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Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations

NOTE:

This draft document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein which will then be considered by the WHO Expert Committee on Biological Standardization (ECBS). The distribution of this draft document is intended to provide information on the proposed document: Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations to a broad audience and to ensure the transparency of the consultation process.

The text in its present form does not necessarily represent the agreed formulation of the ECBS. Written comments proposing modifications to this text MUST be received by 17 September 2021 using the Comment Form available separately and should be addressed to the Department of Health Products Policy and Standards, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland. Comments may also be submitted electronically to the Responsible Officer: Dr Tiequn Zhou at: zhout@who.int.

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the second edition of the *WHO style guide* (KMS/WHP/13.1).

Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations

Guidance documents published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes regulatory considerations for national regulatory authorities (NRAs) and for manufacturers of biological products.

Abbreviations

AESI	adverse events of special interest
COVID-19	coronavirus disease 2019
DNA	deoxyribonucleic acid
dsRNA	double-stranded RNA
ELISA	enzyme-linked immunosorbent assay
GMP	good manufacturing practice(s)
HPLC	high-performance liquid chromatography
IU	International Unit(s)
IVT	in vitro transcription
LNP	lipid nanoparticle
mRNA	messenger RNA
NRA	national regulatory authority
ORF	open reading frame
PCR	polymerase chain reaction
PEG	polyethylene glycol
PEGylation	polyethylene-glycol-ylation
PEGylated	polyethylene-glycol-ylated
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
tRNA	transfer RNA
UTR	untranslated region
WHO	World Health Organization

1. Introduction

Although the immunostimulatory effects of RNA have been known since the early 1960s (1), the possibility of using direct in vivo administration of in vitro transcribed messenger RNA (mRNA) to temporarily introduce genes expressing proteins (including antigens) was demonstrated in 1990 following the direct injection of "naked" nucleic acids (2). Subsequent improvements in stabilizing mRNA, increasing the feasibility of manufacturing RNA-based products and decreasing RNA-associated inflammatory responses have led to significant advances in the development of mRNA vaccines and therapeutics (3–6). There are several reasons why the mRNA platform has emerged at the forefront of vaccine technology. Among these are the rapid speed at which mRNA candidate vaccines can be constructed and manufactured, and the need to rapidly develop vaccines against emerging pathogens, such as zoonotic influenza virus strains, Zika virus and most recently severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19).

A number of publications have now discussed some of the safety, production and regulatory issues associated with this new technology (7–11). In addition, the rapidity with which clinical trials have progressed for COVID-19 candidate vaccines, their emergency use approval or authorization (or conditional marketing authorization) by NRAs, and subsequent widespread use have created a pressing need for WHO guidance on evaluating the quality, safety and efficacy of mRNA products used for the prevention of infectious diseases in humans. Such evaluations must take into account; (a) the inherent immunological, physiochemical and structural properties of mRNA; (b) the need for formulation to ensure stability and efficient delivery; and (c) the novel manufacturing process. Because detailed information is not yet available on the methods used for production, controls are not yet standardized for safe and efficacious mRNA vaccines, and (in the case of candidate vaccines) certain details remain proprietary and thus not publicly available, it is not feasible to develop specific international guidelines or recommendations at this time. Consequently, regulatory flexibility is currently needed. The detailed production and control procedures, as well as any significant changes in them that may affect the quality, safety and efficacy of mRNA vaccines, should be discussed with and approved by the NRA on a case-by-case basis. Nevertheless, the key principles described in this document are applicable to the class of preventive mRNA vaccines for human use in general and are intended to provide guidance until more detailed information becomes available. For mRNA vaccines that target diseases for which there are existing vaccines and corresponding WHO guidance, it may be appropriate to consider the relevant sections of this document for issues specific to mRNA vaccines in conjunction with the corresponding Part B (nonclinical evaluation) and Part C (clinical evaluation) of the respective WHO Recommendations and Guidelines for guidance on issues specific to the evaluation of vaccines against that disease (12).

Any given manufacturer's mRNA vaccines might potentially be viewed as a platform technology in which the gene insert can readily be changed without necessarily having to change the manufacture or control of the resulting new product (except for immunogen-specific tests for identity, stability and potency). However, this will depend on the resulting characteristics of the final vaccine. If significant changes are made to the final vaccine, resulting in changes to the critical quality attributes as well as subsequent cellular interaction, then further consideration of the manufacturing process, controls and testing of the product will be required.

The WHO Expert Committee on Biological Standardization discussed these and related issues at its meetings in August and December 2020, and expressed its support for the development of a WHO guidance document on regulatory considerations in the evaluation of mRNA vaccines, which could be updated as more scientific and clinical data became available (13,14).

2. Purpose and scope

This document provides information and regulatory considerations regarding key aspects of the manufacture and quality control, and nonclinical and clinical evaluation, of preventive mRNA vaccines for human use. Although the most advanced vaccines in this class are COVID-19 vaccines and are used as examples in the text, the document should not be taken as providing guidance specific only to COVID-19 vaccines. However, in light of the current COVID-19 pandemic and corresponding speed of mRNA vaccine development, the document is intended to provide special considerations for this class of preventive mRNA vaccine as rapidly as possible. It should nevertheless be noted that there remain knowledge gaps in the scientific understanding of the pathogenesis of COVID-19 and of precisely what level of immunogenicity is needed for a successful, broadly relevant and durable COVID-19 vaccine. These knowledge gaps are currently being addressed by ongoing research and development efforts.

Because mRNA vaccines are novel and differ from other types of vaccines (even other nucleic acid vaccines such as plasmid DNA vaccines) a short introduction to mRNA-vaccine-specific topics is provided where deemed useful. Due to the novelty of mRNA vaccines and their manufacturing process, a comprehensive approach has been taken to ensure that all relevant aspects can be considered by manufacturers when developing this type of product, and by regulators when evaluating such products.

The scope of the document is limited to mRNA and self-amplifying mRNA (sa-mRNA) packaged in lipid nanoparticles (LNPs) for in vivo delivery of the coding sequences of a target antigen relevant to active immunization for the prevention of an infectious disease. It is acknowledged that mRNA and sa-mRNA products in formulations other than LNPs are also in development, and parts of this document may be applicable to those products as well.

Replicating agents, viral vectors and RNA replicons (packaged in viral proteins or encoded by plasmid DNA) are outside the scope of this document. In addition, mRNA and sa-mRNA products intended for therapeutic purposes (that is, products for the treatment, mitigation or cure of diseases, including infectious diseases, as opposed to active immunization for their prevention) are also outside the scope of this document. In addition, mRNA products expressing monoclonal antibodies (whether for disease prevention or therapy) are also outside the scope of this document. It may be the case that some aspects discussed in section 6 do apply to mRNA-based therapeutic products (including those expressing monoclonal antibodies) as the manufacturing steps of such products may be similar to those described for vaccines. However, because the nonclinical and clinical evaluations of such therapeutic products would need to be based on their therapeutic indication, it is not feasible to include regulatory considerations for them within this document.

As there may be a need to develop multivalent mRNA vaccines or to change the existing vaccine strain for some pathogens (for example, influenza viruses or SARS-CoV-2) then specific considerations are provided in this document where appropriate; in addition, any general WHO guidance of relevance should also be consulted.

Because regulatory pathways for emergency use authorization vary and not all NRAs have such pathways, approval for emergency use is also outside of the scope of the document. However, suggestions are provided, where possible, for rapid vaccine development in the case of priority pathogens during public health emergencies (see sections 7.3 and 8.3).

This document has been developed in light of the available knowledge to date and will need to be updated as new data become available. Given that this is a dynamic field, both in terms of vaccine manufacturing technologies and clinical trial design, this document should be read in conjunction with other relevant recent guidance, including WHO disease-specific guidelines and recommendations, if available.

3. Terminology

The definitions given below apply to the terms as used in this document. These terms may have different meaning in other contexts.

Adjuvant: a substance intended to enhance the relevant immune response and subsequent clinical efficacy of a vaccine.

Biological: a medicine produced by a biological system, as opposed to strictly chemical reactions. These include traditional biologicals (such as live vaccines) and biotechnologically produced

medicines (such as monoclonal antibodies or subunit vaccines such as human papillomavirus vaccines). In other documents, these may be referred to as biologics or biological medicines.

Candidate vaccine: an investigational vaccine that is in the research and clinical development stages and has not been granted marketing authorization or licensure by a regulatory agency in the country in which such authorization or licensure will be sought.

Drug product: see **final vaccine**.

Drug substance: the purified mRNA before final formulation. It is prepared as a single homogeneous production batch, kept in one or more containers designated as such and used in the preparation of the final dosage form (final vaccine or drug product).

double-stranded RNA (dsRNA): fully double-stranded RNA along its entire length rather than in distinct segments (such as the secondary structure of mRNA), and which can be generated as a by-product during the in vitro transcription (IVT) manufacturing process for mRNA vaccines. As viruses with genomes made of dsRNA are sensed by intracellular receptors and can, if present, activate innate immune responses, dsRNA is an impurity that needs to be removed from the mRNA during the manufacturing process, or its amount in the product at least determined and controlled.

Excipient: a constituent of a medicine other than the active substance, added in the formulation for a specific purpose. While most excipients are considered inactive, some can have a known action or effect in certain circumstances. The excipients must be declared in the labelling and package leaflet of the medicine to ensure its safe use. In the context of this document, the lipids that form the LNPs are excipients but the LNPs themselves are defined as intermediates of the **drug product**.

Final formulated bulk: an intermediate in the manufacturing process of the **final vaccine**, consisting of a homogeneous preparation of the final formulation of drug substance(s) and **excipients** at the concentration to be filled into final containers. Alternatively, the final formulated bulk may be stored at a higher concentration and diluted immediately prior to filling. In the context of this document, the term refers to mRNA formulated with LNPs and other excipients as needed.

Final lot: a collection of sealed final containers that is homogeneous with respect to the composition of the product and the avoidance of contamination during filling. A final lot must therefore have been filled from a final formulated bulk in one continuous working session. A final formulated bulk might be filled into more than one final lot.

Final vaccine (or drug product): a final dosage form (for example, a vialled frozen or liquid suspension or lyophilized cake) that contains an active ingredient (drug substance) typically formulated with excipients and packaged for use. In the context of this document, the term refers to a preparation of mRNA formulated with LNPs and other excipients that is filled into final containers. If filled in concentrated form or lyophilized a diluent is needed. Otherwise, the final containers should be filled at the concentration for the clinical dose (though each container might contain multiple doses). Also referred to as "finished product" in other documents.

Good manufacturing practice (GMP): a system that ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.

Immunogenicity: the capacity of a vaccine to elicit a measurable adaptive immune response against a target antigen(s).

in vitro transcribed mRNA: the product of a manufacturing process whereby mRNA is generated in vitro from a linear DNA template using a DNA-dependent RNA polymerase enzyme (for example, a T7, T3 or Sp6 phage RNA polymerase) and nucleoside triphosphates.

Lipid nanoparticle (LNP): a delivery formulation consisting of various lipid components to ensure that the mRNA is stabilized and encapsulated, for example, to avoid extracellular degradation and to facilitate its uptake into cells and release into the cytosol. The LNPs may also have adjuvant activity. To enhance stability, the lipids may undergo modifications such as polyethylene-glycol-ylation (PEGylation).

Marketing authorization or approval: a formal authorization for a medicine (including vaccines) to be marketed. Once an NRA approves a marketing authorization application for a new medicine (different NRAs may give these applications different designations), the medicine may be marketed and may be available for physicians to prescribe and/or for public health use (also referred to as product (drug or biological) licensing, product authorization or product registration).

Mode-of-action and mechanism-of-action: the manner in which the adaptive immune response elicited by the vaccine protects against the pathogen at the cellular (mode) or molecular (mechanism) level – for example, neutralization by neutralizing antibodies, opsonization by opsonizing antibodies or cytotoxicity by T cells.

Modified nucleosides: naturally occurring modified nucleosides (such as pseudouridine) that can be substituted for the usual nucleoside (in this case, uridine) when making mRNA vaccines,

with a resultant potential decrease in inflammatory activity and/or an increase in stability. Another type of modification is methylation. Nucleosides might also contain unnatural modifications.

messenger RNA (mRNA): a single-stranded RNA molecule that is translated in the cytoplasm of a cell into the protein that it encodes. It contains an open reading frame (ORF) that encodes the protein (in the case of vaccines, the target antigen), flanking untranslated regions, a 5′ cap (or alternative) and a 3′ sequence such as a poly(A) tail.

Novel excipient: an **excipient** (for example, a lipid) not used before in any medicine approved or licensed for human use, or if previously used in an approved or licensed medicine for human use then not using the same route of administration (and/or present at a higher concentration) as that approved or licensed. The word "novel" is used in the same way to describe other terms elsewhere in this document.

