Orphan Disease, Biodefense and the Animal Rule: A Thermostabilized Ricin Toxin Vaccine

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ABSTRACT

Objective: Biodefense applications represent orphan populations and approvals for medical countermeasures usually involve pursuit of marketing approval via the "Animal Rule". Using our novel, thermostabilized ricin toxin vaccine, RiVax™, we discuss some of the key requirements to pursuing FDA approval.

Method: Ricin intoxication elicits consistent clinical symptoms across species. RiVax protects two animal species from ricin exposure. Immunogenicity correlates between species (including human) are under investigation, using total antibody levels, neutralizing antibody levels and antibody epitope profiling.

Results: Ricin is a potent toxin which can be easily produced from castor beans. The Centers for Disease Control and Prevention (CDC) has classified ricin as a Category B bioterrorism agent. Currently, there is neither a therapeutic nor a vaccine that can be used to protect against ricin exposure or reverse the effects once exposed. Aerosol exposure is the most potent route of poisoning with even microgram quantities causing death. Exposure to ricin becomes rapidly irreversible within 4 hours making treatment alternatives extremely challenging, if not implausible.

RiVax has demonstrated 100% protection in ricin intoxication models in mice and non-human primates (NHPs). However, total antibody levels do not correlate with protection. Neutralizing antibody levels do not appear to correlate with protection in mice, potentially due to the limits in assay sensitivity. Epitope profiling, using competition between sera from vaccinated individuals with monoclonal antibodies, has demonstrated a potential correlation with protection in mice. Similarly, epitope profiling has demonstrated a consistency in vaccine response between NHPs and humans, further qualifying the NHP model as an efficacy correlate under the Animal Rule (codified by the US Food & Drug Administration at 21 CFR 314.600, for drugs, and 21 CFR 601.90 for biological products).

RiVax has also been demonstrated to be safe in healthy human volunteers in two Phase 1 clinical trials in the absence and presence of alum. Vaccination elicited total and neutralizing antibody responses, as well as similar epitope profiles to NHPs.

These studies form the initial basis for the development and advancement to pivotal studies pursuing approval of RiVax for protection against ricin poisoning. Financial support for the development of RiVax is currently only available through Government sources, with funding up to $25M received to date from the National Institute of Allergy and Infectious Diseases (NIAID).

Conclusions: Biodefense applications constitute a unique subset of orphan diseases, requiring the use of the Animal Rule to obtain approval. The Animal Rule requires the demonstration of efficacy in well controlled animal studies that are known to be highly related to the human clinical disease. The Animal Rule also requires the demonstration of a consistent mechanism of the proposed test agent in both the animal models and in humans. Finally, demonstrated safety in humans is generally also required. RiVax is a heat stable ricin toxin vaccine being developed under the Animal Rule. Results to date have demonstrated consistent efficacy across animal species and safety in humans. The demonstration of correlation in efficacy between humans and animals requires the identification of a biomarker (correlate of immune protection). Studies with epitope profiling suggest that antibody competition assays may provide this key link between animal and human studies.

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BIODEFENSE

Biodefense is focused on providing protection against biological weapons, both for the military and for the broader civilian population. Biological weapons may include toxins, infectious agents and radiation. Some of these may be built similar to common disease, and some may be threats which are not seen obviously.

When the threat is uncommon (e.g., exposure to ricin toxin), the threat is actually an orphan disease. Products addressing biodefense threats are eligible for both Orphan and Fast Track designations.

When the threat is uncommon, clinical trials are not possible and the Animal Rule is used to facilitate FDA review.

ANIMAL RULE

The Animal Rule is applicable when:

- Human efficacy studies are not ethical (e.g., exposing humans to ricin); or
- Clinical trials are not feasible (e.g., field trials after an accidental release are not feasible)

The Animal Rule is generally applicable to biodefense threats, accidental exposures and emerging infectious pathogens.

The Animal Rule requires:

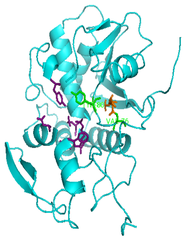
- Safety is evaluated as per standard procedures, including:
  - GLP toxicology studies
  - GLP/non-GLP safety-pharmacology studies
  - Phase 1 clinical studies
- Efficacy is evaluated in animals, including:
  - Pathobiology of the disease is well understood
  - Mechanism of the drug addressing the disease is well understood
  - Disease and drug affect demonstrated in at least one species in a manner predictive of human disease
  - Animal study endpoint is directly relevant to human disease, and usually focused on mortality and major morbidity
  - Kinetics or pharmacodynamics of the drug in the animal species allows prediction of the human dose level

METHODS

Ricin Intoxication and the RiVax Vaccine

Ricin, a plant toxin capable of being weaponized, has well documented toxicity. It is known to be lethal by the aerosol route, resulting in epithelial necrosis within hours of exposure, multifocal hemorrhagic edema and death within 12-36 hours. Ricin contains both an A chain and a B chain, linked by a disulfide bond. The A chain irreversibly inhibits the ribosome, prohibiting protein synthesis and resulting in cell death. The B chain facilitates entry of ricin into cells.

RiVax contains a modified ricin A-chain (RAC), genetically altered to eliminate both the toxicity attributed to the enzymatic activity of ricin (active site modification) as well as the toxicity attributed to vascular leak, which is a secondary toxicity [vascular leak syndrome (VLS) modification].



