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Meeting report

WHO informal consultation on scientific basis for regulatory evaluation of candidate human vaccines from plants, Geneva, Switzerland, 24–25 January 2005[✉]

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Abstract

In January 2005, WHO convened a meeting of leading experts in plant-derived vaccines and experts from regulatory authorities for an informal discussion on the state-of-the-art and to analyse whether specific guidance might be needed for plant-derived vaccines that is not yet provided by regulatory authorities. After a series of individual presentations, a general discussion was held to obtain a consensus on the need for further guidance. Both the presentations and the conclusions are presented. The meeting concluded that existing guidelines for the development, evaluation, and use of vaccines made by traditional methods can be applied to plant-derived vaccines. For plant-derived vaccines some specific issues will have to be addressed. These include, but are not restricted to, containment of the plants including disposal of waste materials. It was noted that plant-derived vaccines have been produced and clinically tested under US investigational new drug application, and all applicable regulatory and good manufacturing practice requirements are in place for this type of product. An innovator wishing to bring a plant-derived vaccine to market should consult closely with regulatory authorities to ensure that all appropriate studies are undertaken.

Keywords: Vaccines; Plants; World Health Organization

1. Introduction

Vaccines are an effective means of reducing the disease burden of infectious diseases in developing countries. To ensure the widest availability of these vaccines in poor countries, approaches are needed with lower production costs and less need of medical personnel to administer the vaccines. A promising avenue is the development of plant-derived vaccines, which can be administered orally or to other mucosal surfaces. Plants are able to produce different classes of proteins of pharmaceutical significance at a high yield, leading to potentially inexpensive products. Oral administration is important as it diminishes the need for needles and syringes which require highly trained immunization staff.

2. Presentations

2.1. The context: what human vaccines are likely to be produced in plants

Dr. C. Arntzen, Arizona State University, Phoenix, USA described his research indicating several potential advantages that are related to plant-derived vaccines, i.e. heat-stable formulation for storage and transport (avoiding cold-chain) and ease of delivery for better compliance leading to a reduced demand for skilled health care professionals in developing countries (and in developed countries, too).

A number of antigens have been expressed in plants since 1992, and the cumulative number reached 45 in 2003. Dr. Arntzen introduced examples of plant-derived hepatitis B antigens delivered in raw potatoes [1], [2] and [3], as well as using *Escherichia coli* heat labile enterotoxin (LT) expressed in corn [4], [5] and [6]. He also noted that plant-derived vaccines may have another potential advantage for protection against biowarfare agents such as *Yersinia pestis* (plague) antigens F1 and V by storing antigen bearing plant tissue and formulating vaccine as needed allowing for rapid response supply in the case of an emergency.

Plant-derived vaccines can offer advantages over traditional vaccines in global immunization programs. The use of vaccines produced by traditional methods are associated with difficulties including cost, requirement for injection, lack of heat stability, lack of mucosal effectiveness, and an increasing need for combination vaccines. There are several technical issues concerning plant-derived vaccines to be resolved before they enter wide-scale use: finalizing the regulatory regime from the laboratory through commercialization; requirement of commercial scale-up from current pilot scale; and securing public acceptance of the technology.

Dr. S. Webb, Dow AgroSciences, Indianapolis, USA described new initiatives of his company in the field of Animal Health Science. Proof of concept was established for evaluation of plant cell culture vaccines. The principles of the concept were demonstrated for the production of antigens from Newcastle Disease Virus, and in the response to this antigen, in specific pathogen-free (SPF) chicks. Plant-cell expressed protein antigens were conformationally correct; reproducible protection against infection could be induced; and mucosal delivery was shown to be immunogenic. The plant cell cultures appeared to be genetically stable through 40 passages, as shown by fingerprint profiles, and products containing expressed antigens appeared to be highly stable. Dr. Webb emphasized that some advantages of plant-derived vaccines produced by a contained and controlled system are no shedding because of the use of non-replicating subunits, free of

adventitious agents as no components of animal origin are being used, and environmentally safe.

