



Testing and Development of Orthopoxvirus Vaccines in the Era of the "Animal Rule"

MRCE

Making a Vaccine Against a Bioweapon Involves the FDA Animal Rule

- To allow appropriate studies in animals in certain cases to provide substantial evidence of the effectiveness of new drug and biological products used to reduce or prevent the toxicity of chemical, biological, radiological, or nuclear substances.
- This rule will apply when adequate and well-controlled clinical studies in humans cannot be ethically conducted and field efficacy studies are not feasible.
- In these situations, ...products ...may be approved for marketing based on evidence of effectiveness derived from appropriate studies in animals and any additional supporting data.

New drug and biological products; evidence needed to demonstrate effectiveness of new drugs when human efficacy studies are not ethical or feasible. Federal Register 2002: 67:37988-37998.

Important FDA Guidance on the Animal Rule

- The challenge agent used in animal studies should be identical to the etiological agent that causes the human disease.
- If the challenge agent is different from the etiological agent then justification must be provided by the sponsor.
- The animal model should use the route of exposure to the challenge agent that is the same as the anticipated human exposure route.
- The response to the etiologic agent manifested by the animal species exposed to that agent should be similar to the illness or injury seen in human.

Guidance for Industry : Animal Models-Essential elements to address efficacy under the animal rule. FDA CDER CBER January 2009

Smallpox - The Disease



Prophylaxis and Therapy



FDA Guidance on Second-Generation Smallpox Vaccines

- Thus, for second-generation smallpox vaccines derived from the NYCBH strain or other strains that have been demonstrated to have field efficacy, efficacy will be based on clinical studies in which the new vaccine is compared with Dryvax with regard to "take" rates and immunologic responses such as neutralizing antibody .
- Stringent animal models of efficacy will not be required for these types of vaccines. Human safety data will have to be collected and evaluated to support licensure.

Karen Midthun, Director, Office of Vaccines Research and Review: Regulatory Requirements for Historical and New Smallpox vaccines. Workshop, Langen Germany, Sept 5-6, 2002

FDA Guidance on Third-Generation Smallpox Vaccines

- Evaluation of third generation vaccines derived from strains with no previous demonstration of efficacy (e.g., Modified Vaccinia Ankara; MVA) is complex and will likely depend on the new final (Animal) rule.
- Efficacy can be based on adequate and well-controlled animal trials if the results establish that the product is reasonably likely to provide clinical benefit to humans.
- The new vaccine will be compared with Dryvax in humans with regard to immunologic responses such as neutralizing antibody.

Karen Midthun, Director, Office of Vaccines Research and Review: Regulatory Requirements for Historical and New Smallpox vaccines. Workshop, Langen Germany, Sept 5-6, 2002

Therapeutics and Prophylactics for Smallpox

Approved:

- Vaccine immune globulin
- ACAM2000
- IMVAMUNE (MVA)

In clinical trials:

- ST-246
- CMX001

FDA Guidance on Fourth-Generation Smallpox Vaccines

- There are no fourth generation products that have been evaluated by the FDA and are now in the clinic.
- Efficacy can be based on adequate and well-controlled animal trials if the results establish that the product is reasonably likely to provide clinical benefit to humans.
- The new vaccine will be compared with Dryvax with regard to immunologic responses such as neutralizing antibody; the T cell comparisons will be more difficult.

Animal Models

- Ectromelia mouse model
- Vaccinia virus mouse model
- Monkeypox rodent models
- Monkeypox and variola viruses non-human primate models

Mousepox Model

- Mouse strain dependent intranasal LD50 from 0.3 to 90 PFU.
- Acute infection , time to death ~7-14 days.
- Generalized rash mouse strain and route dependent ~ 7-11 days p.i.
- Death due to hepatic necrosis???
- Transmission among cage mates

Parker, S., Oberle, C., Hembrador, E., Lanier, R., Painter, G., Robertson, A., Buller, R.M., and Siddiqui, A. M. (2008). Mousepox in the C57BL/6 strain provides a new model for evaluation anti-poxvirus therapies in mammals. *Virology* 385, 11.

Vaccinia Virus Mouse Model

- Intranasal route LD50 10^5 - 10^6 PFU
- Acute infection , time to death ~6-9 days
- No rash in immunocompetent mice
- Cause of death primary bronchopneumonia

Fogg C., Lustig, S., Whitbeck, C.J., Eisenberg, R.J., Cohen, G.H. and Moss, B. (2004) Protective immunity to vaccinia virus induced by vaccination with multiple recombinant outer membrane proteins of intracellular and extracellular virions. J. Virol. 78, 10,230.

