Guidance for Industry

Considerations for Plasmid DNA Vaccines for Infectious Disease Indications

DRAFT GUIDANCE

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For questions on the content of this draft guidance, contact Herbert A. Smith, Ph.D., at (301) 827-3070.

U.S. Department of Health and Human Services
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Contains Nonbinding Recommendations

Draft – Not for Implementation

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This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

In December 1996, FDA issued a guidance document, "Points to Consider on Plasmid DNA Vaccines for Preventive Infectious Disease Indications," to assist the developers of DNA vaccines. That document delineated the manufacturing, preclinical, and clinical issues relevant to the development of DNA vaccines, and described potential safety concerns that we, FDA's Center for Biologics Evaluation and Research (CBER), recommended vaccine developers address prior to the initiation of phase 1 clinical studies. The recommendations for DNA vaccine manufacture and testing provided in that document were based on our experiences with other types of vaccines and DNA-based products, including gene therapy agents.

In the intervening years, we have concurred with the initiation of phase 1 clinical studies of DNA vaccines for a number of infectious disease indications including malaria, hepatitis B, and human immunodeficiency virus (HIV). The initiation of phase 1 clinical studies is predicated on you, the manufacturers and/or sponsors of vaccine clinical studies, documenting the quality and consistency of plasmid manufacture, combined with extensive preclinical safety studies. Considerable clinical experience has been accumulated since the issuance of the above 1996 guidance on plasmid DNA vaccines, and we need to update that guidance. This guidance, when finalized, will update and replace the 1996 guidance document.

FDA helps ensure that clinical studies provide critical information on vaccine safety and immunogenicity without placing undue or unreasonable demands on vaccine study sponsors. Ongoing interactions between FDA and vaccine study sponsors are designed to achieve these goals. This update to the 1996 Points to Consider document describes our current recommendations for the development and testing of DNA vaccines.

For the purposes of this document, DNA vaccines are defined as purified preparations of plasmid DNA designed to contain one or more genes from a pathogen as well as regulatory genetic elements to enable production in a bacterial host system. Typically, these plasmids possess DNA sequences necessary for selection and replication in bacteria. In addition, they contain eukaryotic promoters and enhancers as well as transcription termination/polyadenylation sequences to promote gene expression in vaccine recipients, and may contain immunomodulatory elements. DNA vaccines are biological products as set forth in section 351 of the Public Health Service Act (PHS) (42 U.S.C. 262) and are regulated by CBER. The principal regulations applicable to DNA vaccines are located in 21 CFR Parts 210, 211, 600, 601, and 610. Other guidance documents are available from CBER and may contain information that is relevant to DNA vaccines. Some of these documents are listed below and additional guidance documents may be found on the CBER website (http://www.fda.gov/cber/guidelines.htm) or the CDER website (http://www.fda.gov/cder/guidance/index.htm).

This document is intended to assist you in your development of DNA vaccines to prevent infectious diseases. This guidance is not necessarily applicable to DNA vaccines for the treatment of established diseases (infectious or malignant), since subjects with ongoing disease may require more aggressive therapy with a different margin of safety than prophylactic vaccines administered to healthy individuals. Applications for DNA vaccines designed to prevent or treat infectious diseases should be submitted to CBER's Office of Vaccines Research and Review (OVRR) where primary review responsibility is assigned. Plasmid DNA products intended for non-infectious therapeutic indications are not addressed in this guidance. Applications for these products should be submitted to CBER's Office of Cellular, Tissue and Gene Therapies (OCTGT).

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. MANUFACTURING ISSUES

The following sections describe the manufacturing information we recommend that you submit to us for a new DNA vaccine product for clinical study under an Investigational New Drug Application (IND).

A. Product Manufacture

We recommend that the manufacturing summary describe all components used during manufacture as well as those present in the final product. We recommend that you provide detailed descriptions of the plasmid construction, including the source and diagrams of all plasmids used, and all intermediate recombinant DNA cloning procedures. We recommend that the DNA sequence of the entire plasmid be provided by direct sequencing of the plasmid present in the Master Cell Bank (MCB). During production, other methods of sequence verification, such as restriction enzyme mapping and polymerase chain reaction (PCR) may be employed at intermediate steps.

