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Guideline on quality, non-clinical and clinical aspects of live recombinant viral vectored vaccines

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Executive summary

Vaccines against infectious diseases based on a viral vector expressing the antigen of a heterologous infectious agent have been under development for some time. This guideline applies to such live recombinant vaccines for the prevention and treatment of infectious disease, and provides guidance on quality, non-clinical and clinical aspects.

The present document should be conceived as general guidance and should be read in conjunction with other applicable EU and ICH guidelines.

1. Introduction (background)

Live attenuated vaccines are among the most effective vaccines against human infectious disease due to the broad and long-lived immune response they induce. Live viral vectors that express heterologous antigens are being extensively investigated in the development of novel vaccines and it is believed that these will provide an optimum immune response towards the expressed antigen. Examples of viral vectors being utilised in this way include pox viruses, adenoviruses, alphaviruses, measles virus, yellow fever virus and vesicular stomatitis virus. The heterologous antigens being expressed by these viral vectors generally derive from those infectious agents against which no effective vaccine exists such as human immunodeficiency virus (HIV), malaria, Dengue virus, severe acute respiratory syndrome coronavirus (SARS-CoV) and Ebola virus; in other cases improvements to established vaccines are being sought e.g. tuberculosis (TB). Due to the variety of different strategies and their novelty, combined with a lack of experience with marketed vaccines, there is a need for scientific guidance.

The Scope of the CPMP note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)[1], which came into effect in 2001, includes live recombinant vaccines but provides insufficient regulatory guidance for those developing such vaccines and for those regulators who will be involved in their assessment. Consequently, this guideline has been developed to provide the scientific principles that should be followed in assuring the quality, safety and efficacy of these vaccines and replaces specific related guidance in CPMP/BWP/3088/99[1]. It should be read in conjunction with other guidelines as noted in the text that follows.

2. Scope

The objective of this guideline is to provide recommendations on the quality, non-clinical and clinical studies that should be performed in order to obtain marketing authorisation of a live recombinant viral vectored vaccine intended for use in the prophylaxis of infectious disease in humans. This guideline is intended for products entering the marketing authorisation procedure. However, the principles laid down in the guideline should be considered by applicants entering into clinical trials.

Live recombinant vectored vaccines are live viruses (the vector) that express a heterologous antigen(s). A live recombinant vectored vaccine may also have been genetically modified for other reasons, e.g. attenuation. Live viral vaccines that have been genetically modified but do not express a heterologous antigen are not specifically addressed in this guideline although many of the principles provided would apply to such vaccines.

Live recombinant viral vectored vaccines include those that are based on replication-defective as well as replication-competent viruses. Replication defective vectors may have a natural host-restriction such as the avi-pox vectors, or they may have been attenuated by laboratory passage to the extent that they are no longer considered to be replication-competent in humans, e.g. modified vaccinia Ankara (MVA) (both of the above vectors belonging to the family of Poxviridae), or they may have a specific genetic modification (often the deletion of a gene[s] necessary for replication) that limits their replication to less than a single cycle (abortive replication) as in the case of many adenovirus vectors.

The derivation of the heterologous antigen gene may be of viral, bacterial, parasitic or other pathogen origin. The heterologous gene may comprise sequences coding for an entire antigen, a portion of an antigen, more than one antigen or heterologous antigen genes from more than one infectious agent. In some cases protection against the wild-type virus from which the vector is derived, may also be sought.

Plasmid DNA vaccines, recombinant protein vaccines and vaccines against non-infectious indications, many of which should be more properly termed 'immunotherapeutic' medicinal products, are not within the scope of this guideline. Vaccines based upon bacterial vectors such as salmonella and Bacillus Calmette-Guérin (BCG) are also being developed but are not within the scope of this guideline although many of the principles that apply here could also apply to these vaccines.

3. General considerations

Different approaches are being followed to create live recombinant vaccines, and the design of the vaccine including the viral vector upon which it is based is central to product development, vaccine manufacture and assessment of its safety and efficacy.

The manufacture of a live recombinant vectored vaccine typically will involve the propagation of the vaccine virus on a suitable cell substrate. In some cases it might involve the use of plasmid DNAs to provide required functions or proteins in trans.

The immune response to a live recombinant vectored vaccine will derive from the immunogenicity of the expressed heterologous antigen and the antigens of the vector itself, and in this regard it is emphasised that, besides the heterologous antigen specific immune response, an assessment of the overall immune response elicited by the vectored vaccine will be required. In addition, the virulence of live recombinant vaccines cannot be predicted from that of the viral vector, even when the vector by itself is already attenuated for humans, and so emphasis is placed on this aspect in non-clinical safety studies in appropriate animal models and in clinical development. This will be even more important where a vector of non-human virus origin is employed as information on the pathogenicity of the wild type virus for humans might be limited or absent.