2.2. The challenge: assuring quality, safety, efficacy and acceptability of plant-based vaccines through appropriate regulations

Dr. P. Minor, National Institute for Biological Standardization and Control, Potters Bar, UK focused on the adventitious agents that might be present in plants. Dr. Minor first described lessons from animal and insect derived vaccines. Currently available vaccines and biologicals are derived from cells originated in humans (MRC5, WI38, and others), monkeys (primary kidney), chickens (chick embryo fibroblast), or rodents (hybridomas, Chinese hamster ovary, primary hamster kidney).

Viruses of concern from human cells include human immunodeficiency virus and hepatitis B and C virus. In practice, human viruses have not been a major issue in vaccine production because they are dealt with through verified procedures, although there is a concern about the occasional failure to adhere to good manufacturing practices (GMP).

Animal viruses might be, however, more dangerous, especially if they are capable of infecting humans. Even if they do not infect humans, adventitious animal viruses may adversely affect production. In the case of chicken eggs, it is not proven that avian viruses such as avian retroviruses (e.g. avian leucosis virus in yellow fever vaccines) and other agents excluded from SPF flocks are dangerous. In rodent cells, viruses which may be present are not so well known. The use of rodent cell lines is not free from risks as many serious human viral diseases are transmitted by rodents, including viruses which cause little disease in the normal host. Viruses in animal cells thus can be dangerous. If they are present, it is wise to consider that they may present risks, even if they have not been shown to be hazardous. It is possible to breed virus-free animals provided the viruses which may be present are known, although this is not always the case.

Issues with relation to insect cells include lack of the appropriate expertise on insect viruses except as means of control of pests. Most insect cells may have viruses in them and infection can be hard to detect and difficult to eliminate. Most insect viruses seem not to infect humans. Insect viruses are more of concern for production and yields. Steps should be taken to eliminate them.

Plant viruses are not known to have infected humans but they may affect production. A theoretical risk is that viruses can be introduced from other sources, e.g. from pests breaching the production area.

Dr. M. Moloney, SemBioSys Genetics Inc., USA introduced the quality aspects of products derived from safflower. From an environmental point of view the plant has advantages in the ease of containment and a relatively low environmental impact. Extensive efforts, nevertheless, have been carried out to enhance the containment program. Regulatory guidelines have been issued by the US Department of Agriculture (USDA) and Canadian Food Inspection Agency (CFIA), Canada. According to Dr. Moloney, the US Food and Drug Administration (FDA) does not foresee problems with plant-made proteins derived from safflower. The industry has developed a code of conduct, which include a containment analysis and a critical control plan covering plant host system characterization, personnel training, contingency planning, performance audits and verification, safe transportation, and site security. Following these guidelines, a human insulin analogue has been made in the safflower oilseed.

Dr. Y. Thanavala, Roswell Park Cancer Institute, Buffalo, USA approached the plant-derived vaccines from an immunological point of view indicating that the major source of stimulation of the entire immune system is at mucosal surfaces where the external environment is recognized. However, the body's normal response to harmless gut antigens is localized resulting in systemic immunological tolerance, also known as oral tolerance. In view of this phenomenon, the induction of immunity following an oral antigen might be inappropriate. Vaccine-related immunity can be distinguished from oral tolerance as the former is primarily induced by encapsulated antigen formulations delivered with adjuvants in a specified number of doses. Delivery of an antigen as a soluble protein should be avoided.

Dr. F. Verdier, Aventis Pasteur, France focused on safety issues of plant-derived vaccines, distinguishing between issues related to the environment, to the manufacturing process, and to the final proteins. Safety issues for final proteins relate to the degree of downstream processing ranging from minimal processing (e.g. edible vaccines) to extensive processing (e.g. extracted and purified proteins). The environmental risks are associated with the spreading of foreign transgenes, by dispersal of the seed, pollination of sexually compatible strains, and the disposal of transgenic plant waste material. An important method of risk-reduction might be growing the plants in green houses versus open field conditions. Risks may also be associated with transfer of the genes, i.e. leading to the unexpected transmission of genes coding for resistance to antibiotics or herbicides. These issues would be addressed in plant-derived vaccine development because the standard requirements for all new vaccines are that they go through complete nonclinical and clinical evaluation.