We recommend that you describe the genotype, phenotype, source of the bacterial cells and the procedures to construct master and working cell banks used for production. Specific guidance for the establishment of MCBs and Working Cell Banks (WCBs) is described in the "Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (1993)".[1] We recommend that you test both the MCBs and WCBs to ensure that they are free from bacteriophage and other adventitious agent contamination, and that you establish the genetic stability of the MCB and WCB.

We recommend that the description of the manufacturing process be sufficiently detailed to enable an assessment of the safety of the product. If changes in product manufacture occur during the development of plasmid products intended for clinical studies and preclinical lots manufactured for safety evaluation, we recommend that you provide a clear summary illustrating all differences between lots of vaccine used in preclinical studies and those intended for use in clinical studies.

B. Bulk Plasmid Product Release Testing

If bulk and final product are the same (i.e., if production runs yield one lot and no further steps in formulation are performed), then testing as described below may be redundant and unnecessary. We recommend that you test bulk plasmid products for the properties described below, and that you use standard assay(s) of adequate specificity and sensitivity. We recommend that you evaluate assay methods by testing known amounts of reference materials or spiked samples, or by other appropriate measures, and that you submit to CBER data documenting assay performance. In addition to bulk and final product release testing, we recommend that you also perform in-process testing to ensure manufacturing consistency and product safety. Prior to the initiation of phase 1 clinical studies, we recommend that you initiate stability testing as early as possible to support use of the product for the duration of the proposed clinical investigation.

[1] Document is available on CBER's website at: (http://www.fda.gov/guidelines.htm).

Typically, the bulk release criteria will include tests for visual appearance and plasmid concentration. We recommend that the bulk release criteria describe the extent of circular plasmid present with establishment of a minimum specification. We recommend that you characterize the product for the extent of supercoiled plasmid in the bulk preparation and that you establish a minimum specification (preferably >80%). We recommend that you evaluate bulk plasmid preparations for the presence of bacterial host cell contaminants to include DNA, RNA, and protein and set limits for the maximum level of each of these contaminants. We generally recommend that host cell contaminants be at as low a concentration as is technically feasible. We recommend that you perform a test for pyrogenic substances and that you include the test results with the bulk release documentation. The Limulus Amebocyte Lysate (LAL) test is a sensitive indicator of the presence of bacterial endotoxins and endotoxin contamination should not exceed 5.0 EU/kg body weight for the intended recipients.

We recommend that you include a test to establish the identity of the bulk product by restriction enzyme analysis in the bulk release criteria. When a single manufacturing facility is used to manufacture more than one DNA vaccine product, we recommend that you perform identity tests capable of distinguishing individual plasmids.

We recommend that you develop a potency assay. During early product development, we will allow sponsors considerable flexibility in the selection of potency assays. This could include *in vitro* measures of transfection efficiency that monitor the transcription and/or translation of the encoded gene(s). Assays that monitor *in vivo* immunogenicity of the DNA vaccine are preferred. We recommend that assays be quantitative. We recommend that as product development proceeds towards licensure, you provide evidence that *in vitro* potency correlates with *in vivo* immunogenicity. We recommend that sponsors maintain retention samples of each lot to facilitate comparisons between lots as assay development progresses. The selection and implementation of a potency assay may be discussed with CBER to ensure acceptability of the design.

C. Final Product Release Testing

We recommend that you test the final DNA vaccine product for potency, general safety, sterility, purity, quantity, and identity. The test methods and specifications may be the same as those employed for the bulk product release. To detect extraneous toxic contaminants potentially introduced during manufacture, we recommend that you perform the general safety test in mice and guinea pigs on each final product lot. If the plasmid product is lyophilized we recommend that you perform a test for residual moisture. We recommend that you perform a test for the presence of endotoxin on each lot of final product. In addition to final product release testing, we recommend that you also perform in-process testing to ensure manufacturing consistency and product safety.

We recommend that you establish acceptance criteria and acceptable limits and that you report the results for each lot of vaccine to be used for clinical studies.

III. DNA VACCINE MODIFICATIONS

A. Changes to the Insert or Vector

Changes to the DNA sequence of the insert gene or vector sequences of a DNA vaccine would require the submission of a new IND (See section 351 of the PHS Act and 21 CFR Part 312). We recommend that you include in the IND a description of the manufacturing process and the results from preclinical safety evaluation of the new (modified) DNA vaccine.

