Annex 3

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
DNase	deoxyribonuclease
dsRNA	double-stranded RNA
GMP	good manufacturing practice(s)
HPLC	high-performance liquid chromatography
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
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
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
sa-mRNA	self-amplifying mRNA
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 (*7–14*). In addition, the rapidity with which clinical trials have progressed for COVID-19 candidate vaccines, their approval or 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 special formulations such as lipid nanoparticles (LNPs) to ensure in vivo stability and efficient delivery; and (c) the novel cell-free enzymatic 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 certain details remain proprietary and thus not publicly available, it is not feasible to develop specific international guidelines or recommendations at this time. Consequently, flexibility in the scientific approach to regulating mRNA vaccines 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 an individual case-by-case basis. Nevertheless, the key principles described in this document are applicable to the class of preventive mRNA vaccines against infectious diseases 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 (*15*).

Any given manufacturer's mRNA vaccines might potentially be viewed as a platform technology in which the coding region can readily be changed without necessarily having to change the manufacture or control of the resulting new product (except for antigen-specific tests for identity, potency and stability). 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 on this novel product type became available (*16, 17*).

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 against infectious disease for human use. Sufficient information should be provided as is phase-appropriate, and is expected to increase as product development advances. Although the most advanced vaccines of this type 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 type of preventive mRNA vaccine as rapidly as possible. It should nevertheless be noted that there remain gaps in the scientific understanding of the types and amount of immunogenicity that any given mRNA vaccine might need to achieve for it to be successful, broadly relevant and durably efficacious against the disease it is intended to prevent. Each vaccine will therefore need to be evaluated in terms of its own benefits and risks.

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 current document is limited to mRNA and self-amplifying mRNA (sa-mRNA) packaged in 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 serving as passive immunization 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 and its subsections 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), specific considerations are provided in this document where appropriate. In addition, any general WHO guidance of relevance should also be consulted; a number of WHO documents providing such guidance are listed below in section 4.

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 below).

This document has been developed in light of the available knowledge to date. 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 (or biological product): 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.

Design of experiments: a structured, organized method for determining the relationship between factors affecting a process and the output of that process.

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): some viruses have genomes comprising fully double-stranded RNA along their entire length rather than in distinct segments (such as the secondary structure of mRNA). If present, such dsRNA is sensed by intracellular receptors and can activate innate immune responses. Depending on the manufacturing method, dsRNA can be generated as a by-product during the in vitro transcription (IVT) manufacturing process for some mRNA vaccines, though some segments may be single stranded. This type of dsRNA is an impurity that should be removed from the mRNA during the manufacturing process, or its amount in the product at least determined and controlled. If the manufacturing method does not produce dsRNA, then the control of this as an impurity is unnecessary.

Engineering run: a manufacturing campaign conducted to engineer manufacturing methods in order to improve or confirm those methods for use in good manufacturing practice (GMP)-compliant production. The materials made in such a campaign are not intended for use in humans.

Excipient: a constituent of a medicine other than the active drug 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 the current document, the lipids that form the LNPs are excipients but the LNPs if formed separately from the mRNA are defined as intermediates in the production 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. Note that if more than one **drug substance** is to be combined (as in a multivalent or combination vaccine), their mixing would occur as part of the preparation of this final formulated bulk.

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 one or more drug substances (active ingredient) 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 or modified 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 lipid components may include, but are not limited to, an ionizable/cationic lipid, a helper lipid (for example, phospholipids

and/or cholesterol) and a lipid(s) modified for example by polyethylene-glycolylation (PEGylation). The LNPs and/or lipid components may also have adjuvant activity.

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 use different terms for such applications), 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). Once authorized or approved, the new medicine must be manufactured, controlled and labelled as described in the authorization or approval file.

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 one or more open reading frames (ORFs) that encode the protein (in the case of vaccines, the target antigen), flanking untranslated regions (UTRs), a 5′ cap (or alternative) and a 3′ sequence such as a poly(A) tail.

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 increased in vivo stability. Another type of modification is methylation. Nucleosides used to manufacture mRNA vaccines might also contain unnatural modifications.

mRNA integrity: the proportion of the mRNA that is the correct size and contains the 5′ cap and poly(A) tail. In addition, the correct sequence of the mRNA should be confirmed.