Platform technology: a group of technologies used as a base upon which other applications, processes or technologies are developed. In the context of mRNA vaccines, a given manufacturer might have one or more platforms on which they will develop vaccines (or therapeutics) against various diseases or pathogen strains. At present, experience exists for strain changes but in future experience will be gained in the use of a platform technology to develop new vaccines. A platform would be considered when the manufacturing methods are essentially unchanged, the test methods (except for identity) and specifications are not changed, the immunomodulatory compounds or elements are unchanged, and the compliance with GMP is unchanged. One implication of the use of platform technology to develop new candidate vaccines is that the experience and knowledge gained, data generated (manufacturing, control, stability and nonclinical) and validation of unchanged methods can all be used as supportive data for the more rapid assessment and development of a new candidate vaccine. Clinical data from the platform in terms of safe starting doses or tolerable doses might also be supportive of initiating clinical trials of the new candidate vaccine at doses already known to be tolerable with the platform. If aspects of the platform technology have been changed, along with the mRNA sequence, then justification should be provided as to why data generated with the original platform should be considered supportive of the new candidate vaccine. Because the production and control methods used for mRNA vaccines are not yet standardized between manufacturers, information from other manufacturers would not be supportive of a platform technology. Such information may be considered to be similar to that for a product class and evaluated as being supportive if justification is provided and compelling. Furthermore, regulatory flexibility is justified because of the current lack of standardization even in the face of platform technology use. As always, a case-by-case approach is justified and should be discussed and agreed with the relevant NRA(s).

Self-amplifying mRNA (sa-mRNA): an mRNA vaccine that in addition to encoding the desired antigen(s) also encodes nonstructural proteins of certain alphaviruses (either on the same molecule as the antigen or on a separate molecule). When expressed intracellularly, these ORFs produce the proteins of an alphavirus's replication machinery, enabling the cell to produce multiple copies of the mRNA encoding the antigen protein. The goal of sa-mRNA is to increase the in vivo potency of the mRNA vaccine by increasing the amount of protein antigen made. Other designations have been given to this form of mRNA vaccine but in this document the term sa-mRNA will be used.

Therapeutic: a treatment given after a disease or condition (or signs or symptoms thereof) are evidenced, in contrast to the prevention of disease before exposure to the infectious organism has occurred. Although preventive vaccines are not considered to be therapeutic in this document, it is acknowledged that the definition of therapeutic in some regulatory jurisdictions may differ. Therapeutics as defined above are outside the scope of this document.

transfer RNA (tRNA): an RNA molecule used by ribosomes and which acts as an adaptor involved in translating the codons of the mRNA into a protein.

4. General considerations

As with all vaccines, the intended clinical use of the mRNA vaccine should be described, including the pathogen targeted, the antigen(s) chosen, disease to be prevented and the target population(s). Given the novel structure and manufacturing of mRNA candidate vaccines (in contrast to other already licensed vaccine types with which regulators are familiar) consideration should be given to the following when evaluating mRNA vaccines for their quality, safety and efficacy:

- In particular, the relevant biological characteristics of the specific mRNA technology used should be described – including for example, the capability of the given mRNA to trigger innate immune responses as well as target-antigen-specific responses; the quality, quantity and bias of the immune responses (for example, type 1 T-helper (Th1) or Th2 cell phenotype); and biostability. To justify the vaccine design, all available information on the type of immunity considered relevant to the specific pathogen and disease should also be described.

- The rationale for the selection of the target antigen(s) or parts thereof and of any proteins (for example, cytokines) that are encoded, as well as their contribution to the proposed mode- or mechanism-of-action (proposed protective process) of the vaccine, should be clearly described. Likewise, the rationale for the selection of any coding sequences added to or any modification of the target antigen, such as those to ensure

the folding of the target antigen into a particular conformation, should be provided. The complete annotated sequence identifying all ORFs (including any unexpected ORFs) and all other sequence elements (including their justification for use) should be provided. Justifications for the use of any specific noncoding sequence and of structural elements such as the chosen 5` cap structure should be provided. With regard to sa-mRNA, any viral replicase genes encoded in the vaccine construct to allow amplification of the mRNA in human cells after delivery should be described in detail. The anticipated function and purpose of each gene sequence encoded in the mRNA should be indicated, as well as those of specific noncoding and structural elements, explaining their contribution to the overall mode- or mechanism-of-action.

- The formulation of the final vaccine product and all excipients (including all components used for the generation of LNPs) should be described. An appropriate rationale for the proposed composition of the final vaccine and inclusion of excipients should be provided. Information on the method of production of the LNPs and the final vaccine (drug product) including information on the critical quality attributes of the intermediates and final product, their in-process controls and any sterilization procedure should also be provided.

- For each novel excipient (see **Terminology** for definition) detailed information on the rationale for its inclusion, the method of production (including details and controls on the starting materials, intermediates and raw materials) and data from nonclinical studies on its safety (and if required by a given NRA, safety pharmacology) should be provided.

- The intended dosing, the route of administration, and a description and justification of any novel administration device as well as any required diluent should be provided. Relevant compatibility studies should be performed where necessary.

- Although any given manufacturer’s mRNA vaccine product may be considered to be produced by a platform technology if only the target antigen sequence is changed, the control, nonclinical testing and clinical development of each vaccine should be considered individually, and any special features of that candidate vaccine taken into account. Early consultation with the NRA(s) will be key to ensuring the efficient development of any given candidate vaccine.

- With regard to the development of multivalent candidate vaccines, noting the development of precedents might be helpful. Relevant examples might include: (a) seasonal influenza virus vaccines, which are both multivalent and undergo annual

strain changes; (b) human papillomavirus vaccines such as the quadrivalent vaccine that was changed after initial approval to a nonavalent vaccine, trivalent poliomyelitis vaccines, multivalent rotavirus vaccines and multivalent pneumococcal vaccines, which are used against different strains that cause the same (or related) disease(s); or (c) diphtheria and tetanus-toxoid-containing vaccines or measles, mumps and rubella vaccines, which are combination vaccines used against different disease targets. Available guidance on the development of combination vaccines against multiple diseases may also be considered.

The current document should be read in conjunction with other relevant WHO guidelines such as:

- WHO guidelines on nonclinical evaluation of vaccines (15);
- Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (16);
- Guidelines on clinical evaluation of vaccines: regulatory expectations (17);
- WHO good manufacturing practices for pharmaceutical products: main principles (18);
- Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients (19);
- WHO good manufacturing practices for biological products (20);
- WHO good manufacturing practices for sterile pharmaceutical products (21);
- Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (22);
- *WHO Guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (23);
- Guidelines on stability evaluation of vaccines (24);
- Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (25);
- Guideline on the stability evaluation of vaccines for use under extended controlled temperature conditions (26);
- Guidelines for independent lot release of vaccines by regulatory authorities (27);
- Guidelines on procedures and data requirements for changes to approved vaccines (28); and
- WHO Policy Statement: multi-dose vial policy (MDVP). Handling of multi-dose vaccine vials after opening (29).

5. Special considerations

The mRNA of vaccines that are currently the most advanced in terms of clinical development is produced enzymatically rather than biologically within a cell, and thus differs from the production of most other biologicals (1,30). Manufacturing either starts with linearized DNA

plasmids which have been produced in bacteria (similar to the way in which biologicals such as plasmid DNA vaccines are produced) or with a linear DNA molecule produced by the polymerase chain reaction (PCR). Regardless of whether the manufacture of the RNA starts from a plasmid DNA converted to a linear molecule or otherwise from an already linear DNA sequence, mRNA production occurs in vitro by means of a DNA-dependent RNA polymerase that transcribes the DNA template into an mRNA molecule. The mRNA sequence generally consists of the usual elements of cellular mRNA, such as the coding region, 5′ and 3′ untranslated regions (UTRs) which regulate mRNA translation, a 5′ cap and a 3′ poly(A) tail.

The nucleotides used in manufacture may contain naturally occurring nucleosides or modified or synthetic nucleosides (3,8). Examples of alterations to the naturally occurring nucleoside include the use of pseudouridine or N1-methylpseudouridine in place of uridine (3,4,31). In addition, altering or optimizing codon use (without changing the encoded amino acids) may impact stability and enhance in vivo translation of the mRNA in humans (for example, for translation by transfer RNAs (tRNAs) more frequently found in human cells). Alternatively, codons may be selected for more infrequent tRNAs in order to slow translation of the protein, thus permitting desired protein folding. Some changes to the mRNA are designed both to increase its stability and to moderate activation of the innate immune system *(4)*. Depending on the clinical indication, it may be desirable to decrease innate immune responses that might lead to inflammatory reactogenicity in vivo (3,4,31). For some preventive vaccines, some of the innate immune response may be considered useful for augmenting the desired adaptive immune response, and the mRNA may be designed accordingly. The gene sequence encoding the target antigen should contain start and stop codons and be flanked by 5′ and 3′ UTRs and generally have a 5′ cap and a 3′ poly(A) tail. The cap can be added to the mRNA enzymatically or during in vitro transcription (IVT) using anti-reverse cap analogues (ARCA) (3). Likewise, the 3′ poly(A) tail can be encoded in the DNA template or added enzymatically after IVT. These design features can impact the critical quality attributes and control testing of the mRNA drug substance and/or the final vaccine drug product.

Of relevance to considerations of the safety and efficacy of mRNA vaccines are the structures adopted by the RNA in the vaccine product. Unlike DNA, which is normally double stranded, most RNA is single stranded. However, depending on its sequence, RNA can form a complex structure consisting of short double-stranded segments with various single-stranded loops in between. The reason this is relevant is that truly double-stranded RNA (dsRNA) is a form taken by the genome of some RNA viruses and can induce cells to trigger immune reactivity as an innate response to viral infection. However, endogenous cellular mRNA does not induce such an effect despite containing partial double-stranded segments. The in vivo effects, including potential triggering of innate immunity, of an mRNA candidate vaccine should be characterized and addressed in the vaccine design, nonclinical studies and clinical trials.

RNA-based products can take different forms. The most advanced candidate vaccines take the form of mRNA encoding the target antigen (32,33). Because mRNA (and RNA in general) is subject to degradation by nucleases, the most advanced mRNA candidate vaccines at the time of writing (which include COVID-19 vaccines) are formulated in LNPs, which aids stability and delivery (30,34–40). There are different types of LNPs depending on their composition, the types of lipids employed and the manufacturing process used (41). Some may not yet have been employed for the delivery of mRNA (42–45). Other stabilizing and delivery systems using polymer and polypeptide, as well as other lipid-based systems or combination of polymer and lipid-based systems, may be developed for mRNA delivery in the future. These drug delivery systems could also be surface modified for tailored cellular interactions, where necessary.

It is important to note that the drug substance is the mRNA(s). The lipids which form the LNPs are excipients of the final vaccine or drug product. The manufacture of LNPs from the different lipids is part of the drug product manufacturing process. It is acknowledged that some LNPs, depending on their composition, may also have immunomodulatory effects (44–47) and some lipids may act as adjuvants without being formulated as LNPs. Nonetheless, vaccine adjuvants, which are immunomodulating to the vaccine, are also considered to be excipients. Similarly, as discussed above, RNA itself can be immunomodulating. Consequently, both components (the mRNA and the LNPs) may contribute to the mode-of-action of the vaccine and the implications of this need to be considered in the nonclinical and clinical evaluations.

Some candidate vaccines may contain various mRNAs encoding different antigens. Examples include multiple antigens from the same pathogen, the same antigen representing different strains or serotypes of the same pathogen, or multiple antigens from different pathogens. Such vaccine development is not without precedent, as discussed below in section 5. As with other combination or multivalent vaccines, the amount of mRNA for each target antigen, and the expression efficiency of each and resulting immune responses, must be balanced against the other(s) to avoid interference with expression and immune responses and to ensure the necessary strain-specific immune responses. Furthermore, consideration should be given to achieving an adequate dose for each encoded target antigen without exceeding a maximally tolerable total mRNA (and LNP) dose. Additional consideration should also be given to the manufacture, control and stability of multivalent vaccines to ensure appropriate quality control of the drug product and to ensure the suitability of the analytical procedures used to control each mRNA component in the final vaccine.

Interactions between the mRNA and the LNPs may vary depending on the length and secondary structure of the mRNA, as well as the composition of the LNPs. Therefore, the particle size, morphology and surface properties (for example, charge) of the resultant LNPs containing the mRNA could vary when a different mRNA is employed. Consideration of the critical quality

attributes and physicochemical properties of both the mRNA and the LNPs is therefore necessary to provide an understanding of the desirable properties of the final vaccine.

Some candidate products contain the components needed to permit the mRNA vaccine to be self amplifying (so-called self-amplifying mRNA or sa-mRNA) (8,35,48). These products include the gene sequences for replicase proteins (to date, from alphaviruses) in addition to the target antigen, either on the same or a different mRNA molecule. As a result, the mRNA coding for the antigen can be replicated in vivo, leading to increased expression of the target antigen. Current sa-mRNAs are also formulated in LNPs (35). There may be implications for vaccine safety and efficacy due to the design of the sa-mRNA if the target antigen is encoded either on a separate mRNA molecule or on the same molecule as the replicase gene sequences. For example, the particle size and morphological characteristics of the LNPs may vary depending on the size of the mRNA encapsulated. In addition, the total amount of mRNA needed to achieve the same level of efficacy may vary between the two designs due to differences in the degree of expression efficiency, as well as in the amount of dsRNA, the innate immune response, the half-life of the mRNA and so on – all of which could result in a different safety profile.