Monkeypox Virus Rodent Models

- As a preliminary step before using scarce non-human primates
- Monkeypox virus is used as a surrogate for variola virus

Ground Squirrels (*Spermophilus tridecemlineatus*)

- Can not be bred in captivity
- Subcutaneous route LD50 < 1 PFU
- Acute infection , time to death ~6-11 days
- Few immunological reagents



Sbrana, E., Xiao, S.Y., Newman, P.C., and Tesh, R.B. (2007) Comparative pathology of North American and central African strains of monkeypox virus in ground squirrel model of the disease. *Am. J. Trop. Med. Hyg.* 76: 155-164.

African Dormice (*Graphiurus kelleni*)

- Can be maintained as a breeding colony
- Intranasal route LD50 = 12 PFU
- Acute infection , time to death ~6-12 days
- Transmission among cage mates
- Few immunological reagents



Schultz, D., Sagartz, J., Huso, D., and Buller, R.M. (2008). Experimental infection of an African dormouse (*Graphiurus kelleni*) with monkeypox virus. *Virology* 383: 86-92.

Prairie Dogs (*Cynomys ludovicianus*)

- Can not be bred in captivity
- Intranasal route LD50 = 5,900 PFU
- Extended asymptomatic period, 9-12 days
- Acute infection, generalized rash, time to death variable
- Transmission among cage mates likely
- Few immunologically reagents

Hutson, C.L., et al. (2010) Dosage comparison of Congo Basin and West African strains of monkeypox virus using a prairie dog animal model of systemic orthopoxvirus disease. *Virology* 402: 72-82.



C57BL/6-*stat1*^{-/-} Mice

- Not completely immunocompetent
- Intranasal route LD50 = 47-213 PFU
- Acute infection, time to death ~7-11 days
- Vaccination with Dryvax vaccine was not tolerated, but MVA protected against ectromelia virus challenge

Stabenow, J., Buller, R.M., Schriewer, J., West, C., Sagartz, J.E., and Parker, S. (2010). A lethal mouse model for evaluation of prophylactics and therapeutics against monkeypox virus. *J. Virol.* 84: 3909.

CAST/EiJ Mice

- Intranasal route LD50 = 680 PFU
- Acute infection , time to death ~6-13 days
- Completely immunocompetent
- Vaccination with live Dryvax vaccine was tolerated and protected against vaccinia virus challenge

Americo, J.L., Moss, B., and Earl, P.L. (2010) Identification of wild-derived inbred mouse strains highly susceptible to monkeypox virus infection for use as small animal models. *J. Virol.* 84, 8172.

Non Human Primate Models for Monkeypox and Variola Viruses

- Aerosolized monkeypox virus challenge in cynomolgus monkeys
- Intratracheal monkeypox virus challenge in cynomolgus monkeys
- Intravenous monkeypox virus challenge in cynomolgus monkeys
- Intravenous variola virus challenge in cynomolgus monkeys

Aerosolized Monkeypox Virus Infections of Cynomolgus Monkeys

- LD50 > 10⁵ PFU, survival not dose dependant
- Acute infection, time to death ~6-11 days
- Generalized rash 6-8 days p.i.
- Cause of death primary fibrinonecrotic bronchopneumonia

Nalca, A., et al. Experimental infection of cynomolgus macaques (*Macaca fascicularis*) with aerosolized monkeypox virus. (2010) *PLoS one* 5: 1.

Intratracheal Monkeypox Virus Infections of Cynomolgus Monkeys

- LD50 10⁵ - 10⁶ PFU
- Acute infection, time to death 15-19 days
- Generalized rash 8-10 days p.i.
- Cause of death primary fibrinonecrotic bronchopneumonia

Stittelaar, K.J. et al. (2005). Modified vaccinia virus Ankara protects macaques against respiratory challenge with monkeypox virus. *J. Virol.* 79: 7845.

Intravenous Monkeypox Virus Infections of Cynomolgus Monkeys

- LD50 $10^6 - 10^7$ PFU
- Acute infection , time to death 10-18 days
- Generalized rash 3-6 days p.i.
- Death due to multi-organ failure???

Earl, P.L. et al., (2004) Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. *Nature* 428: 182.
Buchman, G.W. et al., (2010) A protein-based smallpox vaccine protects non-human primates from lethal monkeypox virus challenge. *Vaccine* 28: 6627.

Intravenous Variola Virus Infections of Cynomolgus Monkeys

- LD50 $10^8 - 10^9$ PFU
- Acute infection , time to death 3-6 days
- Generalized rash 2-3 days p.i.
- Death due to multi-organ failure???
- Studies can only be carried out at CDC

Earl, P.L. et al., (2004) Immunogenicity of a highly attenuated MVA smallpox vaccine and protection against monkeypox. *Nature* 428: 182.

Conclusions

- Sufficiently varied orthopoxvirus small animal models exist for rapid generation of preclinical data to support further efficacy testing.
- Currently used non-human primate models of smallpox do not accurately recapitulate human disease.
- It is not clear how the FDA will handle fourth generation vaccines under the Animal Rule.