In other cases, live recombinant vectored vaccines are being administered in conjunction with other vaccination approaches such as recombinant protein or DNA vaccines.

In this guideline, emphasis is placed upon safety issues, such as: the phenotype of the recombinant virus (e.g. level of attenuation and replication, alterations in tropism, and neurovirulence),

the extent of pre-immunity to the vector, the extent of immunity induced to the vector and the potential for re-use of the vector, genetic stability of the recombinant virus, reversion to virulence or recombination with wild type strains, clinical follow-up in a healthy patient population, chromosomal or germline integration (where appropriate).

These aspects will drive the selection and number of different relevant toxicological and pharmacological models. Emphasis is also placed on first-in-use-in-man studies as the vaccines within the scope of this guideline are novel laboratory-derived viruses obtained by recombinant DNA technology and clinical assessment will represent their first contact of humans (see also, the guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMA/CHMP/GTWP/125459/2006)[2]).

Although vaccines containing live-attenuated viruses are typically not adjuvanted, guidance on the co-use of immunomodulators is provided by the guideline on Adjuvants in Vaccines for Human Use (CHMP/VEG/134716/04)[3] and by the accompanying Explanatory Note (CHMP/VWP/244894/2006)[4].

4. Legal basis

This guideline has to be read in conjunction with Directive 2001/83 and its Annex I [5] as amended.

As the drug substances which form the subject of this guideline will qualify as biotechnological products in accordance with Part A of the Annex to Regulation 726/2004[6], applications for Marketing Authorisation for the drug products which contain them will need to be made using the Centralised procedure.

In addition, since the vaccines that fall within the scope of this guideline are likely to be Genetically Modified Organisms (GMOs), the legal requirements for including Environmental Risk Assessments in the Marketing Authorisation application, as outlined in Regulation 726/2004[6], Directive 2001/18/EC[7] and in the guideline on Environmental Risk Assessments for Medicinal Products Consisting of, or Containing, Genetically Modified Organisms (GMOs) (including footnotes thereto) (EMA/CHMP/BWP/473191/2006)[8], will apply (see also the Guideline on scientific requirements for

the environmental risk assessment of gene therapy medicinal products (EMA/CHMP/GTWP/125491/2006)[9]).

Since gene therapy medicinal products do not include vaccines against infectious diseases, the vaccines which form the subject of this guideline should not be considered to fall within the scope of the Advanced Therapies Regulation EC 1394/2007[10].

5. Quality aspects

5.1. General considerations

The requirements contained within the Ph. Eur. general chapter on Vaccines for Human Use (Ph. Eur. Section 6.3)[11] should be followed. Further, although these vaccines are not gene therapy products as defined by Regulation (EC) No 1394/2007[10], where the specific vectors addressed in Ph. Eur. general chapter 5.14 on Gene Transfer Medicinal Products for Human Use[12] are used in a viral vectored vaccine, adherence to the guidance provided is recommended.

5.2. Genetic development

The rationale behind the development of the vaccine should be clearly described and should include information on the choice of vector, the origin of the heterologous antigen gene(s) and the elements concerning the expression of the transgene(s). All intended and unintended genetic modifications such as site-specific mutations, insertions, deletions and/or rearrangements to any component as compared with their natural counterparts should be detailed. For a vaccine construct that incorporates transcriptional or translational elements to control the expression of a transgene in, for example, a temporal or tissue-specific manner, summary evidence should be provided to demonstrate such specificity from a product characterisation and control viewpoint. In a marketing authorisation application, cross-reference should be made to the detailed reports included in the relevant parts of clinical and non-clinical dossiers.

A description of all shuttle vectors and plasmids used in the development of the vaccine, illustrated with a flow diagram should be provided. Cloning of the viral vaccine should be described and the final construct characterised genetically (Section 5.3.2). The impact of selection markers used during screening and development and remaining in the final product should be carefully evaluated. In this respect, the use of antibiotic resistance genes in the vaccine virus should be avoided.

The development of the cell substrate used for propagation and/or packaging should be appropriately described. This should include the history of the cell line, its development for the purposes of the vaccine, identification and characteristics. Special attention should be given during the development and handling of the cells to avoid the possibility of cross-contamination with other cells or viruses and to avoid contamination with adventitious agents. For packaging cell lines, full details of their construction should be given, including the nature and location of the helper viral nucleic acid and its encoded proteins/functions.