In contrast to viral replicons (which are packaged in viral structural proteins) sa-mRNAs are delivered in LNPs or other delivery systems. This means that the cells that take up sa-mRNAs and those that take up viral replicons are likely to differ as viral replicons enter their host cells via the viral receptor, while sa-mRNAs depend on a formulation for intracellular delivery (35). RNA-based products can also be contrasted with both viral-vectored vaccines and with live viral vaccines (for RNA viruses) by their lack of genes encoding the structural proteins of the virus being used as the vector or live vaccine. Thus, there are various similar products in development, the differences between which are largely dictated by biology or design. Other similar technologies include circular RNA products that are in development, mRNAs that use an internal ribosome entry site (IRES) instead of a cap and RNA encapsulated in other drug-delivery systems using polymer and polypeptide systems (or a combination of polymer and lipid-based systems). However, the scope of the current document is limited at present to mRNA or sa-mRNA encapsulated in LNPs.

It should also be noted that current mRNA vaccines are designed to target the mRNA to immune cells, particularly antigen-presenting cells such as dendritic cells. However, their design does not limit targeting to these cells exclusively. It is envisaged that future delivery systems will be designed to potentially target the mRNA to specific cells or tissues – for example, through the use of surface-modified LNPs in which a targeting ligand/motif could be attached to the LNP surface. Any changes to the physicochemical properties that result in different innate immunostimulatory effects may have further implications for the safety or efficacy of the mRNA or sa-mRNA vaccine.

6. Manufacture and control of mRNA vaccines

All mRNA vaccines are regulated as biologicals and as with other biologicals adequate control of the starting and raw materials and excipients and of the manufacturing processes is as important as that of the final product. Regulatory considerations therefore place considerable emphasis on the control strategy for the vaccine manufacturing process as well as on the comprehensive characterization and release testing of the drug substance and the final vaccine itself.

The general guidance contained in WHO good manufacturing practices for pharmaceutical products: main principles (18), Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients (19), WHO good manufacturing practices for biological products (20) and WHO good manufacturing practices for sterile pharmaceutical products (21) should be applied to the design, establishment, operation, control and maintenance of manufacturing facilities for mRNA vaccines. WHO guidance should also be applied to the control of mRNA vaccine filled in the final form, the keeping of records and retained samples (for future studies and needs), labelling, distribution and transport, as well as storage and expiry dating for mRNA vaccines (24–26). Quality control during the manufacturing process relies on the implementation of quality systems, such as good manufacturing practice (GMP) to ensure the production of consistent commercial vaccine lots with product characteristics similar to those of lots shown to be safe and efficacious in clinical trials.

Throughout the process, a number of in-process control tests (with acceptable limits) should be established (including tests to measure critical and non-critical quality attributes) to allow quality to be monitored for each batch or lot from the beginning to the end of production. Release specifications and characterization methods should be agreed with the NRA(s) as part of the clinical trial authorization/approval or marketing authorization/approval. The drug substance and drug product release specifications for marketing authorization/approval should be set based on the testing of product resulting from the commercial manufacturing process as well as the results obtained for the lots used in clinical trials. Such release specifications and characterization methods should cover critical parameters that can provide reassurance on the consistent quality required to provide a safe and effective vaccine. This will include methods to assess content, identity, purity, mRNA integrity, potency, other quality and safety parameters, and stability.

mRNA vaccines for use in clinical trials should also be prepared under GMP conditions appropriate for the stage of clinical development – that is, full compliance may not be possible in initial or early development when manufacturing and control procedures remain in development and may not yet be validated; under emergency conditions, and based on risk–benefit assessment, it may be acceptable to consider using starting materials that were not prepared in complete compliance with GMP. Appropriate attention needs to be given to ensuring the quality and

correct identity of all reagents used in production and control. Particular attention should be given to the sourcing of components of animal (including human) derivation. Attention should also be given to ensuring freedom from or control of potential adventitious agents supported by relevant evidence and risk assessment. Many of the general requirements for the quality control of biological products, such as tests for endotoxin, stability and sterility, should also be applied to mRNA vaccines. The specifications should be defined on the basis of the results of tests on lots that have been shown to have acceptable performance in clinical studies. Additional controls specific to mRNA or sa-mRNA vaccines formulated in LNPs should be described, including controls for raw materials and excipients and in-process controls for manufacturing intermediates.

It should be recognized that the level of detail required by a regulatory authority increases as product development proceeds. During the initial phases of clinical development, the information contained in a clinical trial application should be adequate to allow for an assessment of the risks derived from the drug product and the manufacturing process. This would include, for example, identification of and specifications for all materials used in the process, assessment of risks from biologically sourced materials, certification or phase-appropriate GMP compliance of the manufacturing facility, a brief description of the processes and tests, results of testing of vaccine to be used in the proposed clinical trial and results of preliminary stability testing. As with all vaccines, for pivotal clinical trials the level of detail provided on the quality (manufacturing and controls) of an mRNA vaccine would be expected to increase substantially.

While not every mRNA vaccine can be viewed as being made based on a platform technology, a given manufacturer's technology might to some extent be viewed this way. In other words, if essentially no changes are made to the manufacturing processes, tests (except for identity) or specifications then a new candidate mRNA vaccine might be supported by data from an earlier candidate mRNA vaccine or licensed product. This could be the case when the only changes made are to the sequence and these changes do not significantly change the size or secondary structure of the resultant new mRNA or its interaction with the LNP. Supportive data might include data gained on the manufacturing processes, tests, specifications, and nonclinical and clinical safety.

Any changes made to product composition (for example, change in the mRNA sequence, enhanced valency, change in excipients or addition of preservatives) or manufacture (for example, change in process, site or scale) during the development of clinical lots should be adequately described. Depending on the way in which the final product composition was changed (for example, addition of novel excipients) new nonclinical studies might be warranted (see section 7 below). For changes to the manufacturing process (such as scale-up or change to the purification process) the comparability of the resulting drug substance and drug product with those produced using the previous process should be evaluated. Such comparability studies

might be based on immunogenicity data from animal models, results from physicochemical analyses, studies of process and product-related impurities, and stability data. The WHO Guidelines on procedures and data requirements for changes to approved vaccines (28) should be consulted in this regard. All changes made to the product post-approval should follow the requirements listed in these same Guidelines (28).

Defined recombinant nucleic acids used as active drug substances in vaccines, whether of biological or synthetic origin, could be assigned an international nonproprietary name (INN) upon request (49,50).

6.1 General manufacturing overview

Vaccines based on mRNA represent a new class of biological product and are manufactured differently from traditional biologicals. Most such biologicals are propagated or produced in a cell-based (or organism-based) system whereas the mRNA component is manufactured enzymatically via IVT. The production process normally involves the use of a linear DNA template to direct DNA-dependent RNA transcription with recombinant enzymes and nucleoside triphosphates. The choice of sequence or structure not only of the ORF but also of the UTRs, the cap and the poly(A) tail length should be justified.

Generally, once the mRNA has been transcribed the template DNA is digested using deoxyribonuclease (DNase) prior to purification of the mRNA. If the cap and poly(A) elements are not added during the IVT process, or if a longer poly(A) tail is required, these can be added enzymatically following synthesis but prior to purification (8,31,51,52) In addition to removing the DNA template, the unattached caps, unincorporated nucleotides and the enzymes (such as RNA polymerase) used in production, all process-related and product-related impurities (for example, dsRNA and incorrectly sized mRNA molecules) should also be removed to the extent feasible. Attention should also be paid to the removal of enzymes possibly involved in DNA template generation, such as DNA polymerase and restriction enzymes. The methods of purification and their purposes should be described and justified. Validation of the purification processes should be demonstrated. Whenever protein digestion with proteinase(s) is an impurity-reduction step, this step should be validated.

In most cases, the purified mRNA would be considered to correspond to what is termed for other vaccines “the purified bulk antigen” – even though the mRNA is not the actual antigen but instead matches the transcript encoding the antigen. This could also be thought of as the bulk biological substance or bulk active substance and is referred to in this document as the drug substance in order to use terminology familiar to most manufacturers and regulators to describe the active biological element of the vaccine.

As would be expected for any vaccine, a flowchart of production should be provided that indicates each process step, the samples taken at that process step and the in-process control tests for which the samples are taken. The process flowchart should also clarify the steps in the process at which manufacturing reaches the stages of drug substance, final formulated bulk and final filled vaccine (drug product), and at which steps in the flowchart samples are being taken for in-process control and release testing. The tests carried out at each of these steps should also be indicated. The duration of storage of the concentrated purified mRNA (drug substance) or any intermediates (such as the final formulated bulk) that are held or stored should be supported by hold-time/stability studies. An agreed-upon number of lots of the drug product should be placed on a stability programme, as would be expected for any vaccine.

The mRNA (drug substance) is not suitable for clinical use unless it is protected and delivered by a given formulation (part of the drug product). The formulations chosen for the most advanced mRNA vaccines so far are based on LNPs. Although there are other approaches to encapsulating mRNA-based products, this document only details systems that use LNPs. The formulation both stabilizes the mRNA and facilitates its entry into cells and release into the cytosol, which could be achieved by either active or passive uptake. The LNPs may also provide an adjuvant activity (44,46,47). In order to protect the mRNA from degradation by nucleases, the LNPs must be made inaccessible to such nucleases, but must also be able to release the mRNA once inside the target cell. The LNPs must also be of a suitable size range with desirable surface properties for optimal uptake by target cells. Hence, product development data concerning the optimization of both the formulation and the manufacturing process should be provided. For example, consideration should be given to the concentrations of the different lipids, the mRNA–lipids ratio, pH of buffers/solvents, mRNA encapsulation efficiency, and the flow rate and mixing rate of the lipids and mRNA, as well as the thawing temperature of the different components, as these will all have an impact on the quality of the final vaccine (drug product). In this way, the process of encapsulation into the LNPs can be carefully controlled and the production methods and control measures adequately described and suitably validated.

Although sa-mRNA contains the coding sequences (viral nonstructural genes) for additional proteins that permit its in vivo amplification (but not its packaging, which requires viral structural genes), the method of manufacture in which IVT is followed by purification and formulation in LNPs is essentially the same as that described above. For sa-mRNA with the additional coding sequence on the same molecule as the target antigen coding sequence the control measures required for the manufacturing processes might also be similar or the same as those for an mRNA vaccine. However, if the replicase gene(s) is encoded on a separate mRNA molecule then additional manufacturing processes and quality controls may be required, and these should be described to ensure that the required mRNA(s) are adequately encapsulated in the LNPs. The degree of expression efficiency might also vary between the two approaches (for

example, using two mRNAs as opposed to a single one) and this might have implications for the expected safety and efficacy of the vaccine design due to differences in the amount of dsRNA, the innate immune responses elicited, the half-life of the mRNA and so on. If the separate mRNA molecules are encapsulated separately and not mixed prior to encapsulation then this would also need to be described and may involve additional manufacturing processes and quality controls to ensure adequate final mixing and an appropriate ratio of the two (or more) mRNAs. Likewise, for multivalent mRNAs the mixing step(s) either before or after encapsulation need to be described and controlled appropriately.

The key quality control points should include:

a. Starting and raw materials and excipients – including, but not limited to: (a) a linear DNA template which could be a PCR-generated product or a plasmid DNA that has been linearized (generally by restriction endonucleases); (b) nucleotides; (c) enzymes (for example, DNA-dependent RNA polymerase (which is usually the T7 polymerase), capping enzyme, 2′ O-methyltransferase, poly(A) polymerase and DNase); (d) buffers; (e) solvents; and/or (f) column resins (if column chromatography is used in purification).

   - In particular, any animal-derived (including human-derived) starting or raw materials or excipients, or any starting or raw materials or excipients that were themselves produced using animal-derived (including human-derived) raw materials should be subject to control by appropriate sourcing, by control testing and by risk assessment. Materials of animal origin (including human origin) should comply with the current *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (23).

   - Attention should be given to ensuring freedom from or control of potential adventitious agents supported by relevant evidence and risk assessment.

b. In-process controls of the manufacturing processes – including the processes used to manufacture the bulk mRNA substance (drug substance), as well as the formulation (the LNP manufacture and encapsulation steps), final formulated bulk and filling of the final formulated bulk (drug product); also including either controls for or validation of the consistency of LNP formulation (size and polydispersity), consistency of mRNA encapsulation, and removal of partial mRNAs, dsRNA impurities and excess lipids.

c. Release of the mRNA vaccine drug substance and final filled vaccine (drug product) following manufacture.

d. Process validation – processes should be validated to control and assure the consistency and stability of the final drug product.

