

An Immunoadhesin Therapy for Inhalation Anthrax



CREATE-IGERT Symposium

October 16, 2008

Keith Wycoff, PhD, Research Director

Planet Biotechnology Inc

Planet Biotechnology's Business

Produce antibodies in transgenic tobacco
for treatment of disease

➤ CaroRx: Blocks adhesion of *Streptococcus mutans*

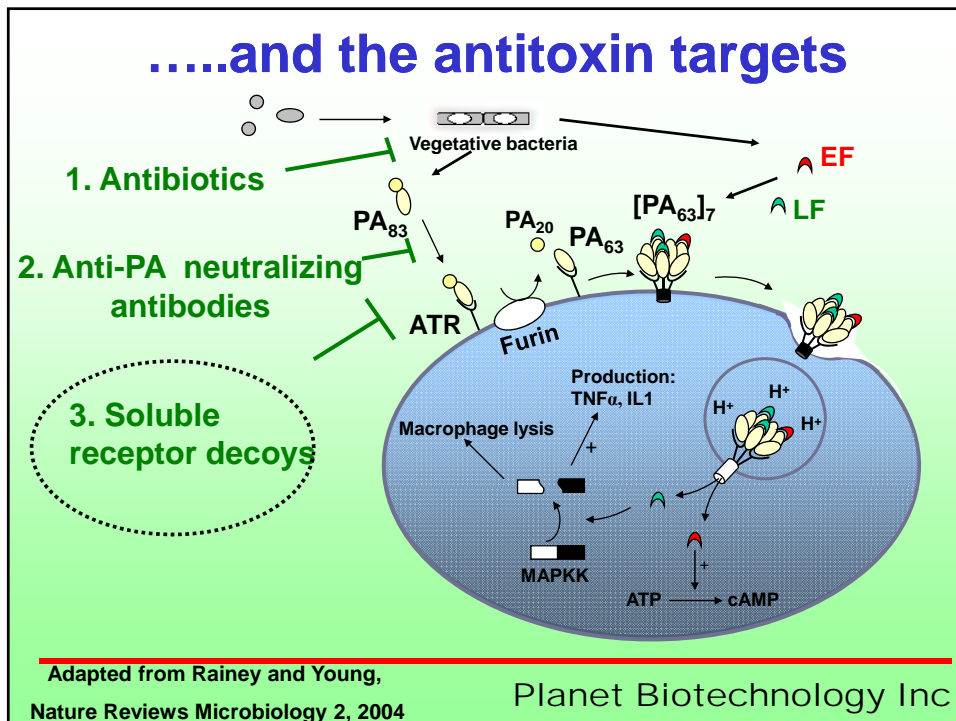
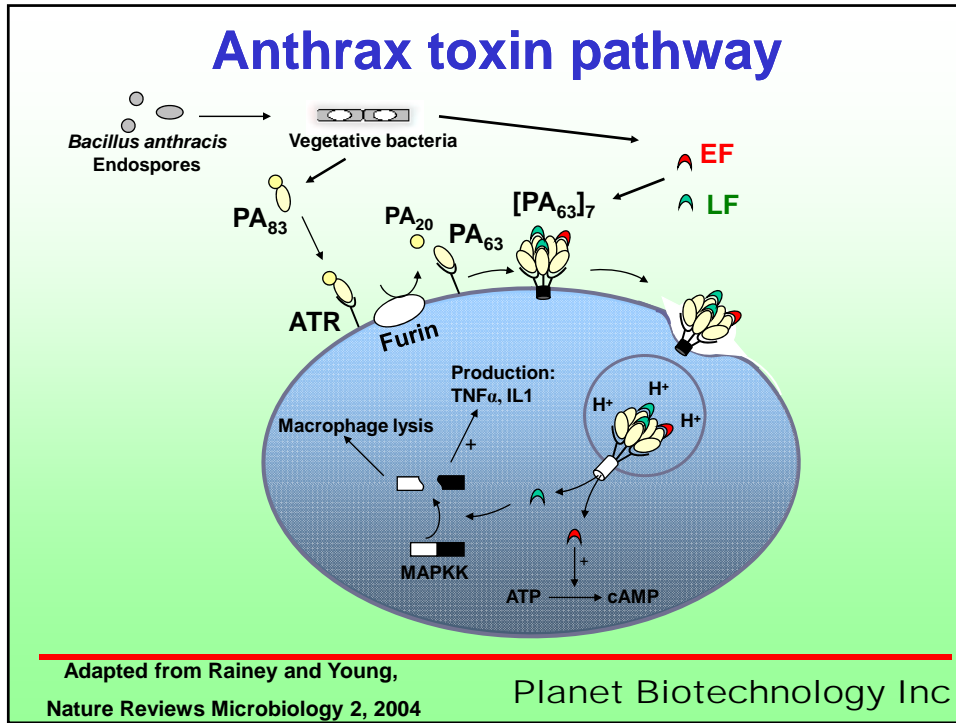
First plant-made pharmaceutical approved as Class I
medical device in Europe

