ASU AzBio
Arizona Biodesign Institute
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Delivery of Plant-Derived Oral Vaccines

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"Biomimetic Vaccine" Technology

Mimic nature's protein stabilization during seed development:

(Fertilization, embryo development and desiccation are developmentally regulated (to a "glassy state")

Dehydration of plant tissues mimics nature to protect pharmaceutical proteins in the tissues

(Monoclonal antibodies are stable and can be purified from air-dried alfalfa leaves; Biotechnol. Bioeng. 64: 135-143, 1999)

Antigen Stabilization By Dehydration

Contained batch production and harvest

Batch process;
Freeze drying

Powderize, sieve, blend for uniformity.

(Add additional excipients, adjuvants, sweeteners, etc.)

Approved Clinical Trial For Processed Plant Material:
Norwalk Virus Capsid Protein From Tomatoes

Tomato bioengineering: Dr. Hugh Mason (Associate Professor; Arizona Biodesign Institute)

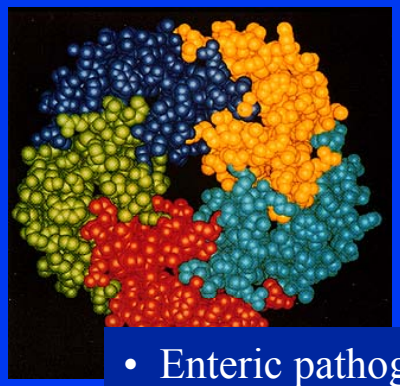
Clinical trial design: Dr. Carol Tacket (Center for Vaccine Development; U. of Maryland, Baltimore)

Sample process design: Dwayne Kirk (PhD student, ASU)

Dose: dried tomato fruit in gelatin capsules plus/minus oral adjuvant (food grade saponins from *Quillaia saponaria)*

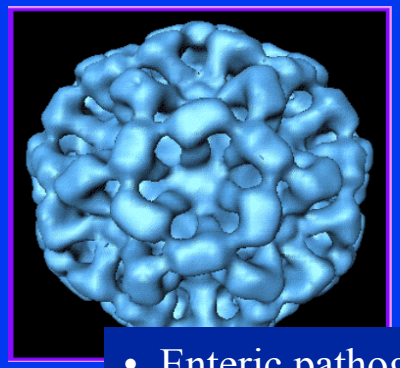
Previous Clinical Trials With Plant-derived Antigens

- **Tacket, C.O., Mason, H.S., Losonsky, G., Clements, J.D., Levine, M.M., C.J. Arntzen.** 1998. Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. Nature Medicine, 4:607-609.



- Enteric pathogen

- **Tacket, C.O., H.S. Mason, G. Losonsky, M.K. Estes, M.M. Levine, C.J. Arntzen**. 2000. Human immune responses to a Novel Norwalk virus vaccine delivered in transgenic potatoes. The Journal of Infectious Diseases. 182:302-305.



- Enteric pathogen

- Thanavala, Mason, and Arntzen (unpublished). Oral delivery of Hepatitis B Surface Antigen boosts parenteral immune response in humans.

- Non-enteric pathogen

Why Potatoes?

Plant Engineering:

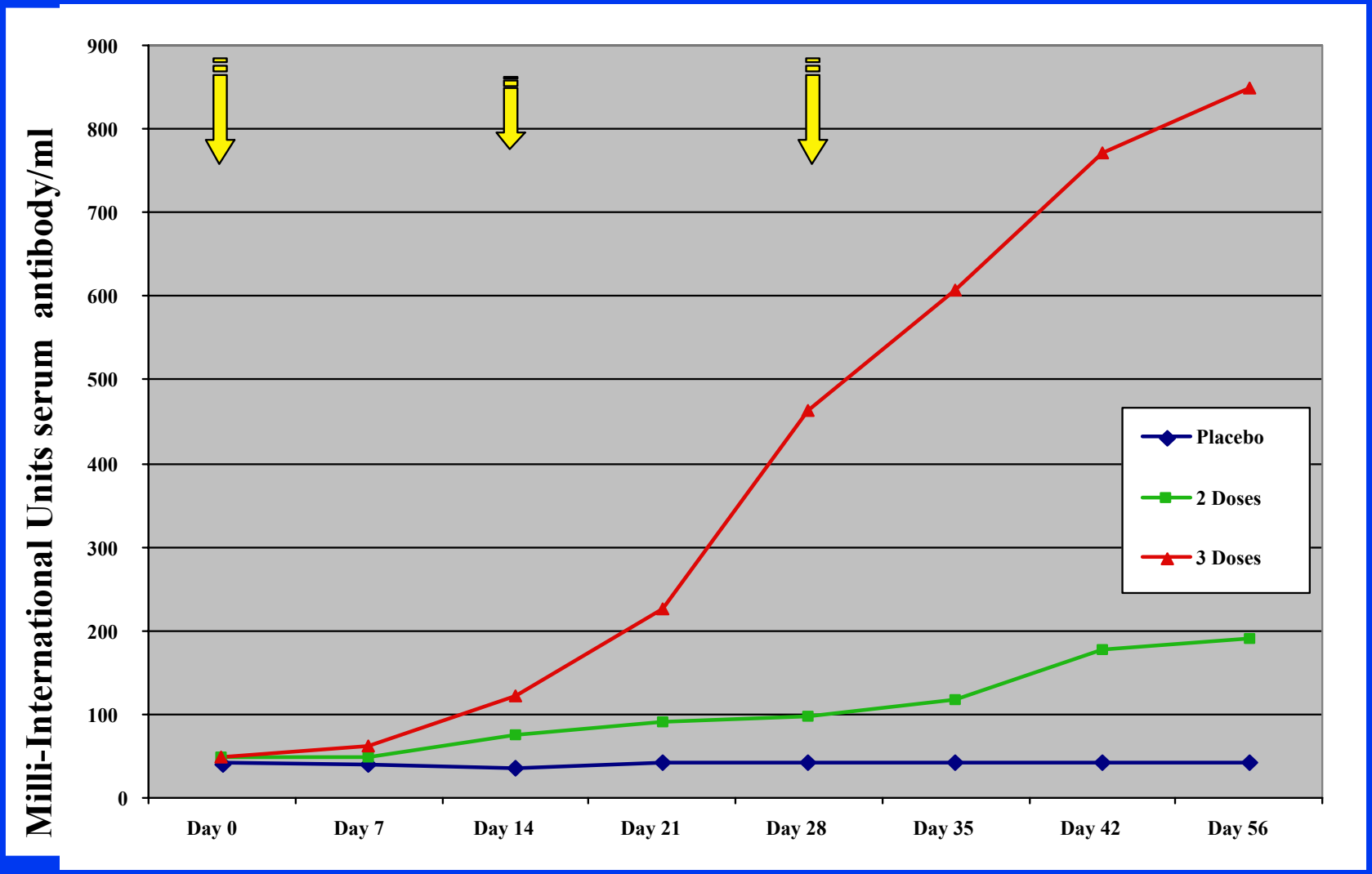
- Facile transformation system
- 3-5 months to significant biomass
- Clonally propagated

Regulatory:

- Pre-clinical studies were straight forward
- Well defined food

Hepatitis B Boosting Trial

Average mean IgG titers for all volunteers



Tomatoes are Scalable Production Systems

Greenhouse production, in USDA approved facilities, to meet Standard Operating Procedure protocols for genetic containment (to protect food crops). Production under pharmaceutical standards.

Tomato-based Vaccine Production

Raw Material Cost Estimates

- USDA Web site: Average value at U.S. "farmer's gate" for processing tomatoes is $54 per ton ($0.05/kilo)

- 20 doses (2 mg antigen for oral delivery) per kilo fresh weight (at present), or 1/4 cent per dose in U. S.

- Alternative systems are being evaluated for antigen production

- Alfalfa tablets (Fe^{++} supplement) are $0.012 in US health food stores

Plant Tissue Production In Fermentation

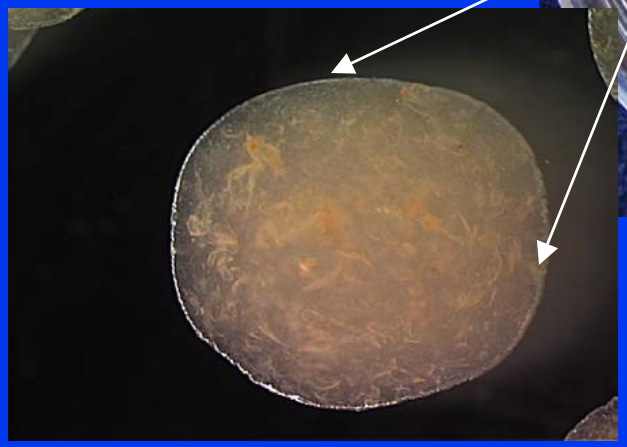
In recognition of GM Food debates, and potential problems therein…..

- Root cultures have been developed from vaccine producing plants and adapted to liquid culture

- Dried roots = 100 fold concentration of antigen over fresh plant tissue

Encapsulation of Plant Extracts for Stability, Uniformity, and Blended Contents

- Tomato Juice can be encapsulated in alginate
- Dried particles are stable
- pH sensitive coatings are possible



Fresh

Air Dried

Food process technology is applicable

Plant-Derived Vaccines

What is possible:

1. Heat stable formulation for oral delivery
2. Vaccines suitable for long term storage (Example: strategic biodefense vaccine reserves)
3. "Scalable" biomanufacturing to limit initial capital cost requirements as vaccine demand is established
4. Suitability for multiple antigens