Analytical methods that might be considered for assessing some of these key quality control points are discussed in the literature – though precise methods and specifications are not yet in the public domain. As of the time of writing this document these are considered by manufacturers to be proprietary and confidential. The following methods may be considered as examples of potential means for characterization or control at various key quality control points (53–55).

Purpose(s)	**Examples of techniques**
Identity	DNA template sequencing; mRNA sequencing
Identity and quantification	reverse transcriptase quantitative PCR
Quantification and purity	ultraviolet spectroscopy; fluorescein-based assays
Quantification, size, RNA integrity, surface charge and percentage encapsulation	agarose or acrylamide gel electrophoresis, including capillary electrophoresis
Quantity of mRNA, quantity of lipids, quality of mRNA and nanoparticle integrity	chromatographic assays such as size-exclusion, anion-exchange, affinity or reverse-phase
Quantity and nanoparticle integrity	mass spectrometry
Other quality parameters and purity	dsRNA blot; tests for percentage capping; percentage transcripts with (and size of) poly(A) tail
Potency and correct protein	cell-free translation or cell-based expression systems
Particle size distribution (purity, consistency, safety)	light scattering such as dynamic or static light scattering; nanoparticle tracking analysis; electron microscopy; size-exclusion chromatography
Particle surface characterization (including size, polydispersity and zeta potential)	laser doppler electrophoresis; dynamic light scattering analysis
Physicochemical characterization (including surface and morphological properties)	electron microscopy; atomic force microscopy; X-ray diffraction; differential scanning calorimetry analysis

Safety parameters	Tests for sterility, endotoxin content

For clinical trial use, mRNA candidate vaccines should be manufactured under GMP conditions appropriate for the stage of clinical development. It is still expected that clinical trial material should be released on the basis of meeting appropriate quality control standards. Full compliance with GMP would be expected for clinical trial material used in pivotal trials and for commercial manufacture.

Any manufacturing changes made during clinical development, particularly if made following completion of pivotal safety and efficacy trials but prior to seeking licensure, need to be described and justified. A comparative analysis with the clinical efficacy lots should be made. For post-approval changes, compliance would be expected with the WHO Guidelines on procedures and data requirements for changes to approved vaccines (28).

6.2 General information and description of vaccine construct and composition

Information should be provided that describes the mRNA drug substance and the formulated mRNA vaccine in terms of its design and construction, its composition (for example, lipids and other excipients), the quantities of each excipient used and the mRNA sequence. The rationale for and function of the chosen excipients should also be provided in the description. Where relevant, information on the structure and molecular weight of the lipids employed and on their use in the vaccine formulation should be included. In other words, both the mRNA and the LNP components should be considered when describing the properties of the mRNA vaccine.

6.2.1 mRNA sequence and arrangement of elements

a. The annotated sequence of the DNA template should be provided. The sequence and position or length of all elements contained within the mRNA, including start and stop codons, flanking UTRs, regulatory elements (for example, promoter for the RNA polymerase) and 5′ cap and 3′ poly(A) tail, should be provided, as well as the ORF for the target antigen. If any additional proteins are encoded (such as those for a self-amplifying construct or a cytokine) their sequence should be provided (see points d and e below). The presence and function of any additional sequences included in the construct should be described.

b. Because vaccine mRNA can be manufactured containing nucleosides that are naturally occurring or modified or synthetic, the sequence information should include the specific nucleosides used.

c. Additionally, optimized codons (for example, codons that either better match the frequency of the appropriate tRNAs in human cells or that are used to attain a specific secondary or tertiary structure of the mRNA) may be used rather than the native codons in the pathogen's genome (for example, to increase the stability of the mRNA). The use of such optimized codons should be described and justified.

d. As noted above, in addition to coding for the target antigen(s) sa-mRNA also codes for a viral RNA-dependent RNA polymerase complex. Such a construct constitutes a replicon with the result that multiple copies of the mRNA coding for the antigen can then be made in vivo upon delivery to and uptake by the cells of the vaccine recipient, thus potentially increasing the efficacy of the vaccine. The sequences for any such replicon should be provided and their functions explained. If the replicase is provided on a separate mRNA molecule from the target antigen then the manufacture and control of each component should be illustrated and narratively described. Generally, these coding sequences are present on the same molecule but if separated then additional controls may be required and should be described.

e. If an mRNA vaccine includes sequences that code for any other immunomodulator (such as a cytokine) or non-coding sequences intended to act as an immunomodulator then such sequences and information on their purpose should be provided.

6.2.2 Formulations and components

a. **Batch formula**: the batch formula for commercial production should be provided. The amounts of each component in a single vaccine dose should be listed. The total volume of a batch should be defined.

b. **Chemical nature and formulation**: the mRNA is formulated principally for increased stability and to aid cellular uptake. While several potential types of delivery agents exist (such as protamine complexes, cationic liposomes and lipid-, polymer- or lipid/polymer-based nanoparticles) the mRNA candidate vaccines currently in use or in the most advanced clinical trials are encapsulated into LNPs. Characterization of these formulations, both chemically and in terms of the physical parameters of the structural formulation (such as nanoparticles), is required and should address characteristics such as the consistency and stability of the formulation and final product. Considerations of the critical quality attributes of the lipids and the drug product should also be included. Sufficient characterization of the mRNA-LNP complex and of its uptake into target cells should be provided. This may include an understanding of the surface chemistry, size, polydispersity, shape, charge and protein-binding properties of the resultant mRNA-LNP

in order to ensure that adequate protection of the mRNA and the required stability of the vaccine are achieved. Where the LNPs are shown to have inherent immunomodulatory effects, relevant data on the potential benefits and drawbacks should be presented. Thus any characteristics of the formulation that might impact the toxicity, adverse events, immune responses and efficacy of the vaccine can be described and their effects (positive or negative) considered.

c. **Additional immunomodulators or adjuvants**: the mRNA might also encode specific immunomodulatory molecules such as cytokines. Furthermore, a separate adjuvant or immunomodulatory (stimulatory or suppressive) compound not encoded in the mRNA might be added to the formulation or as part of the LNP. As a general principle regarding vaccines formulated with adjuvants, a demonstration of the contribution of such an addition to vaccine immunogenicity should be provided (16). Quality aspects of the separate adjuvant, if included, should also be addressed and described.

d. **Additional sequences**: if additional sequences are included to target the mRNA to antigen-presenting cells or to increase the release of the mRNA from the endosome, the sequence and function of these additions need to be described and evidence provided of their function to support their proposed mechanism-of-action.

e. **Additional excipients (such as preservatives)**: the composition, necessity for and (in the case of preservatives) the preservative efficacy of such additional excipients should be described and shown not to adversely affect the properties of the LNP.

6.3 Control of starting and raw materials and excipients

As with any vaccine, appropriate attention needs to be given to the sourcing and quality of all reagents used in production (19). The reagents/raw materials should be procured from vendors/suppliers approved by the manufacturer through the internally defined quality systems. Suppliers of such materials should be managed by an appropriate qualification programme.

6.3.1 Quality of starting and raw materials and excipients

The starting and raw materials and excipients, including those used to produce the mRNA (such as the DNA template, nucleotides (which may contain modified nucleosides), enzymes, buffer, solvents, any columns for purification and so on) should be described. Information should be provided on their provenance, quality, control, stability and role, including the point at which each material is used in the manufacturing process. The materials should be suitable for use in GMP production, and reference to internationally accepted pharmacopoeias or details on their specifications should be provided. The process used for the derivation of any reagent or starting

material (such as the linear DNA template) should also be described. With respect to the LNPs, the source and quality of the lipids used in their manufacture (especially of novel lipids present in LNPs that have not previously been studied nonclinically or clinically) should be sufficiently detailed to permit meaningful assessment of their safety and quality. Suitable specifications should also still be provided for any such excipient not considered to be novel. In the case of novel excipients (for example, cationic lipids) details of the manufacturing process and control of the novel lipids (including the starting materials and intermediates) should be provided, where feasible. This will include information on and relevant justification of the proposed starting materials and any intermediates used in the synthesis of the novel excipients. Details of the manufacturing site(s) and manufacturing process, along with the required process controls and specifications of the starting materials, raw materials (for example, reagents and solvents), intermediates and final excipients (that is, lipid) should be provided. Consideration should also be given to the use and control of solvents, and to the potential for contamination with elemental impurities (56–58). Where the recycling of materials/reagents/solvents is proposed, this should be justified and appropriately controlled. The level of impurities associated with the excipients should also be suitably controlled and justified. Any purification and isolation steps should be detailed. To assure the quality of the proposed novel excipients, their manufacturer should also have available relevant information on the analytical methods used for the characterization and batch analyses of the materials. Since PEGylation plays a critical role in providing stability and enhancing the cellular interaction of LNPs (39) adequate controls (for example, of molecular weight and polydispersity) should be in place for the PEGylated lipid.

The linear DNA template is considered to be the starting material for the GMP production of the mRNA. Procedures for establishing the cell banks and the manufacture of the plasmid DNA should be performed in a manner consistent with the production of material for use in subsequent GMP manufacture.

A cell bank system should be established, described and tested. The genetic stability of the seed bank must be demonstrated. A purification process also needs to be in place to reduce impurities from the DNA plasmid (such as RNA, host-cell DNA, protein, lipids and polysaccharides). The manufacturing process needs to be set up in such a way as to minimize the risk of microbiological contamination.

Testing of DNA plasmids and the linear template should include tests for genetic identity by sequencing, for integrity (including confirmation of the desired encoded antigen sequence and regulatory/controlling sequences) and for percentage linear DNA, as well as tests for residual genomic DNA, RNA and protein, sterility or bioburden, and endotoxin levels.

In early clinical development it may be acceptable to use well-qualified material on the understanding that greater control will be expected to support pivotal trials and commercial manufacture.

6.3.2 Release of starting and raw materials and excipients

As with any vaccine, certificates of compliance (if applicable) and certificates of analysis should be provided for all raw materials and a clear indication given of which testing is performed by the mRNA manufacturer or whether the material is accepted on the basis of the certificate of analysis provided by the manufacturer of the raw material. An internal policy shall be defined based on criticality risk ranking for the in-house testing and release of raw materials used in the manufacturing process. Starting materials should be released in accordance with the requirements and specifications for use in GMP manufacture.

6.4 Process development and in-process controls

The development history of the manufacturing process should be provided. Tests and acceptance criteria for critical steps of the manufacturing process should be developed and justified to ensure, and provide feedback on, the control of the process. In cases where a well-established platform technology is being used, knowledge gained from the manufacture of approved products can be considered.

Validation of the manufacturing processes should demonstrate that they comply with their critical, non-critical and key parameters, and yield a product that consistently meets the predefined quality attributes. This should include demonstration of the reproducible and consistent clearance of process- and product-related impurities to levels acceptable for intended use in humans.

Process validation is not generally required for a candidate vaccine used in preliminary clinical trials, though critical steps such as aseptic processing and sterility of the drug product should be validated or appropriately demonstrated to be controlled during the manufacture of clinical materials.

6.5 Product characterization

A summary of the characterization of the mRNA (drug substance) and the final vaccine (drug product) should be provided in addition to in-process and lot-release testing. Rigorous characterization using a range of orthogonal chemical, molecular, physical and biological methods will be essential. Characterization refers to studies and analyses that are not performed routinely on every lot but which allow the manufacturer to gain important knowledge of the

structure, performance and safety of their product in order to guide process and analytical test development and improvement. This is in contrast to the in-process and lot-release testing performed on every lot. Justification of the choice of analytical methods for the determination of various parameters should be considered, particularly when a different outcome would likely be obtained using alternative techniques – for example, particle size measurement using different methods. It is for this reason that orthogonal methods are recommended.

The sequences of the population of manufactured mRNA should be determined and the degree of consistency of the proper sequence defined. Consistency of manufacture is discussed further in section 6.6 below. The degree of consistency of the capping and polyadenylation processes should also be characterized. Demonstration of expression of the complete encoded protein(s) without truncated or alternative forms should be provided. In particular, if expression of truncated or alternative forms of the target antigen is demonstrated during characterization studies and these alternative forms would result in neo-antigens or unwanted immune responses then this may require a redesign of the mRNA sequence. The degree of consistency of encapsulation of the mRNA in the LNPs should also be addressed during characterization, while particle uptake studies could assist in characterizing potential potency measures. During characterization, it should be determined whether any of these characteristics should be controlled as critical quality attributes and/or stability-indicating parameters.

Certain aspects of the LNPs should be very carefully characterized. These include particle size as determined by different analytical techniques to explore the morphological and dimensional characteristics of the LNPs containing the mRNA. Information on the density and distribution of polyethylene glycol (PEG) within the LNPs would also be useful to help understand the surface properties of the mRNA-LNP complex as these will affect the stability, cellular interaction and immunological response properties of the product; such information would also help to confirm the consistency of the manufactured vaccine. Measurement of surface charge (for example, zeta potential) should also be considered as a method for characterizing the LNPs.