The information provided should specify the origin of all genetic parts of the vaccine and their function, and overall this should allow a clear understanding of the functionality of the vaccine and how it is attenuated (where attenuation is the result of genetic engineering).

The quality of materials used in development of the vaccine should be appropriate to the stage of development. Vaccine to be used in clinical development should comply with the requirements for manufacture according to the Clinical Trials Directive[13].

5.3. Vaccine seed lots

5.3.1. General

There is considerable experience in the manufacture and use of live attenuated virus vaccines and useful guidance can be found in World Health Organisation (WHO) recommendations and Ph. Eur. monographs for individual vaccines.

Production of vaccines will typically involve a seed lot system involving a master seed lot (MSL) and a working seed lot (WSL). Seed lots are likely to be prepared by passage of the recombinant virus in the substrate to be used in vaccine production. This might involve a cell substrate, in ovo propagation or

transfection of a cell line with DNA plasmids. The number of passages from the initially developed recombinant vaccine virus to the master and working seed lots should be limited in order to minimise the possibility of genetic and phenotypic changes; any changes would indicate genetic instability and should be investigated.

Where DNA plasmids are involved in the manufacturing process, a characterised lot of plasmid DNA will be required. This needs to derive from a characterised cell bank of the host bacterial cell (see below).

5.3.2. Characterisation of vaccine seed lots

The MSL should be characterised as fully as possible. The nature and extent of seed lot characterisation will depend on a variety of factors including the nature of the vector, the origin and nature of the heterologous gene(s), the nature of genetic modifications, the history of the substrate used in seed lot preparation, the application of clonal selection and the nature of the reagents used in seed lot production.

MSL characterisation will include a description of the genetic and phenotypic properties of the vaccine virus. This should include a comparison with the parental vector and is particularly important where vector modification might affect attenuation, pathogenicity, tissue tropism or species specificity of the vaccine virus compared with the parental vector.

Genetic characterisation will involve nucleotide sequence analysis of the vaccine virus; restriction mapping, southern blotting, polymerase chain reaction (PCR) analysis, or DNA finger-printing will also be useful adjuncts. Individual elements involved in expression of the heterologous gene(s) (including relevant junction regions) should be described and delineated. Genetic stability of the vaccine seed to a passage level comparable to a production batch should be demonstrated. Genetic stability studies should ensure especially that no changes occur to regions involved in attenuation (where known), replication deficiency, antigen expression and antigen structure. Attention should be paid to the stability of the site of insertion, copy number and orientation for certain types of vectors, e.g. vectors based upon pox and herpes viruses. Also, effects of gene insertion/deletion on the vector such as gene silencing or over-expression should be considered, e.g. for adenovirus vectors.

Phenotypic characterisation should focus on the markers for attenuation/modification and expression of the heterologous antigen(s), and should be performed in vitro and in vivo (unless otherwise justified) under conditions allowing detection of revertants (including the emergence of replication competent vectors from replication incompetent vectors). However, other studies including antigenic analysis, infectivity titre, in vitro yield and in vivo growth characteristics in a suitable animal model should form part of the characterisation. For some vaccines, particle number may be measured instead of or in addition to infectivity titre.

A subset of the above studies should be applied to the working seed lot and justification for the chosen subset should be provided. Details of the projected life span of master and working vaccine seed lots should be provided and the anticipated frequency of derivation of new working seed lots. Details of the proposed qualification strategy for future working seed lots should be provided. The condition of storage of the seed lots should be described and data provided on the stability of the seeds upon storage.

5.3.3. Adventitious agent safety

Ph. Eur. chapter 5.1.7[14] should be consulted and tests for extraneous agents should be performed according to Ph. Eur. chapter 2.6.16[15]. The testing for specific viruses should take into account the susceptibility of the substrate used for the generation of the seed and/or for production.

Where materials of ruminant origin are used in preparation of the master and working seeds, compliance with relevant TSE note for guidance[16] is required (see also Public statement on the evaluation of bovine spongiform encephalopathies (BSE) - risk via the use of materials of bovine origin in or during the manufacture of vaccines (EMEA/CPMP/BWP/476/01)[17]). The EU guideline on the use of bovine serum (CPMP/BWP/1793/02)[18] should also be consulted, where appropriate.

Where virus vectors, helper viruses or other recombinant viruses are used to generate the seed lot, the potential for contamination by such viruses should be considered and where necessary the seed lot should be tested for such contaminants. It is important that assays can distinguish between vaccine virus and such contaminants.