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 this same way to describe other terms used 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

(separate individual vaccines or a combination vaccine) or pathogen strains against the same disease (separate monovalent or mixed multivalent vaccines). The term could also be applied to a particular drug-delivery system (such as LNPs containing the mRNA) where identical lipids, concentrations, methods of preparation and purification and so on are used. Use of the term "platform technology" would be considered appropriate when: (a) the manufacturing methods are essentially unchanged (but may be optimized for each specific candidate vaccine); (b) the test methods (except for identity, potency and stability) and acceptance criteria are unchanged; (c) the immunomodulatory compounds or elements are unchanged; and (d) 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 and nonclinical 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, flexibility in the scientific approach to regulating mRNA vaccines is justified because of the current lack of standardization even in the face of platform technology use. As always, an individual 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 viruses (such as alphaviruses), either on the same molecule as the antigen or on a separate molecule. When expressed intracellularly, these ORFs produce the proteins of the viral replication machinery thus 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.

Target antigen(s): the protein(s) or portion thereof, encoded within the mRNA of an mRNA vaccine, for which an immune response to vaccination is expected to result in protection against one or more pathogens or strains.

That is, protection against disease (or infection) caused by a pathogen(s) may be conferred by the resulting immune response against the target antigen(s) following vaccination.

Therapeutic: a treatment given after a disease or condition (or signs or symptoms thereof) is evidenced, in contrast to the prevention of disease before exposure (or in rare cases following exposure but before the onset of signs or symptoms) to the infectious pathogenic 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 here are outside the scope of the current document.

Transfer RNA (tRNA): an RNA molecule used by ribosomes and that 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 target 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 in vivo stability. To justify the vaccine design, all available information on the type of immunity (protective and immunopathogenic) 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 or specially designed noncoding sequence (including poly(A) tail) and of structural elements (such as the chosen 5′ cap structure or alternative) should be provided. With regard to sa-mRNA, any viral replicon 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. Toxicological and immunogenicity data on the LNP 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 and/or human clinical studies on its safety and, if required by a given NRA, on its safety pharmacology (see section 7.2.d below) 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 combination or 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 (that is, nine-valent) 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 (*18*);
- Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (*19*);
- Guidelines on clinical evaluation of vaccines: regulatory expectations (*20*);
- WHO good manufacturing practices for pharmaceutical products: main principles (*21*);
- Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients (*22*);
- WHO good manufacturing practices for biological products (*23*);
- WHO good manufacturing practices for sterile pharmaceutical products (*24*);
- Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (*25*);
- *WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products* (*26*);
- Guidelines on stability evaluation of vaccines (*27*);
- Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (*28*);
- Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions (*29*);

- Guidelines for independent lot release of vaccines by regulatory authorities (*30*);
- Guidelines on procedures and data requirements for changes to approved vaccines (*31*); and
- WHO policy statement: multi-dose vial policy (MDVP). Handling of multi-dose vaccine vials after opening (*32*).

5. Special considerations

The mRNA of vaccines that are currently the most advanced in terms of clinical development or that are currently in widespread use against COVID-19 is produced enzymatically rather than biologically within a cell. This approach thus differs from the production of most other biologicals with which manufacturers and regulators are familiar (*1, 33*). Manufacturing either starts with linearized DNA plasmids that 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 enzymatically using the polymerase chain reaction (PCR) or other synthetic methods. Regardless of whether the manufacture of the RNA starts with a linearized molecule generated from a plasmid DNA or from an already linear DNA sequence, mRNA production occurs enzymatically in vitro by means of a DNA-dependent RNA polymerase that transcribes the linear 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) that 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 that might be made to the naturally occurring nucleoside include the use of pseudouridine or N1-methylpseudouridine in place of uridine (*3, 4, 34*). In addition, altering or optimizing codon use (without changing the encoded amino acids) may impact in vivo 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 in vivo 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, 34*). 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 appropriate cap analogues. 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, the manufacturing methods and the control testing of the mRNA drug substance(s) 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, RNA is often represented diagrammatically as being 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 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 therefore 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 and the widely used COVID-19 vaccines take the form of mRNA encoding the target antigen (*35, 36*). Because mRNA (and RNA in general) is subject to degradation by nucleases, the most advanced mRNA candidate vaccines and widely used COVID-19 vaccines at the time of writing are formulated in LNPs, which aids in vivo stability and delivery (*33, 37–43*). There are different types of LNPs depending on their composition, the types of lipids employed and the manufacturing process used (*44*). Some may not yet have been employed for the delivery of mRNA (*45–48*). 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 (*47–50*) 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 lipids in the LNPs) may

