Published in final edited form as:
Vaccine. 2010 April 1; 28(16): 2801–2805. doi:10.1016/j.vaccine.2009.11.025.

FDA Guidance on Prophylactic DNA Vaccines: Analysis and Recommendations

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Abstract

The FDA has been regulating the conduct of prophylactic DNA vaccine trials in the US for nearly 15 years. This work describes the evolution of FDA policy over that period, the status of current regulatory guidance, and provides recommendations for further changes to facilitate development in this field.

Keywords

DNA vaccines; guidance; clinical trials

1) Introduction to DNA vaccines

The prevention of communicable diseases is a public health priority and the primary goal of vaccine research. One promising strategy to achieve this goal involves the use of antigen-encoding DNA plasmids to induce protective cellular and humoral immune responses against pathogenic viruses, parasites and bacteria [1-3]. DNA vaccines are composed of an antigen-encoding gene whose expression is regulated by a strong mammalian promoter expressed on a plasmid backbone of bacterial DNA [1;3-5]. These plasmids incorporate DNA sequences necessary for selection and replication in bacteria, plus various promoters, enhancers, and other elements designed to increase expression of the encoded protein in vaccine recipients. Cells transfected with DNA vaccines transcribe, translate, and express the encoded protein(s) in the context of self MHC [1;3;6]. While influenced by the nature and route of plasmid DNA delivery, professional antigen presenting cells (APCs) play a dominant role in the resultant induction of immunity. APCs directly transfected in the skin or muscle migrate to the primary lymphoid organs where they initiate an immune response [7-9] and cross-present antigen produced by transfected non-immune cells (such as muscle cells) [3;10-14].

DNA vaccines intended for prophylaxis against infection have proven safe and effective in a number of animal studies [3;7;9;10]. Multiple phase I clinical trials involving DNA vaccines

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have been conducted [15-17]. Results from those trials indicate that although DNA vaccines appear safe, the immune response they elicit in humans is modest [15-20].

2) FDA Regulatory policy

2.1) Development of regulatory policy

Continued progress towards the clinical development of DNA vaccines is influenced by the regulatory environment created by the Center for Biologics Evaluation and Research of the US Food and Drug Administration (CBER/FDA). CBER sets and implements vaccine policy in accordance with it's interpretation of relevant Federal statutes, laws and guidelines. Existing CBER guidelines reflect the agency's experience in the regulation of other types of vaccines and biological agents. This conservative approach helps maintain consistency in product regulation, thereby insuring compliance with the US Code of Federal Regulations. CBER policy mandates that sufficient preclinical data be obtained in one or more “relevant” animal models to conclude that a DNA vaccine is likely to be safe and immunogenic before that vaccine proceeds into human clinical studies. Typically, pre-clinical studies are performed in mice to determine whether a vaccine is immunogenic and in rabbits to determine whether the vaccine causes acute or chronic toxicity. A variety of studies are also required to establish that the vaccine can be synthesized reproducibly, and that it remains stable under prolonged storage. Results from such pre-clinical studies influence decisions concerning the number, timing, and dose of vaccine that can be administered to humans.

The regulation of prophylactic DNA vaccines has evolved since clinical trials of these agents were initiated in the mid 1990's. The accumulation of pre-clinical and clinical experience, including information concerning plasmid manufacture, vector construction, vaccine immunogenicity, and safety, informed changes to regulatory guidelines. In 2007, the FDA updated it's guidance document concerning the manufacture and testing of DNA vaccines designed to reduce susceptibility to infectious diseases [21]. DNA vaccines intended for other uses, such as the treatment of autoimmune disease or cancer, were not covered by that document. This reflected differences in the level of risk deemed acceptable for products used to treat pre-existing illnesses versus prophylactic vaccines intended for use by the general public. The FDA's initial approval of phase I clinical trials of prophylactic DNA vaccines relied on evidence that plasmids could be manufactured consistently, coupled with extensive pre-clinical safety data. Early recommendations concerning DNA vaccine manufacture and testing were largely based on FDA experience involving other types of vaccines and DNA based products [22]. Since that time, considerable additional information and experience has accumulated concerning DNA vaccine manufacture, activity and safety [21;23]. That new information formed the basis for revisions in FDA regulatory guidelines [21].