Dr. P. Macdonald, CFIA, Canada described regulation of the environmental release of plants producing human vaccines including such issues as protecting humans and livestock, and environmental safety. The CFIA Plant Biosafety Office has experience in regulating plant-derived material, which is called Plant Molecular Farming, defined as "the use of plants with novel traits (PNTs) for the production of a pharmaceutical or industrial biomolecules rather than for food or feed". Areas of concern are the protection of the environment, avoiding gene flow and keeping PNTs out of conventional commodities. Dr. MacDonald indicated that despite the best efforts of industry towards high level of stewardship, compliance problems can still occur. To maintain public confidence in the technology, it is important that regulatory policy proceed cautiously and thoroughly. The CFIA recommends establishing a licensing system for "molecular farmers." The proceedings of a workshop which has been held to look at the applicability of existing systems to plant molecular farming are available at a website (<http://www.inspection.gc.ca/english/plaveg/bio/mf/worate/reprape.shtml>).

2.3. Existing regulatory perspectives

Ms. Y. Maruyama, WHO, Geneva, introduced the WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants [7], and quality-control methods for medicinal plant materials [8]. Under the overall context of quality assurance and control of herbal medicines, WHO GACP guidelines provide general technical guidance on obtaining medicinal plant materials of good quality for the sustainable production of herbal products classified as medicines. WHO GACP guidelines are also related to WHO's work on the protection of medicinal plants, aiming for promotion of sustainable use and cultivation of medicinal plants. A publication of quality-control methods for medicinal plant materials provides recommended test procedures for assessing the identity, purity, and content of medicinal plant materials. The manual, which is intended to assist

national laboratories engaged in drug quality control, responds to the growing use of medicinal plants, the special quality problems they pose, and the corresponding need for international guidance on reliable methods for quality control.

Dr. J.W. van der Laan, National Institute for Public Health and the Environment, Bilthoven, the Netherlands described principles of WHO guidelines on nonclinical evaluation of vaccines and highlighted important issues. Nonclinical testing of vaccines is a relatively new area and the WHO guideline was recently published [9]. The guidelines stress the need for the proof-of-concept in animal studies, if an animal model exists. Toxicity studies might focus on expected and unexpected immunogenicity. The nonclinical guidelines mention also the use of the appropriate route of administration in animal studies. New legislation concerning the need for safety studies with genetically modified food may serve as a model for plant-derived oral vaccines.

Dr. I. Knezevic, WHO, Geneva, described principles for clinical evaluation of vaccines as part of overall vaccine regulation. Regulatory issues should be considered at all stages of vaccine development, production, pre-licensing evaluation and post-marketing surveillance. Dr. Knezevic emphasized the need to work closely with the national regulatory authority (NRA) of the country in which a trial will be carried out in addition to taking into account the policies and guidelines of the US FDA, European Medicinal Evaluation Agency (EMA), or general guidelines. It is important to make early contact with the local regulatory authorities to get advice on planned studies and involve regulators in the development of appropriate testing procedures for new and novel vaccines. Vaccines of assured quality, by definition, rely on a strong NRA [10], therefore, regulators should play an active role from early stages of vaccine development through the whole vaccine life.

Clinical trial approval takes different forms such as investigational new drug (IND) application in the US and the investigational medicinal product dossier (IMPD) application, which is now implemented in the European economic area. These are in addition to ethical clearance, which is required in all countries. To help regulators review clinical trials, WHO standards, such as standards for quality control of clinical lots and for the measurement of immune response in clinical trials, are available [11].

