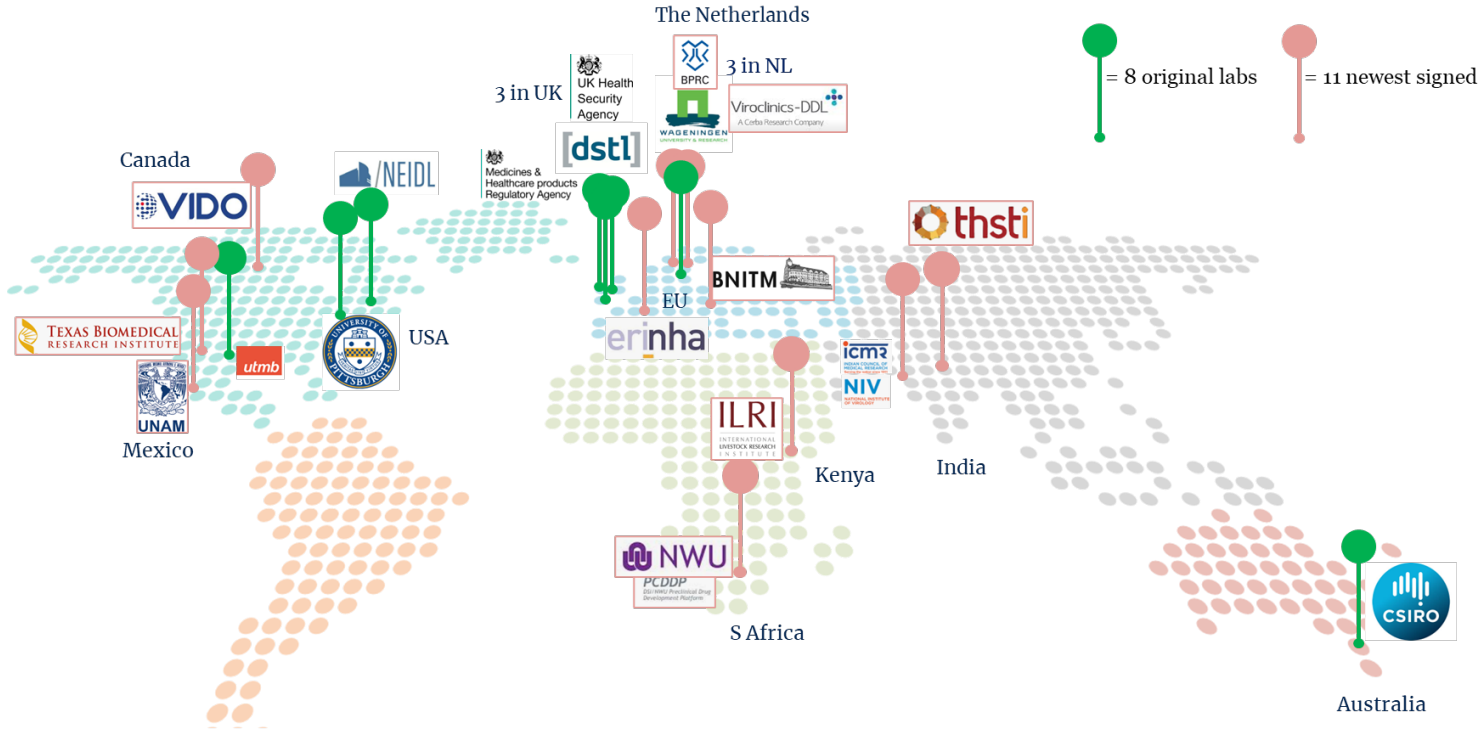


Slide credit:
Mark Williams, NIAID

The CEPI Preclinical Model Network (PMN)

The PMN is a global network of laboratories that serve CEPI preclinical needs from model development through IND-enabling vaccine testing

- BSL-2 laboratories for early immunogenicity testing, including vaccine down-selection, and expanding toxicology capacity
- BSL-3 and -4 laboratories to handle CEPI priority pathogens (SARS-CoV, SARS-CoV-2, MERS-CoV and other coronaviruses, CHIKV, NiV, LASV, RVFV, Filoviruses, and various Disease X families) + other pathogens, including influenza viruses
- Expanding breadth into non-animal models (i.e., *ex vivo*, organoids, organ-on-a-chip, etc.)
- Small and large species, including NHP and agricultural species
- Compliance with high animal ethics standards https://www.nc3rs.org.uk/
- High-quality deliverables supported by established quality systems or well-documented through protocol-specified methods



National Centre for the Replacement Refinement & Reduction of Animals in Research