Ribbon diagram of RiVax (Protein Data Base, PDB,1RTC):

- Active site in purple
- VLS site in orange
- Residues mutated (Y80, V76) to inactivate each site in green
- Active site residue Y80 was changed to A and the VLS V76 residue was changed to M

RESULTS

Ricin Intoxication: Animal Models

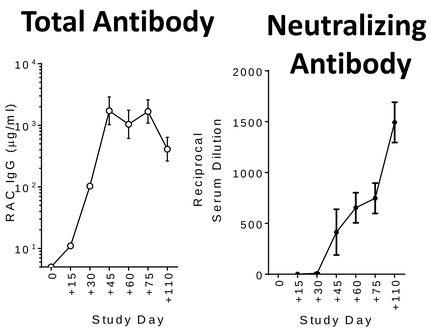
There has been no reported human experience with exposure to aerosolized ricin, although case reports of ingestion of the native castor bean are known.

Ricin is a mitochondrial toxin and as such, its effects on cells and on exposed organs are very consistent, increasing the predictability of human disease secondary to aerosol exposure. Animal models in rodents (mice and rats) and primates (rhesus macaques) have demonstrated nearly identical results:

Rodent Aerosol	Rhesus Aerosol
• Lethal exposure 1-3 μg/kg • Death within 1-3 days • Lung damage within 6-12 hr • Intra-alveolar edema and inflammatory exudate	• Lethal exposure 5-15 μg/kg • Death within 1-2 days • Lung damage within 6-12 hr • Intra-alveolar edema and inflammatory exudate

The rhesus macaque model of aerosolized ricin exposure has been extensively characterized, as the immune system of primates is most related to humans and most likely to predict the response of the human immune system to vaccination.

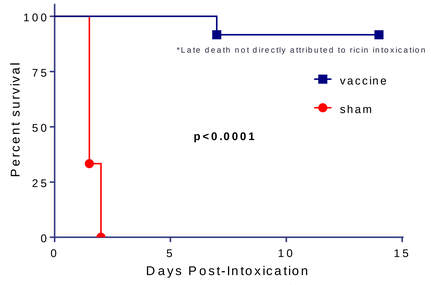
Product Mechanism: Immune response to RiVax



- Neutralizing antibodies believed to correlate with protective efficacy.
- Neutralizing antibodies consistently observed after 3 vaccinations.
- Neutralizing antibody response on Day 110 measured prior to ricin exposure *and resulted in 100% protection.*

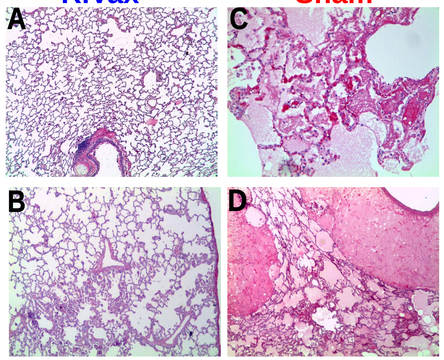
RESULTS

Product Efficacy: Thermostabilized RiVax (RiVax-TR) provides 100% Protection against Aerosolized Ricin



Roy et al. 2015 PNAS 112: 3782-3787

Product Efficacy: Significantly Less Lung Damage in RiVax Vaccinated Animals by Histopathology



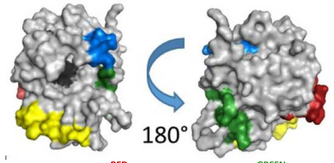
Histopathology of lungs from animals either vaccinated with RiVax (Panels A, B) or sham vaccinated (Panels C, D) and then exposed to a lethal dose of aerosolized ricin (Roy et al. 2015 PNAS 112: 3782-3787):

- Panel A (RiVax): otherwise unremarkable normal lung;
- Panel B (RiVax): mild hyperplasia and focal inflammation;
- Panel C (Sham): marked edema and lung fibrin accumulation; and
- Panel D (Sham): massive edema and associated inflammation.

Magnification: A, B, and D, 20×; C, 100×

RESULTS

Product Mechanism Concurrence: Epitope Profile in NHPs Similar to Humans

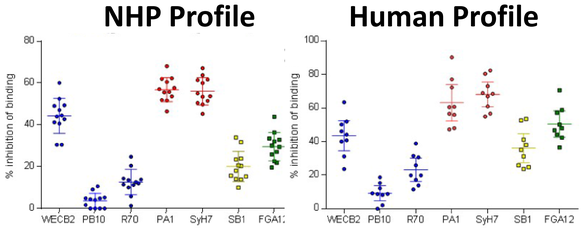


BLUE:
WECB2 (non-linear, neutralizing)
PB10 (linear, neutralizing)
R70 (linear, neutralizing)

RED:
PA1 (non-linear, neutralizing)
SyH7 (linear, neutralizing)

GREEN:
FGA12 (linear, non-neutralizing)

YELLOW:
SB1 (linear, non-neutralizing)



Roy et al. 2015 PNAS 112: 3782-3787

- Mouse monoclonal antibodies, recognizing defined epitopes, were used in a competitive ELISA with NHP or human sera to determine the epitope profile.
- Human samples obtained from a Phase 1 clinical study with RiVax formulated as a liquid alum suspension.
- For both the human and NHP profiles, WECCB2 vs PB10/R70 and PA1/SyH7 vs PB10/R70 are statistically significantly different ($p<0.001$).
- In mice, a linear epitope (aa 97-108) defined by Mabs PB10/R70 is immunodominant.

CONCLUSIONS

- Biodefense indications generally meet the definition of Orphan Diseases and are eligible for Orphan and Fast Track designations.
- The Animal Rule provides a path to approval where clinical trials are not feasible and are specifically intended for Biodefense applications.
- Use of the Animal Rule requires well-characterized animal models with demonstrated concurrence in both disease processes and product mechanism of action across species.
- Soligenix is developing a heat stable ricin toxin vaccine, RiVax, using the Animal Rule and has shown:
  - Concurrence of disease processes;
  - Concurrence of drug mechanism of action; and
  - 100% protection in a large animal model.

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