DNA Vaccine Development: Practical Regulatory Aspects

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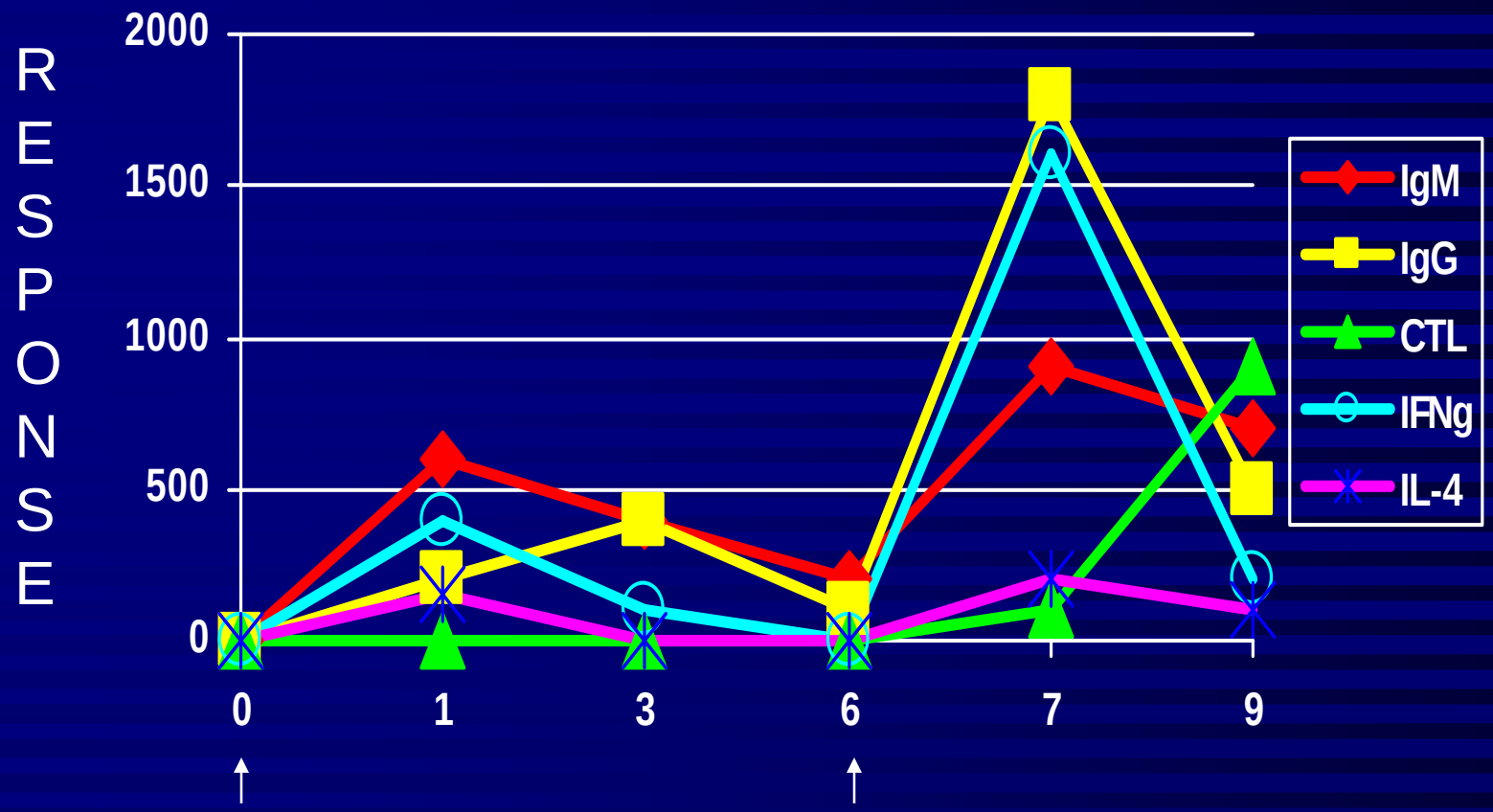
Plasmid DNA Vaccines

- DNA plasmids are designed so that a strong promoter drives the expression of one or more genes encoding the protein(s) of interest.
- The immunogenicity of DNA plasmids promised to revolutionize vaccine development:
  - Eliminated roadblocks to vaccine development:
    - Pathogen isolation, growth, purification and attenuation
    - Protein identification, production and purification
  - Tools of molecular biology used to isolate/clone relevant genes.
- Animal studies indicate that DNA vaccines can induce protective antibody and CTL responses *in vivo.*

Immune Responses Induced by DNA Vaccines



Considerations for Manufacturing Process Development

Vaccine Production and Quality Control

Principles common to all vaccine manufacture:

- Detailed manufacturing procedures: consistency of production
- Defined compatible components
- Product characterization: specifications
- Adventitious agent testing
- Examination for extraneous materials
- Stability, including genetic stability
- Recommendations for lot release testing

