Ricin Toxin Vaccine: Using Monoclonal Antibodies as Biomarkers to Satisfy the FDA's Animal Rule in Orphan Disease

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ABSTRACT

Objective: Biodefense indications often require the use of the "Animal Rule". This demands the use of biomarkers which correlate with protection in animals and are consistent with those in humans. We have developed novel biomarkers using monoclonal antibodies to determine antibody profiles in all species.

Method: Serum antibody epitope profiling using competition for binding with known monoclonal neutralizing antibodies yields a sensitive, predictive and species-independent assay of immunological protection to subsequent ricin intoxication in mice and non-human primates (NHPs).

Results: Ricin is a highly toxic plant-derived toxin that causes a rapidly progressive respiratory syndrome when inhaled. Ricin toxin is easily derived from castor bean production and constitutes a serious biological threat agent. Soligenix is developing a ricin-toxin vaccine derived from the A-chain moiety of ricin toxin (RiVax®), adjuvanted with aluminum and thermostabilized via lyophilization in conjunction with glassifying excipients. RiVax has demonstrated 100% protection in a rhesus macaque model of aerosolized ricin exposure and safety in two Phase 1 clinical studies.

Development of a ricin-toxin vaccine will require use of the "Animal Rule" for FDA approval [21 CFR 314.600 through 314.650 (drugs) or 21 CFR 601.90 through 601.95 (biological products)]. Use of the Animal Rule dictates the identification of well-defined immune correlates of protection that can be correlated between human studies and animal models. Recent studies have evaluated immunogenicity measures, including total anti-ricin immunoglobulin G (IgG), neutralizing antibody levels, and epitope competition profiles as potential immune correlates of protection. Epitope competition assays have been specifically developed using neutralizing monoclonal antibodies with known recognition sites (epitopes) on ricin/RiVax. Studies in mice, NHPs and humans have suggested that the epitope competition profiles are similar across species and that threshold levels of epitope competition are predictive of survival to subsequent ricin challenge. Studies of different monoclonal antibodies (with different epitope recognition sites) have identified a specific antibody and threshold of competition yielding a prediction accuracy greater than 70% for survival to subsequent intoxication in mice.

Using these same antibodies, the stability of these epitopes on the RiVax protein itself is also related to (indicative of) RiVax drug product potency and stability.

Conclusions: Several medical countermeasures for biodefense applications constitute a unique subset of orphan diseases, in many cases requiring the use of the Animal Rule to obtain approval. The Animal Rule requires 1) the demonstration of efficacy in well-controlled animal studies that are known to be highly related to the human clinical disease 2) the demonstration of a consistent mechanism of the proposed test agent in both the animal models and in humans and 3) demonstrated safety in humans. RiVax is a ricin A-chain toxin vaccine being developed under the Animal Rule (21 CFR 601.90 through 601.95). Results, to date, have demonstrated consistent efficacy and safety across animal species including humans. The ultimate demonstration of correlation in efficacy between humans and animals requires the identification of a biomarker(s) (correlate(s) of immune protection). Studies with epitope profiling suggest that antibody competition assays with particular epitope recognition sites may provide this biomarker and could be the key link between animal and human studies. Also, RiVax protein integrity studies (determined via antibody recognition and binding) demonstrate the conformational stability of the RiVax protein in certain epitope regions, as well as the antibody generation to these epitopes, may be key in monitoring RiVax protein integrity and potency. Moreover, in aggregate, these results suggest that epitope recognition may be a powerful tool for vaccine development, particularly under the Animal Rule.

RiVax has received orphan drug designation in the United States from the US Food & Drug Administration (FDA) and in Europe from the European Medicines Agency (EMA). Upon approval, the RiVax program may be eligible for a Priority Review Voucher. This research was supported with funding from National Institute of Allergy and Infectious Diseases (NIAID) grant U01AI082210 and NIAID contract #HHSN272201400039C awarded to Soligenix, Inc.