5.4. Vaccine manufacture

5.4.1. Vaccine production

The production of a live recombinant viral vectored vaccine is likely to follow that of other live viral vaccines in that in many cases there will be minimal downstream processing and the basic requirements for manufacture and control will be essentially similar. There is considerable experience in the manufacture of live attenuated virus vaccines and guidance provided by WHO recommendations and Ph. Eur. monographs for individual vaccines provide useful information.

The method of production will involve the growth of the vaccine virus in the production cell line/substrate. Depending on the scale of production, it may be necessary to expand an ampoule of working seed before inoculation of the production cell culture. In some cases, DNA plasmids might be used to transfect the production cell culture in addition to or instead of infection with the vaccine virus. Whatever the case, the method of production should be clearly defined and a flow chart will be a useful adjunct for this.

The number of passages between the working seed lot and vaccine production should be kept to a minimum and shall not exceed that used for production of the vaccine shown in clinical studies to be satisfactory, unless otherwise justified and authorised.

Different substrates might be used for production including primary cells, diploid cells, continuous cell lines and embryonated hen's eggs. The rationale for the use of a particular substrate should be provided. Diploid and continuous cells should comply with Ph. Eur. general chapter 5.2.3 Cell Substrates for the Production of Vaccines for Human Use[19]. Reference should also be made to WHO Requirements for the use of animal cells as in vitro substrates for the production of biologicals[20].

With the exception of primary chick embryo cells, the use of primary cells should be avoided or their use fully justified. General guidance on the use of primary cell cultures is provided in Appendix 1 of ICH Topic Q5D Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products[21]. Chicken flocks used for the source of chick embryo cells should comply with Ph. Eur. general chapter 5.2.2[22]. There is considerable experience in the manufacture of live attenuated virus vaccines on chick embryo fibroblast (CEF) primary cultures (e.g. measles, mumps) and, for additional guidance, manufacturers making use of CEF cultures are referred to WHO requirements for measles, mumps and rubella vaccines, especially Section A.4.1.3 on the derivation of avian-embryo cell cultures[23].

Where genetically engineered cells are used for production, reference is made to appropriate sections within ICH Q5D Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products[21].

Control cells/eggs should be used as per Ph. Eur. general chapter on Vaccine for Human Use (Ph. Eur. Section 6.3)[11] and associated relevant general chapters and monographs, unless appropriately justified.

All materials used during production should be described and be of suitable quality. Approved animal serum may be used provided that residual levels in the final vaccine have been reduced to acceptable limits. Draft guidance on the use of bovine serum should be consulted and material of animal origin should comply with the relevant TSE guideline. Penicillin, all other β -lactam antibiotics and streptomycin should neither be used during production nor added to the final product.

5.4.2. Harvesting

The method of harvesting the vaccine virus should be described. An identity test should be performed on the harvested virus; this should include identity of the expressed heterologous antigen and of the vector virus. The virus titre should be determined by a validated method, preferably an infectious titre expressed for example in CCID₅₀ (cell culture infectious dose) or plaque forming units. A reference preparation should be included to validate the titration assay. Minimum acceptable titres should be established for use of a single virus harvest in the preparation of a virus pool or final bulk.

Tests for extraneous agents should be performed on each single harvest according to Ph. Eur. 2.6.16[15] and should be designed to take into account the need to neutralise the vaccine virus. Sensitive molecular methods could be used as alternatives to test for the presence of specific viral

genomes. The nature of any specified viruses being sought should take into account the method of production, viz. bankable cell culture, primary cells, embryonated eggs or use of animal serum.

Tests for replication competent viruses may be necessary for certain replication defective vaccine viruses, e.g. replication defective adenovirus vectored vaccines.

Single virus harvests should also be subject to sterility tests[24] and tests for mycoplasma[25] according to the Ph. Eur.

5.4.3. Virus pools

Depending on the unit size of production, single virus harvests may be pooled and from these virus pools, the final bulk will be prepared. The strategy for pooling of single virus harvests should be described. Only single virus harvests that comply with the tests outlined in Section 5.4.2 should be used. A virus pool should be subject to a clarification step and may be concentrated to obtain the required virus titre. All processing of the virus pool should be described in detail. Virus pools should be subject to sterility tests according to the Ph. Eur.[24].

5.4.4. Final bulk vaccine

The final bulk vaccine can be prepared from one or several virus pools or it may be derived from a single virus harvest. Only single virus harvests and virus pools that comply with the tests outlined in Sections 5.4.2 and 5.4.3 should be used.

Formulation of final bulk vaccine

Substances such as diluents or stabilisers or any other excipient added during preparation of the final bulk vaccine should have been shown not to impair the efficacy and safety of the vaccine in the concentrations employed. Freeze-drying may be suitable for long-term storage. For reconstitution of a freeze dried vaccine, the addition of a colorant in the reconstitution medium could be considered, provided it does not impair the safety, potency and in-use stability of the reconstituted vaccine.

