The European Agency for the Evaluation of Medicinal Products
Evaluation of Medicines for Veterinary Use

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NOTE FOR GUIDANCE[1]:

DNA VACCINES NON-AMPLIFIABLE IN EUKARYOTIC CELLS FOR VETERINARY USE

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[1] This note for guidance will be developed as needed and as understanding of the issues evolves.

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INTRODUCTION

The use of plasmid DNA as a form of vaccination has progressed very rapidly in the last few years and several trials of this type of vaccination are in progress. DNA vaccination involves the inoculation of a gene(s) encoding a relevant antigen against which an immune response is desired, under the control of a promoter, which will permit its expression in the vaccinated animal. This gene construct is contained, for manipulation and for manufacturing purposes, within a bacterial plasmid DNA molecule. This type of vaccine has potentially important advantages over the direct inoculation of the antigen itself, e.g., it may provide a much wider stimulation of the immune system, including the stimulation of a cytotoxic T cell response. It can also have advantages over the use of a live attenuated micro-organism, e.g., the avoidance of breakthrough of disease arising from inadequately attenuated infectious agents. Furthermore, the manufacture of a plasmid DNA vaccine is likely to be simpler than for more traditional forms of vaccine and DNA vaccines are likely to have greater stability.

SCOPE OF THE DOCUMENT

This document is intended to provide advice to manufacturers seeking marketing authorisation for a nucleic acid vaccine for use in animals when the vaccine consists of a bacterial DNA plasmid. This document is applicable to vaccines consisting of plasmid DNA, non-amplifiable in eukaryotic cells. New developments involving plasmid DNA delivered by live vectors (e.g. bacteria) or capable of amplification in the vaccinated animal by any mechanisms, are not within the scope of this document.

As most of the DNA vaccines will be composed of a mixture of plasmids coding for different immunogens isolated from a single pathogen (virus, bacteria or parasites) or from different pathogens (mono- and multivalent DNA vaccines), DNA vaccine, as defined in the document, should be understood as a range of vaccine plasmids destined for use in a given species to induce an immune response.

The guideline should be read in conjunction with European Directives and other specialised guidelines, since all the appropriate standard requirements for veterinary vaccines are applicable, also, to the products within the scope of this document. The production of other biological products can provide the necessary experience on which a plasmid DNA biological product should be controlled. Therefore, these guidelines will focus on particular aspects relevant to this novel form of vaccination, developmental testing and control of DNA vaccines keeping in mind that each vaccine should be considered on a case-by-case basis. It is intended as guidance to help manufacturers to define assays to be carried out and to build up the application dossier.

SPECIAL ISSUES

There are several aspects of the use of a DNA vaccine, which have to be considered at the preclinical safety stage:

1. The plasmid DNA which is internalised by the cells of the vaccinated animal may integrate into the chromosomes of the vaccinated animal and disrupt the normal replicative state of that cell, causing uncontrolled cell division and tumourigenesis:

   After injection of DNA into an animal, a small proportion of the DNA molecules enters cells. The probability of any DNA molecule integrating into the chromosome is also low and given that oncogenesis is a multi-factorial event, the risk of insertional mutagenesis is exceedingly low. So far the integration of plasmid DNA into chromosomal DNA of a vaccinated animal has not been observed. However, probability of integration events occurring may differ according to the tissue type, the route of application, the amount of plasmid administered and the age of the vaccinated animal.

2. The method of vaccination may result in undesirable immune reactions:

   The mechanism of the immune response to an antigen, which is expressed due to injected DNA, is poorly understood. This raises concerns about possible adverse effects on the immune system, including auto-immune reactions.

   Although DNA can have a very low immunogenic potential, bacterial DNA can have a mitogenic or immunostimulatory effect. This property may be used to advantage in some DNA vaccines and is under active investigation. As with the co-use of cytokines, the specific incorporation of immunostimulatory sequences in any form should proceed with care.

3. The additional use of genes encoding cytokines or co-stimulatory molecules may pose additional risks:

   There is considerable interest in the co-administration of a gene encoding a cytokine in order to direct a specific type of immune response. However, this could have detrimental effects especially if the cytokine has been introduced on an expression plasmid whose expression cannot be terminated. Furthermore, it will be important to avoid the induction of immunity towards an encoded cytokine, which could have untoward and undesirable consequences for a vaccine recipient.

4. The expressed antigen may itself have undesirable biological activity:

   An encoded antigen may exhibit undesirable biological activity and if this is the case appropriate steps may have to be taken (e.g. deletion mutagenesis) to eliminate the activity while retaining the desired immune response.