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:

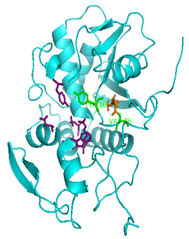
- Safety is evaluated as per standard procedures, including:
  - ✓ GLP toxicology studies
  - ✓ GLP/non-GLP safety-pharmacology studies
  - o Phase 1/2 clinical studies (final studies planned in 2019)
- Efficacy is evaluated in animals, requiring that:
  - ✓ Pathobiology of the disease is well understood
  - ✓ 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
  - o *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):

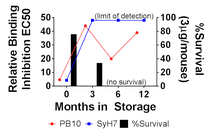
- Active site in purple
- VLS site in orange
- Residues mutated (Y80, V76) to inactivate each site in green
- Active site residue Y80 was changed to A and the VLS V76 residue was changed to M

RESULTS

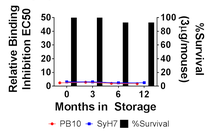
In Vitro Binding and Protective Efficacy in Mice

- Relative *in vitro* EC_{50} binding competition between RTA and RiVax recorded as a function of RiVax storage time and temperature revealed the stability of the thermostabilized vaccine and the instability of the liquid-adsorbed vaccine formulation when evaluated with the SyH7 or PB10 monoclonal antibodies.
- *In vivo* vaccination challenge studies in mice further revealed a complete loss of protective efficacy in the liquid adsorbed formulation stored at 40°C at the 6 month timepoint.

Liquid-Adsorbed Vaccine 40°C



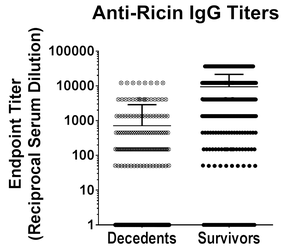
Lyophilized Vaccine 40°C



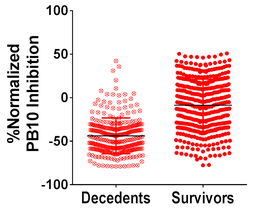
Immunogenicity Correlates in Mice

- Immunogenicity and survival data was compiled from 4 individual studies (689 female BALB/c mice) evaluating efficacy/potency of various RiVax formulations using the same experimental design (i.e., vaccinations on Days 0 and 21, blood draws on Day 30 and intraperitoneal challenge with 5xLD_{50} ricin on Day 35 with monitoring to Day 42).

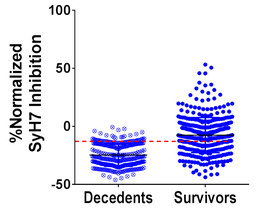
Anti-Ricin IgG Titers



EPICC Assay - PB10



EPICC Assay - SyH7



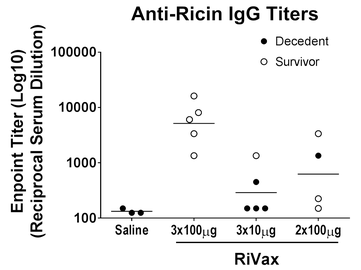
RESULTS

Protective Efficacy in Non-Human Primates

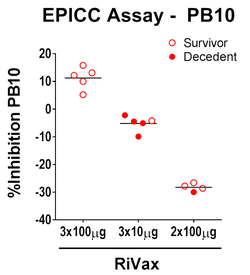
- Vaccinations (lyophilized formulation) were administered on Days 0, 30 and 60 with blood collection on Day 105 and aerosolized ricin challenge (3xLD_{50}) on Day 110 with survival monitored to Day 124 (4).

Group	N (M/F)	Dose (µg protein)	Vaccination Schedule	%Survival (3xLD_{50} aerosol)
Saline	1/2	0	Days 0, 30, 60	0
RiVax	2/3	100		100
RiVax	2/3	10		20
RiVax	2/2	100	Days 0, 30	75

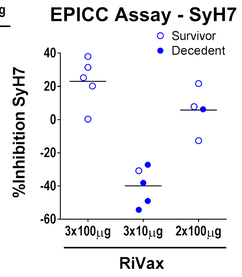
Anti-Ricin IgG Titers



EPICC Assay - PB10



EPICC Assay - SyH7



RESULTS

Predictive Accuracy of the EPICC SyH7 Assay

- Using a training set of 143 mice, a binary classification model was derived and applied to the prospective test set (remaining 546 mice), yielding an overall accuracy of 74%.