Addition of antibiotics as antimicrobial preservatives is normally not acceptable. For multidose preparations, the need for effective antimicrobial preservation should be evaluated taking into account possible contamination during use and the maximum recommended period of use after opening the container or reconstitution of the vaccine. If an antimicrobial preservative is used, it should not impair the safety or efficacy of the vaccine. During development of the formulation, the efficacy of the antimicrobial activity should be demonstrated in accordance with the Ph. Eur. (5.1.3)[26].

Testing of final bulk vaccine

The titre of the final bulk vaccine should allow for titre loss during filling, freeze drying and shelf life. Each final bulk vaccine should be tested for sterility according to the Ph. Eur.[24]

Until filling into final containers, the final bulk vaccine should be stored under conditions that have been shown to retain the activity of the virus.

5.5. Control of final vaccine

Testing of the final vaccine should be appropriate for the nature of the vaccine, its pharmaceutical form and its route of administration. Samples of containers from each batch of final vaccine should be subjected to tests for sterility, identity and, potency; a test for thermal stability should be performed unless otherwise justified. Endotoxin levels should fall within limits based on the results of production batch analyses. For a freeze-dried vaccine, a test for water content should be included.

Qualitative and quantitative analyses on final vaccine should include tests for residual host cell protein, tests for residual reagents, a test for residual animal serum proteins (e.g. BSA) where relevant, and values should fall within acceptable specifications. When production is based upon a continuous cell line, tests for residual host cell DNA will be required[20]. Where these tests have been carried out with satisfactory results on the final bulk vaccine or on purified virus pools, the test may be omitted on the final lot.

5.5.1. Identity

The identity of each batch of the drug product (final formulated vaccine) should be determined. The approach used should be explained and justified.

5.5.2. Potency assays

The potency of each batch of final vaccine should be determined and should ideally reflect infectivity of the vector and expression of the heterologous antigen. The particular approach to potency determination should be explained and justified. Ideally it will involve measurement of an infectivity titre. In some cases it may be more appropriate to also or solely measure particle number. When both infectivity and particle number are measured, the particle to infectivity ratio can be determined and this should form part of the release specification. In other cases a potency assay might be based upon expression of the heterologous antigen in vitro or immunogenicity in vivo. The potency to be established as the release specification should be justified by the preclinical and/or clinical data obtained for the developed vaccine. A reference preparation should be established and should be included in each potency determination to validate the assay.

5.5.3. Stability

An end of shelf life specification should be defined and adequately justified. Appropriate stability indicating methods should be applied and selected (in advance) on the basis of relevant stress tests. Maintenance of potency throughout the period of validity should be demonstrated. Any loss of potency during storage should be assessed and trends or spontaneous losses even within the limits of acceptable potency may indicate that the vaccine is unstable.

5.5.4. Consistency of Production

Data from successive production lots should demonstrate consistency of production and be used to set limits on relevant parameters.

6. Non-clinical immunological and safety requirements

6.1. General considerations

The guidance provided in CPMP/SWP/465/95 Preclinical Pharmacological and Toxicological Testing of Vaccines[27] is applicable to live recombinant viral vectored vaccines. The advice provided in this section is supplemental to that guidance, which should be consulted alongside this document. Appropriate information is also available in the WHO guideline on non-clinical evaluation of vaccines[28]. In line with the latter guideline, safety pharmacology studies are generally not considered necessary, unless there is a cause for concern.

An important aspect is the choice of animal species to be used in the non-clinical assessment programme of the vaccine. The animal model (if available) should mimic as closely as possible the disease in humans against which protection is being sought, and protection against the disease with the vaccine should be demonstrated (proof-of-principle concept). If an animal model for the disease cannot be found, an infectivity model that demonstrates protection against infection may be appropriate. Furthermore, a relevant model ideally is an animal where the extent of replication of the vaccine, its virulence and its spread within the animal, will be as close as possible to the situation anticipated in humans. If feasible, studies of safety and protection should be carried out in the same animal model.

Pre-existing immunity needs careful consideration as it might influence the outcome of non-clinical and clinical studies. Non-protective immune responses might already be present due to cross-reactivity with related or wild type versions of the vector and/or the source of the heterologous antigen insert. Conversely post-immunization contact (for naïve organisms) with such versions might trigger safety concerns and require additional non-clinical testing. Ideally, the animal model should be able to reflect these immunological properties. In the absence of any suitable model, appropriate consideration needs to be given to clinical studies, especially post-vaccination studies.