2.2) Assessment of regulatory policy

The development of pharmaceutical products is envisioned as a linear process, wherein drugs or biologics discovered through basic research progress to pre-clinical animal testing and then into phase I - III clinical trials. The development of DNA vaccines has not followed this linear route, as immunogenicity concerns have short-circuited efforts to progress from small phase I to larger phase II/III immunogenicity and efficacy studies. To date, numerous phase I studies have been conducted to distinguish among the various plasmid components, sequence motifs, adjuvants, sites/methods of administration, and other variables in terms of their impact on vaccine immunogenicity [15-18]. Indeed, rather than developing a single product, those involved in DNA vaccine trials commonly design multiple “candidate” constructs (simultaneously or in succession) to identify elements that can be incorporated to improve the immunogenicity of subsequent vaccines.

While existing CBER guidelines seek to maintain consistency in product regulation and maximize compliance with the Code of Federal Regulations, such policy does not recognize or accommodate to the exigencies of DNA vaccine development. Thus, the conservative nature of current regulations may hinder efforts to improve vaccine performance. As noted above, most phase I DNA vaccine trials utilize "candidate" plasmids (or plasmid/adjuvant combinations) that are unlikely to proceed further towards licensure. An optimized regulatory policy for DNA vaccines would therefore facilitate the conduct of multiple phase I trials, with each trial involving only a small number of human volunteers.

2.3) Recommended changes to regulatory policy

Several policy changes would significantly lower the cost and accelerate the initiation of phase I studies:

i. CBER should require less extensive toxicology (including integration) testing for "candidate" vaccines entering phase I trials. The requirement for such data could be shifted to vaccines intended for phase II/III study.

RATIONALE: The authors recognize that this policy change might be perceived as increasing the risk to subjects participating in phase I trials. However even extensive pre-clinical animal testing does not necessarily reduce subject risk, as the predictive value of rodent studies for DNA vaccines used in humans is unreliable. Indeed, strong protective immune responses have been repeatedly achieved in mice but not in Man. In terms of the safety and toxicity studies performed in mice and/or rabbits, such studies uniformly indicate that DNA vaccines are safe [24-27]. In the absence of a DNA vaccine that causes toxicity, the reliability of either animal model in predicting an adverse clinical outcome cannot be ascertained. Thus, the added value of extensive toxicology testing for "candidate" vaccines is unclear. Moreover, the safety of DNA vaccines is by now well established in human clinical trials [17;20;26;28;29].

ii. CBER should require less demanding product manufacturing data for "candidate" vaccines entering phase I trials. The requirement for such data could be shifted to vaccines intended for phase II/III study.

RATIONALE: The safety profile of DNA vaccines has been excellent. Manufacturers need to establish that candidate vaccines used in phase I studies are sterile and free of endotoxin. As "candidate" vaccines are administered to only a handful of volunteers, issues of lot-to-lot consistency and purity become important primarily for those plasmids that proceed into phase II trials. Any potential risks associated with such a relaxation in regulatory requirements could be managed by conducting dose-escalation studies that minimized the number of volunteers exposed to novel vaccines.

iii. Increase the access of biotech and academic investigators to the FDA for advice on trial design/conduct. This would include increasing the number of pre-IND meetings allowed and permitting sponsors to contact FDA reviewers for advice "on the record" concerning their product. Mirroring the European regulatory model, the FDA could receive reimbursement for such broadened access, enabling the organization to hire and train the additional personnel needed to provide these services.

RATIONALE: Pre-IND meetings facilitate the open exchange of data and ideas between those producing new DNA vaccines and those regulating such agents. These meetings have proven extremely useful to manufacturer's and CBER reviewers alike. The number of pre-IND meetings was restricted years ago when CBER resources were limiting. New resources have been provided to the FDA, and should be channeled into supporting more of these highly effective interactions. Similarly,

allowing FDA reviewers to provide reliable advice on an ongoing basis would be of considerable benefit to vaccine manufacturers.

iv. Define “candidate” vaccines as “well characterized products”. This would allow the biochemical analysis of plasmids to substitute for biological potency assays of “candidate” vaccines. Similarly, the conduct of comprehensive biodistribution and integration studies could be shifted to those products entering phase II trials, speeding and reducing the cost of phase I studies.

