DNA Vaccines: Regulatory Considerations and Safety Aspects

Anne Ingeborg Myhr

GenØk – Centre for Biosafety, SIVA Innovation Centre, PB 6418, N-9294 Tromsø, Norway.
Corresponding author: anne.myhr@genok.no

DOI: https://doi.org/10.21775/cimb.022.079

Abstract
DNA vaccines have great potential as preventive or therapeutic vaccines against viral, bacterial, or parasitic diseases as well as cancer, and may also be used as gene therapy products. Although many human and veterinary DNA vaccines have been investigated in laboratory trials, only four of these have been approved for commercial use. In this paper an overview of the regulatory requirements for the development of DNA vaccines is given. The regulatory process in EU and USA is described. A discussion concerning the relevance of national regulations on gene technology is included. In addition the main safety concerns associated with DNA vaccines, relating to unwanted side effects in the vaccinated mammal or fish, are presented. Finally, the need for greater openness regarding the assessment information is discussed.

Introduction

DNA vaccines have several advantages over other vaccination strategies including the ability to induce both cellular and humoral immunity. In addition these vaccines may also cause less side-effects than, for example, vaccines based on attenuated pathogens (Pereiro *et al.*, 2014). The process required for the development of DNA vaccines is relatively inexpensive and simple with the advantage that, when fully established, it can be adapted and applied to other systems (Wharen and Liu, 2014).

In 2003, the US Centers for Disease Control and Prevention developed and obtained approval for the use of an experimental vaccine to be used for the vaccination of endangered wild Californian condor against West Nile virus (Chang *et al.,* 2007). Two years later, in 2005, a derivative of this experimental vaccine was licensed by the US Department of Agriculture to to protect horses against West Nile virus (Chang *et al.,* 2007). In 2005 the Canadian Food Inspection Agency (CFIA) approved a DNA vaccine against Infectious Haematopoietic Necrosis Virus (IHNV) in farmed salmon (Salonius *et al.,* 2007). In 2010 the US Department of Agriculture granted approval for the use in dogs of the first anti-cancer DNA vaccine (Wharen and Liu, 2014). The first DNA vaccine for use in gene therapy was approved in 2008 by the Australian Pesticides and Veterinary Medicines Authority. This vaccine, consisting of a

Box 1. List of abbreviations.

BLA	Biologics License Application
CFIA	The Canadian Food Inspection Agency
CBER	Center for Biologics Evaluation and Re search (FDA)
CFR	Code of Federal regulations
EMA	European Medicines Agency
FDA	Food and Drug Administration
GCP	Good clinical practise
GHRH	Growth hormone releasing hormone
GTMP	Gene therapy medicinal product
IHNV	Infectious Haematopoietic Necrosis Virus
IND	Investigational New Drug
MAA	Marketing authorisation application
OCTGT	CBER Office of Cellular, Tissue and Gene Therapies
OVRR	CBER Office of Vaccine Research and Review
PD	Pancreas disease
VRBPAC	Vaccines and Related Biological Products Advisory Committee
VHP	Voluntary harmonisation procedure
WHO	World Health Organization

growth hormone releasing hormone (GHRH) product for use in swine, is used as a gene therapy measure to increase the number of pigs weaned by breeding sows (Racz *et al.*, 2014). Recently, the European Medicines Agency (EMA) recommended granting approval for the first DNA vaccine in Europe. This DNA vaccine is to be used to protect salmon against pancreas disease (PD) (EMA, 2016).

Thus, all currently licensed DNA vaccines are for veterinary use only. Several experimental human DNA vaccines are currently undergoing Phase I, II and III clinical trials in order to investigate their efficacy and safety (see WHO International Clinical Trials Registry Platform and U.S. National Institutes of Health database on clinical trials). Among the vaccines being tested include DNA vaccines that could offer protection against infectious diseases such as malaria, HIV, influenza, tuberculosis and Ebola virus, and anti-tumor and gene therapy vaccines (Felber *et al.,* 2014; Wharen and Liu, 2014). To date none of these has met the regulatory requirements necessary for approval and commercialisation.

The main safety concern with DNA vaccines relates to unwanted side-effects in the vaccinated mammal or fish. Unwanted side-effects include the development of autoimmunity, host cell genome integration, injection site inflammation, antibiotic resistance due the presence of antibiotic resistance genes within the construct, and tissue destruction due to cytotoxic responses Evensen and Leong, 2013; Gillund *et al.,* 2008; Myhr and Dalmo, 2005; Pereiro *et al.,* 2014). Environmental safety concerns includes the potential of vaccine shedding to the environment and spread via predatory animals (Gillund *et al.*, 2008; Lorenzen and LaPatra, 2005). In terms of human safety, although with a low probability, there is potential adverse effects caused by self-injection by vaccinators.