6.2. Pharmacodynamic studies (protection and immunogenicity)

Immunogenicity data derived from a relevant species are expected before starting clinical trials. The investigations should include a quantitative and qualitative evaluation of immune responses according to dose and dose interval. These data should characterise the humoral, cell mediated and innate immune responses as appropriate to both the intended protective antigen as well as to the antigens of the vector used, as the latter constitute part of the immune response to the vaccine and are important in estimating the possibility of re-use of the vector virus in another vaccine.

Any correlation between an antibody or other immune response such as alterations to local cytokine responses and the level of protection from infection or disease should be evaluated. These key data are expected during development of the product. Pre-existing immunity to the vector or to the heterologous antigen within the vectored vaccine should be taken into account.

Disease markers such as viral shedding, body weight changes and other relevant clinical signs are important endpoints. These data are expected early during development of the product.

6.3. Non-clinical safety studies (toxicity testing)

6.3.1. Single and repeated dose toxicity

For the virus from which the vector is derived, knowledge of its route of infection, of the specific tissues and cells where it replicates, and of the shedding of infectious virus should be taken into account when determining the strategy for safety testing and help support the relevance of the selected animal model.

Single and/or repeated dose toxicity studies should assess the toxicity of the vaccine compared to formulation control. Information on the vector itself without active insert might also be valuable and should be provided.

In addition, when vector immunity is found to interfere with the response to subsequent doses, potentially compromising the results of a repeat dose toxicity study, a single dose toxicity may suffice provided that the intended human schedule is also single dose.

A case-by-case approach is advised in devising the strategy of testing.

6.3.2. Distribution studies

The biodistribution of a live-recombinant vaccine virus should be studied in a full set of tissues and organs including the brain, especially for the intranasal route of administration. One species is considered sufficient if scientifically justified. Distribution studies might include recovery of infectious virus, detection of viral antigens or detection of viral genetic material. Crossing of the blood-brain-barrier might be an indication of potential neurovirulence. Studies making use of the virus upon which the vector is based (including those published in the public domain with sufficient detail) might provide useful additional information.

The need for tests for inadvertent germline transmission should be assessed and the guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMA/273974/2005)[29] consulted.

6.3.3. Reproduction and developmental toxicity studies

The need for reproductive toxicity studies is mainly dependent on the potential use of the vaccine during pregnancy. Consideration should be given to available clinical and/or epidemiological data on infection by the virus upon which the vector is based; however, a change in tropism brought about by

the heterologous antigen may cause unknown and unexpected effects on the foetus. When required, the study design should reflect the application of the vaccine during the most sensitive period.

Reproductive studies should also be considered if biodistribution by the intended clinical route suggests replication in reproductive organs.

6.3.4. Local tolerance

Local tolerance studies are required, but might be combined with other toxicity studies (see also, the guideline on Non-clinical Local Tolerance Testing of Medicinal Products [CPMP/SWP/2145/00][30]).

7. Clinical

7.1. Introduction

Comprehensive guidance for the clinical evaluation of vaccines is provided in EMEA/CHMP/VWP/164653/2005[31]; the advice provided in this section is supplemental to that guidance, which should be consulted alongside this document.

In all instances sponsors are strongly encouraged to seek advice from EU Regulators at an early stage to discuss the most appropriate clinical development programme. The following explains the points that are or are not considered within this clinical section.

i) With respect to the foreign antigen(s) expressed by a live viral vector:

The assessment of the immunogenicity of the foreign antigen(s) expressed by the vector and the protective efficacy conferred against the infectious disease(s) the vaccine is intended to prevent should follow the principles laid down in EMEA/CHMP/VWP/164653/2005[31]. These principles will not be repeated in the sections that follow.

The principles laid down in EMEA/CHMP/VWP/164653/2005[31] also apply if:

- Administration of the viral vectored vaccine is intended to precede or to be followed by another vaccine (which may or may not be a viral vectored vaccine and may or may not employ the same viral vector) in order to optimise protection.
- The sponsor wishes to claim that the vaccine also confers some protection against disease caused by wild-type viruses related to the viral vector itself.

ii) With respect to the viral vector component of the vaccine:

There is a need to assess the immunogenicity of the vector itself and any potential there may be for:

- natural immunity to the vector in vaccinees, or
- immunity elicited by administration of the vectored vaccine.

Any pre-existing or acquired immune response to the viral vector may possibly interfere with immune responses to, and hence the potential protection afforded by, the foreign antigen(s) expressed.

