

# Current Concepts for Development of the Nonclinical Toxicology Package for Novel Preventive Vaccines

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## Types of vaccines

- Microorganisms inactivated by chemical/ physical means that retain appropriate immunogenic properties.
- Living microorganisms that have been selected for their attenuation but retain immunogenic properties.
- Antigens extracted from microorganisms, secreted by them or produced by recombinant DNA technology.
- Chimeric microorganisms.
- Antigens produced *in vivo* in the vaccinated host following administration of a live vector or nucleic acid or antigens produced by chemical synthesis *in vitro*.

## How vaccine antigens are presented to the body

- Antigens may be in their native state, truncated or modified following introduction of mutations, detoxified by chemical or physical means and/or aggregated, polymerized or conjugated to a carrier to increase immunogenicity.
- Antigens may be presented alone or in conjunction to an adjuvant, or in combination with other antigens, additives and other excipients.



# General Considerations for Vaccine Safety

## Vaccines: benefits vs. risks

- Introduction of preventive vaccines has resulted in marked survival and quality of life for much of humanity.
- Decrease of human morbidity has resulted in complacency.
- Unlike almost all other medicines, vaccines are routinely administered to large numbers of otherwise healthy individuals.
- Certain vaccine under development (biodefense, pandemic influenza) may never be used; therefore risk/benefit ratio is difficult to estimate.
- *Poison is in everything, and no thing is without poison. The dosage makes it either a poison or a remedy – Paracelsus.*
  - Vaccine safety may not follow this paradigm.
  - Like any other medicine, vaccines have an inherent risk.
  - **No vaccine is 100% safe.**

## Can we “overload” the immune system?

- Can the immune system be quantitatively overloaded?
  - Potential immune repertoire of neonate is approximately  $10^9$  to  $10^{11}$  antigens
  - Capacity is therefore approximately  $10^5$  vaccines simultaneously, assuming 100 antigens/vaccine
  - Based on standard 11 vaccine regimen, children are now exposed to 125 vaccine antigens (not simultaneously)
  - In 1960, there were over 3,200 antigens per five vaccines total
- No evidence of differential response to single immunization vs. multiple vaccinations.
- Vaccination can result in short-term hyporesponsiveness to in vitro immune tests, but **not** to infection.

Source: Offit et al., 2002

## Does vaccination lead to autoimmunity?

- Multiple sclerosis due to molecular mimicry attributed to:
  - Hepatitis B: extremely unlikely, since HBsAg is dissimilar to MBP
  - Influenza: theoretically plausible due to similarity of antigen in Influenza A with MBP
  - Paradoxically, influenza vaccine prevents, rather than exacerbates, symptoms of multiple sclerosis
- Type 1 diabetes due to viral infections.
  - Evidence of a link in natural infection
  - No clinical association with vaccination
- Rheumatoid arthritis: Lyme disease vs. Lyme disease vaccine.

## Does vaccination lead to increases in asthma/allergy?

- So-called “Hygiene Hypothesis” states that preventing childhood infections results in a stronger Th2 response.
- Immunological fallacy:
  - Vaccines do not prevent most “childhood infections”
  - Other strong Th2 conditions (e.g., pregnancy) do not predispose to allergies
  - Helminth infections decrease, rather than increase, allergies
  - Increased prevalence of Th1 (autoimmune) and Th2 (allergic) conditions are not geographically exclusive
- Several major, well-controlled clinical trials have failed to detect a correlation between allergy and vaccination.

## Allergic reactions to vaccines can occur

- Reactions to vaccines are rare (few cases per every 10,000 vaccinations).
- Reactions occur to various vaccine components:
  - Animal proteins (ovalbumin, chicken proteins, gelatin, calf lymph)
  - Yeast proteins (recombinant products)
  - Preservatives (thimerosal)
  - Adjuvants (aluminum)
  - Antibiotics
- Reactions occur to packaging (latex in stoppers).