RATIONALE: All biological agents entering clinical trial should be sterile and non-reactogenic. However most “candidate” DNA vaccines will not progress beyond phase I study. In such cases, requirements concerning the nature and extent of product characterization should be limited.

3) Description of Current Regulatory Guidance

3.1) Evolution of current regulatory guidance

In December 1996, the FDA issued a guidance document to assist DNA vaccine developers entitled, “Points To Consider on Plasmid DNA Vaccines for Preventive Infectious Disease Indications” [22]. That document provided recommendations concerning the manufacture, pre-clinical and clinical issues relevant to the development of DNA vaccines, and raised safety concerns to be considered by vaccine developers prior to the initiation of clinical studies. Thus guidance was revised in 2007 as greater understanding of the manufacturing, pre-clinical, and clinical issues associated with DNA vaccines accumulated [21;23]. An overview of the key changes in regulatory perspective between the original Points to Consider document and the revised Guidance document on prophylactic DNA vaccines is provided below, accompanied by commentary on additional regulatory strategies that might further promote DNA vaccine development.

3.2) Manufacturing and Lot Release Issues

An important goal of FDA guidance is to insure that the methods, processes and facilities used for vaccine manufacture yield a product that is consistently safe, pure, and potent [30]. As noted above, a decade of experience with DNA vaccines should allow CBER to designate these agents as “well characterized products”. This would speed and simplify the production of “candidate” vaccines for clinical testing. Based on evidence that the backbone of a DNA plasmid may influence vaccine activity [4;5], combined with technological advances that substantially reduced the time and cost of DNA sequencing, manufacturers should continue to provide a plasmid's complete sequence prior to the initiation of phase I trials. This sequence should be fully annotated, and any differences from the predicted sequence identified and explained.

When initially produced by bacteria, plasmids tend to be full-length and in a super-coiled conformation. Subsequent steps in manufacture and storage can result in the plasmid being nicked or cut, which converts the super-coiled plasmid into open circular and eventually linear forms. Opinion is split concerning the degree to which degradation from super-coiled to closed circular forms of plasmid impact vaccine immunogencity. The FDA elected to err on the side of caution and recommend that lot release criteria include a minimum specification that ≥80% of the product be composed of super-coiled plasmid. However, if a sponsor can document that a specific vaccine remains highly immunogenic despite a lower super-coiled plasmid content, this criterion can be relaxed.

Recommendations concerning the potency assays used to assess product quality were revised in the 2007 Guidance. Historically, potency was evaluated by monitoring vaccine immunogenicity *in vivo*. The FDA elected to provide sponsors with considerable flexibility in

the selection of potency assays during initial clinical development (phase I and early phase II). In addition to *in vivo* measures of immunogencity, it was deemed acceptable to evaluate potency using *in vitro* measures of transfection efficiency, including studies that monitor the uptake, transcription, and/or translation of the encoded gene(s). As product development proceeded towards licensure, the development of quantitative potency assays that evaluate protein production and/or immunogenicity *in vivo* were considered most relevant.

3.3) Preclinical Safety Issues

Potential safety concerns were identified by the FDA prior to the first DNA vaccine entering clinical trial. Those safety issues were largely derived from previous experience with other types of vaccines, and included the possibility that DNA vaccination might i) stimulate the production of autoantibodies against the plasmid's DNA, potentially inducing or accelerating the development of systemic autoimmune diseases such as systemic lupus erythematosus (SLE); ii) induce a local inflammatory response against cells expressing the vaccine encoded antigen, facilitating the development of organ-specific autoimmune disease through epitope spreading; iii) result in the development of tolerance rather than immunity to the encoded antigen (which was expressed by host cells and presented in the context of self MHC), thereby increasing the vaccinee's risk from infection; iv) selectively enhance the hosts' Th1 cytokine response due to CpG motifs present in the plasmid backbone and/or v) result in the integration of plasmid DNA into the genome of host cells, increasing the risk of carcinogenesis or other genetic abnormalities [31-36].