WHO guidelines for clinical evaluation of vaccines are available to assist in evaluation of clinical trials as a part of the regulatory overview [12]. The guidelines outline the data that should be obtained during the different stages of vaccine development to support a marketing approval. In addition to general principles that apply to all existing vaccines, several specific issues should be considered as prerequisites for initiation of clinical trials of plant-derived vaccines. First, consistency of production should be carefully monitored and appropriate parameters for a vaccine in question identified during the development of a vaccine. Since there is not much evidence on vaccine quality at the stage of clinical trial approval, consistency of production is an essential element in assuring vaccine quality. Second, characterization of starting materials as well as intermediates and final products is of great importance. In particular, genetic stability of a plant-derived vaccine should be demonstrated. Third, justification of the use of plants instead of currently used substrates should be provided. Given that safety of new plant substrate is not known and tools for safety assessment at pre-licensing stage are limited, it is important to consider potential benefits of a plant-derived vaccine in comparison with currently available vaccine or the one being developed using another substrate and another technology.

Dr. C. Tacket, Center for Vaccine Development, University of Maryland, Baltimore, USA, participated by teleconference and introduced experience with three IND applications for clinical trials of plant-derived vaccines in the USA. The IND applications were for vaccines produced in whole transgenic potato or for vaccines produced in corn germ. None of the INDs was put on clinical hold. Rather, all three generated multiple queries from FDA similar to INDs for other vaccines made by traditional methods [13].

Transgenic plant-derived vaccine product-specific queries included definition of lot, definition of stages of manufacturer analogous to a master seed, working seed, or production lot, definition of lot release criteria including microbial limit test, definition of the date of manufacture, DNA sequence of the heterologous gene in the plasmid construct, assurance that agrobacterium is not included in the plants used to manufacture the whole-vegetable vaccine, presence of antibiotic resistance genes in whole-vegetable vaccine, tests for residual antibiotics, certificates of analysis for pesticides used, residual pesticide analysis, and compliance with regulations from USDA regarding transport, storage, and disposal of transgenic plants. Other important queries related were how to ensure control of inventory and disposition of viable seeds to be sure they are not used for food or feed production, description of the green house, growth conditions, other plants present, and transportation of fruits or vegetables to a processing plant.

This experience demonstrates that pre-IND consultation with the US FDA can greatly decrease the number of queries after IND submission, and that investigators and vaccine producers may have to be innovative in applying FDA regulations to plant-derived biologics.

2.4. Opinions of regulatory authorities

Dr. A. Nolte, European Medicines Agency, provided an overview of the EU regulatory framework applicable to medicinal products from transgenic plants. These products are regulated by Directive 2001/83/EC¹ on medicinal products [14]. The release into the environment of transgenic plants used in the production of medicinal products is regulated by Directive 2001/18/EC [15].

Medicinal products containing biological active substances manufactured using transgenic higher plants are clearly 'biotechnological' as defined in Part A of the Annex to Council Regulation (EEC) 2309/93² [16], with the result that applicants for marketing authorization for such products are required to use the European centralized application procedure described in the regulation. Existing relevant European and International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guidelines have to be taken into account for the development of medicinal products derived from transgenic plants. In addition, a European guideline on the quality of medicinal products containing biological active substances produced by stable transgene expression in higher plants is currently being developed. A first draft of this guideline was published for consultation in April 2002. This draft is currently being revised. The quality, including biological safety, issues pertaining to medicinal products containing biological active substances (i.e. recombinant proteins and peptides) produced by the expression of one or more transgenes stably located in the genomes of whole higher plants constitute the scope of this guideline. Production using transiently transfected plants production using plant cell culture fall outside the scope of this guideline.

The guideline applies primarily to plant-derived products intended for parenteral administration to human beings. Products intended for oral non-parenteral administration are expected to need to take only a subset of the guidance offered into account. Guidance is offered on production using plant lines cultivated under closed conditions or in open fields. The document will provide guidance on the following issues which are under discussion among European experts: development genetics, manufacture and controls, freedom from contamination with viral and non-viral adventitious agents.