Section 7.2 outlines the assessments that are additional to those discussed in EMEA/CHMP/VWP/164653/2005[31].

iii) With respect to the entire viral vectored vaccine:

The general assessment of safety should follow the principles laid down in EMEA/CHMP/VWP/164653/2005[31] and in the guideline on the conduct of pharmacovigilance for vaccines for pre- and post-exposure prophylaxis against infectious diseases (EMEA/CHMP/PhVWP/503449/2007)[32]. Some of the principles laid down in CHMP/GTWP/367513/2006[33] may also be applicable.

There are some issues that require special attention for viral vectored vaccines over and above those applicable to other vaccines that are discussed in section 7.3. These are predominantly virological issues such as the potential for reversion to virulence or for recombination or reassortment with circulating wild-type viruses. Special attention to these matters is needed because the construct of the viral vectored vaccine may result in a safety profile that cannot be predicted from any past experience

there may be with the virus(es) (wild-type or laboratory-modified) from which the vector has been derived. In addition, the same viral vector may not demonstrate an identical safety profile when expressing different foreign antigens.

7.2. Immunogenicity

The humoral and cell-mediated immune responses to the foreign antigen(s) expressed and to the viral vector should be studied as early as possible in the clinical development programme. The extent of investigations will to some extent depend on non-clinical study findings and what is already known about a vector if it has been used previously in other vaccines.

The following issues should be addressed as appropriate for the vector under consideration:

- Pre-existing immunity to the vector (e.g. due to natural exposure to viruses related to the vector, which could be very variable between different geographical locations or risk groups; due to previous doses of a vaccine containing the vector virus or components thereof; or due to maternal antibody).
- The nature of the immune response to the vector (e.g. neutralising and non-neutralising antibody, CMI and an investigation of the duration of measurable immunity carried over into the post-approval period as necessary).
- Any demonstrable correlation (positive or negative) between immune responses to the vector and immune responses to the foreign antigen(s) expressed.
- Consideration of, and if possible acquisition of data on, the overall immunological effects of concomitant or sequential administration of licensed viral vectored vaccines that use the same vector but express different foreign proteins.

7.3. Safety

Assessment of the specific issues relating to safety as detailed below should commence as early as possible during the clinical development programme and should continue as necessary into the post-approval period. Several of the matters that need to be considered are the same as those that would apply to any live vaccine.

The extent of investigations will to some extent depend on the degree of reassurance on certain issues provided by the quality data, the non-clinical study findings and what is already known about a vector if it has been used in other vaccines previously. The specific investigations will also need to be tailored to the known properties of the vector.

The following issues should be addressed as appropriate for the vector under consideration:

- the potential for the generation of replication competent virus from a replication defective viral vector; measurement of the immune response to an antigen present in replication-competent viruses but absent in replication-defective viruses might help in identifying such a situation,
- the potential for reversion of the viral vector to virulence; this might also occur during manufacture of a batch of vaccine or in vaccine recipients,
- the potential for recombination or reassortment with other infectious agents that might co-incidentally occur in vaccinees around the time of dosing,
- the incidence of viraemia,
- consideration of specific adverse events that might reflect distribution of the vector to specific body sites,
- assessment of the extent and duration of vaccine shedding and the potential for transmission of the live vectored vaccine to contacts,
- the potential for integration of genes derived from the vector into the host genome[29],
- the potential for vaccination to trigger autoimmune diseases,
- the potential for certain adverse reactions to occur only in subsets, e.g. those with a particular genetic predisposition,
- the potential for increased susceptibility to infection by the agent against which protection is being sought due to high levels of immunity to the vector virus.