contribute to the mode-of-action of the vaccine and the implications of this need to be considered in the critical quality attributes and 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 above in section 4. As with other combination or multivalent vaccines, each mRNA would be considered to be a separate drug substance that will be combined into a final drug product. Also, as with other combination or multivalent vaccines, the amount of mRNA for each target antigen, the expression efficiency of each and the resulting immune responses, must be balanced against the other(s) in order to avoid interference with the expression of (and thus immune responses to) all the target antigens, and to ensure the necessary strain- or antigen-specific immune responses to the total vaccine. Furthermore, consideration should be given to ensuring an adequate dose of mRNA for each target antigen using appropriate nonclinical toxicity studies to evaluate the highest tolerable total mRNA and LNP doses, and if applicable, justified by previous clinical experience. See sections 7.3 and 8.1 below for further discussion of this point.

Additional consideration should also be given to the manufacture, control and stability of combination or multivalent vaccines to ensure appropriate quality control of each drug substance and the drug product and to ensure the suitability of the analytical procedures used to control each mRNA component (that is, each drug substance) 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 lipid composition of the LNPs. Therefore, the particle size, morphology, surface properties (for example, charge) and encapsulation efficiency of the resultant LNPs containing the mRNA could vary when a different mRNA is used. 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, 38, 51*). These products include the gene sequences for replicon 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 (*38*). 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 replicon 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 sa-MRNA, and so on – all of which could result in a different safety profile, and hence all of which needs to be considered in the vaccine design and evaluation.

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 (*38*). 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).

As described above in sections 1 and 2, and in order to develop WHO guidance as rapidly as possible, the scope of the current document is limited to mRNA or sa-mRNA encapsulated in LNPs.

It should also be noted that in the case of current mRNA vaccines, various cell types at the site of injection take up the mRNA. However, future delivery systems may be designed that selectively target the mRNA to specific cell types 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 but these are beyond the scope of the current document.

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 (*21*), Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients (*22*), WHO good manufacturing practices for biological products (*23*) and WHO good manufacturing practices for sterile pharmaceutical products (*24*) should be applied to the design, establishment, operation, control and maintenance of manufacturing facilities for mRNA vaccines. The primary guidance on GMP requirements for mRNA vaccines used to prevent infectious diseases is provided in WHO good manufacturing practices for biological products (*23*). This document advises that for recombinant or biotechnology products, GMP compliance is expected from the starting materials through to the filled, finished product. 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 (*27–29*). Quality control during the manufacturing process relies on the implementation of quality systems, such as 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 monitoring and/or control tests (with acceptable limits) should be established through a risk-based approach (including tests to measure critical and non-critical quality attributes, as applicable) in order 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 attributes 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, potency, quality and safety attributes, 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 benefit–risk 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 materials 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 commercial 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 lot(s) (and if applicable, for a clinical trial application, placebo or other comparator) 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 (other than process optimization for each candidate vaccine), tests (except for identity or potency) or specifications, then a new candidate mRNA vaccine might be supported by data from an earlier candidate mRNA vaccine or licensed product. For example, this could be the case when the only changes made are to the sequence and these changes do not 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, stability and nonclinical and clinical safety.

Details of 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 provided. 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/or stability data. The WHO Guidelines on procedures and data requirements for changes to approved vaccines (*31*) should be consulted in this regard. All changes made to the product post-approval should follow the requirements listed in these same Guidelines (*31*); other relevant guidance may also be considered such as the ICH Harmonised Guideline on pharmaceutical product lifecycle management (*52*).

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 (*53, 54*).

6.1 General manufacturing overview

Vaccines based on mRNA represent a new type 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, 34, 55, 56). 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 (if not controlled at the level of the DNA template). The methods of purification and their purposes should be described and justified. Any purification processes – such as protein digestion with proteinase(s) as an impurity-reduction step – should be validated at the appropriate phase of development (see section 6.4 below).