➤ RhinoRx: Blocks infection by human rhinovirus

➤ Biologics for bio-defense applications:

Block Anthrax and Botulinum toxin mediated cell death

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Anthrax Toxin Receptors

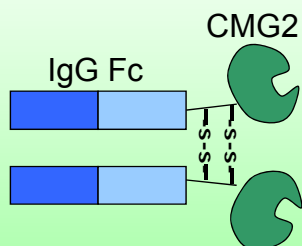
1. Tumor endothelial marker 8 (TEM 8)
2. Capillary morphogenesis protein 2 (CMG2)

Soluble forms of both receptors {extracellular domain related to von Willebrand factor A (VWA) domain} inhibit the action of lethal toxin

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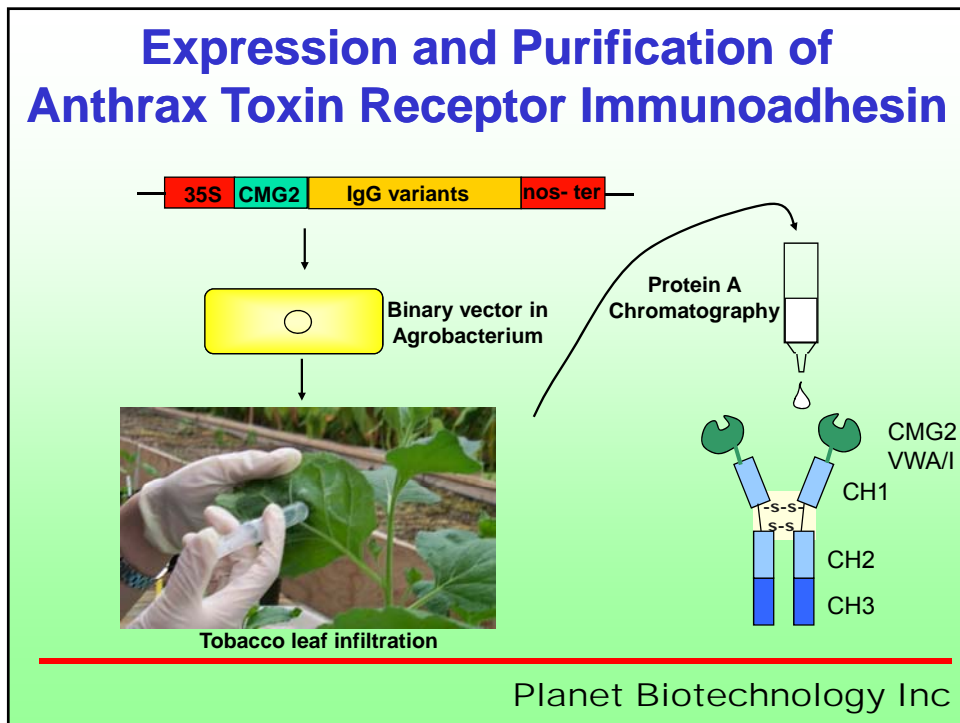
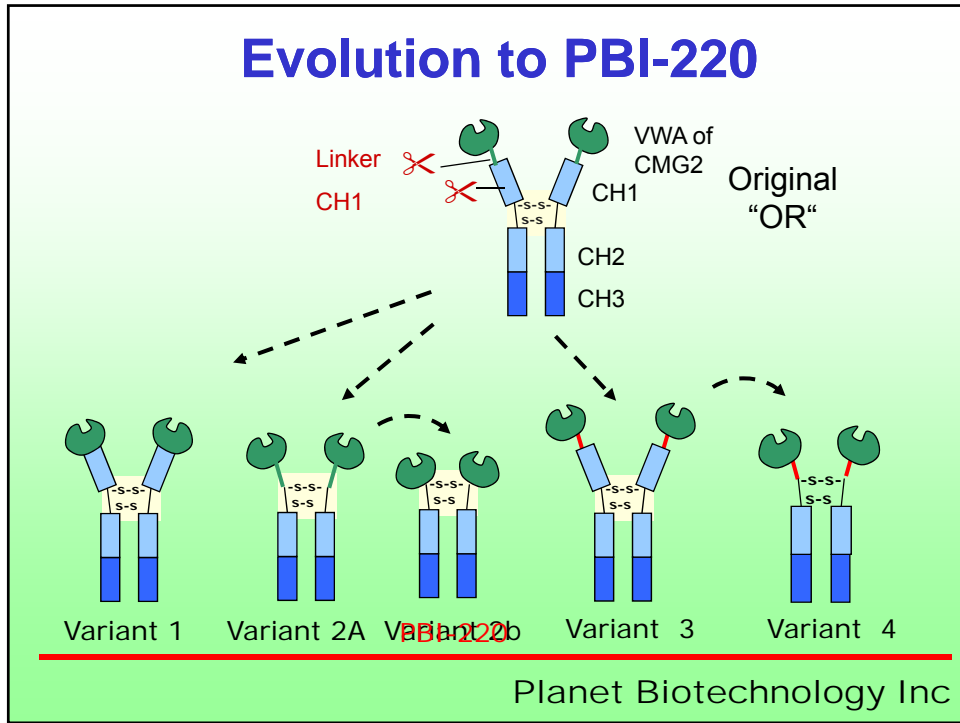
PBI-220: Anti-Anthrax Immunoadhesin made in Tobacco