B. DNA Sequence Analysis

An issue of product identity of particular relevance to DNA vaccines concerns the degree to which plasmids should be sequenced before the initiation of phase 1 clinical studies. In 1996, we recommended that manufacturers provide (at a minimum) the sequence of the protein-encoding gene insert. Based on evidence that the plasmid backbone may influence vaccine activity, and recognizing that technological advances since 1996 have facilitated DNA sequencing, we recommend that manufacturers provide the complete sequence of the plasmid before initiating phase 1 clinical studies.

Some DNA vaccines contain a complex mixture of plasmids, with each plasmid carrying a gene encoding a different antigenic protein. For example, a vaccine may contain multiple variants of a highly mutable gene (such as the gene encoding the envelope of HIV-1) or the entire genome of a microorganism may be 'shotgun cloned' into a common plasmid backbone. We advise you to establish the identity and amount of each plasmid component in the vaccine preparation to ensure lot-to-lot consistency. However, there may be instances when technical limitations prevent complete sequence information from being obtained on a heterogeneous mixture of plasmids before initiation of phase 1 clinical studies. In such instances, the amount of sequence information required will be evaluated on a case-by-case basis.

IV. PRECLINICAL IMMUNOGENICITY AND SAFETY

A. General Considerations

Preclinical safety evaluation is required for all new vaccines, including DNA vaccines, prior to their use in clinical studies (21 Code of Federal Regulations (CFR) 312.23). We recommend that you perform preclinical safety studies on every novel DNA vaccine or DNA vaccine/adjuvant combination. We may modify the preclinical safety evaluation requirements in specific situations where multiple variants of a specific gene (such as HIV-1 Env) are cloned into the same plasmid vector on which a complete safety evaluation has already been performed. We recommend that you consult with CBER well in advance of IND submission to evaluate the adequacy of preclinical safety studies and prior human experience to support the investigational vaccine product. Pivotal animal safety studies must be performed in accordance with Good Laboratory Practice (GLP) regulations (21 CFR Part 58).

B. Immunogenicity

We recommend that you develop assays to assess immunological potency in animal models. This could include the evaluation of antigen-specific antibody titers, seroconversion rates, activation of cytokine secreting cells, and/or measures of cell-mediated immune responses. Optimally, these studies are designed to collect information regarding the duration of the immune response. For complex DNA vaccines encoding multiple antigens, we recommend that you assess the immune response generated against a representative subset of the encoded antigens.

C. Autoimmunity

Published preclinical studies indicate that DNA vaccination can activate autoreactive B cells to secrete IgG anti-DNA autoantibodies (See Section VI, References). However, the magnitude and duration of this response appears to be insufficient to cause disease in normal animals or accelerate disease in autoimmune-prone mice. These preclinical studies helped to establish that systemic autoimmunity is unlikely to result from DNA vaccination. Similarly, the absence of an immune response against cells expressing the vaccine-encoded antigen (including muscle cells and dendritic cells) suggests that an autoimmune response directed against tissues in which such cells reside is unlikely. Based on these findings, we will no longer expect that you perform preclinical studies to specifically assess whether vaccination causes autoimmune disease.

The possibility persists that DNA vaccines might idiosyncratically cause or worsen

organ-specific autoimmunity by encoding antigens (including cryptic antigens) that cross-react with self. Thus, we recommend that you continue to monitor the general well being of animals participating in preclinical immunogenicity and toxicity studies, and of all human trial participants. In cases of immunity developing against a transgene product (such as a cytokine), we recommend that you examine potential cross-reactivity with the corresponding endogenous protein. Studying an animal model using a construct containing the analogous animal gene is recommended to evaluate potential adverse effects.

D. Tolerance

Published studies to address whether DNA vaccines could induce neonatal tolerance yielded divergent results (see Section VI, References). Most DNA vaccines did not induce tolerance in neonatal animals, but idiosyncratic examples of neonatal tolerance have been observed (see Section VI, References). Tolerance has never been observed following vaccination of mature animals. Taken together, these studies suggest that the capacity of a DNA vaccine to induce tolerance may depend on the nature of the encoded antigen and the age at which, and frequency with which, the vaccine is administered. Based on these findings and other considerations, we recommend that prior to use of a DNA vaccine in children or newborns that: i) you first test the vaccine for safety and immunogenicity in adults, and ii) you utilize appropriate preclinical models to evaluate the potential of such vaccines to induce neonatal tolerance.