Regulatory agencies such as the EMA, the World Health Organization (WHO) and the Food and Drug Administration (FDA) have published guidelines for DNA vaccines. In addition, many countries have national regulations, including those on gene technology, which must be taken into consideration before any DNA vaccine can be made available at a commercial level.

DNA vaccine: definition and development

DNA vaccines are usually produced from purified bacterial plasmid preparations. Recently, DNA vaccines have been developed not only for prophylactic purposes but also to target non-infectious health issues such as cancer and rheumatism (Juan *et al.,* 2015, Pol *et al.*, 2014).

The bacterial plasmid functions as a vehicle for gene delivery. It contains one or more DNA sequences that express a protein antigen (antigens) or peptides capable of inducing immune response (Pereiro *et al.,* 2014), flanked by a eukaryotic promotor/enhancer and a transcription/polyadenylation sequence to promote gene expression in the vaccine recipients. Some of these plasmids also contain an antibiotic resistance gene to be used for selection during the production process. The plasmid is grown in bacteria, purified, and dissolved in a solution (e.g. PBS/saline), before being administrated to the animal or human. There are different routes for administration of the vaccine: intramuscular; in the skin (subcutaneous or intradermal); and by particle mediated administration. In addition there have been a few experimental studies on oral DNA vaccine delivery. After administration of the plasmid it is usually taken up by a cell (myocytes), the DNA is transcribed and mRNA is translated to protein(s) by the cells own apparatus. Expression of the gene encoding the antigen induces an immune response resulting in disease protection (Ballesteros *et al.,* 2012).

Before any DNA vaccine can be submitted for approval to the regulatory agencies the following steps need to be carried out in order to test its safety and performance (Klug *et al*., 2012):

- Laboratory demonstration of the proof of concept
- Design and establishment of the manufacturing process
- Demonstration of adequate quality and non-clinical safety
- Clinical trial approval
- Demonstration of clinical safety and efficacy
- Marketing authorisation application

The use of animal models

All DNA vaccines are tested in animal models. To test the immunological properties of veterinary DNA vaccines against infectious diseases, the animal model need to be appropriate to establish

a proof-of concept. In the case of human vaccines against infectious diseases, tests should be carried out in animal models that have an immunogenic/biological response similar to that of humans (Klug *et al.,* 2012).

The testing in animal models are usually carried out in specific facilities that are approved by national authorities and that follow national guidelines. These national regulations are found under Acts on Animal Welfare and Guidelines for the use of research animals (see for example USDA, 1966; EU Directive 2010/63/EU; Norwegian Animal Welfare Act, 2009).

The use of human clinical trials

DNA vaccines intended for human use have to undergo a clinical development program, which include investigation and data gathering on immune response, persistence of immunity, appropriate dose and vaccination procedure.

Human clinical trials must follow guidelines and procedures that have been approved by the regulatory authorities and ethics committees [see for example EU Directive 2001/83/EC and Code of Federal Regulations Title 21 (FDA, 2016)]. The WHO has published a guideline for national authorities and vaccine manufacturers regarding the clinical assessment of vaccines (WHO, 2004). In this guideline an outline of the international regulatory expectations to the different stages of vaccine development and for marketing approval are described. In addition, most countries demand that clinical trials be performed in line with good clinical practise (GCP) (International Conference on Harmonization, 1997; EMA, 2007 and 2012a; EU Directive 2001/20/EC), and according to the Declaration of Helsinki (1966).

Informed consent to clinical trials

Before becoming commercially available for use in humans, new medicinal products must undergo clinical trials that involve testing on humans. Participation in clinical trials must be voluntary, and volunteers have to make an informed decision before hand (See Box 2).

Informed consent to participate in a clinical trial, implies that the volunteer has been informed about the purpose of the study, the potential impacts of participation on their daily life, how the research will be carried out (research protocol), the likely risks, if any, to participant health, and the possible side-effects. Volunteers should also be informed that they may withdraw from the study at any time.

The information concerning informed consent and guidelines has to be approved by the national ethics committees before being presented to volunteers. The WHO has developed templates that can be used for obtaining informed consent. Templates can also found under the EU Directive 2001/20/EC and FDA (2014).