Dr. J. Daugherty participated by teleconference and gave the perspective of the US FDA regarding the regulatory pathway for licensure of human vaccines produced in bioengineered plants. The Coordinated Framework for Regulation of Biotechnology established, in 1986, that genetically engineered products would be regulated under existing legal authorities. Also, safety assessments would be based on product characteristics and not by the process by which the product was developed. The FDA's regulatory authority over vaccines derives from the Food Drug and Cosmetic Act and the Public Health Service Act. Because human vaccines derived from bioengineered plants are regulated under existing statutes and regulations, a new regulatory pathway is not currently needed. Sponsors should consult the applicable parts of Title 21 US Code of Federal Regulations and available FDA guidance for clinical development planning of their product(s) [17].

Dr. Daugherty described some of the key issues to be considered by sponsors during the manufacture of vaccines derived from bioengineered plants. These include ensuring the stability of the transgene and its expressed antigen, ensuring consistency of manufacture, and ensuring appropriate environmental monitoring/control during vaccine manufacture, transport and storage. A permit from the Animal and Plant Health Inspection Service (APHIS) of the USDA may be required for growth of bioengineered plants under non-contained conditions.

The dosage form of a vaccine can have significant effects on bioavailability of the vaccine antigen. Also, immune tolerance to the vaccine antigen(s) may occur following administration by the oral route. Although vaccines derived from bioengineered plants and intended for human use are regulated as biologics, and not foods, FDA recommends that any packaging materials for this type of product conform to the existing regulations for packaging of foods (not including labeling regulations). Finally, sponsors should consult with FDA if by-products or residues generated from the manufacture of human vaccines from bioengineered plants are intended for human or animal feed.

Dr. E. Griffiths, Health Canada, summarized Canadian regulatory expectations of plant derived human vaccines. These products are regulated under the Food and Drug Act. A switch in production process for an existing vaccine from conventional systems to plants would require a new approval, regardless of any previous approval. Regulatory challenges arising from novel biotechnologies are to ensure public safety but not to inhibit development and innovation, since adequate regulations are needed to safeguard recipients against adverse effects and to ensure they receive full benefits of scientific innovation and knowledge. Much of existing regulatory guidance for biotechnology products and vaccines in general applies.

Issues in Canada related to development genetics are similar to those of other regulatory authorities. Issues at the stage of cultivation and harvesting are related to defined production and purification strategies. Downstream processing and product characterization issues may be affected by the type of vaccine and mode of administration. Highly purified antigen administered parenterally may

need rigorous characterization, while minimally purified products administered orally or mucosally may need less rigorous characterization. Particular attention should be paid to controlling levels of unwanted immunogenic substances, since plant protein processing patterns, differ from microbial or mammalian systems. Many plant glycans contain fucose or xylose residues that do not occur in humans.

In terms of freedom from microbial contaminants, bioburden rather than complete sterility can be considered unless there is a concern that microbial proteases may compromise antigen quantity and quality. For plant-derived antigens intended for oral delivery, it would be helpful to assess the applicability of WHO guidelines on the production and control of inactivated oral cholera vaccine [18].

Containment regulations to protect the environment or for workplace health and safety would be carried out by the CFIA. From the regulatory view, plant growth conditions should approach conditions equivalent to those of cell culture based production for parenteral products. Health Canada will be involved in regulating the production process from the start of the growing season and will include the regulatory status of fields, greenhouses, and barns.

When facing regulatory challenges of novel biologics, sponsors should ensure sound science based regulatory decisions, and regulators should ensure regulatory positions are clear and adequate to reflect scientific advances. Science and commerce in biologics fields are truly international, as are public health concerns to which they give rise. Innovators, manufacturers and regulators are on a learning curve, and need to strive for harmonized international approach to regulation of this exciting field of molecular farming. Dr. Griffiths closed by noting: "Be prepared for unanticipated events—expect the unexpected".