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 mimics 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. As with any vaccine, an agreed-upon number of lots of the drug product should be placed on a stability programme.

The mRNA (drug substance) is not suitable for clinical use unless it is protected and delivered by a given formulation (the preparation of which is part of drug product manufacture). 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, the current document only covers 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 (*47, 49, 50*). In order to protect the mRNA from degradation by nucleases, it is incorporated into the LNPs to make it inaccessible to such nucleases – however, the LNPs must also 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–lipid 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 replicon genes are encoded on a separate mRNA molecule, then additional manufacturing processes and quality controls may be needed to ensure that the required mRNAs are adequately encapsulated in the same LNPs, and these additional processes should be described. The molar ratio of the two encapsulated mRNAs should be provided and justified, and the method of validation described. 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, 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. For sa-mRNA in which the mRNA encoding the replicon and the mRNA expressing the target antigen are encoded on different molecules, it will be important that these two RNAs are co-encapsulated in order for them to be taken up by the same cell in vivo. Therefore, if the two RNAs are encapsulated separately and then mixed, a justification for this approach will be required.

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 enzymatically or synthetically generated (for example, by PCR) 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 RNA polymerase), capping enzyme, 2′ O-methyltransferase, poly(A) polymerase, DNase and proteinase K); (d) buffers; (e) solvents; (f) column resins (if column chromatography is used in purification); and/or (g) lipids. The linear DNA template is considered to be the starting material for the manufacture of the drug substance. The other listed items (along with any not listed but also used in manufacture) would be considered to be raw materials. Excipients are those raw materials that are present as inactive ingredients in the final drug product/vaccine. For the manufacturing of excipients,

compliance is expected with WHO Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients (*22*).

- 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* (*26*).
- 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 and intermediates – 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 (regarding, for example, their size and polydispersity), consistency of mRNA encapsulation, and removal of partial mRNAs and dsRNA impurities.

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

d. Process validation – processes should be validated to demonstrate the consistent manufacturing of the commercial final drug product with the desired quality profile (see section 6.4 below).

Analytical methods that might be considered for assessing some of these key quality control points are discussed in the literature – though detailed analytical procedures and acceptance criteria for tested attributes are not yet standardized or in the public domain. As of the time of publication of the current document, these are considered by manufacturers to be proprietary and confidential. The methods shown in Table 1 may be considered as examples of possible means for characterization or control at various key quality control points (*57–59*).

For clinical trial use, mRNA candidate vaccines should be manufactured under GMP conditions appropriate for the stage of clinical development. It is 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 (*18, 19*).

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 with the WHO Guidelines on procedures and data requirements for changes to approved vaccines (*31*) would be expected, though other relevant guidance might also be considered such as the ICH Harmonised Guideline on pharmaceutical product lifecycle management (*52*).

Table 1

Examples of possible methods for characterization or control at various key quality control points, by potential use(s)

Examples of possible method	Potential use(s)
DNA template sequencing; mRNA sequencing	Identity
Quantitative reverse transcription PCR (qRT-PCR)	Identity and quantification
Ultraviolet spectroscopy; fluorescein-based assays	Quantification and purity
Agarose or acrylamide gel electrophoresis, including capillary electrophoresis	RNA quantification, RNA size, RNA integrity, LNP surface charge and percentage encapsulation
Chromatographic assays such as size-exclusion, anion-exchange, affinity or reverse-phase	Quantity of mRNA, quantity of lipids, quality of mRNA and nanoparticle integrity
Mass spectrometry	Quantity and nanoparticle integrity
dsRNA blot; tests for percentage capping; percentage transcripts with (and size of) poly(A) tail	Purity and other quality attributes
Cell-free translation or cell-based expression systems	Potency and expression of correct protein
Light-scattering techniques such as dynamic or static light-scattering analysis; nanoparticle tracking analysis; electron microscopy; size-exclusion chromatography	Particle size distribution (purity, consistency, safety)
Laser Doppler electrophoresis; dynamic light-scattering analysis	Particle surface characterization (including size, polydispersity and zeta potential)

Table 1 *continued*

Examples of possible method	Potential use(s)
Electron microscopy; atomic force microscopy; X-ray diffraction; differential scanning calorimetry analysis	Physicochemical characterization (including surface and morphological properties)
Tests for sterility, endotoxin content	Safety attributes
pH determination; gravimetric, azeotropic or titrimetric method to test residual moisture	Quality attributes

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, sequence and construction, its composition (for example, lipids and other excipients), and the quantities of each excipient used. 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 role in the vaccine formulation should be included.