Authorisation of human clinical trials

Clinical development of DNA vaccines follows a sequence of phases and starts with a Phase I study (WHO, 2004; Klug *et al.*, 2012). This aims to assess the immunogenic efficacy and safety of the vaccine. Studies are carried out on a limited number (20-80) of volunteers. If the results from the Phase I study look promising, the data generated are adapted for entering Phase II study with a higher number of volunteers (100-300). The decision to proceed to Phase III trials is based on the safety and immunogenicity data obtained in Phase II. The intention in Phase III trials is to increase the safety database and to compare the protective efficacy of the vaccine with other treatments (for example older versions of vaccines). The number of volunteers in Phase III trials various between 1000-3000 individuals. Phase IV trials concerns post-marketing studies, and are carried out after approval of the marketing authorisation application (MAA) has been granted. These are used to gather information about the optimal use, efficacy and potential unexpected adverse health effects of the vaccine.

Within Europe the conduct of a clinical trial is based on submission of an application to the competent authority of the individual member state (list at http://www.ema.europa.eu/ema/

> Informed Consent is the decision, which must be written, dated and signed, to take part in a clinical trial, taken freely after being duly informed of its nature, significance, implications and risks and appropriately documented, by any person capable of giving consent or, where the person is not capable of giving consent, by his or her legal representative; if the person concerned is unable to write, oral consent in the presence of at least one witness may be given in exceptional cases, as provided for in national legislation.

Box 2. Definition of informed consent in the European Directive 2001/20/EC.

index.jsp?curl=pages/medicines/general/general_content_000155.jsp). If the clinical trial is to be conducted in three or more member states a voluntary harmonisation procedure (VHP) must be submitted. This voluntary harmonisation procedure will be evaluated in a single procedure by the competent authorities from the member states where the clinical trial is planned. In this single procedure questions regarding the protocol and the product from the competent authorities will be discussed and clarified in the same procedure. However, authorisation will eventually be granted individually by each of the competent authorities.

The EMA has published guidelines that provides a comprehensive overview of the clinical development of vaccines (EMA, 2005). These guidelines include requirements to the information that should accompany a MAA concerning the DNA vaccine, the antigens and the clinical data. In addition, the EMA has also developed guidelines for clinical trials performed outside Europe where the intention is to apply for a MAA that includes Europe (EMA, 2007 and 2012a).

Vaccine clinical development within the USA follows the general pathway established for drugs and other biologics by the FDA. The regulations pertaining to food and drugs are found in section 21 of the Code of Federal regulations (CFR) (FDA, 2016). Application for clinical trials are termed Investigational New Drug (IND) application (FDA, 2007). The FDA assess both the application for clinical trial as well as the MAA.

Marketing authorisation application (MAA)
When the safety and efficacy of a DNA vaccine has been demonstrated in animal models and through Phase I-III clinical trials, the next stage is the MAA. This should be submitted to the national competent authority in the country where the vaccine will be commercially available. Such a MAA must fulfil the requirements that are found in common technical documents and these documents are published on the homepages of the various countries national competent authorities.

In Europe the European Medicines Agency (EMA) operates a centralised system for MAAs for human and veterinary medicines. All DNA vaccines MAAs have to be made through this system (EU, 2004). Once submitted to the EMA, the MAA for a DNA vaccine will be processed either by the Committee for Medicinal Products for Human Use or by the Committee for Medicinal Products for Veterinary Use i.e. according to the intended final use.

Once submitted the MAA is handled according to a strict timetable. For example, the DNA vaccine quality, non-clinical and clinical data must be assessed within 210 days. The Committee assesses this and other relevant information concerning the quality, safety and efficacy of the DNA vaccine. The Committee's opinion is forwarded to the European Commission which makes the decision on whether to grant a marketing authorisation in the EU.

In the USA the applicant has to submit a Biologics License Application (BLA) to the FDA. The license application needs to contain information about the efficacy and safety of the DNA vaccine that is considered necessary for the assessment of risk and benefit. The FDA's Center for Biologics Evaluation and Research (CBER) is responsible for regulating vaccines in the USA. The FDA makes a distinction between purpose: DNA vaccines against infectious diseases are regulated by the CBER Office of Vaccines Research and Review (OVRR), while DNA vaccines that are intended for non-infectious therapeutic uses fall under the responsibility of CBER´s Office of Cellular, Tissue and Gene Therapies (OCTGT).