Future

- Smallpox vaccines based on T cell peptides, protein subunits, and/or DNA expression plasmids.
- Approaches that can increase the immunogenicity of IMVAMUNE with a goal of reducing doses from 2 to 1.
- Development of IMVAMUNE or other candidates as a vaccine platform for use to prevent other diseases.

Collaborators

Saint Louis University

- Jill Schriewer
- Scott Parker
- Ed Hemabrador
- John Long
- Hollyce Hartzler
- Past lab members

7th Wave

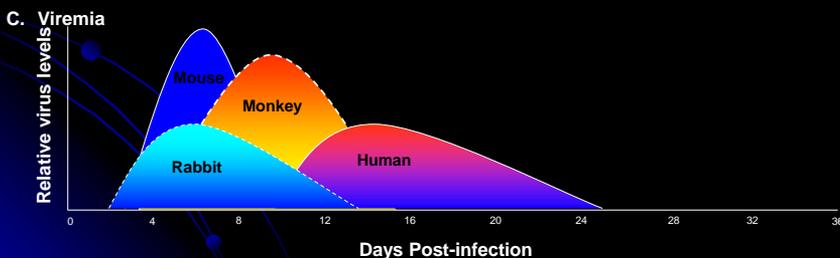
- John Sagartz

Institute for Immunology and Informatics

- Annie DeGroot and colleagues

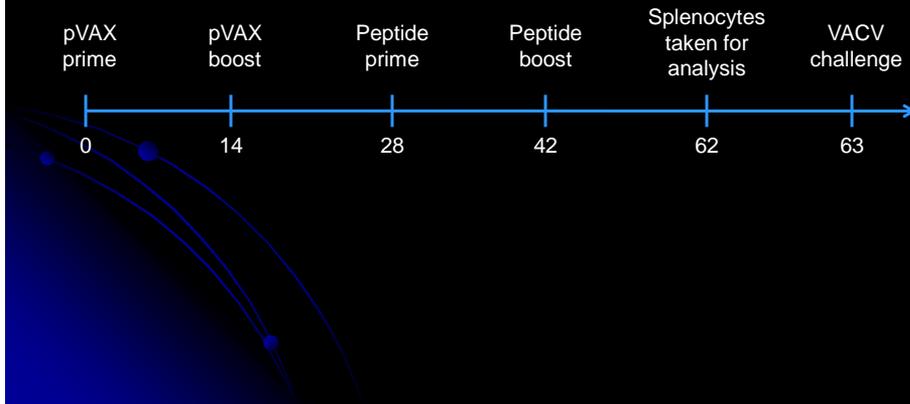
R21 AI-064647, N01-AI-15436, and U54 AI057160 MRCE

Orthopoxvirus Disease Models



Courtesy of Dennis Hruby, SIGA Technologies, Inc

Evaluation of the Efficacy of a DNA-prime, Peptide Boost, Multi-T-cell Epitope Poxvirus Vaccine in HLA DR3 mice



VennVax Protects Against a Lethal Intranasal Challenge with Vaccinia Virus

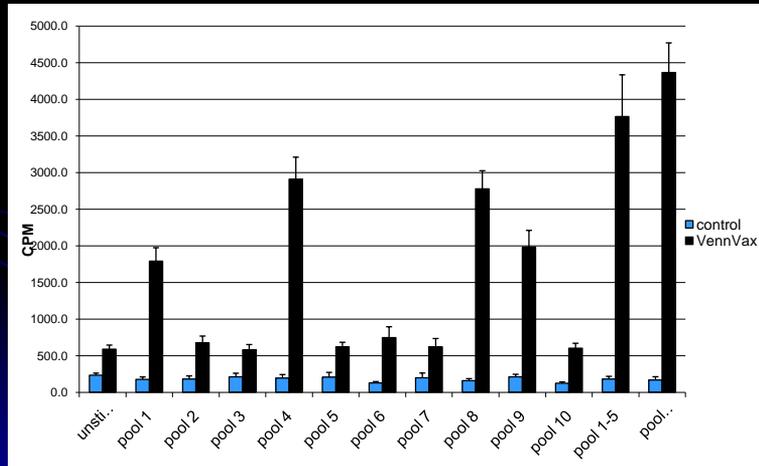
Control plasmid and irrelevant peptide:

13/16 dead = 81.3% mortality
mean time to death = 13.8 +/- 0.3 days post infection

Test plasmid and Test peptides:

0/18 dead = 0% mortality

Specific Proliferation of Splenocytes to VennVax A and VennVax B Epitopes



IFN-Gamma Secretion of Splenocytes to VennVax A and VennVax B Epitopes