Accumulated data from animal studies showed that DNA vaccines could increase the production of anti-DNA autoantibodies, but that the magnitude of this effect was insufficient to accelerate or increase disease severity in lupus-prone mice or induce autoimmunity in normal animals [33;34]. Other studies indicated that an immune response was induced against cells expressing the vaccine-encoded antigen, but that non-transfected cells in the same tissue were not targeted for elimination by the immune system [34]. Finally, articles describing the safety of DNA vaccines used in clinical trials provided no indication that systemic or organ-specific autoimmune diseases was being elicited in vaccine trial participants. Based on these findings, the 2007 Guidance document concluded that sponsors no longer need to perform preclinical studies to specifically assess the effect of vaccination on autoimmunity. Rather, established clinical monitoring procedures were sufficient to determine if vaccination induced any adverse events, including the induction of autoimmune disease.

There is no evidence from pre-clinical or clinical trials that DNA vaccines result in the development of tolerance in adults. The possibility that such vaccines might induce tolerance in newborns or young children remains a matter of dispute. Despite evidence that long-term tolerance can develop following DNA vaccination of neonatal mice [32;34], immunity is the more likely outcome when newborns are vaccinated [37-40]. The revised Guidance documents concludes that once vaccine-induced protection is achieved in adults, careful pre-clinical studies using an appropriate animal model should enable studies in progressively younger subjects.

3.4) Plasmid Integration issues

DNA is considered a contaminant of “conventional” (non-DNA) vaccines. Manufacturers typically minimize or remove extraneous DNA during the production process to minimize human exposure to potentially injurious material. Not surprisingly, concerns that plasmid DNA might integrate into the host genome, increasing the likelihood of malignant transformation, genomic instability, or cell growth dysregulation were raised when DNA vaccines were first proposed for clinical use [31;36;41]. Based on advice from the Vaccine's Advisory Committee,

the FDA set guidelines designed to insure that the frequency of plasmid integration would be substantially lower than the spontaneous mutation rate [42].

Initial efforts to examine whether DNA vaccines could integrate into the host genome involved monitoring the biodistribution and persistence of plasmids *in vivo*. In most studies, DNA was isolated from tissues of vaccinated animals and the presence of plasmid examined using sensitive Q-PCR assays. Integration was then assessed by size fractionating high molecular weight genomic DNA free from smaller unintegrated plasmids (this sometimes required restriction endonuclease digestion to eliminate plasmid concatemers) [24;43;44]. Q-PCR and/or RAIC-PCR were then used to detect and quantify residual plasmid present in the genomic DNA preparation. Results showed that conventional intra-muscular, subcutaneous, intra-dermal, and particle-mediated delivery of DNA plasmids rarely resulted in the long-term persistence of plasmid in tissues distal from the site of vaccine administration [24;43-46]. In contrast, tissue at or near the site of administration (such as muscle and the overlying skin) commonly contained thousands or even millions of copies of plasmid per microgram of host DNA shortly after delivery [24;43-49]. Over time, the vast majority of this plasmid disappeared [43-49]. Based on studies involving multiple different plasmid backbones, and of the same plasmid backbone with various inserts, the revised FDA Guidance document indicated that biodistribution/ persistence studies could be waived for vaccines prepared using a plasmid vector previously documented to have an acceptable biodistribution/integration profile. For novel plasmids or methods of formulation and delivery, integration studies would be required when plasmid persists at levels exceeding 10,000 copies per ug of host DNA. This recommendation reflects the expectation that only a tiny fraction of persisting plasmid will integrate into the host genome, regardless of the method of delivery [45].

In evaluating the potential harm of plasmid integration, it should be noted that the risk of introducing plasmids with strong regulatory regions into the host genome far exceeds that associated with random point mutations [43;50]. Moreover, the technology used to detect plasmid persistence does not examine the frequency with which short fragments of plasmid integrate. In this context, sections of DNA as short as 7 bp can affect rates of integration or recombination. Examples include the VDJ recombination signal sequence and related sequences, chi-like elements and minisatellites, ALU sequences, a recombinase signal present in hepatitis B and mammalian genomes, and topoisomerase II recognition sites [43].