Lot Release Testing

- Sterility - to detect bacterial or fungal contaminants
- General safety test - performed in guinea pigs and mice to detect extraneous toxic contaminants
- Identity tests:
  - Plasmid size,
  - Restriction endonuclease digestion pattern
  - Percent of plasmid that is circular or supercoiled
- Purity – freedom from protein, RNA, endotoxin and bacterial DNA contamination
- Potency - *in vivo* or *in vitro* test to assess immunogenicity or transfection/translation efficiency
- Tests for removal of process contaminants

Safety Issues Associated with DNA Vaccines

- Induction of autoimmunity
  - Local inflammatory responses (myositis)
  - Organ-specific autoimmunity
- Persistence and integration of plasmid DNA
  - Sites of uptake and expression
  - Persistence of plasmid and protein product
  - Integration into the host genome
- General toxicity

Current CBER Perspective

- No systemic or organ-specific autoimmunity has been observed in DNA-vaccinated volunteers.
- CBER will no longer mandate that pre-clinical studies examine whether DNA vaccines induce autoimmune disease.
- If the formulation or content of a specific DNA vaccine raises concern that immunization may induce autoimmunity, specific pre-clinical and phase I clinical assessments will be requested on a case-by-case basis.

Concern: Integration of Plasmid DNA may cause Genetic Toxicity

- Vaccine-derived promoters/enhancers may alter the expression of host genes (including oncogenes).
- Genomic instability (breaks or rearrangements)
- Inactivation of tumor suppressors.
- Integration into reproductive tissue may result in germline alteration.

Persistence of DNA Vaccines *in vivo*

- Initial vaccine uptake is influenced by transfection efficiency and the method/dose of plasmid delivered.
  - Vaccine is primarily localized to the site of injection.
- The amount of plasmid decreases by several orders of magnitude over time.
- Typically, <30 copies of plasmid/million host cells persist long-term, corresponding to an integration rate 1,000-fold lower than the natural mutation rate.
- No long-term persistence has been reported for reproductive organs.

Current CBER Perspective

- Integration studies will be required only if the plasmid persists at high copy number (>300 copies/10^6 host genomes) *in vivo.*
- Biodistribution/persistence studies will be waived for DNA vaccines demonstrably similar to those already approved for clinical trial.
- Sponsors should contact the FDA for advice concerning:
  - **New or significantly modified plasmids**
  - **When changes in formulation or method/route of delivery significantly alter plasmid uptake or distribution**
  - **If differences in the behavior of "approved" plasmids are observed.**

Toxicity Evaluation

- Serum chemistries including liver and renal function tests (ALT, AST, creatine kinase, BUN)
- Hematologic analyses (CBC and differential)
- Clinical assessments (general health, injection site observation, limb use impairment)
- Necropsy (gross pathology and histopathology)
  - Acutely, 2-3 days after the final immunization
  - Chronically, 2-3 weeks after the final immunization.

General Safety of DNA Plasmids

Animals immunized twice/month for 5 months.

- No lasting change in immune milieu.
- No deaths
- No weight loss
- Normal serology and urinalysis
- No macroscopic or microscopic changes in:
  - **spleen** **lymph nodes**
  - **liver** **kidney**
  - **intestine** **heart**
  - **lungs** **adrenals**

Proposed Revisions to CBER Guidelines

- Preclinical safety studies should be performed on every novel DNA vaccine or vaccine/adjuvant combination.
- Toxicity studies should use the highest dose of vaccine planned for clinical administration.
- Vaccine can be delivered on an accelerated schedule:
  - Vaccination intervals shorted to Q 3 - 4 weeks
  - Immunize with N + 1 doses of vaccine.
- CBER may modify the requirements for preclinical safety evaluation in select situations:
  - Where multiple variants of a specific gene are cloned into a common plasmid vector
  - When a complete safety evaluation has already been performed on a similar plasmid construct.

DNA Plasmids: Safety Profile in Man

- DNA plasmids have been introduced into many hundred normal volunteers.
- No serious adverse events have been reported.
- Local reactogenicity has been mild.
- Multiple immunizations are required to elicit even modest immune responses.
- Ongoing efforts are directed towards improving immunogenicity in Man.

Future Concerns

- Improvements in vaccine formulation/delivery may increase plasmid dissemination, cellular uptake, persistence, and the risk of integration or toxicity.
  - Intranasal, oral and i.v. routes may more efficiently disperse plasmid throughout the body.
  - Liposome encapsulation or electroporation may increase plasmid uptake and the range of cells being transfected
  - Changes in vector/gene may increase the risk of integration.
  - Changes in CpG content may alter toxicity
- Dose escalation increases all risks:
  - 20 ug ---> 7,500 ug per subject.
  - Multiple doses of multiple plasmids are being administered.
- Use of novel cytokine encoding plasmids.

Conclusion

As CBER accumulates experience with novel types of DNA vaccine, novel vaccine/adjuvant formulations, and novel vaccination strategies, our science-based review of these products will continue to evolve.