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, if sequences are changed from the native sequence in order to optimize codons, these changes should be described and justified. Codons may be altered for several reasons, including to better match the frequency of the appropriate tRNAs in human cells, to attain a specific secondary or tertiary mRNA structure, to

reduce innate immune responses or to increase the in vivo stability of the mRNA.

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 potency of the vaccine. The sequences for any such replicon should be provided and their functions explained. If the replicon is encoded 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. If more than one mRNA molecule (drug substance) is included in the drug product (final vaccine), this should be described, including whether the different mRNA molecules are encapsulated in a single LNP at the same time or encapsulated separately in LNPs that are subsequently mixed.

b. Chemical nature and formulation: the mRNA is formulated principally for increased in vivo 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 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 attributes 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 quality of the

lipids and critical quality attributes of the drug product should also be included. Sufficient characterization of the mRNA-LNP 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 safety, immunogenicity and efficacy of the vaccine should be described and their effects (positive or negative) should be considered during formulation development.

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 (*19*). Quality aspects of the separate adjuvant, if included, should also be addressed and described.

d. **Additional peptides/proteins**: if additional peptides/proteins are included to target the mRNA to antigen-presenting cells or other specific cell types 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 materials used in production (*22*). The 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) and the lipids in the LNP 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 processes used for the derivation of the linear DNA template and raw materials should also be described. Although the starting material for the production of mRNA vaccines is the linear DNA template, that template may be derived from upstream materials such as a DNA plasmid propagated in a recombinant cell bank (see section 6.3.1.1 below).

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. Consideration should be given to performing a nitrosamine risk assessment on the (cationic) lipid(s), if relevant.

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, enzymes, buffers and solvents), intermediates and final excipients (for example, lipids and salts) should be provided. Consideration should also be given to the use and control of solvents, and to the potential for contamination with elemental impurities (*60–62*). Where the recycling of materials/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, stability monitoring and batch analyses of the materials. Since inclusion of a PEGylated lipid plays a critical role in providing in vivo stability and enhancing the cellular interaction of LNPs (*42*), adequate controls (for example, of molecular weight, polydispersity and mole percent) should be in

place for the PEGylated lipid. For the manufacturing of excipients, compliance is expected with WHO Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients (*22*).

6.3.1.1 Quality of linear DNA derived from plasmid as starting material

As stated above, the linear DNA template is considered to be the starting material for the GMP production of the mRNA vaccine. If the linear DNA is prepared from plasmid DNA, then the procedures for establishing the cell banks and the manufacture of the plasmid DNA should be performed in accordance with the requirements for the production of material for use in subsequent GMP manufacture.

A cell bank system should be established, described and tested for microbial purity (freedom from bacterial and fungal contamination) and identity. The genetic stability of the seed bank must be demonstrated. If the poly(A) tail is encoded in the plasmid DNA, then that region on the DNA plasmid in particular should be tested for the rate of recombination. 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 (if used to generate the linear DNA) 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 (using appropriate reference standards) for residual genomic DNA, RNA and protein, sterility or permissible bioburden, and endotoxin levels. In early development, testing might be carried out only on the DNA plasmid (if used) or on the linear DNA.

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/vendor/supplier of the raw material. An internal policy should 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 subsequent GMP manufacture.

6.4 Process development and in-process controls

The development history of the commercial manufacturing process should be provided. Tests and acceptance criteria for alert and action limits 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 in the justification.

Validation of the manufacturing processes should demonstrate that they comply with their 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 attributes 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 and may need to be validated (see section 6.4 above). 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. Particle-uptake studies could assist in characterizing potential potency measures through identification of cell types that take up the particles, mode or mechanism of uptake, and efficiency of uptake, and thus guide selection of the type of cell-free or in vitro method that best allows for assessment of these activities. During characterization, it should be determined whether any of these characteristics should be controlled as critical quality attributes and/or stability-indicating attributes.