POINTS TO BE ADDRESSED FOR A DNA VACCINE

As indicated above, the standard data requirements for veterinary vaccines have to be addressed in the application dossier. The information must be presented in accordance with the format set out in the Notice to Applicants. The following are given as examples of the level of detail and points to be addressed, as appropriate, in the various sections of the dossier.

A) ANALYTICAL SECTION:

A detailed description on the development of the vaccine plasmid should be provided. This should include details of the gene encoding the protein, against which an immune response is sought, information on the construction of the entire plasmid and the host bacterial cell.
The origin of the gene of interest should be described in detail, such as the name of the micro-organism or cell from which the gene was derived, the origin of the source, its species, passage history, subtype and isolation strategy followed. The rationale for the use of the gene(s) should be discussed and the sequence of the wild-type gene and the antigenic properties of the encoded protein in its natural state should be provided.

The steps in the construction of the entire vaccine plasmid should be described, including the source of the plasmid(s) used and subclones generated during the cloning procedure. Functional components such as regulatory sequences (origins of replication, viral/eukaryotic promoters, introns, termination sequences) and selection markers should be clearly indicated and information on the source and function of these elements should be provided when appropriate. Sequence data on the entire plasmid will be required and the use of all specific elements or regions of DNA should be justified. DNA sequence homology checks of the plasmid with all published DNA sequence data of the target species should be performed and the information given in the application dossier. An informative restriction map of the vaccine plasmid should be presented. Special attention should be given to the nature of a selection marker. The use of certain selection markers such as resistance to therapeutic antibiotics as well as certain sequences such as retroviral-like long terminal repeats (LTRs) and oncogenes should be avoided as far as possible; if not, a justification should be given in the dossier. The rationale for the choice of the host bacterial cell used for production of plasmid should be provided along with a description of its source, phenotype and genotype. It should be demonstrated that the host cell is free from bacteriophage and other adventitious agent contamination.

The identity of the vaccine plasmid after transfection into the bacterial cell to be used for production and the phenotype of the transfected cell should be confirmed. Since rearrangements of the plasmid are unacceptable, data on the stability of the plasmid within the bacterial cell will be required. The expression of prokaryotic genes, such as a selection marker, in a eukaryotic cell line should be investigated.

Cell seed

The production of plasmid DNA vaccines should be based on a well defined master cell seed (MCS) and working cell seed (WCS) system. The cloning and culturing procedures used for the establishment of the MCS should be described. The origin, form, storage, use, and expected duration at the anticipated rate of use must be described in full for all cell seeds. The MCS should be fully characterised and specific phenotypic features which form a basis for identification should be described. The sequence of the entire plasmid should be established at the stage of the MCS. WCSs should be adequately characterised and meet established acceptance criteria. The viability of the host-vector system in the MCS and WCS under storage and recovery conditions should be

determined. It should be demonstrated that MCS and WCS are free from extraneous microbial agents.

Production, validation and in process testing

Procedures and materials used during production fermentation and harvesting should be described in detail. Data on consistency of fermentation and harvesting conditions, culture growth and plasmid yield should be presented. Relevant in-process controls should be identified and rejection criteria during fermentation and harvesting should be established.

The minimum and maximum level of cell growth to be accepted during production should be defined and should be based on information concerning the stability of the host cell/plasmid system up to the maximum level of fermentation used in production. At the end of fermentation and harvesting, bacterial cell/plasmid characteristics should be investigated. This may include restriction fragment analysis, and the yield of both cells and plasmid.

Any methods used to extract the plasmid DNA and remove and/or reduce the concentration of unwanted materials must be described in detail and the process explained and validated.

Clearance capacity for removal of contaminants will be established for the purification process by the difference in contaminant levels before and after each purification step. Batch acceptance will be established on the basis of compliance with the upper acceptance limits defined for each contaminant. Special attention should be given to the removal of endotoxin.

Validated in-process controls for any potential contaminants of concern should be developed and routine batch test upper acceptance limits established, based on data from tests showing the safety of that concentration.

Routine control of bulk vaccine and finished product

Identity

The molecular form of each batch of plasmid should be assessed using a suitable technique, e.g. agarose gel electrophoresis, and an informative restriction analysis(es) should be performed.

Characterisation of in-vitro expression of the correct antigen should be documented through the use of specific assays, such as Western Blot, IFA or ELISA. For vaccines, which include plasmids encoding non-antigenic biologically active molecules, these plasmids should also be expressed *in vitro* and expression assessed with an appropriate bioassay.