# Strategies for Ensuring Vaccine Safety

## Regulatory guidance

- FDA/CBER
  - “Guidance for Industry: Evaluation of Combination Vaccines for Preventable Diseases”, 1997
- CPMP/EMEA
  - “Note for Guidance on Preclinical Pharmacological and Toxicological Testing of Vaccines”, 1998
- WHO
  - “Guidelines on Nonclinical Evaluation of Vaccines”

**Regardless of “guidance”, it is prudent to involve the relevant agency in the design of the study prior to its implementation.**

**This pays dividends when filing the IND.**

## FDA Guidance for Industry - Considerations for Plasmid DNA Vaccines for Infectious Disease Indications

- Immunogenicity
  - Tested in a relevant animal model
  - May include the evaluation of antigen-specific Ab titers, seroconversion rates, and/or induction of CMI responses.
- Cytokines
  - Important when immunomodulatory genes are included as part of the construct
  - Studies should be in animal species responsive to the encoded human cytokine(s) or models using homologous animal gene(s).
  - Look for adverse consequences, such as generalized immunosuppression, chronic inflammation, autoimmunity or other immunopathology.
- Prime/Boost Strategies
  - Should submit information supporting the safety and tolerability of the dose, schedule, and route of administration of each component proposed for use in the heterologous prime-boost regimen.
  - If existing data are deemed adequate to characterize the potential risks of the prime-boost regimen to study participants, additional toxicology studies may not be necessary.

## FDA Guidance for Industry - Considerations for Plasmid DNA Vaccines for Infectious Disease Indications

- Autoimmunity
  - No longer recommended that preclinical studies be performed to specifically assess induction of autoimmune disease.
  - When an immune response is induced by a transgene product encoding self-antigen should examine potential cross-reactivity with the corresponding endogenous protein.
- Local Reactogenicity and Systemic Toxicity Studies
  - Studies for systemic toxicity and local site reactogenicity may be combined. injection site reactogenicity should include detailed clinical observations of the injection site(s) following each vaccine administration and histological evaluations of injection-site tissue.
  - Evaluate both short-term and persistent toxicity, preferably by studying separate cohorts of animals 2 to 3 days and 2 to 3 weeks after the final vaccination.
- Biodistribution, Persistence, and Integration Analysis
  - A typical biodistribution/persistence study assesses the presence of plasmid collected from a panel of tissues at multiple time points ranging from a few days to several months post administration.

## The “easy” part: formulation

- Production under CGMP ensures reproducible product.
- Elimination of animal-derived products (especially bovine) reduces or eliminates risk of allergy and BSE-related disease.
- Elimination of “legacy” issues (e.g., SV40).
- Appropriate lot-release assays reduce risk associated with microbial contamination, endotoxin, or foreign matter.
- Reduction or elimination of preservatives, antibiotics/antimicrobials, and toxic components.
- Studies should include:
  - Potency
  - Stability
  - Batch release



## Considerations for choosing animal model(s)

- Selection of appropriate animal model is **crucial**.
- Must be similar to human in response to vaccine.
  - Parameters should include information on antibody titers (unless available from proof of concept/immunogenicity studies).
- Must be similar to human in susceptibility and pathology to targeted organism.
- Rodents (particularly mice) and nonhuman primates are the most common species; rabbits are also used but are less common.
- In general, only one species is necessary for toxicity studies.
  - May require multiple species if significant species differences are known or suspected in response to the antigen.
  - Not all models fit all purposes.
- Basic toxicology study design considerations apply.

## Toxicology study design

- Studies should be done GLP whenever possible
- Single-dose (acute) toxicity study may not be necessary if multiple doses have been evaluated in early proof of concept immunogenicity studies.
- Repeat-dose toxicity (pivotal study).
  - Dose level selection: “human-equivalent dose”, and possibly a multiple of this dose (contingent on dose volume)
  - Vaccine formulation and route of administration should be the same as the intended clinical route
  - Dose schedule should exceed the human schedule (at least “plus one”)
  - Timing of dose administrations should closely follow human course, although it may be accelerated (episodic, not daily)
  - Recovery group is recommended

## Standard endpoint evaluation

- Clinical observations
- Body weight
- Clinical pathology (hematology and clinical chemistry)
  - Some changes are expected (e.g., globulin levels)
- Immunological assessment (immunogenicity/response to vaccine)
- Anatomic pathology (organ weights, macro- and microscopic evaluation)
  - “Pivotal” organs, including lymphoid tissue
  - Administration site
- Assessment of local tolerance (reactogenicity) using a human-equivalent dose
  - Difficult due to volumes needed (up to 0.5 mL/dose)