The decision to recommend or reject the approval of a DNA vaccine is done by an interdisciplinary review team, that includes microbiologists, chemists and biostatisticians. If the application is recommended it will be submitted to the Vaccines and Related Biological Products Advisory Committee (VRBPAC). This is an expert committee consisting of scientists and a consumer representative that provides advice to the FDA. If this expert committee also recommends approval, a decision will be made by the FDA on whether to grant a marketing authorisation in the USA.

DNA vaccines against infectious versus therapeutic use
The regulatory authorities in Europe and in the USA have established slightly different regulatory pathways for plasmid DNA vaccines for

prophylaxis against infectious diseases versus therapeutic use.

As mentioned above, in the USA, DNA vaccines against infectious diseases are regulated by the OVRR, while DNA vaccines that are intended for non-infectious therapeutic purposes are regulated by the OCTGT.

In the European Union, all DNA vaccines, regardless of whether they are to be used for therapeutic purposes or against infectious disease are submitted to the EMA (EU, 2004). As mentioned previously DNA vaccine against infectious diseases will be assessed according to the intended final use i.e. for human use or for veterinary use. In addition, anti-infectious disease vaccines are explicitly excluded from the GTMP definition found in the EU Directive 2009/120/EC. However, a therapeutic DNA vaccine is considered to be a gene therapy medicinal product (GTMP) so are assessed differently (see below).

Relevance of GMO regulations
The MAA for a therapeutic DNA vaccine through the centralised procedure must be accompanied by an environmental risk assessment as specified in EU Directive 2001/18/EC. The objective of this risk assessment is to identify and assess on a case-by-case basis the potential harmful effects of a GMO for humans, animals (domestic and wildlife), plants, microorganisms and the environment. In the case of GTMPs it will therefore be important to investigate potential risk of vaccine shedding to the environment and transmission to other organisms in the environment.

The EMA has developed two specific guidelines for the preparation of the environmental risk assessment. These facilitate the adoption of the requirements and the methodology of the Directive to GMO-containing medical products. Although these guidelines have mainly been used for MAA of GM vaccines, they may provide necessary information and procedures that can also be used to perform environmental risk assessment of DNA vaccines.

Technical guidelines and safety aspects of DNA vaccines
Various national and international authorities have developed technical guidelines for DNA vaccines. The clinical guidelines of DNA vaccines covers issues such as characterisation of the immune response, investigation of the appropriate dose, assessment of the persistence of detectable immunity, and consideration of the need for and response to booster doses (see for example EU Directive 2001/83/EC; FDA 2007; WHO, 2004).

There are also technical guidelines that cover other safety aspects. These include the FDA CBER guidance for industry (FDA, 2007), the WHO technical report (WHO, 2007), and the EMA guidelines for the veterinary use of DNA vaccines (EMEA, 2001). In the case of the latter, the EMA is currently in the process of revising and updating their guidelines with the aim of providing specific guidance regarding the use of DNA vaccines in humans and for veterinary use (EMA, 2007, 2012b, 2016).

The EMA guidelines for the veterinary use of DNA vaccines (EMEA, 2001), state that several aspects must be considered in order to conduct a risk assessment of DNA vaccines. This includes:

1. The possibility of plasmid DNA integrating into the chromosome,
2. Concerns about possible adverse effects on the immune system,
3. Risks posed by the additional use of genes encoding cytokines or co-stimulatory molecules, and
4. Undesirable biological activity by the expressed antigen itself.

The FDA guidelines include the above and also recommend that safety testing should include (FDA, 2007):

1. Tests on vaccine immunogenicity, effects from cytokines and other immunomodulatory genes and autoimmunity,
2. Tests of local reactogenicity and systemic toxicity, and
3. Studies of bio-distribution, persistence and integration.

Safety aspect and relevant studies

Information about the plasmid and the gene of interest
According to the technical guidelines a detailed description of the DNA plasmid is compulsory

and must include information concerning the origin and nucleotide sequence of the gene(s) encoding the protein (or peptide), the choice of selection marker, the identity of the micro-organism or organism from which the gene was derived, and the origin of the microorganism that will be used (Klug *et al.*, 2012).

Biodistribution/Persistence

When a DNA vaccine is administered to mammals and fish several events may happen (Bureau *et al.*, 2004). These include; i) uptake of the DNA by cells at the administration site, ii) DNA remains extracellularly located at the administration site, iii) degradation of DNA by endonucleases at the administration site, and iv) distribution of DNA by blood, cells and lymph to various tissues. For example, in fish plasmid DNA has been detected in tissues such as the heart and kidney following injection (Seternes *et al.*, 2007; 2016; Tonheim *et al.,* 2008).