Blocks binding of PA to its cellular receptor

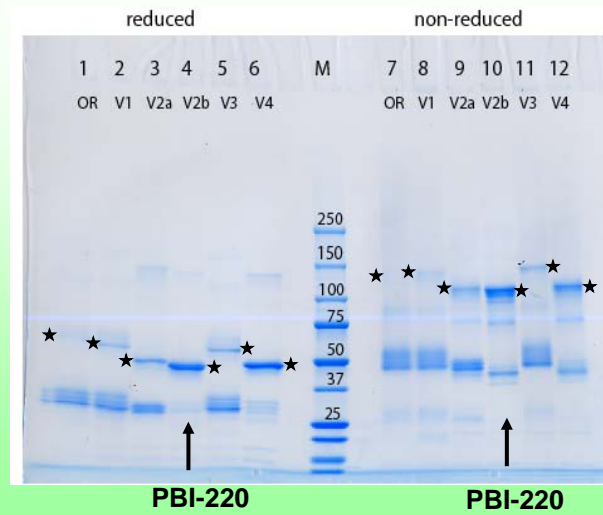


A genetic fusion of capillary morphogenesis protein 2 (CMG2) and IgG Fc

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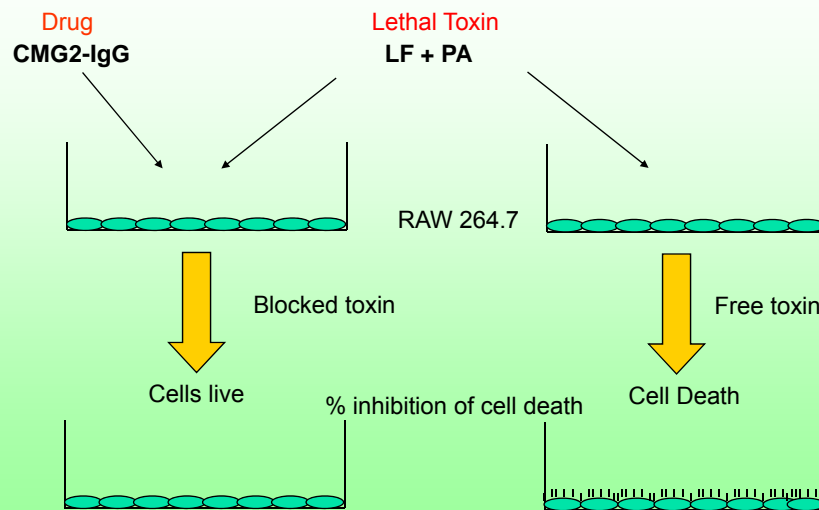


SDS-PAGE of immunoadhesins



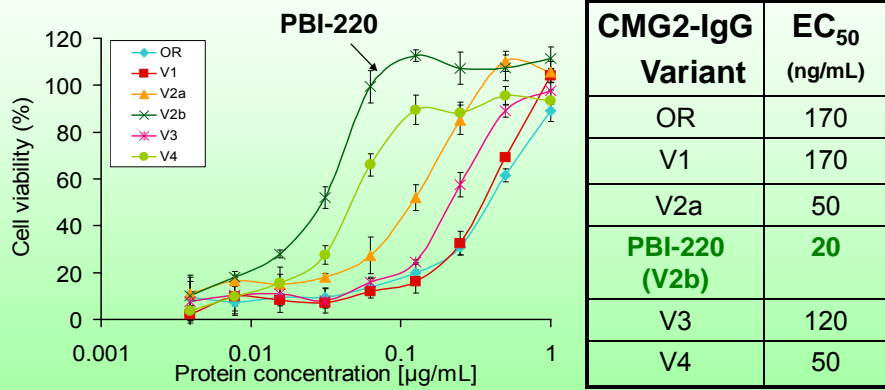