## Non-standard endpoint assessment

- Special immunological investigations:
  - Ex: potential vaccine induced immunosuppression (V antigen of *Y. pestis*)
  - Ex: effect of live vaccine in immunocompromised individuals
- Developmental toxicology
- Genotoxicity/carcinogenicity:
  - Ex: genomic integration by DNA vaccines
- Safety pharmacology:
  - Ex: Potential neurotoxicity of anti-botulinum vaccine
- Pharmacokinetics (generally not needed)
  - Ex: Persistence of a live vector or vaccine in select tissues

## Other considerations

- Adjuvants:
  - Must be separately addressed as an NCE and tested for toxicity
  - Need for adjuvant should be demonstrated
  - Compatibility with the vaccine antigen must be addressed early
  - Special case: due to their intended activity, potential for immunotoxicity must be considered
- Additives (excipients, preservatives): toxicity must be assessed
  - Stand-alone toxicity of all components must be addressed
- Devices – especially important for novel delivery devices



## Special cases

- Live attenuated vaccines:
  - Degree of attenuation and the stability of the attenuation phenotype should be addressed.
  - If the wild-type organism is neurotropic a test for neurovirulence should be performed at least at the level of the vaccine seed.  
**Reversion to full virulence is crucial parameter.**
  - If the live attenuated vaccine is based on a genetically modified organism, then an environmental risk assessment may be required as part of the preclinical evaluation.
- Combination vaccines:
  - Physiochemistry is important (interaction of Ags with each other; effect of combination on adjuvant, etc.)
  - Immunological response should be tested separately and in combination

## Reproductive toxicology of vaccines

- Necessary when vaccine may be administered to females of child-bearing potential
- Standard reproductive toxicology testing for drugs is not strictly appropriate for vaccines
- Studies should use appropriate animal model
  - Vaccine should have demonstrated immunogenicity in the animal
  - Understanding of the kinetics of antibody formation (and vector clearance for living vaccines) is crucial
- Conducted using Phase 3 human dose and schedule
- Study design should evaluate embryo-fetal and neonatal toxicity
- Male reproductive toxicity not necessarily considered

## Assessment of clinical toxicity

- For preventive vaccines, the use of healthy volunteers significantly limits the tolerance for risk.
- Acceptance criteria for volunteers, and evaluation of health during the clinical trial portion of the development life-cycle, is tightly controlled.
- Certain vaccines may require very large Phase 3 studies given the importance of ensuring safety.
- Safety assessment continues through all phases of clinical trials, including post-licensure.
  - Provisions must be made in the life-cycle to accommodate this testing.
  - Safety data trending especially important in vaccines.

## Example of clinical abnormalities

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
<b>Pain</b>	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or interferes with daily activity	Emergency room (ER) visit or hospitalization
<b>Tenderness</b>	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
<b>Erythema/Redness</b>	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
<b>Induration/Swelling</b>	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

**Similar tables apply to urine, hematology, clinical chemistry, vital signs and systemic illness.**



# Case Study 1: Live Bacterial Vaccine

## Tularemia vaccine

- Tularemia Live vaccine strain (LVS) developed by DoD from USSR source material.
- LVS is an “attenuated” organism, although the mechanism of attenuation is unknown.
- LVS existed previously only as an IND product.
- Early development was empirical; no standardized toxicology; non-GMP; limited route/dose studies.
- Efficacy was tested in humans by aerosol exposure.



## “Acute” toxicity evaluation

- Establish safety and tolerability of LVS by various potential clinical routes.
- New Zealand White (NZW) rabbit used as a model:
  - Appropriate susceptibility
  - Commonly used for toxicity testing
  - Previous studies with rabbits inoculated percutaneously or subcutaneously with up to  $10^9$  cfu LVS resulted in no deaths
- Study designed in close cooperation with FDA.

## Acute toxicity study design

- Male rabbits (n=5/group) given a single 0.1mL dose of LVS by one of three routes:
  - SC, ID, or scarification
- Highly characterized LVS used as vaccine
- Dose range from  $10^5$  to  $10^9$  cfu in log increments
- PBS administered as vehicle control
- Clinical observations through day 34
- Gross necropsy performed day 35



## Acute toxicity study results

- Vaccine was well tolerated at all doses
- No deaths or adverse clinical signs
- Dose-related reactogenicity in LVS-treated rabbits
  - Erythema/eschar most persistent in ID cohort
- Gross necropsy findings within normal limits
- LVS not detected in liver or spleen at necropsy