Studies of biodistribution of the DNA vaccine and persistence in the mammal or fish following administration must therefore be carried out. This can be done using nucleic acid detection techniques as for example PCR. In addition biodistribution studies should also address the risk for germ line transmission of the DNA vaccine.

Unintended immune responses following DNA vaccination

DNA vaccination has the potential to cause unintended immune response. These include the induction of autoimmune responses, e.g. antibodies to mammalian or fish DNA (Gillund *et al.,* 2008). In addition since the DNA backbone of a DNA vaccine is a plasmid of bacterial origin it may also have intrinsic immunostimulatory activity. For example, the unmethylated CpG motifs found in bacterial DNA can activate the innate immune system in mammals and fish and induce the production of proinflammatory cytokines.

At present there are no requirements for the assessment of the development of autoimmune responses in the technical guidelines developed by national and international authorities (Klug *et al.*, 2012). However, it is important to monitor for unintended immunogenic effects at all stages i.e. in animal models, during clinical trials and after the approval has been granted.

Chromosomal integration

Another safety concern for DNA vaccines is their potential to integrate into the host´s genome resulting in a disruption of the host gene expression (Faurez *et al.*, 2010). It is thought that the probability for integration may be dependent on administration routes. However currently very little is known about this process. Therefore, there is a need for further research to investigate integration; this includes methods for detection and research on potential mechanisms for integration, as well as on strategies to avoid it to occur. Such studies are also of regulatory importance.

Environmental release of DNA vaccines

The potential for DNA products to be released unintentionally into the environment is another safety concern. This could happen because of accidents during vaccine production or during the vaccination process. Plasmid DNA can be resistant to breakdown in the environment. The use of antibiotic resistance selection markers may be a concern if plasmids containing such genes are entering the environment (Williams *et al.*, 2009). Therefore it may be necessary to study potential environmental effects such as, for example, the survival of plasmid DNA in the environment and the potential uptake of this DNA by other organisms in the environment.

A study in Norway on potential adverse effects by DNA vaccines

In a study by Gillund *et al.* (2008) Norwegian scientists within academia and industry and representatives from governmental authorities was asked about potential adverse consequences from DNA vaccination.

The respondents identified the following unintended immune responses: (i) destruction or damage of cells, tissues and organs due to the induced inflammatory response caused by the DNA vaccine, (ii) autoimmune responses, (iii) allergic reactions, (iv) development of tolerance to DNA vaccines, (v) development of new diseases, and (vi) other pathological changes.

The respondents identified also possible adverse effects from tissue and organ distribution of intact or fragmented plasmid DNA that included; (i) integration of plasmid DNA in the recipient genome, including the possibility of integration in the gonadal chromosome and inheritance by offspring(s), (ii) recombination, (iii) somatic

mutations, autoimmunity, and (iv) uptake of the plasmid DNA in microorganisms.

Some of the respondents also identified transfer of the DNA vaccine or parts of the plasmid DNA to microorganisms as a possible adverse effect of environmental release of intact DNA vaccines. This issue was also linked to potential to transfer of antibiotic resistance to bacteria if antibiotic resistance genes were used as marker genes in the vaccine.

However, most of the respondents also emphasised that they considered the probability for the potential adverse effects to occur to be negligible, especially concerning consequences from environmental release.

Policy relevance of persistence of DNA vaccines

The EU regulation on GM food and feed (1830/2003) specifies that products of animals treated with GM medicinal products shall not to be labelled as GM food. However, it is not clear if this labelling exemption is limited to the medicinal products authorized as GMOs, or if it includes DNA-vaccinated products.

The GMO issue may only be of concern for countries that have specific GMO legislation and that demands that GMOs and products thereof need to be labelled. In the USA and Canada there are no requirements for labelling of food containing GMOs, and they do not have specific GMO legislation.

Norwegian authorities may, for example, due to uncertainties with regard to whether DNA vaccines persist in host tissues and organs following vaccination consider treating labelling DNA vaccinated animals (to be used as food) as a GMO (Foss and Rogne, 2003). It is also unclear if Europe will regard DNA vaccinated animals as GMOs with the result that they must be regulated under Directive 2001/18/EC. Labelling vaccinated animals as a GMO may influence consumer willingness to buy these foodstuffs. Ultimate these uncertainties may prevent the use of DNA vaccines. It is therefore extremely important that research focusing on the stability of the DNA construct, bio-persistence and whether the DNA construct may become integrated into the chromosome of the recipient organism are performed.