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Toxin Neutralization assay



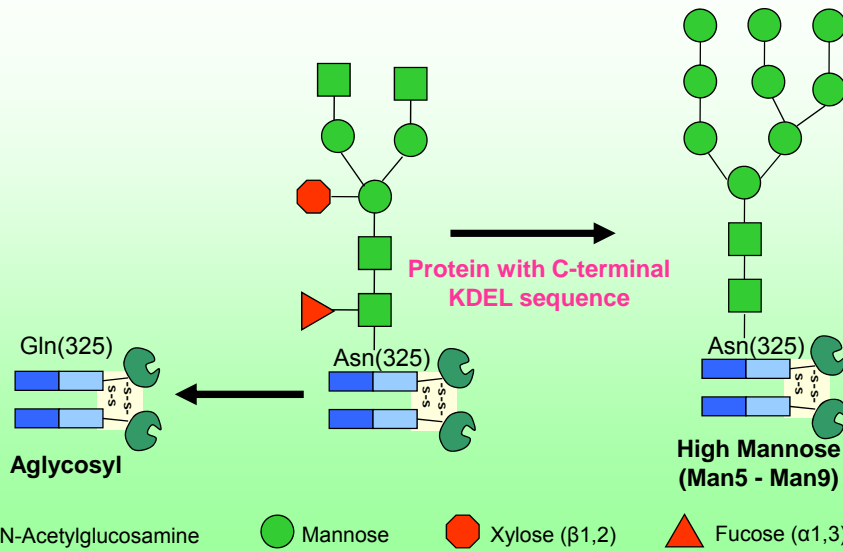
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Neutralization of lethal toxin activity by variants of CMG2-IgG



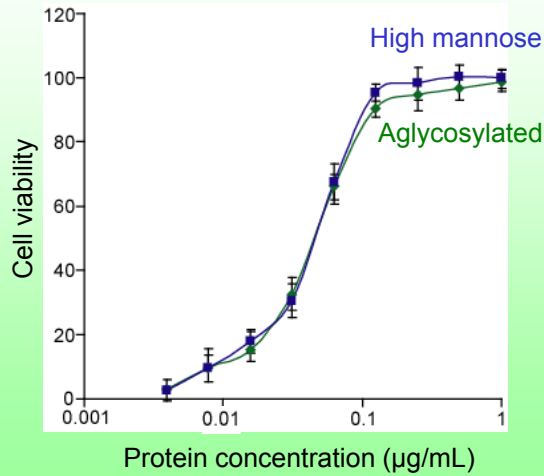
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PBI-220 is a glycoprotein