The immunogenicity elicited by the mRNA-encoded target antigen should be characterized in nonclinical studies in order to characterize and understand the product. Additionally, if the LNPs have inherent immunomodulatory effects these should also be characterized. Whenever other immunomodulatory elements or genes are included in the mRNA, their contribution to the mode-of-action (for example, immunogenicity) of the mRNA-encoded target antigen should also be determined in nonclinical studies in order to justify their inclusion in the characterized product design (see section 7 below).

Potential impurities in the starting materials and in the purified mRNA should be described and investigated. Such impurities may include residual bacterial host-cell proteins (if used to

manufacture the DNA template), endotoxins, residual bacterial host-cell RNA and chromosomal DNA (if bacteria were used to manufacture the DNA template), enzymes (such as DNA and RNA polymerases and restriction enzymes), unincorporated nucleotides, mis-folded RNA, dsRNA, incomplete or differently sized RNA, and other materials used in the manufacturing process. Data should be provided on the impurities present in the purified mRNA, with specifications set for their maximum acceptable or lowest achievable levels. For impurities and residuals with known or potential toxic effects, a toxicological risk assessment is expected to be carried out. Degraded mRNA may be assessed as part of analytical procedures such as polyacrylamide or agarose gel electrophoresis, high-performance liquid chromatography (HPLC) and/or capillary gel electrophoresis. The degree of consistency of the sequence and structure of the mRNA, and its expression of a consistent protein when transfected into cells in vitro, are important characteristics to be determined for the drug product.

Any potential impurities (both process- and product-related) that may arise from the lipids used in the formulation of the drug product should also be characterized and investigated. Any specification limits proposed should be suitably controlled and within the clinically determined acceptable range.

6.6 Consistency of manufacture

As with other biologicals, prior to seeking marketing authorization a number of consecutive batches should be tested and analyzed using validated methods to determine the consistency of manufacture. Any differences between one batch and another outside the accepted range for the parameters tested should be noted and investigated. The data obtained from such studies, combined with product and process knowledge and evaluation of the criticality of variations in specific attributes, should be used as the basis for justification of the chosen specifications.

During preliminary clinical development few lots will have been made and demonstration of production consistency may be limited or not possible. The ability to demonstrate consistency will increase as manufacturing experience is gained during product development. Confirmation of the consistency of lots is generally done during advanced development (for example when the manufacturing process has been scaled up for commercial manufacture) but prior to submission of application(s) for marketing authorization. However, in some cases, scale-up for commercial manufacture may be undertaken while marketing authorization is being sought for clinical trial-scale material. Whenever changes to the manufacturing process are implemented, the comparability of lots, especially to those used in pivotal studies and made by the intended commercial process, should be demonstrated. Comparability protocols and strategies for demonstrating comparability are discussed in the WHO Guidelines on procedures and data requirements for changes to approved vaccines (28).

6.7 Manufacture and control of bulk purified mRNA (drug substance)

As stated above in section 6.1, an overview of the development and manufacture of the mRNA should include a justification for the selection of the target antigen gene, other gene(s) contained in the mRNA sequence, UTRs, 5′ cap, 3′ poly(A) tail and regulatory elements used. Any gene expression or other optimization modifications should be described. Annotated sequences of the complete DNA template and mRNA should be provided. Both an illustrative and annotated flowchart and a narrative description of the manufacture, in-process controls and release tests should be provided. The detailed production and control procedures along with any significant changes in them that may affect the quality, safety and efficacy of the mRNA vaccine should be discussed with and approved by the NRA.

In the case of sa-mRNA, if the replicase and target antigen are expressed on separate mRNA molecules, this should be described and clearly illustrated in the provided flowchart, which should also include any additional manufacturing processes and/or quality control tests. For example, consideration should be given to controls such as the ratio of replicase-encoding mRNA molecules to target-antigen-encoding mRNA molecules, or to methods to ensure (or controls to determine whether or not) both molecules are encapsulated into the same LNP, if applicable.

6.7.1 Control of bulk purified mRNA (drug substance)

Specifications for critical quality attributes for the identity (see section 6.7.1.1 below), purity (section 6.7.1.2), quantity and physical state (section 6.7.1.3), safety (section 6.7.1.4) and quality (section 6.7.1.5) of the bulk purified mRNA should be established and justified. Descriptions of the analytical methods used should be provided, the acceptance limits defined and assay validation information described. The results of testing of all batches produced at commercial scale should be summarized and provided. Specifications should also be established for stability under storage conditions.

Early in development, to support clinical trial authorization, results from testing batches made in accordance with GMP (18–21) and, if available, engineering runs performed to establish manufacturing procedures should be summarized and provided. Although specifications may be limited and have somewhat wide acceptance criteria in early development, these should be reviewed and tightened, when appropriate, as experience in the manufacturing process and analytical methods is gained. Not all of the tests conducted during product characterization need to be carried out on each batch of vaccine as release testing. Some tests are required only to obtain product and process knowledge on a limited series of batches to establish the methods and consistency of production. Thus, a comprehensive analysis of the initial commercial-scale

production batches should be undertaken to establish consistency with regard to the identity, purity, quality, safety and stability of the drug substance; thereafter, a limited series of tests may be appropriate for quality control, as agreed with the NRA.

As experience is gained in manufacturing consistency, post-approval changes might permit reducing the testing and the amount of supporting information required through the use of process validation, product characterization and/or a comparability protocol (28).

6.7.1.1 Identity

Each batch of bulk purified mRNA should be tested to confirm its identity. Confirmation of identity could include determination of the mRNA sequence by direct RNA sequencing, sequencing (or determining the presence or absence) of a reverse transcription PCR (RT-PCR) product or high-throughput sequencing. If identity is based on an RT-PCR amplicon that represents only a portion of the complete mRNA sequence then the sequence chosen should be unique to that mRNA product (including accessory and regulatory regions) and not be common to any others that might be manufactured in the same facility or using the same equipment. However, it might be more appropriate to sequence the entire mRNA as this approach could serve to address both identity and purity.

6.7.1.2 Purity and impurities

Each batch of bulk purified mRNA should be tested for purity and the result should be within the allowable limits established. The control of impurities should also address the materials introduced during manufacture, such as the DNA template, unincorporated nucleotides, unincorporated caps, enzymes, mRNA fragments and dsRNA. This may be achieved through process validation to establish the removal of process-related impurities or through release tests for the residual impurities. Consideration of the necessity of testing for dsRNA should take into account the design of the manufacturing process as not all processes produce dsRNA. The analyses should include sensitive and reliable assays for process- and product-related impurities, and strict upper (maximum allowable) limits should be specified for their content in the bulk purified mRNA. Chromatographic detection methods may be considered. Residual DNA template might be quantified by quantitative PCR. It is important that the techniques used to demonstrate purity are based on as wide a range of physicochemical, biological and/or molecular properties as possible. Consideration of the results of methods such as forced degradation studies may guide decisions on which impurities will need to be tested for and/or measured by purity tests during production, at release and/or in stability protocols.

Tests for residual levels of process- or product-related impurities as part of quality control may be reduced or discontinued once production processes have been adequately validated for their

suitable removal, and production consistency has been demonstrated, if agreed to by the NRA. Plans and specifications for the periodic revalidation of processes should be described. Until the processes are validated, impurities should continue to be tested for and/or measured in a number of lots as agreed to by the NRA. In the case of major changes to manufacturing, revalidation or continued measurement would be expected for the number of lots agreed with the NRA. Container-closure system compatibility, leachables and extractables should also be assessed and discussed in the application for marketing authorization.

6.7.1.3 Quantification and physical state

The integrity of the structure of the mRNA is considered to be a critical quality attribute for release of the mRNA. Thus, control is needed of mRNA integrity, 5′ capping efficiency, 3′ poly(A) tail presence or length, percentage intact mRNA, percentage mRNA fragments, percentage of dsRNA and so on. The need to measure 3′ poly(A) tail presence or length depends upon the way in which this sequence is added to the mRNA. If encoded in the DNA template then all full-length mRNA should include the poly(A) tail, but if it is added enzymatically after IVT then it would be appropriate to address this parameter through testing or process validation. Likewise, the presence of dsRNA depends on whether the processes used are capable of producing it as an impurity. Tests such as gel electrophoresis, PCR or chromatographic detection methods might be considered for these purposes. It should be borne in mind that quantification of the mRNA is the basis for vaccine dosing and the presence of intact mRNA is key to the mechanism-of-action of the vaccine. Thus, the methods used for quantifying the mRNA (for example, ultraviolet spectrophotometry) and for quantifying the intact mRNA (for example, gel electrophoresis) should be described.

6.7.1.4 Safety parameters

Relevant safety tests should be described. These may include tests for endotoxins along with either bacterial and fungal sterility testing (including demonstration of lack of bactericidal or fungicidal activity of the test article) or bioburden testing (including quantity, identification and freedom from specified unwanted organisms). If required by the NRA, a test for pyrogenicity may be performed (which may be the monocyte activation test). However, animal testing should be avoided whenever alternative satisfactory testing is available and allowed. For scientific and ethical reasons, it is desirable to apply the 3Rs concept of “Replace Reduce Refine” to minimize the use of animals in testing and consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation, as well as for other product tests. In particular, manufacturers and regulators should take note of the decision of the WHO Expert Committee on Biological Standardization in 2018 to discontinue the inclusion of the general safety (innocuity) test in routine lot release testing requirements for all vaccines in WHO Recommendations,

Guidelines and other guidance documents for biological products (59). This test should therefore not be required or requested.

6.7.1.5 Additional quality parameters

Additional important quality parameters should be established and controlled (such as appearance, pH and, if relevant, viscosity). In addition, there are a number of critical quality attributes relevant to mRNA vaccines (such as poly(A) tail length and capping efficiency) which need to be controlled, as mentioned above in regard to purity and impurities (see section 6.7.1.2).

6.7.1.6 Reference materials

An in-house reference preparation (that is, working standard) should be established for use in assay standardization. Information on the reference standards or reference materials used for testing of the bulk purified mRNA should be provided by the time of application for marketing authorization.

A suitable batch (that is, one that has been clinically evaluated) should be fully characterized in terms of its chemical composition, purity, biological activity and complete sequence, and an adequate sample retained for use as a chemical and biological reference material. The reference material should be formulated in an appropriate form. Storage should be under conditions under which the reference material has been shown to be stable and a routine programme for monitoring such stability should be implemented. A plan for replacing the initial reference material upon exhaustion should be agreed with the NRA.

In early development (for example, preliminary clinical trials) an engineering run batch or a batch from which the lot of mRNA vaccine evaluated in the pivotal nonclinical studies was made may serve as a reference until a suitable clinical trial batch has been identified and characterized for use as a reference in advanced development (for example, pivotal clinical trials) and commercial manufacture.

6.7.1.7 Stability

A stability assessment should be conducted in accordance with the WHO Guidelines on stability evaluation of vaccines (24). The types of studies conducted, the protocols followed and the study results should be summarized in an appropriate format such as tables or graphs along with a narrative document. The summary should include results as well as conclusions with respect to appropriate storage conditions or shelf-life. Data on stability to support the shelf-life of the bulk and any future extension of it should be based on long-term real-time stability studies under actual conditions.

6.8 Manufacture and control of final formulated vaccine (drug product)

As stated above in section 6.1, an overview of the development and manufacture of the vaccine should include both an illustrative and annotated flowchart and a narrative description of the manufacture, in-process controls and release tests. The methods used to assure the proper formation of LNPs should be detailed. Any proposed hold-time of the bulk formulation or bulk LNPs should be appropriately specified and validated. Adequate consideration should be given to ensuring physicochemical stability and microbial control during such hold-times. The methods used for final formulation, fill and finish should also be described and suitably validated.

6.8.1 Composition

The final composition of the vaccine, including the active drug substance (mRNA) and all excipients (for example, lipids), should be described along with the quantity of the components in each presentation – particularly if marketing authorization is being sought for more than one dosage or dosage form. The function of each of the components should also be described.

6.8.2 Manufacture and control of LNPs and encapsulation of mRNA

The methods used to assure the proper formation of LNPs should be described. Appropriate product development data should be provided to support the rationale for their proposed formulation and manufacturing process. All critical quality attributes of the LNPs and final mRNA-LNPs should be investigated. Where suitable, a Design of Experiment approach could be adopted. Their size and polydispersity, and in turn stability, are all influenced by both the flow dynamics of the lipid and aqueous phase and the shear stress induced during the manufacturing process. Thus, relevant studies that explore the critical processing parameters and their operational ranges optimal for mRNA-LNP formulation and stability of the final formulated vaccine should be performed. This will ensure that the product is consistently manufactured to the required quality. Any proposed hold-time of the bulk LNPs or bulk formulation should be appropriately specified and validated. Adequate consideration should be given to ensuring physicochemical stability and microbial control during such hold-times.