Need of transparency

Currently all DNA vaccine applications to EU and USA are confidential. This lack of openness and availability of assessment information may reduce the public credibility and confidence in decisions regarding DNA vaccines.

The data and the risk assessments supporting the application for DNA vaccines against West Nile Virus (in the USA) and the IHNV (in Canada), are not publicly available. In Canada the CFIA provided a public report on the environmental assessment of the IHNV DNA vaccine. This was, however, not made available to the public until after the approval of the vaccine was made. This lack of transparency is also found in the EU, where medicinal products lack the public openness issues that are otherwise central to non-nucleic acid drug applications.

Although patent rights linked to DNA vaccines may hamper openness and transparency during development and testing in animal models and clinical trials, authorities should consider how to increase the transparency concerning the regulatory process of DNA vaccine applications. Increased transparency may help to ensure the integrity of the process by disclosing the authorities' evaluation, and provide a mechanism to improve the knowledge base upon which to make future policy decisions.

Conclusion

At present only four veterinary DNA vaccines have been approved for commercial use. The regulatory framework varies between countries and the requirements to be fulfilled generally are based on whether the vaccine is for human or veterinary use. With more experience - the time from research, development, and risk assessment to commercial approval may decrease, and new DNA vaccines for protection against viral, bacterial, or parasitic diseases may be available. At the same time there are safety aspects that need to be investigated, and, although some of these fall outside the regulatory scope, they are nevertheless of immense importance. The same applies to concerns regarding whether the DNA construct may become integrated into the chromosome of the recipient organism. A GMO label on DNA vaccinated animals may hamper the widespread use of DNA vaccines, hence one should carefully investigate the genes to be used in the vaccine and avoid using constructs that

may become integrated in the chromosome. A future goal should be to prove more transparency around risk assessments of health and environmental aspects concerning DNA vaccines.

References

Ballesteros, N.A., Saint-Jean, S.S.R., Perez-Prieto, S.I., and Coll, J.M. (2012). Trout oral VP2 DNA vaccination mimics transcriptional responses occurring after infection with infectious pancreatic necrosis virus (IPNV). Fish Shellfish Immun. *33*, 1249-1257. http://dx.doi.org/10.1016/j.fsi.2012.03.016

Bureau, M.F., Naimi, S., Torero Ibad, R., Seguin, J., Georger, C., Arnould, E., Maton, L., Blanche, F., Delaere, P., and Scherman, D. (2004). Intramuscular plasmid DNA electrotransfer: biodistribution and degradation. Biochim. Biophys. Acta *1676*, 138–148. http://dx.doi.org/10.1016/j.bbaexp.2003.11.005

CFIA (Canadian Food Inspection Agency) (2007). Veterinary Biologics guideline 3.2 E. Guideline for the regulation of veterinary biologics produced by biotechnology. http://www.inspection.gc.ca/english/anima/vetbio/info/vb302e.shtml, accessed 15 January 2016.

Chang, G.J. J., Davis, B.S., Stringfield, C. and Lutz, C. (2007). Immunization of the endangered California condors (*Gymnogyps californianus*) protects this species from lethal West Nile virus infection, Vaccine *25*, 2325-2330. http://dx.doi.org/10.1016/j.vaccine.2006.11.056

Declaration of Helsinki (1996). World medical association Declaration of Helsinki, ethical principles for medical research involving human subjects, http://www.wma.net/en/30publications/10policies/b3/17c.pdf, accessed 15 December 2015.

EMA (European Medicines Agency) (2005). Note for guidance on the clinical evaluation of vaccines. Scientific opinion of the panel on animal health and welfare.
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003875.pdf, accessed 14 January 2016.

EMA (European Medicines Agency) (2007). Concept paper on guidance for DNA vaccines. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003855.pdf, accessed 7 November 2015.

EMA (European Medicines Agency) (2012a). Reflection paper on ethical and GCP aspects of clinical trials of medicinal products for human use conducted outside of the EU/EEA and submitted in marketing authorisation applications to the EU Regulatory Authorities. http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2012/04/WC50012 5437.pdf, accessed 16 January 2016.

EMA (European Medicines Agency) (2012b). Committee for the Medicinal Products for Human Use: Concept paper on guidance for DNA vaccines. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/03/WC500124898.pdf, accessed 14 January 2016.

EMA (European Medicines Agency) (2016). Committee for Medicinal Products for Veterinary Use (CVMP) Meeting of 19-21 April 2016.http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2016/04/news_detail_002514.jsp&mid=WC0b01ac058004d5c1, accessed 28 June 2016.