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Aglycosylated and High mannose PBI-220 are comparable *in vitro* (TNA)



EC50 (PBI-220):

Aglycosyl: 0.040 µg/mL

High mannose: 0.035 µg/mL

Li H. et al., J. Immunol. Methods (2008) Planet Biotechnology Inc

In Vivo Animal model for Anthrax

Study in Collaboration with Peterson Lab

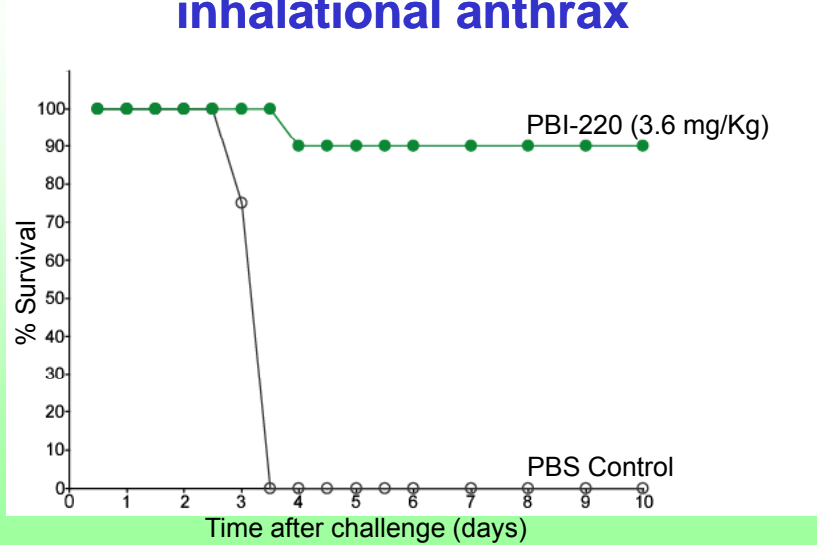
Dutch-belted Rabbits challenged
intranasally, with 100LD₅₀ (1x10⁸ cfu/mL)
of *B. anthracis* Ames spores

PBI-220



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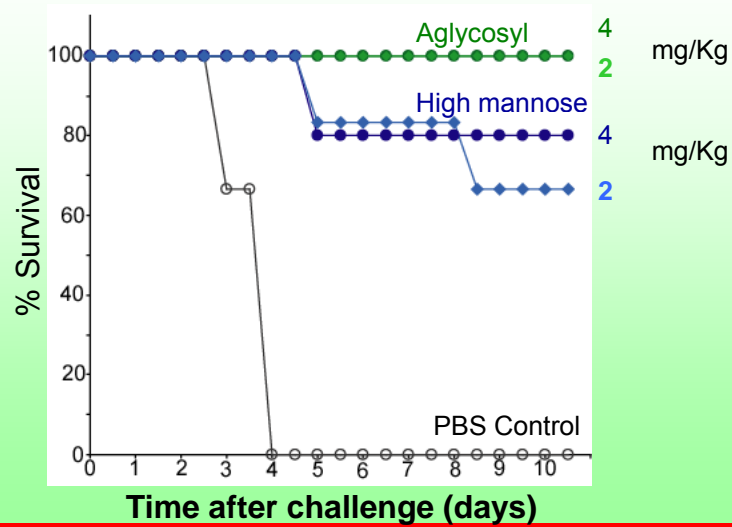
Algycosyl PBI-220 protects rabbits from inhalational anthrax