The preparation of the lipids, the complexation of mRNA and lipids, dilution and any purification steps, and subsequent filling into suitable containers should be described and the process validated to meet the necessary in-process specifications. Various filtration techniques (for example, tangential flow filtration) should be considered for the removal of raw materials used in the preparation of LNPs. Specific attention should be given to minimizing the degradation of the mRNA during encapsulation with the LNPs and under manufacturing

conditions known to influence the stability of the LNPs and final mRNA-LNP vaccine product (for example, the impact of thawing of the mRNA and the freezing rate of the LNPs or mRNA-LNPs). Likewise, if lyophilized, the conditions for freeze-drying and reconstitution should be considered and justified.

Suitable controls for the LNPs should also be specified and would typically include: (a) identity, quantity and purity (including impurities) of the lipids; (b) particle size and distribution; (c) polydispersity; and (d) RNA encapsulation efficiency/proportion encapsulated. In some cases, the surface properties (for example, charge) may also need to be specified to ensure consistency and stability of the product.

It will also be important to consider the subsequent impact that any change made to the mRNA drug substance (for example, change in sequence, length or secondary structure) may have on the critical quality attributes of the LNPs (for example, particle size and distribution, morphology, and surface properties) and ultimately on the final vaccine product (for example, percentage of encapsulation and cellular interaction/uptake). Relevant developmental data are expected to demonstrate product consistency and to support the product optimization process.

6.8.3 Manufacture of final vaccine (drug product), filling and containers

An annotated flowchart should be provided that illustrates the manufacturing steps from the bulk purified mRNA (drug substance) to the final vaccine (drug product). The chart should include all steps (that is, unit operations) such as dilution of the final formulated bulk, identification of materials and intermediates, and in-process and quality control tests. A narrative description of each process step depicted in the flowchart should be provided. Information should also be included on, for example, its scale, buffers and other additives, major equipment and process controls (including in-process tests and critical process operational parameters with acceptance criteria that are justified by relevant development data). Details of the sterilization process and microbial control should also be included.

The general guidance concerning filling and containers provided in WHO good manufacturing practices for biological products (20) should be applied to vaccine filled in the final form. The aseptic fill process of the mRNA-LNP should be adequately validated to ensure all critical quality attributes are maintained and meet the required specifications. Care should be taken to ensure that the materials of which the containers and closures (and, if applicable, the transfer devices) are made do not adversely affect the quality of the vaccine. To this end, a container-closure integrity test and assessment of extractables and/or leachables for the final container-closure system are generally required for the qualification of containers and may be needed as part of stability assessments.

If multi-dose vaccine vials are used and the vaccine does not contain preservative then their use should be time restricted, as is the case for reconstituted vaccines such as bacillus Calmette–Guérin (BCG) and measles-containing vaccines (29). In addition, the multi-dose container should prevent microbial contamination of the contents after opening. Relevant simulation studies (for example, multi-puncture tests) of the container-closure system may be required to demonstrate the suitability of the proposed system. Multi-dose vials should be designed to meet the label claim, with acceptable overfill to allow for correct dosing. Multi-dose vaccine vials should be evaluated for the maximum anticipated vial septum punctures to assess the risk of compromising vial integrity and the potential for vial contamination. The extractable volume of multi-dose vials should be validated. If multi-dose vaccine vials are supplied as concentrate, an additional compatibility study should be conducted using the proposed reconstitution solutions and an appropriate post-dilution hold-time should be established. The pre-dilution and post-dilution specifications should be set out and justified. Manufacturers should provide the NRA with adequate data demonstrating the stability of the product under appropriate conditions of storage, distribution and during use.

When a final vaccine contains more than one mRNA species (for example, in a multivalent vaccine or an sa-mRNA consisting of separate mRNAs) there may be additional considerations in the manufacture of that final vaccine. One such consideration will be ensuring the appropriate ratio of the different mRNAs in the formulation to optimize the expression of each and to minimize immune interference (in the case of multivalent vaccines). Another consideration will be whether the mRNAs will be mixed prior to encapsulation in the LNPs or whether each mRNA will be encapsulated in LNPs and then a mixture of mRNA-LNPs prepared. In either case, the approach selected should be described.

6.8.4 Control of final vaccine (drug product)

Samples should be assessed from each final vaccine lot. All tests and specifications should be approved by the NRA. Specifications for the final vaccine should be established and justified by the manufacturer. As a principle, the final specifications should be defined on the basis of the relevant batch data on lots that have been shown to have acceptable performance in clinical studies. Descriptions of analytical methods and acceptance limits for the vaccine should be provided, including information on method validation. It is recommended that testing should include an assessment of identity (see section 6.8.4.1 below), purity (section 6.8.4.2), content (section 6.8.4.3), safety (section 6.8.4.4), additional quality parameters (section 6.8.4.5) and potency (section 6.8.4.6).

Although specifications may be limited and have somewhat wide acceptance criteria in early development, these should be reviewed and tightened, when appropriate, as experience in the manufacturing process and analytical methods is gained.

A summary of the results of the testing of all lots produced at commercial scale should be provided. Early in development, to support clinical trial authorization, results from testing lots made in accordance with GMP (18,20) and, if available, engineering runs performed to establish manufacturing procedures should be summarized and provided.

Not all of the tests conducted during product development need to be carried out on every lot of vaccine produced at commercial scale. Some tests are required only to obtain product and process knowledge on a limited series of lots to establish consistency of production, as discussed in sections 6.5 and 6.6 above. Several consecutive lots of vaccine, in final dosage form, should be tested and analysed using validated methods to confirm manufacturing consistency. Any differences between one lot and another should be noted and investigated. The data obtained from such studies, as well as clinical trial outcomes with various lots, alongside product and process knowledge and evaluation of the criticality of variations in specific attributes, should be used as the basis for defining the vaccine specifications and acceptance criteria to be used for routine lot release. Thus, a comprehensive analysis of the initial commercial production lots should be undertaken to establish consistency with regard to the identity, purity, strength/content/quantity, safety, additional quality parameters, potency and stability of the mRNA vaccine but thereafter a more limited series of tests may be appropriate, if agreed with the NRA.

When a final vaccine contains more than one mRNA species (for example, in a multivalent vaccine or a sa-mRNA consisting of separate mRNAs) there may be additional considerations in the control of that final vaccine. Some of these considerations will be based on the approach taken in manufacture – for example, whether the mRNAs were encapsulated together as a mixture or were encapsulated separately and then the different mRNA-LNPs mixed. This may then affect the size, charge and polydispersity of the LNPs. In addition, validating the consistency of mixing is crucial to ensuring that each dose contains the appropriate ratio of each of the mRNAs. Ensuring the proper ratios in the total mRNA content of the final vaccine will be critical as the total mRNA content is the basis for dosing. Identity testing should address the inclusion of each mRNA, while still differentiating the vaccine from other products made in the facility. If one drug substance or component (for example, the mRNA encoding the replicon) is used in more than one vaccine or product made in the facility then such identity testing will also be crucial in preventing mix-ups.

As experience is gained in manufacturing consistency, post-approval changes might permit reducing the testing and amount of supporting information required through the use of process validation, product characterization and/or a comparability protocol (28).

6.8.4.1 Identity

Each lot of vaccine should be subjected to an appropriate test to confirm the identity of the final product and distinguish it from other products made in the same facility or using the same equipment. Confirmation of the identity by sequence analysis should be considered (see section 6.7.1.1 above).

6.8.4.2 Purity and impurities

The purity of each lot of final vaccine should be assessed and shown to be within the specified limits. Consideration should be given to potential impurities resulting from any component of the delivery system and to controlling aspects of impurity such as oxidation and degradation in the final vaccine. It is unlikely that a single test will be sufficient to detect all potential impurities. Tests for mRNA integrity, particle size, lipid/polymer impurities and the proportion/efficiency of mRNA encapsulated in the LNPs should be considered. Container-closure system compatibility, leachables and extractables should also be assessed and discussed in the application for marketing authorization (see also section 6.7.1.2 above).

6.8.4.3 Content, strength or quantity

mRNA vaccines are dosed based on quantity of the mRNA by weight. Therefore, in addition to assessing potency (see section 6.8.4.6 below), a quantification method for the mRNA should be described (see section 6.7.1.3 above).

6.8.4.4 Safety parameters

Each lot of final vaccine should be tested for sterility. If the vaccine is to be administered by a non-parenteral route then omission of the sterility test and inclusion of an appropriate alternative bioburden test needs to be appropriately justified. Further, a test for endotoxin should be conducted on each lot and appropriate specifications defined. If required by the NRA, a test for pyrogenicity may be performed (which may be the monocyte activation test). However, animal testing should be avoided whenever alternative satisfactory testing is available and allowed. For scientific and ethical reasons, it is desirable to apply the 3Rs concept of “Replace Reduce Refine” to minimize the use of animals in testing and consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation and other product tests. In

particular, manufacturers and regulators should take note of the decision of the WHO Expert Committee on Biological Standardization in 2018 to discontinue the inclusion of the general safety (innocuity) test in routine lot release testing requirements for all vaccines in WHO Recommendations, Guidelines and other guidance documents for biological products (59). This test should therefore not be required or requested.

6.8.4.5 Additional quality parameters

Other important quality parameters should also be established and controlled. These can include appearance (including presence of both visible and sub-visible particulate matter), extractable volume and pH. Depending on the product characteristics, the control of other parameters such as osmolality or viscosity may also be important. For the final vaccine (drug product) additional parameters should include mRNA integrity, lipid/polymer identification and content, nanoparticle size, mRNA–lipid ratio, encapsulation efficiency and polydispersity index.

With respect to nanoparticle size, multiple point control should be adopted similar to the control of nanoparticle-based therapeutic products, and the test used for measurement of particle size should be specified as the results will be dependent upon the analytical method employed. The degree of encapsulation of the mRNA in the LNP should also be regarded as a critical quality attribute as non-encapsulated mRNA is considered to be unstable. Confirmation should be provided that the structure of the final product does not change due to freeze-thawing and dilution. Techniques such as gel or capillary electrophoresis and/or HPLC already being performed for purity or for identity may also be useful in assessing some quality parameters.

Other tests (such as a test for residual moisture if the vaccine is lyophilized) may be required to confirm the physical characteristics of the product as well as the formulation. Validation of the analytical methods used should be described to assure the control of the identified critical quality attributes of the drug product.

6.8.4.6 Potency

The potency of each lot of the final vaccine should be determined using a suitably quantitative and validated functional method(s). Different tests may be required to control various aspects of potency (including functionality) which will likely be disease specific. Immunogenicity in the vaccine recipient is a complex function of the final vaccine properties, including delivery to target cells by its formulation as well as expression of the mRNA-encoded protein(s) (which may include a self-amplifying replicon component). Thus, potential in vitro potency assays may include cell-based transfection systems or cell-free assays. Such methods would demonstrate that the correctly sized protein of correct identity is expressed from the mRNA. However, because potency should be analyzed on the basis of not only the product type (in this case, mRNA

vaccines) but also the clinical indication of the disease to be prevented, it is not possible to indicate a particular assay method that should be used to measure potency. Scientific justification for the potency test(s) selected to control the product should be provided.

When a vaccine against a new strain(s) is developed, consideration should be given to ensuring that the potency assay(s) address the strain change. If the potency assay cannot distinguish between strains (for example, those differing by only a few nucleotides) then use of a combination of the potency assay(s) and sequence confirmation may be justified.

The potency specifications for mRNA vaccines should be set based on the minimum dose used to demonstrate efficacy in clinical trials plus human immunogenicity data. An upper limit should also be defined based on available human safety data.

Animal-based assays tend to be highly variable and difficult to validate. It is also desirable to apply the 3Rs concept of "Replace Reduce Refine" to minimize the use of animals in testing. Consideration should therefore be given to the use of appropriate in vitro alternative methods for potency evaluation. It is envisaged that, as with plasmid DNA vaccines, a combination of biochemical or biophysical measures (such as nucleic acid quantity, mRNA integrity and genetic sequence) might be used to establish and monitor the potency of mRNA vaccines. Manufacturers are encouraged to work towards the goal of employing in vitro assays that are suitably quantitative and assess function. However, it needs to be acknowledged that these measures only account for the mRNA and not the impact of any formulation, adjuvant, immunomodulators and so on, and the potency assessment of mRNA vaccines will thus need to be considered on a case-by-case basis. Therefore, discussion of appropriate potency measures and reaching of agreement with the NRA is advised.

6.8.4.7 Reference materials

A suitable lot of the final vaccine that has been clinically evaluated should be fully characterized in terms of its chemical composition, purity, biological activity and full sequence, and retained for use as an internal reference material. This material should be used as the basis for evaluation of product quality for commercial production lots (see also section 6.7.1.6 above).

In the future, national standards may be prepared and provided by the NRA while international standards may become available from WHO. Should such international standards become available it will be important to calibrate the internal or national reference material against them. In this way, comparisons can be made in a more reliable and less variable way whenever new reference materials need to be prepared. In addition, the expression of results in a common unit

(such as IU), when appropriate, will also allow for the comparison of test results obtained from different laboratories and for different products.