EMA (European Medicines Agency). National competent authorities (human). http://www.ema.europa.eu/ema/index.jsp?curl= pages/medicines/general/general_content_000155.jsp, accessed 30 July 2016.

EMEA (European Medicine Agency) (2001) Committee for Veterinary Medicinal Products, Note for guidance: DNA vaccines non-amplifiable in eukaryotic cells for veterinary use. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500004614.pdf, accessed 14 January 2016.

EU (2003). Regulation (EC) no 1829/2003 on GMO food and feed. http://eur-lex.europa.eu/eli/reg/2003/1829/oj, accesses 16 January 2016.

EU (2004). Regulation (EC) no 726/2004 on Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:136:0001:0033:en:PDF, Accessed 18 September 2016.

EU Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use (Consolidated version: 16/11/2012), http://

ec.europa.eu/health/files/eudralex/vol-1/dir_2001_83_cons/dir2001_83_cons_20081230_en.pdf, accessed 5 January 2016.

EU Directive 2001/20/EC of the European Parliament and of the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implemen-tation of good clinical practice in the conduct of clinical trials on medicinal products for human use, http://ec.europa.eu/health/files/eudralex/vol-1/dir_2001_20/dir_2001_20_en.pdf, accessed 5 January 2016.

EU Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC, http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32001L0018, accessed 05 January 2012.

EU Directive 2009/120/EC (2009) Amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use as regards advanced therapy medicinal products, http://ec.europa.eu/health/files/eudralex/vol-1/dir_2009_120/dir_2009_120_en.pdf, accessed 05 January 2012.

EU Directive 2010/63/EU (2010). Legislation for the protection of animals used for scientific purposes. http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm, accessed 5 January 2016.

Evensen, O., and Leong, J.A. (2013). DNA vaccines against viral diseases of farmed fish. Fish Shellfish Immunol. *35*, 1751-1758. http://dx.doi.org/10.1016/j.fsi.2013.10.021

Faurez, F., Dory, D., Le Moigne, V., Gravier, R., and Jestin, A. (2010). Biosafety of DNA vaccines: New generation of DNA vectors and current knowledge on the fate of plasmids after injection. Vaccine *28*, 3888-3895. http://dx.doi.org/10.1016/j.vaccine.2010.03.040

FDA (Food and Drug Authorities) (2007). Guidance for industry: Considerations for plasmid DNA vaccines for infectious disease indications. http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm074770.html Accessed 5 January 2016.

FDA (Food and Drug Authorities) (2014). Informed consent information sheet. http://www.fda.gov/RegulatoryInformation/ Guidances/ucm404975.htm, Accessed 14 January 2016.

FDA (Food and Drug Authorities) (2016). Title 21- Food and Drugs. http://www.ecfr.gov/cgi-bin/text-idx?SID=3ee286332416f26a91d9e6d786a604ab&mc=true&tpl=/ecfrbrowse/Title21/21tab_02.tpl, Accessed 14 January 2016.

Felber, B.K., Valentin, A., Rosati, M., Bergamaschi, C. and Pavlakis, G.N. (2014). HIV DNA Vaccine: Stepwise improvements make a difference. Vaccines *2*, 354-379. http://dx.doi.org/10.3390/vaccines2020354

Foss G.S., and Rogne, S. (2003). Gene modification or genetic modification? The devil is in the Details. Nature Biotech. *21*, 1280-1281. http://dx.doi.org/10.1038/nbt 1103-1280

Gillund, F., Tonheim, T., Seternes, T., Dalmo, R.A. and Myhr, A.I. (2008). DNA vaccination in aquaculture –Expert judgments of impact on environment and fish health. Aquaculture *284*, 25–34. http://dx.doi.org/10.1016/j.aquaculture.2008.07.044

International Conference on Harmonization (IHC) (1997). Good clinical practice. http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html, accessed 5 January 2016.

Juan, L., Xiao, Z., Song, Y., Zhijian, Z., Jing, J., Kun, Y., Yuna, H., Dongfa, D., Lili, D. and Liuxin, T. (2015) Safety and immunogenicity of a novel therapeutic DNA vaccine encoding chicken type ii collagen for rheumatoid arthritis in normal rats. Hum. Vaccines Immunother. *11*, 2777-2783. http://dx.doi.org/10.1080/ 2164 5515.2015. 1073425

Klug, B., Reinhardt, J. and Robertson, J. (2012) Current status of regulations for DNA vaccines. In J. Thalhamer et al. eds. Gene Vaccines, (Wien: Springer-Verlag), pp. 285-295.