E. Challenge/Protection, Cytokines, Prime/Boost

When appropriate and where possible, we encourage animal challenge/protection studies with the corresponding infectious agent early in development to demonstrate the rationale for the use of the investigational vaccine. For DNA vaccines that co-express cytokine genes, you should consider specific preclinical studies in animal species responsive to the encoded human cytokine or models using the analogous animal genes to assess whether modulation of the cellular or humoral components of the immune system might result in unintended adverse consequences, such as generalized immunosuppression, chronic inflammation, autoimmunity or other immunopathology. When plasmid DNA vaccines are used in vaccination strategies employing a corresponding subunit vaccine, such as in prime and boost study designs, we recommend that you submit specific preclinical information to support the safety and tolerability of the proposed dose, schedule, and route of administration of each vaccine combination.

F. Local Reactogenicity and Systemic Toxicity Studies

Studies designed to assess systemic toxicity may be combined with assessment of local

site reactogenicity. We recommend that you conduct these studies using the highest dose of vaccine planned for clinical use. You may conduct studies of additional doses to provide further support for vaccine safety. An accelerated schedule of vaccine delivery will be considered (preferably vaccination intervals of 3 to 4 weeks), and should include at least one immunization beyond that planned for clinical use. We recommend that the assessments written into the preclinical study protocols include toxicity to potential target organs, including the hematopoietic and immune systems. We recommend that preclinical studies also include clinical pathology assessments (serum chemistry, hematology, and coagulation tests), and histopathology, encompassing both gross and microscopic assessment of tissues.

We recommend that studies of local site reactogenicity include detailed clinical observations of the injection site(s) following each vaccine administration and histological evaluations of injection-site tissue obtained from biopsies or term necropsy samples. We recommend that you 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.

G. Biodistribution and Integration Analysis

Plasmid biodistribution, persistence and integration studies were initially recommended by CBER to determine whether subjects in DNA vaccine trials were at heightened risk from i) the long-term expression of the encoded antigen either at the site of injection or an ectopic site, and/or ii) integration of the plasmid that might increase susceptibility to malignant transformation. Publications resulting from the use of DNA vaccines in clinical studies under IND indicate that intramuscular, subcutaneous, intradermal, or particle-mediated delivery does not result in long-term persistence of plasmid at ectopic sites, and that $\leq$30 copies of plasmid per 10^5 host cells persist at the site of injection after 60 days (see Section VI, References). Before conducting biodistribution/persistence studies, you should contact FDA for advice concerning the need for these studies in particular, when: i) new or significantly modified plasmids are proposed for clinical use, and/or ii) the formulation of the DNA vaccine and/or its method/route of delivery may significantly increase cellular uptake or alter plasmid distribution.

We recommend that you conduct biodistribution/persistence studies when modifications to the vector, inserted gene, method of delivery, route of administration, or formulation significantly impact cellular uptake or immunogenicity. We recommend that all preclinical immunogenicity, toxicity and biodistribution/persistence studies evaluate the formulation and method of administration proposed for the clinical study. This would include assessing any adjuvant or active excipient in the vaccine, and/or the use of a device to administer the vaccine. A typical biodistribution study will assess the presence

of plasmid collected from a panel of tissues at intervals of 7, 30, and 60 days post-administration. The panel of tissues typically includes the blood, heart, brain, liver, kidney, bone marrow, ovaries/testes, lung, mesenteric lymph nodes, spleen, adrenal gland, muscle at the site of administration and subcutis at the injection site. The presence of the DNA vaccine is typically evaluated using a semi-quantitative real time polymerase chain reaction (Q-PCR) study validated for sensitivity and specificity. We recommend that such assays be able to detect 1 copy of plasmid in DNA from 10^5 host cells.

We have determined that integration studies are not necessary when biodistribution/persistence studies demonstrate that plasmid DNA does not persist in any tissue of any animal at levels exceeding 30 copies per 10^5 cellular genomes at 60 days post vaccination. If the DNA plasmid persists at significantly higher copy number at any site in any animal, we recommend that you study whether the DNA has integrated into the genome of the vaccinated animal. Theoretical concerns regarding DNA vaccine integration include the risk of mutagenesis if plasmid insertion reduces the activity of a tumor suppressor or increases the activity of an oncogene. In addition, integration of a DNA vaccine may result in chromosomal instability through the induction of chromosomal breaks or rearrangements. Typically, Q-PCR is used to detect plasmid DNA in genomic DNA preparations. Specifically designed PCR primers may be used to distinguish between integrated and non-integrated plasmids.