6.8.4.8 Stability testing, storage and expiry date

The relevant guidance provided in WHO good manufacturing practices for biological products (20), WHO good manufacturing practices for sterile pharmaceutical products (21) and WHO Guidelines on stability evaluation of vaccines (24) appropriate for the respective mRNA vaccine should apply. Furthermore, the WHO Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (26) might also apply. The statements concerning storage temperature and expiry date that appear on the primary and secondary packaging should be based on experimental evidence and should be submitted to the NRA for approval. For guidance regarding vaccine vial monitors, the WHO *Getting started with vaccine vial monitors* and related WHO guidance should be consulted (60,61).

6.8.4.8.1 Stability

Adequate stability studies form an essential part of vaccine development. The stability of the final product in the container proposed for use should therefore be determined and the results used to set a shelf-life under appropriate storage conditions. Parameters that are stability-indicating should be measured and these may include appearance (including visible and sub-visible particulate matter), mRNA quantity, vaccine potency, mRNA integrity, degree of encapsulation, particle size, polydispersity and impurities associated with the mRNA and lipids. The parameters to be measured should be described and specifications defined and justified. Real-time stability studies should be undertaken for this purpose; though accelerated stability studies at elevated temperatures may provide additional and complementary supporting evidence for the stability of the product and confirm the stability-indicating nature of the assays used to determine stability.

In addition, accelerated and stress testing data as well as platform data can be taken into account to support the shelf-life. Stability data that support clinical use, such as data on stability at elevated temperatures for short-term storage and dispensing, should be generated. For multi-dose vials, in-use stability data will be needed to provide assurance of the required microbial quality and stability of the vaccine under in-use conditions (29).

During initial clinical development limited stability information would be expected. For example, some regulators accept 3 months of real-time stability of the lot to be used in the proposed clinical trial, or one produced in the same manner and meeting the same specifications, at the time of application for clinical trial authorization, but this should be agreed with the NRA.

If deep-freeze conditions are recommended for long-term storage then alternative short-term storage conditions (such as frozen and/or refrigerated) should be explored to support vaccine distribution and dispensing. Similarly, temperature excursion studies or transportation simulation studies may also be expected. Container-closure system compatibility with storage stability (including with regard to leachables and extractables) should be assessed and discussed. The stability assessment should comply with WHO Guidelines on stability evaluation of vaccines (24). Consideration should be given to the development of vaccine formulations that are more thermostable to improve their global utility.

6.8.4.8.2 Storage conditions

Storage conditions should be validated. The vaccine should not be stored for a length of time and/or at a temperature greater than that shown by the manufacturer to be compatible with a minimal loss of potency before being distributed by the manufacturing establishment or before being issued from a storage site. The maximum duration of storage should be fixed with the approval of the NRA based on the results of stability studies, and should be such as to ensure that all quality specifications for the final product, including the minimum potency specified on the container or package, are maintained until the end of shelf-life. During clinical trials, this period should ideally be at least equal to the expected duration of the vaccine administration stage in the fully enrolled clinical trial.

6.8.4.8.3 Expiry date

The expiry date should be defined on the basis of the shelf-life of the final container supported by stability studies and should be approved by the NRA. The expiry date should be based on the date of blending of the final formulated bulk, the date of filling or the date of the first valid potency test on the final lot, as appropriate, and agreed with the NRA.

6.9 Records

The relevant guidance provided in WHO good manufacturing practices for pharmaceutical products: main principles (18) should apply, as appropriate to the level of development of the candidate vaccine.

6.10 Retained samples

A sufficient number of samples should be retained for future studies and needs. These needs may include but are not limited to manufacturing investigations or development, nonclinical studies or future bridging clinical trials. A vaccine lot used in a pivotal clinical trial may serve as a

reference material and a sufficient number of vials should be reserved and stored appropriately for that purpose. Advanced planning is required to enable the retention of an appropriate number of containers of the pivotal clinical trial lot.

6.11 Labelling

The guidance on labelling provided in WHO good manufacturing practices for biological products (20) should be followed as appropriate. The label of the carton enclosing one or more final containers, or the leaflet accompanying the container, should include, at a minimum and as agreed with the NRA:

- the common and trade names of the vaccine;
- INN, if applicable;
- the names and addresses of the manufacturer and distributer;
- lot number;
- nature and content of the active substance;
- product composition, including list of excipients;
- a statement that specifies the nature and content of adjuvant contained in one human dose, if any;
- dosage form and appearance;
- the immunization schedule and the recommended route(s) of administration;
- the number of doses, if the product is issued in a multi-dose container;
- the name and concentration of any preservative added;
- a statement on the nature and quantity, or upper limit, of any antibiotics present in the vaccine;
- a statement on the trace amounts of any other residuals of clinical relevance;
- the temperature recommended during storage and transport;
- container-closure information;
- the expiry/retest date;
- any special dosing schedules;
- any special instructions for in-use handling – for example, necessity for gloves to prevent exposure of product to RNases when handling multi-dose vials, or stability on mixing of contents; and
- contraindications, warnings and precautions, and information on concomitant vaccine use and on known adverse events.

6.12 Distribution and transport

The guidance provided in WHO good manufacturing practices for biological products (20) appropriate for the vaccine should apply. Further guidance is provided in WHO Model guidance

for the storage and transport of time- and temperature-sensitive pharmaceutical products (25). Shipments should be maintained within specified temperature ranges, as applicable, and packages should contain cold-chain monitors, if applicable (26).

7. Nonclinical evaluation of mRNA vaccines

The nonclinical evaluation of candidate mRNA vaccines should be considered on a product-specific basis taking into account the intended clinical use. The design, conduct and analysis of nonclinical studies including selection of appropriate studies relating to the pharmacology (immunogenicity and proof-of-concept) and toxicology of the product should be based on the following WHO guidelines:

- WHO guidelines on nonclinical evaluation of vaccines (15); and
- WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (16).

There are several potential concerns that might be specific to mRNA vaccines. Some concerns may stem from the candidate vaccine design while others arise because they have been seen clinically with other RNA-based products (for example, candidate therapeutic products). Because of the novelty of this product class and for the sake of inclusiveness, numerous issues are listed in this section. Not all of these issues will necessarily be relevant to mRNA vaccines, depending on their design. However, it is incumbent upon the vaccine developer/manufacturer to provide evidence demonstrating the proof-of-concept (for example, immunogenicity and challenge protection) and safety of their candidate vaccine. The types, design and number of studies expected should be agreed with the NRA.

7.1 Pharmacology/immunology/proof-of-concept

In addition to the types of studies discussed in the WHO guidelines above (15,16), additional issues that the NRA might expect nonclinical studies to address may include:

a. Durability of immune responses or immune cell phenotypes that suggest durability, particularly those that are proposed to be related to the candidate vaccine's induction of protection. To assess the durability of immune responses, characterization of immune cell phenotypes and/or cytokine expression could be helpful in investigating persistence and memory responses.

b. Induction of innate immune responses by RNA (such as induction of type I interferon), which have been reported to decrease translation of the target antigen or that could affect the need for (or timing of) boosts or subsequent doses.

7.2 Safety/toxicity in animal models

In addition to the expectations outlined in the WHO guidelines listed above (15,16), consideration should be given to whether studies need to be designed to address the following:

a. **Biodistribution and persistence**: developing a database of evidence about this potential concern will permit the more rapid development of future candidate vaccines (3,62–67). This issue may also depend on whether the vaccine migrates to specific cells or tissues. Nonclinical studies that address whether the mRNA and the LNPs (or lipid components) distribute away from the tissue into which the vaccine was administered, into which tissues they distribute and how long they persist may be expected by the NRA. Agreement on these studies should be sought from the NRA.

b. **Inflammation**: RNA is inflammatory via a number of pathways, particularly via the innate immune system with its numerous sensors for RNA. In mRNA vaccines, both the mRNA molecules and the LNPs (which enable successful delivery and cellular uptake) have properties that can influence and trigger the innate immune system (68,69). While some of this activity may be beneficial for the immune response to the vaccine, it will be important to monitor for both systemic and local toxicity and inflammatory responses. Nonclinical study design needs to take into account any immune responses, reactogenicity or toxicities that might predict immune indictors (68,69) for serious adverse events or adverse events of special interest (AESI) in humans. Additionally, other components added to aid delivery, such as PEG, although relatively benign, can also influence the physicochemical properties and thus the safety profile (70–73). It is therefore important to understand the overall product profile including the formulation and how physicochemical properties (which may vary) can influence inflammation and the safety profile.

c. **Unexpected and serious toxicities from modified nucleosides**: some antivirals and anti-cancer drugs that contained unnatural nucleoside analogues have caused mitochondrial toxicities, resulting in myopathy, polyneuropathy, lactic acidosis, liver steatosis, pancreatitis, lipodystrophy and even fatality. However, some of these clinically observed toxicities were not observed in the nonclinical animal models. While the modified nucleosides used in the most advanced mRNA vaccines (against COVID-19) are naturally occurring, future candidate vaccines may contain modifications that are unnatural. Thus, particularly for mRNA vaccines that include unnatural nucleoside

modifications, careful consideration will need to be given to how these potential toxicities might be observed in appropriate animal models and nonclinical studies during safety evaluation (74–76).

d. **Novel lipids and novel LNPs**: because the lipids used to formulate the LNPs affect the overall charge of the particle, when using LNPs made with novel lipids or when the LNPs are themselves modified (for example, altered ratios or modified processes) and these LNPs have not previously been nonclinically and clinically tested in mRNA products encapsulated in LNPs then evaluation of the toxicity of the new formulation containing the novel lipids (or any novel excipients) may be required. Furthermore, the NRA may require that the genotoxicity and systemic toxicity of the novel lipid component be assessed, similar to the expectations for novel adjuvants set out in the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (16) and/or those for new chemical entities in the ICH guideline S2 (R1) (58).

e. **Novel formulations**: likewise, for formulations (other than LNPs) containing novel excipients, data on and assessment of the systemic toxicity and genotoxicity of the formulation may be expected.

f. **Potential immune anergy**: the induction of anergy (that is, unresponsiveness to antigens rather than immunity to them) has been observed when an antigen is expressed in cells other than professional antigen-presenting cells (77). As a result, the potential induction of immune tolerance is a theoretical risk. Depending on the target antigen encoded and the immunological pathways it might induce, the decision whether or not to evaluate this theoretical risk for any given mRNA product should be agreed with the NRA. Consideration of the product formulation or delivery system and the cells it targets may guide the decision on the necessity for such a study.

It should be noted that early theoretical concerns during plasmid DNA vaccine development regarding the potential for integration of vaccine nucleic acids into the host genome do not apply to mRNA vaccines for the following reasons:

- The only known mechanism by which RNA can integrate into the host genome requires the presence of a complex containing reverse transcriptase and integrase.
- Further, the design of candidate mRNA vaccines should be considered so that they do not include specific RNA-binding sites for primers required for the reverse transcriptase to initiate transcription. In addition, the RNA would have to be relocated to the nucleus after reverse transcription for the resulting product to be integrated.

- Finally, the vaccine mRNA degrades within a relatively short time once taken up by the body's cells, as does the cell's own mRNA. During that entire time, the mRNA vaccine is expected to remain in the cytoplasm, where it will be translated and then degraded by normal cellular mechanisms.

Therefore, nonclinical studies do not need to be performed to specifically address integration or genetic risks as these are considered to be theoretical issues for mRNA vaccines.

As with any vaccine that is anticipated to be used widely in pregnant women or women of childbearing potential, the guidance provided in section 4.2.2 of the WHO Guidelines on nonclinical evaluation of vaccines (15) and section D.2.3 of the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (16) should be consulted. The necessity for such studies will be based on the target population for the given clinical indication of the vaccine. Often, if required, these studies are performed during or after pivotal clinical trials have been performed with the candidate vaccine produced using commercial manufacturing methods and scale.

If clinical data from similar candidate vaccines based on the same platform technology are available then concurrence from the NRA on whether such data are scientifically sufficient to preclude the need for further nonclinical studies should be obtained.

7.3 Accelerating nonclinical evaluation in the context of rapid vaccine development against a priority pathogen during a public health emergency

In the case of the rapid development of vaccines against a priority pathogen during a public health emergency and when the new candidate vaccines are based on a given manufacturer's platform technology, consideration may be given to an abbreviated nonclinical programme as follows:

- Where changes are made to the sequence of the target antigen encoded in an mRNA vaccine that has already been clinically tested (for example, in the case of a pandemic influenza strain when a seasonal or other potential pandemic strain antigen has been tested, or where a variant SARS-CoV-2 spike protein arises), where the same LNPs are used (that is, same lipid composition and mRNA–lipid ratio, and where the total amount of mRNAs and LNPs per dose remain equal to or below that clinically tested) and where an approved manufacturing process is used then, depending on NRA requirements, the nonclinical programme might be limited to an immunogenicity study (or studies) or a challenge-protection study (or studies) in a relevant animal model, if available. As much safety information as feasible should be collected during these immunogenicity or challenge-protection studies given that such nonclinical

proof-of-concept studies are performed without full compliance to good laboratory practices (GLP). If safety information on veterinary vaccines expressing related antigens is available then this might also be useful and should be provided. Any other information concerning the safety of the platform technology used should also be provided for NRA consideration, for example, prior toxicology and biodistribution study data.