Lorenzen, N., and LaPatra S.E. (2005). DNA vaccines for aquacultured fish. Rev. Sci. Tech. *24*, 201–213.

Norwegian Animal Welfare Act (2009). https://www.regjeringen.no/en/dokumenter/animal-welfare-act/id571188/, Accessed 25 January 2016.

Myhr, A.I., and Dalmo, R. (2005). Introduction of genetic engineering in aquaculture: Ecological and ethical implications for science and governance. Aquaculture *250*, 542–554. http://dx.doi.org/10.1016/j.aquaculture.2004.12.032

Pereiro, P., Dios, S., Boltana, S., Coll, J., Estepa, A., Mackenzie, S., Novoa, B., and Figueras, A.

(2014). Transcriptome profiles associated to VHSV infection or DNA vaccination in turbot (*Scophthalmus maximus*). PlosOne *9*. http://dx.doi.org/10.1371/journal.pone.0104509

Pol, J., Bloy, N., Obrist, F., Eggermont, A., Galon, J., Hervé Fridman, W., Cremer, I., Zitvogel, L., Kroemer, G. and Galluzzi, L. (2014) Trial watch. OncoImmunol. *3*, e28185. http://dx.doi.org/10.4161/onci.28185

Racz, R., Li, X.N., Patel, M., Xiang, Z.S., and He, Y.Q. (2014). DNAVaxDB: the first web-based DNA vaccine database and its data analysis. BMC Bioinformatics *15*. http://dx.doi.org/10.1186/1471-2105-15-S4-S2

Salonius, K., Simard, N., Harland, R., and Ulmer, J.B. (2007). The road to licensure of a DNA vaccine. Curr. Opin. Invest. Drugs *8*, 635-641.

Seternes, T., Tonheim, T.C., Løvoll, M., Bøgwald, J., and Dalmo, R.A. (2007). Specific endocytosis and degradation of naked DNA in the endocardial cells of cod (*Gadus morhua* L.). J. Exp. Biol. *210*, 2091-2103. http://dx.doi.org/10.1242/jeb.003186

Seternes, T., Tonheim, T.C., Myhr, A.I., and Dalmo, R.A. (2016). A plant 35S CaMV promotor induces long-term expression of luciferase in Atlantic salmon. Sci. Rep. published online 6 June 2016. http://dx.doi.org/10.1038/srep25096

Tonheim, T.C., Dalmo, R.A., Bogwald, J., and Seternes, T. (2008). Specific uptake of plasmid DNA without reporter gene expression in Atlantic salmon (*Salmo salar* L.) kidney after intramuscular administration. Fish Shellfish Immun. *24*, 90-101. http://dx.doi.org/10.1016/j.fsi.2007.09.006

USDA (U.S. Department of Agriculture) (1966). Animal Welfare Act. http://awic.nal.usda.gov/government-and-professional-resources/federal-laws/animal-welfare-act, accessed 15 January 2016.

U.S. National Institutes of Health. Clinical trials database. https://clinicaltrials.gov/ct2/search/index, accessed 22 July 20166.

Wharen, B. and Liu, M.A. (2014). DNA vaccines: Recent developments and the future. Vaccines *2*, 785-796. http://dx.doi.org/10.3390/vaccines2040785

WHO (World Health Organization) (2004). Guidelines on clinical evaluation of vaccines: regulatory expectations, TRS 924, Annex 1. http://www.who.int/biologicals/publications/trs/areas/vaccines/clinical_evaluation/035-101.pdf?ua=1, accessed 14 December 2015.

WHO (World Health Organization) (2007). WHO expert committee on biological standardization, Technical Report Series No. 941/20, http://apps.who.int/iris/bitstream/10665/43594/1/WHO_TRS_941.pdf, accessed 14 January 2016.

WHO (World Health Organization). Informed Consent Form Templates , http://www.who.int/rpc/research_ethics/informed_consent/en/, accessed 14 December 2015.

WHO (World Health Organization). International Clinical Trials Registry Platform, http://apps.who.int/trialsearch/, accessed 22 July 2016.

Williams, J.A., Carnes, A.E, and Hodgson, C.P. (2009) Plasmid DNA vector design: impact on efficacy, safety and upstream production. Biotechnol. Adv. *27*, 353-370. http://dx.doi.org/10.1016/j.biotechadv.2009.02.003