Dr. T. Kurata, National Institute of Infectious Diseases, Tokyo, Japan, presented research on antigen or antibody expression in genetically modified plants in Japan and pointed out some safety issues related to plant-derived vaccines. In the case of vaccines co-expressed with adjuvant molecules such as cholera toxin B subunit (CTB) or heat-labile toxin B subunit (LTB), not only targeted vaccine antigens but also adjuvant molecules need to be evaluated. Transgene technology related safety issues may include site-specificity of gene integration, stability of the integrated gene, and controlling the extent of expression. Questions were raised on a possibility of dissemination of transgenes derived from transgenic plants into blood cells of an ingested host or to the fetus. These concerns were based on previous biodistribution studies using phage M13 DNA ingested to mice [19], [20] and [21]. Issues arising from unwanted transfer of transgenes need to be addressed in comparison with what is naturally taking place.

2.5. Production and quality-control issues

Dr. Chung K. Lee, Seoul, Korea, Rep. described the applicability of GMP guidelines to plant-derived vaccines. The approval of plant-derived vaccines should follow essentially the same general process for other human vaccines: preclinical research and development, INDs for clinical trials, licensing and post-marketing surveillance. At present guidelines for GACP may provide basic guidance for manufacturing plant-derived vaccines. This guideline includes agricultural practice, collection practice and some limited post-harvest operations. A similar draft guideline has been issued by USDA and FDA [17].

Dr. R. Barry Holtz, Vacaville, CA, USA, described a successful program for development, validation and control of process for plant-made pharmaceuticals (PMP), e.g. monoclonal antibodies for cancer therapy. Dr. Holtz highlighted Process Analytical Technologies (PAT) and potential compliance issues for oral vaccine development. Dr. Holtz described the opportunity to make oral vaccines using PMP technology. Expression of protein antigens has already been successfully demonstrated. The problem to date is the production of a single dose delivery that is made under current GMP (cGMP) type manufacturing control. Using food protein manufacturing techniques, the antigens could be extracted and concentrated using low cost high throughput methods. The antigen concentration could be adjusted to provide a precise dose. The extract could be dried using low impact, low-temperature drying techniques such as spray drying. Spray drying is commonly used to make milk powder without denaturing milk proteins. The final product could be a dried antigen, of precise potency, delivered in a food excipient and packaged in a single dose packet that could be administered to the patient without necessity for injection as a drink or other food preparation. The inherent safety of the plant background would not be problematic in the oral delivery system. Using other food protein production techniques, the antigens could be agglomerated into larger particles or microencapsulated to prevent degradation in the stomach before getting to the mucosal lining of the gut. The raw materials could be successfully grown in greenhouses thereby obviating concerns about containment and genetic drift in plants.

3. Conclusions

At the second day of the meeting, the participants engaged in a detailed discussion to reach a consensus about the state of the art with respect to regulatory principles for plant-derived vaccines. The discussion addressed important subjects selected from points raised by the presentations in the first day.

The scope of the discussions was set to define the need for specific guidance for heat-stable oral or mucosally delivered plant-derived vaccines. A first principle, is to differentiate plant-derived vaccines from food products. Plants should be taken as the production system of proteins, which are potent antigens in animals. Arguments of cost-effectiveness might be important in the use of plants as production system, but costs were not considered at this informal meeting.

3.1. Developmental genetics

Description of the developmental genetics for recombination products is commonly required and guidelines are available in general, which can be applied also to plants. For example, WHO has written guidelines for products developed by recombinant DNA technology [22]. In this respect there is no difference between crops that will be cultivated in a greenhouse or on the field. Several methods for molecular characterization are available (e.g. checking with Southern blotting on the number of copies). This check is also applicable to sexual multiplication. The tissue specificity of the expression of the genes should be studied. The use of a promoter to induce is an option. If this is the case further characterization is needed.