J. Peterson et al., UTMB

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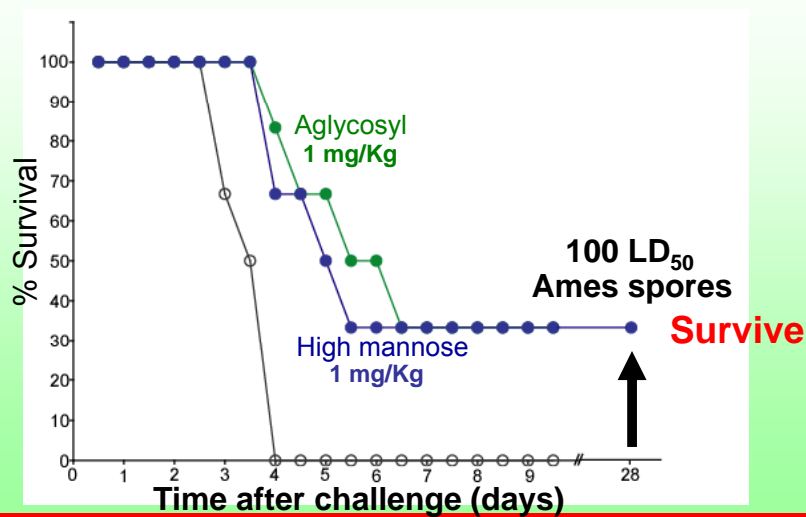
Algycosyl PBI-220 at 2 mg/Kg protects rabbits from inhalational anthrax



J. Peterson et al., UTMB

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Rabbits protected by PBI-220 survives re- challenge



J. Peterson et al., UTMB

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Broad spectrum protection with Immunoadhesins

Study in Collaboration with Leppla Lab

Protective Antigen: 83 kD protein

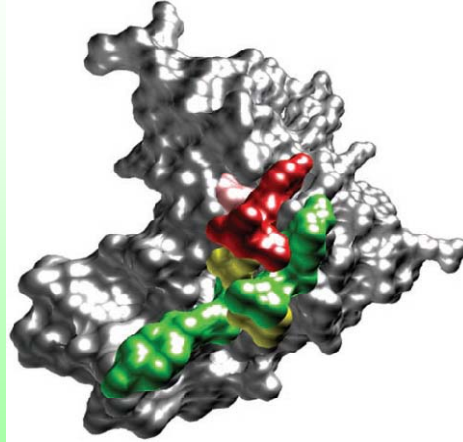
Domain 4 (596 – 735): binding to cellular receptors

(anti-PA neutralizing monoclonal antibodies have binding sites within domain 4)

Alanine scan mutants that lie within this domain

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In Vitro generated Variants of PA



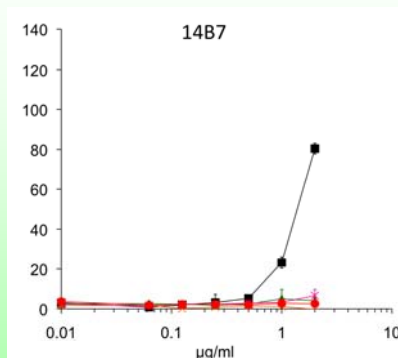
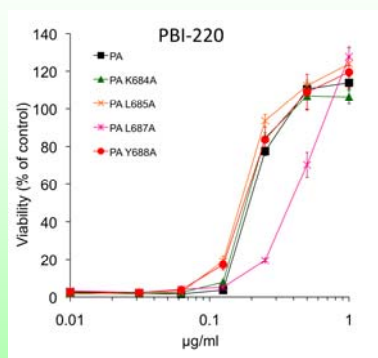
However, the substitutions have greatly reduced binding to Mab14B7

Substitutions of Lys684, Leu685, Leu687, and Tyr688 (*green*) all had only minor effects on cell binding and toxicity.

Rosovitz et al. 278 (33): 30936. (2003)

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PBI-220 protects against functional PA



PBI-220 neutralizes mutant forms of PA.

Mab 14B7 does not.

(Anthim™ is affinity-matured version of 14B7)

Leppla Lab study

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Summary

1. PBI-220, made in tobacco, protects rabbits at 2mg/Kg, from inhalation anthrax
2. PBI-220 offers protection against functional but antigenically altered forms of PA
3. PBI-220 is a viable alternative to neutralizing anti-PA monoclonal antibodies

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