Other tests as appropriate and depending on the method of purification and production and the nature of the plasmid should also be applied.

DNA content

A quantitative test for total DNA content per ml or per dose should be carried out on each batch of finished product.

Tests for contaminants

The purity of each batch of plasmid vaccine must be assessed and the level of contaminants shall be within specified limits to be set for any identified contaminant of bacterial-cell origin. Each

batch of product must also be tested for endotoxin, unless it is demonstrated that the bacterial host is endotoxin negative and that there is no endotoxin identified in three successive batches, and shown to be within the limit established as safe for the product. Additional tests may be required depending on the production process used, the results obtained from purification, validation and safety studies.

Batch potency test

An appropriate assay for the potency of the plasmid vaccine will be required. The most appropriate approach will vary depending on the composition of the vaccine, the nature of the disease, the expressed antigen(s) and the immune response being sought. Thus, the design of a potency assay will require careful consideration by the manufacturer and will be assessed on a case-by-case basis. Whatever the assay, an approved in-house reference preparation will be required and should be established from appropriately characterised batch of vaccine. Quantification of expression of the correct antigen *in vitro* should be performed, using qualitative and quantitative test methods (e.g. ELISA).

Fully validated *in vitro* expression assays can be considered sufficient for establishing batch potency and are preferred, wherever possible, to assays using animals, provided that correlation has been shown for the reference preparation between antigen expression *in-vitro* and potency *in-vivo.*

Batch safety test

The routine batch safety tests should be conducted with ten doses of product administered to the target species

B) SAFETY TESTING

Safety testing should be carried out in accordance with requirements of Part 7 of the Annex to Council Directive 81/852/EEC as amended. The following are examples of specific points, which should be addressed as appropriate under the headings indicated. Batches with maximal DNA content and potency should be used. The overdose studies should be conducted with 10 doses of the finished product unless justification can be provided for the use of a fewer number.

Distribution studies

Distribution studies data will be derived for the DNA vaccines (as defined in 1). Distribution data obtained with one type of plasmid should also be applicable to all other plasmids sharing the same backbone and differing only by the cloned antigenic gene provided that the inserts are approximately the same size.

The route of DNA inoculation as well as the amount of DNA administered may influence the distribution of the DNA in the body. Localisation studies should be designed to determine the distribution of the DNA after administration via the proposed route and employing the proposed method of inoculation. Using the most sensitive methods available, the extent of DNA distribution to and the cellular uptake by the target and the surrounding tissues including the draining lymph nodes should be analysed at various time points (e.g. day 1, day 7 and one month after vaccination or at a longer time, when appropriate). The timing of sampling should take into

account information on the duration of gene expression and the persistence of the DNA in the body of the vaccinated animal.

Integration and tumourigenesis

These studies, if necessary, should be undertaken with the DNA vaccine (as defined in 1) and not with each individual plasmid, when combined.

A step by step analysis should be carried out. As a first step, checks should be undertaken for the presence of plasmid DNA in the target tissue and draining lymph node. If, or when, plasmid DNA is detected, the most sensitive methods available should be used to investigate their integration. If integration is detected or suspected, carry out a test for tumourigenicity in a susceptible laboratory animal system. The incidence of tumors in the target species, particularly at the site of injection and in the target tissue, should be recorded at the end of each experiment in the safety and efficacy studies. Post marketing, any reports of tumours in the target species should be carefully monitored, as part of pharmacovigilance.

Reproductive toxicity

Standard studies on impact on reproductive performance will be conducted for DNA vaccines as for other types of vaccine.

The possibility of migration of the DNA to gonadal tissues and potential DNA transfer into germ line cells of vaccinated male and female animals should be considered. If necessary, the distribution studies, mentioned above, should be extended to provide information on this point.

Examination of immunological functions

Specific studies should be conducted to address the possibility of adverse effects on the immune system, particularly if cytokine genes are used as adjuvants.

C) EFFICACY STUDIES

Efficacy field studies should be carried out according to Part 8 of the annex to Council Directive 81/852/EEC as amended.

The standard requirements for efficacy testing of veterinary vaccines are applicable to these products. Tests should be conducted with batches with minimum DNA content and potency.

For these DNA vaccines, information will be required on the duration of the immune response (e.g. stability of antibody levels) and on the correlation between the immunological parameters measured *in-vivo* and protection. The data generated can be used to support the recommended vaccination schedule.