3.2. Banking

Following the concept of a master seed, a banking system should be established, while the set-up of such a banking system depends on the method of production, whether this is sexual or vegetative. It is important to ensure traceability and to

monitor genetic drift. Drift can be checked by fingerprints not only for the transgene but also the rest of the genome. There is a need for specific guidance for plant-derived vaccine seeds including amplification interval and storage condition.

3.3. GMP

As far as GMP for the production of vaccines and other biologicals in transgenic plants is concerned, it is generally agreed that the principle of the existing GMPs for drugs/biologics should be applied in general. However, considering the unique aspects of manufacturing vaccines in transgenic plants, additional requirements and modification of the existing regulations should be implemented. For example, the detailed GMPs for early parts of manufacturing such as agricultural and collection activities should be added. GMP should apply from the beginning, i.e. plant banking system, to the final product.

3.4. Cultivation

The meeting participants agreed that process validation under GMP is more feasible under greenhouse conditions, and greenhouse cultivation should therefore be recommended for the production of vaccines from plants. Important elements are the recording of all specific events needed to start the cultivation of the plants and source description of the material. The description of the training of personnel is important, as the consistency of handling the material depends eventually on the quality of the technicians. Key parameters are described in the FDA draft guidance document [17].

3.5. Harvesting

Plants being used as carriers for vaccine production can be seen as medicinal plants and should be harvested during the optimal season or time period to ensure the production of the raw materials and the finished product of the best possible quality. Attention has to be paid to stability too, as harvesting will lead to damage to the plant. Consistency is an important issue, as it depends strongly on growing conditions. Criteria for rejection should be defined therefore. As pooling of harvests is to be expected to occur, the final batches should be checked for mutual homogeneity and similarity. The WHO GACP guidelines [7] can be referred to, although not written per se for this type of products. Other aspects are the check for the identity of the product as a "validation" as discussed in the FDA draft guidance document [17].

3.6. Administration to a recipient: nonclinical safety criteria

From the WHO guidelines on nonclinical evaluation of vaccines it can be seen that there is the need to show the proof-of-concept in animals. Ideally this should be done in a species that resembles the human illness when exposed to this pathogen (e.g. the ferret in case of influenza virus). Additionally, it is important to show the effects on the immune system, preferably on the innate immunity as well on the humoral and cellular immunity. A second or subsequent exposure may lead to a response differing from the first dose: this should be assessed in the study design.

Toxicity testing may be of relatively lesser importance because of the low dose of proteinous material, especially with an oral dose. Toxicity testing should be conducted preferably in the same species and at the same level of exposure as used to show immunogenicity.

Risk of allergenicity should be considered especially if constituents of the plant are known to be allergic. The risk on allergenicity might be higher if using an adjuvant (e.g. aluminium-based) to enhance the immune response to the antigen. With regard to the risk of inducing immunotolerance, soluble proteins of the type known to induce this phenomenon should be avoided.

The present EU guidelines on nonclinical pharmacological and toxicological testing (CPMP/SWP/465/95) [23] and the guideline on adjuvants (CPMP/VEG/17/03) [24] are fully applicable as far as they describe the testing of oral vaccines.

The insertion of the transgene might influence the expression of plant toxins or other host proteins, and this possibility should be checked under quality or toxicity studies.

Risk of dissemination of the genetically modified organisms (shedding) is considered to be low for plant-derived vaccines.

3.7. Administration to a recipient: clinical criteria

The WHO guidelines on clinical evaluation of vaccines are applicable for clinical criteria. Two aspects came up specifically: (i) it is important to define and control the dose for an orally delivered inactivated vaccine. Robustness of dose of the vaccine is important in terms of potency and stability; (ii) furthermore with respect to the oral intake the choice of site of trial that may be influenced by intestinal environment (flora and status) of the local population and the traditional food in that area. These aspects should be included in the development of a new oral plant-derived vaccine.

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¹ Amended by Directive 2004/27/EC.

² To be replaced by Regulation (EC) No. 726/2004.