3.5) General toxicity issues

The 2007 Guidance recommends that local reactogenicity and systemic toxicity be thoroughly evaluated prior to the initiation of phase I studies, and that such studies test the highest dose of vaccine planned for human use, delivered “N + 1” times (where N = the number of planned human vaccinations). The Guidance recommends that both short-term and persistent toxicity be evaluated in separate cohorts of animals 2 - 3 days and 2 - 3 weeks after final vaccination. Recommended pre-clinical toxicity studies include serum chemistry, hematology, and coagulation tests plus gross and microscopic histology (with special attention to organs potentially targeted by DNA vaccination, such as the hematopoietic and immune systems). Of note, the FDA has accumulated enormous experience concerning the safety profile of DNA vaccines. Thus, their requirement that complete toxicology studies be performed prior to the initiation of phase I clinical studies deserves re-evaluation. There are no reports indicating that these expensive and time consuming studies (typically performed in rabbits) have lowered the risks associated with human DNA vaccination. Rather than require such studies prior to the initiation of small phase I studies involving “candidate” plasmids, this requirement could reasonably be shifted to products entering phase II trials.

4) Human Clinical Studies

Results of clinical trials involving plasmid DNA vaccines for the prevention and/or treatment of influenza, HIV, malaria, hepatitis B, SARS and many other infectious agents have been reported [15;20;29;51;52][16;17;19;26;28;53]. Doses of plasmid up to 5 mg have been administered, with some vaccinees receiving a cumulative dose of 12 mg following multiple injections [15;20;29;51;52]. These DNA vaccines have typically been delivered intramuscularly or intradermally, using needle and syringe, needleless injectors (such as the Biojector device), or gene gun [15;16;19;20;29;51-53].

These phase I clinical studies were designed to determine whether DNA vaccines could safely induce immune responses in trial participants. Various immunogenicity parameters were monitored, including the amount and isotype of antibody produced against the vaccine-encoded protein, the activation and/or proliferation of PBMC, the production of cytokines, and/or the development of antigen-specific cytotoxic activity. While evidence of both B and T cell stimulation has been reported, the magnitude of the immune responses elicited in phase I DNA vaccine trials has been modest [15;17;20;29;51;52]. Efforts are underway to improve DNA vaccine immunogenicity by boosting the uptake, expression and/or persistence of plasmids *in vivo*, co-administering immune adjuvants, or re-immunizing with protein or viral vectors.

All participants in vaccine trials have been carefully monitored to detect clinical, hematologic or biochemical abnormalities. Major adverse events were not reported following DNA vaccination. Transient mild - moderate inflammation at the injection site, characterized by pain, swelling and redness, were the most typical adverse reactions [15;16;19;20;29;51-53].

5) Conclusions

This work provides an overview of the FDA's current guidance on the manufacture and testing of prophylactic DNA vaccines, and the impact of that guidance on DNA vaccine development. The FDA's guidance evolved over time, reflecting the understanding gained concerning issues associated with DNA vaccine manufacture, immunogenicity and safety. Many of those changes involved a relaxation in regulatory requirements, such as i) providing sponsors with greater flexibility in the selection of potency assays for lot release testing during early clinical development, ii) eliminating the requirement for pre-clinical studies designed to specifically evaluate whether DNA vaccines induce autoimmunity, and iii) reducing the necessity for performing biodistribution, persistence and/or integration studies of certain DNA plasmids.

DNA vaccines currently undergoing clinical evaluation have elicited only modest immune responses in humans. Sponsors are therefore pursuing novel strategies designed to increase vaccine immunogenicity by modifying the plasmids, their methods of delivery, and/or by using them in combination with other types of adjuvant or vaccine. While it is hoped that these novel approaches will impact immunogenicity, there is little evidence that such strategies will reduce safety. It is therefore timely to reconsider the FDA requirement for detailed toxicology studies and extensive product manufacturing, stability and consistency data prior to the initiation of phase I studies. Shifting the requirement for such data to products entering phase II clinical trials would alleviate the need to perform time consuming and expensive studies on “candidate” plasmids unlikely to progress into licensable products, thereby accelerating the development of novel DNA vaccines.

Acknowledgments

The assertions herein are the private ones of the authors and are not to be construed as official or as reflecting the views of the National Cancer Institute, the National Institutes of Health, the Food and Drug Administration, or the US government.

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