Actual Outcome	Predicted Outcome		Total	%Accuracy (Predicted /Total)
	Survivors	Decedents		
Survivors	212	104	316	67
Decedents	36	194	230	84
Overall (Survivors + Decedents)			546	74

- Preliminary model (not yet fully optimized) predicts decedents very accurately, facilitating clinical use as an indicator of individuals needing an additional "booster" shot.

CONCLUSIONS

- Previous studies demonstrated stability and potency of lyophilized thermostabilized formulations of RiVax, consistent with results shown here.
- IgG anti-ricin and neutralizing titers have limited correlative ability to predict survival, while specific competition assays with monoclonal antibodies yield significantly more sensitivity.
- Enhanced sensitivity of the SyH7 antibody in both *in vitro* and *in vivo* assays suggests the importance of the "cluster 2" domain of RiVax to protective efficacy.
- Qualitative consistency of response across species suggests that immunogenicity endpoints, particularly those utilizing the monoclonal antibody competition responses, may be used to demonstrate feasibility of animal models to mimic the human condition, as required by the Animal Rule.
- Quantitative consistency of immunogenicity response will enable definition of a human equivalent dose upon completion of planned Phase 1/2 clinical study with the thermostabilized formulation.

REFERENCES

1. Westfall, J., Yates, J.L., Van Slyke, G., Ehrbar, D., Measey, T., Straube, R., Donini, O., Mantis, N.J. 2018. Thermal stability and epitope integrity of a lyophilized ricin toxin subunit vaccine. Vaccine 36(40): 5967-76.
2. Donini O, Haulenbeek A, Arumugham R, Schaber C. Orphan Disease, Biodefense and the Animal Rule: A Thermostabilized Ricin Toxin Vaccine. Poster Presentated at the National Organization for Rare Diseases (NORD) Rare Diseases and Orphan Products Breakthrough Summit. October 17-18, 2016.
3. Roy, C.J., Brey, R.N., Mantis, N.J., Mapes K., Pop, I.V., Pop, L.M., Ruback, S., Zilleen, S.Z., Doyle-Meyers, L., Vinet-Oliphant, H.S., Didier, P.J., Vitetta,, E.S. 2015. Thermostable ricin vaccine protests rhesus macaques against aerosolized ricin: Epitope-specific neutralizing antibodies correlate with protection. PNAS 112(12): 3782-87.
4. Vitetta, E. S., Smallshaw, J.E. Schindler, J. 2012. A Small Phase IB Clinical Trial of an Alhydrogel-Adsorbed Recombinant Ricin Vaccine (RiVax). Clin Vaccine Immunol 19(10:)197-199.
5. Vance, D.J., Rong, Y., Brey, R.N., Mantis, N.J. 2015. Combination of two candidate subunit vaccine antigens elicits protective immunity to ricin and anthrax toxin in mice. Vaccine 33(3): 417-21.
6. Wang, G., Mega, W., Overheim, K.A., Hutt, J.A., Kuehl, P.J., Benson, J.M., Measey, T., Donini., O. Verification of Lethal Doses of Inhaled Ricin in Rhesus Macaques to Support Vaccine Development. Abstract Number/Poster Board number: 2695/P623. 56th Annual Meeting of the Society of Toxicology. Baltimore MD. March 12-16, 2017.
7. Toth iV, R., Angalakurthi, S.K., Van Slyke, G., Vance, D.J., Hickey, J.M., Joshi, S.B., Middaugh, C.R., Volkin, D.B., Weis, D.D., Mantis, N.J. High-Definition Mapping of Four Spatially Distinct Neutralizing Epitope Clusters on RiVax, a Candidate Ricin Toxin Subunit Vaccine. Clin. Vaccine Immunol. Oct. 18, 2017. doi: 10.1128/CVI.00237-17. [Epub ahead of print]

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