- Where the LNPs have been tested clinically with an unrelated mRNA such that the target antigen is novel (that is, not related to another antigen that has been clinically tested) then the approach of limiting nonclinical studies to an immunogenicity or challenge-protection study might not be sufficient. The decision regarding what type of nonclinical safety/toxicology information should be required might be guided by consideration of what and how much is known about the natural disease in terms of its pathology. If the natural disease is associated with immunopathology due to cross-reactivity, molecular mimicry, autoimmunity, allergenicity or immunity-associated disease enhancement then toxicology studies would likely be needed to ensure that the novel target antigen was not associated with these effects. Where natural disease is not associated with immunopathology or where little is known about the natural disease, discussion with the NRA should be undertaken on how the nonclinical programme might be abbreviated.

- Finally, where the LNPs and the encoded target antigen (and hence the mRNA structure and sequence) are both novel, nonclinical evaluation may be more complex and more extensive studies may be required; thus, discussion with the NRA should also be undertaken and it may not be possible to abbreviate the nonclinical programme. However, it may be possible to initiate clinical studies while some of the required nonclinical studies are being performed in parallel with (or slightly ahead of) clinical development.

Decisions on abbreviating the nonclinical programme should always take into account what is already known about related and previously tested products, particularly if based on the same platform technology. If clinical data from a related product(s) are available, these data are likely to be more meaningful for evaluating the safety of the candidate vaccine in humans than data from any given animal or in vitro human model.

8. Clinical evaluation of mRNA vaccines

The clinical evaluation expectations for clinical trial authorization or marketing authorization will be driven by the disease against which the mRNA vaccine is being or has been developed

and the vaccine mode-of-action (or mechanism-of-action). If an immune correlate of protection has been identified this may change the expectations compared to what might be expected in the absence of such a correlate. Clinical studies should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (22) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (17). Post-marketing pharmacovigilance is also discussed in the latter guidelines. Furthermore, these same guidelines provide considerations in evaluating dosing regimens, clinical development plans, collection of safety data, designs for pivotal efficacy trials (including potential end-points), standardizing immunogenicity assays (including use of IS and reporting of data in IU) and immunobridging to infer efficacy (17). Considerations for studies during pregnancy are also discussed in these same guidelines.

Clinical trials should capture safety, immunogenicity and efficacy data, as expected for any other type of vaccine, but with particular consideration given to the potential concerns outlined below, as these may be more relevant for mRNA vaccines than for other types of vaccines that might already be licensed.

8.1 Safety and immunogenicity evaluation

Sufficient data should be obtained from immunogenicity studies to permit evaluation of the following safety and immunological aspects that may be particularly relevant to mRNA vaccines:

a. **Adverse immune effects**
   Transient decreases in lymphocytes (Grades 1–3) a few days after vaccination were reported in the interim human clinical trial results of an mRNA COVID-19 vaccine, with lymphocytes returning to baseline within 6–8 days in all participants and with no associated clinical observations (78). Such transient drops have been observed for other vaccines and have resulted in no significant deleterious effect on the immune response (79,80). Because RNA induces type 1 interferons, which have been associated with the transient migration of lymphocytes into tissues, the phenomenon of any effect on lymphocyte counts in blood may need specific attention in preliminary clinical trials (64,81–83). Nonetheless, because this phenomenon may be important for the immune response to the candidate vaccine, it may be important to observe whether changes in leukocyte counts and subsets are associated with any adverse clinical signs or symptoms. Thus, the monitoring of appropriate reactogenicity parameters in the immediate post-vaccination period is paramount.

b. **Types and scope of immune responses**
   In addition to the type and scope of immunogenicity described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (17), in studies in which

immunogenicity is measured, additional facets of the safety and immunogenicity of mRNA vaccines may include:

- whether the mRNA candidate vaccine biases towards certain types of immune responses. To date, two clinical studies of COVID-19 mRNA vaccines have noted a Th1-type bias (34,40). This information may be useful for predicting and understanding the impact of the immune responses for a particular disease.

- as with any new vaccine, any instances or evidence of AESI as defined in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (17) or of any other novel adverse event should be captured in clinical trials and in post-marketing evaluation. If so, then investigations should be conducted into associations and potential causes, such as whether unwanted immune responses against vaccine components (such as RNA or lipids) are generated or, if pre-existing in the vaccine recipient, are increased or exacerbated. Alternatively, epitopic mimicry due to the responses to the expressed antigen(s) may need to be investigated.

Consideration should also be given to the total dose of mRNA (especially if the vaccine is multivalent or where separate mRNAs are used in an sa-mRNA vaccine) and to the total dose of LNPs with regard to maximally tolerable doses during the development of mRNA vaccines. If boosting following a primary dose or series is being considered due to waning effectiveness then careful evaluation of any increased frequency or severity of local or systemic reactions should be performed.

It should be noted that during clinical trials or emergency use situations involving COVID-19 mRNA vaccines, immunologically relevant adverse events of particular note (such as anaphylaxis or anaphylactoid reactions) have been observed (84). Anaphylaxis is known to occur very rarely with all vaccines and is not unique to mRNA vaccines. It is not yet known what aspect of the formulation is associated with immunological adverse events and it is advised, as with other vaccines, that individuals with known allergies to specific vaccine components should not be vaccinated with vaccines containing such components (85–88). It should further be noted that recent publications by several regulatory authorities provide useful relevant information, including publications by the European Medicines Agency (67), (89), the Medicines & Healthcare products Regulatory Agency (85,90) and the US Food and Drug Administration (91)(92).

In line with the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (17) the establishment and implementation of active pharmacovigilance plans is recommended. In the specific case of COVID-19 or other vaccines deployed in the context of a public health

emergency, consideration should also be given to running a public awareness campaign on potential adverse events. All adverse events potentially associated with COVID-19 vaccines are currently being assessed further as part of pharmacovigilance activities.

Given the short period for and limited scope of safety studies as part of the efficacy studies that have led to the current permitting of investigational mRNA COVID-19 vaccines for emergency use, and the still unknown long-term safety impacts of LNPs formulated with mRNAs in large human populations, it will be important to continue monitoring and recording rare adverse events that have an unknown relationship with the use of such vaccines. Regulatory agencies should analyze such data for vaccines made by different manufacturers to provide a better clinical understanding and a more precise safety profile for mRNA vaccines in the current formulation designs. Furthermore, manufacturers and public health agencies should consider conducting post-introduction vaccine effectiveness studies, addressing questions of effectiveness among specific risk groups, the duration of protection, and effectiveness against both infection and transmission. As stated above, this is a rapidly evolving area and significant new data are emerging on an ongoing basis.

When international standards expressed in IU are available for standardizing the immune assays used in clinical evaluation of the vaccine, they should be used to calibrate internal standards or other working reference materials, and results should be reported in IU to improve the comparability of results across vaccines, across studies and across different assay platforms.

8.2 Efficacy evaluation

Efficacy evaluation will depend upon the disease against which the candidate vaccine is intended to protect, and the clinical indication determined in clinical trials. Factors that should be considered in the evaluation of vaccine efficacy are described in the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (17).

It should be acknowledged that in countries in which COVID-19 mRNA vaccines are currently receiving emergency use authorization or approval such vaccines remain investigational, that is, under development. The ethical considerations regarding the conducting of ongoing COVID-19 vaccine trials with placebo controls were discussed in open public meetings held in December 2020 (93,94). Trial design issues (including the selection of appropriate comparators) are discussed in the above WHO Guidelines (17). Further guidance is also provided in the outcome document of a WHO Expert consultation on the use of placebos in vaccine trials (95). As with all candidate vaccines, both the scientific merits and ethical considerations should inform the trial design and decisions must be made in the current risk–benefit context of the country in which regulatory authorization is being sought (96,97). In addition, WHO has now published more than

70 Guidelines and Recommendations for vaccines against specific diseases, any one or several of which may provide relevant guidance on the evaluation of any given mRNA vaccine (12).

8.3 Efficacy evaluation in the context of a public health emergency in which immune-escape and other variants arise

As discussed in section 5.6.2 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (17) it may be feasible to consider immunobridging between the manufacturer's original candidate vaccine and a variant candidate vaccine in order to infer efficacy of the variant mRNA candidate vaccine based on a manufacturer's given platform technology in which clinical end-point efficacy has been demonstrated for the original candidate vaccine. The immunobridging may have to be supported by justification of how comparable antibody titres for the prototype and variant vaccines would translate into similar efficacy. Consideration must be given to the following two scenarios: (a) the variant candidate vaccine will replace the original candidate vaccine; or (b) the variant and original candidate vaccines will be combined (that is, in a bivalent or multivalent vaccine) or administered simultaneously or in sequence. Collection of comparative safety data during such immunobridging studies will also be expected. Overall, the considerations for immunobridging studies may depend upon factors such as the disease, pathogen and induced immune response(s) – trial designs and data requirements should thus be decided on a case-by-case basis.

In the specific case of COVID-19 vaccines, consideration may be given to the guidance provided by WHO (98), the European Medicines Agency (67) (89), the Medicines & Healthcare products Regulatory Agency (85), (90), the US Food and Drug Administration (91) (92) and other regulatory authorities (99–101).

In future, mRNA vaccines against influenza viruses may be developed and any proposed strain changes may have to take into consideration current practices for inactivated or live attenuated influenza virus vaccines. The WHO recommendations to assure the quality, safety, and efficacy of influenza vaccines (human, live attenuated) for intranasal administration (102) and WHO Recommendations for the production and control of influenza vaccine (inactivated) (103) should be consulted.

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The first draft was then prepared by a WHO drafting group comprising Dr R. Sheets, consultant, the USA; Dr M. Liu, consultant, ProTherImmune, the USA; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr K. Peden, United States Food and Drug Administration, the USA; Dr S. Sankarankutty, Health Sciences Authority, Singapore; and Dr T.Q. Zhou, World Health Organization, Switzerland. The resulting draft document was then posted on the WHO Biologicals website during December 2020 and January 2021 for a first round of public consultation. Comments were received from: Dr S. Acharya and Dr F. Atouf, US Pharmacopeia, the USA; Dr A. Adisa, Therapeutic Goods Administration, Australia; Dr L. Bisset, Dr A. Cook, Dr V. Ganeva and Dr K-W. Wan, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr R.M. Bretas, Agência Nacional de Vigilância Sanitária, Brazil; Dr G. Cirefice and Dr C. Milne, European Directorate for the Quality of Medicines & HealthCare, France; Dr I. Feavers, consultant, the United Kingdom; Dr D. Feikin, IVB/WHO (on behalf of the Covid-19 Vaccine Effectiveness working group); Dr G. Frank, Biotechnology Innovation Organization, the USA; Dr E. Griffiths, consultant, the United Kingdom; Dr R.A. Hafiz, Saudi Food & Drug Authority, Saudi Arabia; Dr W. Jaroenkunthum, Department of Medical Sciences, Thailand; Dr K. Karikó, Dr A. Kuhn, Dr U. Blaschke, Dr C. Blume, Dr C. Lindemann and Dr J. Diekmann, BioNTech, Germany; Dr D.C. Kaslow, PATH Vaccine Development Global Program, the USA; Dr M. Kucuku, National Agency for Medicines and Medical Devices, Albania; Dr U. Loizides and Dr R.B. Mattavelli, International Nonproprietary Names Programme and Classification of Medical Products, World Health Organization, Switzerland; Professor S. Lu, University of Massachusetts Medical School, the USA; Dr I. Mahmood Al-Sabri, Ministry of Health, Oman; Professor S.F. Malan, University of the Western Cape, South Africa; Dr J. Maslow, GeneOne Life Science Inc., the USA; Dr S. M. Morales Sánchez, National Food and Drug Surveillance Institute, Colombia; Professor E.E. Ooi, Duke-NUS Medical School, Singapore; Dr C. Pohl, Schlosspark-Klinik, Germany; Dr I. Prawahju, National Agency for Drug and Food Control, Indonesia; Dr R. Rabe, Norwegian Medicines Agency, Norway; Professor M. Rizzi, University of Piemonte Orientale, Italy; Dr J.S. Robertson, consultant, the United Kingdom; Dr N. Rose and Dr S. Schepelmann, National Institute for Biological Standards and Control, the United Kingdom; Professor P. Roy, London School of Medicine & Tropical Hygiene, the United Kingdom; Dr M. Savkina, Federal State Budgetary Institution Scientific Centre for Expert Evaluation of Medicinal Products, Russian Federation; Dr L. Tesolin, Dr K.

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