V. CONCLUSION

This document is intended to inform manufacturers/sponsors about current CBER recommendations related to the development of DNA vaccines. We recommend that manufacturers/sponsors of these products concentrate their efforts on the pivotal preclinical safety issues. CBER recommends early consultation to further discuss the issues related to the development of their vaccine.

VI. REFERENCES: REGULATIONS AND APPLICABLE GUIDANCE DOCUMENTS, AND RELEVANT PUBLICATIONS

- **U.S. CODE OF FEDERAL REGULATIONS**
  - 21 CFR PART 50 - Protection of Human Subjects
  - 21 CFR PART 56 - Institutional Review Boards
  - 21 CFR PART 58 - Good Laboratory Practice for Nonclinical Laboratory Studies
  - 21 CFR PART 210 - Current Good Manufacturing Practice in Manufacturing, Processing, Packing, or Holding of Drugs; General
  - 21 CFR PART 211 - Current Good Manufacturing Practice for Finished Pharmaceuticals
  - 21 CFR PART 312 - Investigational New Drug Application
  - 21 CFR PART 600 - Biological Products: General
  - 21 CFR PART 601 - Licensing
  - 21 CFR PART 610 - General Biological Products Standards

- **POINTS TO CONSIDER DOCUMENTS**
  - Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology (4/85)
  - Supplement to the Points to Consider in the Production and Testing of New Drugs and Biologicals Produced by Recombinant DNA Technology: Nucleic Acid Characterization and Genetic Stability (4/92)
  - Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals (7/93)
  - Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use (2/97)

- **INTERNATIONAL CONFERENCE ON HARMONIZATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE (ICH) DOCUMENTS**
  - ICH; Guideline for Industry: Detection of Toxicity to Reproduction for Medicinal Products (9/94)
  - ICH; Guideline for Industry: Detection of Toxicity to Reproduction for Medicinal Products: Addendum on Toxicity to Male Fertility (4/96)
  - ICH; Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products (2/04)

- **FDA GUIDELINES**
  - Guideline for Submitting Documentation for the Stability of Human Drugs and Biologics (2/87)
  - Guideline on Sterile Drug Products Produced by Aseptic Processing (6/87); Draft Guidance for Industry: Sterile Drug Products Produced by Aseptic Processing-Current Good Manufacturing Practice (8/03) (This draft guidance when finalized, will replace the 1987 Industry Guideline on Sterile Drug Products Produced by Aseptic Processing.)
  - Guideline on Validation of the Limulus Amebocyte Lysate Test As An End-Product Endotoxin Test for Human and Animal Parenteral Drugs, Biological Products and Medical Devices (12/87)
  - Guideline for the Determination of Test Residual Moisture in Dried Biological Products (1/90)
  - Guideline on the Preparation of Investigational New Drug Products (3/91)
  - FDA Guidance Concerning Demonstration of Comparability of Human Biological Products, Including Therapeutic Biotechnology-Derived Products (4/96)
  - Guidance for Industry: Guidance for Human Somatic Cell Therapy and Gene Therapy

- **PUBLICATIONS RELEVANT TO THE ISSUE OF DNA VACCINE INDUCED NEONATAL TOLERANCE**:
  - Bona C, Radu D, Kodera T. Molecular studies on the iversification of hemagglutinin-specific human neonatal repertoire subsequent to immunization with naked DNA. Vaccine. 2004 Apr 16; 22(13-14): 1624-30.
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  - Wang Y, Xiang Z, Pasquini S, Ertl HC. Immune response to neonatal genetic immunization. Virology. 1997 Feb 17; 228(2): 278-84.
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- **PUBLICATIONS RELEVANT TO THE ISSUE OF PLASMID DNA BIODISTRIBUTION AND PERSISTENCE:**

- Kim BM, Lee DS, Choi JH, Kim CY, Son M, Suh YS, Baek KH, Park KS, Sung YC, Kim WB. In vivo kinetics and biodistribution of a HIV-1 DNA vaccine after administration in mice. Arch Pharm Res. 2003 Jun; 26(6): 493-8
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