References

1. Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products (CPMP/BWP/3088/99)
<http://www.ema.europa.eu/pdfs/human/bwp/308899en.pdf>
2. Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products (EMA/CHMP/GTWP/125459/2006)
<http://www.ema.europa.eu/pdfs/human/genetherapy/12545906enfin.pdf>
3. Guideline on adjuvants in vaccines for human use (EMA/CHMP/VEG/134716/04)
<http://www.ema.europa.eu/pdfs/human/vwp/13471604en.pdf>
4. Explanatory note on immunomodulators for the guideline on adjuvants in vaccines for human use (EMA/CHMP/VWP/244894/2006)
<http://www.ema.europa.eu/pdfs/human/vwp/24489406en.pdf>
5. Directive 2001/83/EC – on medicinal products for human use
http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-1/dir_2001_83_cons/dir2001_83_cons_20081230_en.pdf
6. Regulation 726/2004 - authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency
http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/vol-1/reg_2004_726/reg_2004_726_en.pdf
7. Directive 2001/18/EC on deliberate release of GMOs
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2001:106:0001:0038:EN:PDF>
8. Environmental risk assessments for medicinal products consisting of, or containing, genetically modified organisms (GMOs) (EMA/CHMP/BWP/473191/2006 - Corr)
<http://www.ema.europa.eu/pdfs/human/bwp/47319106en.pdf>
9. Guideline on scientific requirements for the environmental risk assessment of gene therapy medicinal products (EMA/CHMP/GTWP/125491/2006)
<http://www.ema.europa.eu/pdfs/human/genetherapy/12549106enfin.pdf>
10. Regulation EC 1394/2007 on advanced therapy medicinal products
<http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:324:0121:0137:EN:PDF>
11. Vaccines for Human Use. European Pharmacopoeia
12. Gene Transfer Medicinal Products for Human Use. European Pharmacopoeia 5.14
13. Directive 2001/20/EC – Clinical trials directive
http://europa.eu/eur-lex/pri/en/oj/dat/2001/l_121/l_12120010501en00340044.pdf
14. Viral safety. European Pharmacopoeia 5.1.7
15. Tests for extraneous agents in viral vaccines for human use. European Pharmacopoeia 2.6.16
16. Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products. EMA/410/01 Rev. 4 – for consultation
http://ec.europa.eu/enterprise/pharmaceuticals/pharmacos/docs/doc2009/2009_02/draft_nfg_bse_tse_rev4_2008_02_21.pdf
17. Public Statement on the evaluation of bovine spongiform encephalopathies (BSE) - Risk via the use of materials of bovine origin in or during the manufacture of vaccines (EMA/CPMP/BWP/476/01)
<http://www.emea.europa.eu/pdfs/human/press/pus/047601en.pdf>
18. Note for guidance on the use of bovine serum in the manufacture of human biological medicinal products (CPMP/BWP/1793/02)
<http://www.ema.europa.eu/pdfs/human/bwp/179302en.pdf>
19. Cell substrates for the production of vaccines for human use. European Pharmacopoeia 5.2.3
20. WHO: Requirements for the use of animal cells as *in vitro* substrates for the production of biologicals (Requirements for Biological Substances No. 50). WHO Technical Report Series, No. 878. 1998; Annex 1.
http://www.who.int/biologicals/publications/trs/areas/vaccines/cells/WHO_TRS_878_A1Animalcells.pdf

21. ICH Topic Q5D Quality of Biotechnological Products: Derivation and Characterisation of Cell Substrates Used for Production of Biotechnological/Biological Products (CPMP/ICH/294/95)
<http://www.ema.europa.eu/pdfs/human/ich/029495en.pdf>
22. Chicken flocks free from specified pathogens for the production and control of vaccines. European Pharmacopoeia 5.2.2
23. Requirements for measles, mumps and rubella vaccines and combined vaccine (live) (Requirements for Biological Substances No. 47). WHO Technical Report Series, No. 840, 1994; Annex 3.
http://www.who.int/biologicals/publications/trs/areas/vaccines/mmr/WHO_TRS_840_A3.pdf
24. Sterility. European Pharmacopoeia 2.6.1
25. Mycoplasmas. European Pharmacopoeia 2.6.7
26. Efficacy of antimicrobial preservation. European Pharmacopoeia 5.1.3
27. Note for guidance on preclinical pharmacological and toxicological testing of vaccines (CPMP/SWP/465/95)
<http://www.ema.europa.eu/pdfs/human/swp/046595en.pdf>
28. WHO: Guidelines on nonclinical evaluation of vaccines.
http://www.who.int/biologicals/publications/nonclinical_evaluation_vaccines_nov_2003.pdf
29. Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors (EMA/273974/2005)
<http://www.ema.europa.eu/pdfs/human/swp/27397405enfin.pdf>
30. Note for guidance on non-clinical local tolerance testing of medicinal products (CPMP/SWP/2145/00)
<http://www.ema.europa.eu/pdfs/human/swp/214500en.pdf>
31. Guideline on clinical evaluation of new vaccines (EMA/CHMP/VWP/164653/2005)
<http://www.ema.europa.eu/pdfs/human/vwp/16465305enfin.pdf>
32. Guideline on the conduct of pharmacovigilance for vaccines for pre- and post-exposure prophylaxis against infectious diseases (EMA/CHMP/PhVWP/503449/2007)
<http://www.emea.europa.eu/pdfs/human/phvwp/50344907en.pdf>
33. Concept paper on the development of a guideline on clinical monitoring and follow-up of patients exposed to gene therapy/gene transfer medicinal products (CHMP/GTWP/367513/06)
<http://www.ema.europa.eu/pdfs/human/genetherapy/36751306en.pdf>