## Reactogenicity of LVS in rabbits

**PBS (SC)**



**SCARIFICATION**



**SUBCUTANEOUS**



**INTRADERMAL**



## “Definitive” toxicity study design

- NZW rabbits, (n=3 animals/sex/group)
- A single 0.1mL dose of  $10^6$  or  $10^8$  cfu LVS given via scarification or SC; PBS used as vehicle control
- Two data collection points within each group
  - Clinical observations through Day 15 or 49
  - Necropsies performed on Day 16 or 50
- Endpoints: mortality/morbidity, clinical observations, reactogenicity, body weight gains and food consumption, clinical pathology, anatomic pathology

## Definitive toxicity study results

- No deaths or adverse clinical signs observed.
- Local reactogenicity in LVS-treated groups.
- No toxicologically-significant pathology (clinical or anatomic); few clinical chemistry changes probably due to the immune response and not toxicity.
- LVS not detected in liver or spleen at either time point.
- Microscopic findings consistent with transient response expected with administration of a live bacterial vaccine.
- Doses up to  $10^8$  cfu by well tolerated with either route of administration.
- Histiocytosis observed in the lungs (confirmed by peer review); relevance unknown.



# **Case Study 2: Recombinant Peptide Vaccine**

## Recombinant botulinum vaccine (rBV A/B)

- Codon and transcription/translation signal optimized for expression in the methylotrophic yeast *Pichia pastoris*.
- Expression system gives high yield at BSL-1.
- Bivalent formulation of A and B antigens in aluminum-containing adjuvant.

## rBV A/B nonclinical study overview

- Protection:
  - Determine protection against botulinum neurotoxin A or B following a single vaccination with 2 to 20  $\mu\text{g}$  in mice
- Immunogenicity:
  - Determine immunogenicity following administration with or without adjuvant
- Preclinical safety preparatory to human Phase 1:
  - Repeat-dose toxicity (with recovery) in mice
  - Reactogenicity in rabbits
  - Immunogenicity in mice
  - Functional observational battery in mice

## rBV A/B nonclinical study outcomes

- Protection
  - A single vaccination with 2 to 20  $\mu\text{g}$  fully protected mice from challenge with 1,000  $\text{LD}_{50}$  of botulinum neurotoxin A or neurotoxin B by day 28 post-vaccination.
- Immunogenicity
  - Repeated administration and inclusion of adjuvant enhanced the immunogenicity of rBV A/B as determined by serum neutralizing antibody concentrations
- Safety
  - Transient reactogenicity only in animals receiving adjuvanted vaccines (not adjuvant only or antigen only)
  - Multifocal subacute subcutaneous infiltrates (similar in description to macrophagic myofasciitis)



## **Case Study 3: “Para-vaccine”**

## **Vaccinia Immune Globulin – Intravenous (VIG-IV)**

- Licensed product derived from human hyperimmune serum (volunteers immunized with Dryvax smallpox vaccine).
- Intended to counter rare adverse events associated with vaccination with live vaccinia vaccine.
- Model development for FDA-mandated bioactivity study involved administration of live vaccine in immunocompetent and immunocompromised mice.
- **Study illustrated vaccine safety issue in an unexpected way: vaccination in immunodeficient individuals.**

## Vaccination in immunodeficient individuals

- Live vaccines can *theoretically* become fulminant in immunodeficient individuals.
- For replication-competent organisms, the degree of host immunocompromise that would allow breakthrough has not been determined.
- Mechanism of resistance not always apparent (e.g., vaccinia in SCID mice).
- Possibility of sequestration in immunologically-privileged site during immunization and re-emergence during immunodepressed state.
- Best mechanism for prevention of serious effects is proper attenuation of the organism.



## Vaccinia in normal and immunosuppressed mice



**Immunocompetent**

**Immunodeficient**



## Conclusions

- Although responsible for immeasurable improvements in human health, all vaccines have inherent risks.
- Due to biological differences in the diseases, and state of the science in animal models, designing animal safety studies continues to be done on a case-by-case basis.
- Vaccines exhibit numerous characteristics that make safety assessment unique. These include diversity of physiochemical characteristics, nonstandard dose-response relationships, and (with live agents) self-replication.
- Study design for toxicity and overall safety of vaccines must consider routine endpoints, as well as specialized parameters that are unique to the vaccine.