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. Measurement of surface charge (for example, zeta potential) should also be considered as a method for characterizing the LNPs. These, and other properties, will affect the in vivo stability, cellular interaction and immunological response properties of the product; such information would also help to confirm the consistency of the manufactured vaccine.

The immunogenicity elicited by the mRNA-encoded target antigen is a critical characteristic of the product that should be characterized in nonclinical studies as a means to 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). Consideration of these aspects is important in gaining understanding and knowledge of the product in order to optimize its design and develop appropriate control methods.

Potential impurities that might be introduced by the starting materials, and potential product- or process-related impurities 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, 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 in order to justify the 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. This will permit justification of the specification limits proposed so that these impurities are suitably controlled and are 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 attributes 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 (*31*).

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 replicon 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 replicon-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. For sa-mRNA in which the mRNA encoding the replicon and the mRNA expressing the target antigen are encoded on different molecules, it will be important that these two RNAs are co-encapsulated in order for them to be taken up by the same cell in vivo. Therefore, if the two RNAs are encapsulated separately and then mixed, a justification for this approach will be required.

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 (*21–24*) 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 (*31*).

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 (including accessory and regulatory regions), then the sequence chosen should be unique to that mRNA product 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 potentially purity, depending on the sequencing method used.

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 and to measure impurities 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 product-related impurities will need to be tested for and/or measured 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 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 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 attribute through testing or process validation. Likewise, the presence of dsRNA depends on whether the processes used are capable of producing it. 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 attributes

Relevant safety tests should be described. These may include tests for endotoxins along with testing either for bacterial and fungal sterility (including demonstration of lack of bactericidal or fungicidal activity of the test article) or microbial bioburden (including quantity, identification of microbe species and freedom from specified unwanted organisms). Such testing is generally not required by an NRA for the drug substance, but if required a test for pyrogenicity may be performed on the drug product (which may be the monocyte activation test). 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 (*63*). This test should therefore not be required or requested for either the drug substance or the drug product.

6.7.1.5 Additional quality attributes

Additional important quality attributes should be established and controlled (such as appearance, pH and, if relevant, viscosity).

6.7.1.6 Reference materials

An in-house reference preparation (that is, working standard) should be established for use in assay standardization and comparability assessment. 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 at which the reference material has been shown to be stable. A routine programme for monitoring the stability of the material 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. Whatever approach is taken should be clearly described.

6.7.1.7 Stability

A stability assessment should be conducted in accordance with the WHO Guidelines on stability evaluation of vaccines (*27*). The types of studies conducted, the protocols followed, and the study results should all 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 (or stored intermediates) and any future extension of it should be based on long-term real-time stability studies under actual conditions. For the transportation of intermediates or drug substance, it is expected that shipment validation will be conducted at appropriate storage temperatures and 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 Experiments (DoE) 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. If stored, these are important intermediates in the production of the final vaccine and should be controlled appropriately.

Adequate consideration should be given to ensuring physicochemical stability and microbial control during such hold-times.

The preparation of the lipids, the encapsulation of mRNA with the lipids into LNPs, 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 into 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. If applicable, the diluent or reconstitution solution should be described.

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 (polydispersity); and (c) RNA encapsulation efficiency/proportion encapsulated. In some cases, the surface properties (for example, charge), lipid molar ratio, or cationic lipid to mRNA ratio (for example, nitrogen to phosphate ratio) 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. Likewise, if platform data are intended to support development of new candidate vaccines, the impact of the new mRNA drug substance on the critical quality attributes of the final vaccine product should be determined.

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 (*23*) 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 a 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 (*32*). 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 combination or 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 combination or multivalent vaccines). Another consideration will be whether the mRNAs will be mixed prior to encapsulation in the LNPs or whether each mRNA will be separately encapsulated into LNPs and then a mixture of the two or more mRNA-LNPs prepared. In either case, the approach selected should be described and justified with relevant data.

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 attributes (section 6.8.4.5) and potency (section 6.8.4.6). Stability will also need to be established to justify the requested expiry dating.

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 (*21–24*) 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 6.4–6.6 above. Several consecutive lots of vaccine, in final dosage form, should be tested and analysed using validated methods to confirm manufacturing consistency. Any statistically significant or scientifically meaningful 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 combination or multivalent vaccine, or an 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 at the same time 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 (*31*).

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. If the vaccine contains more than one mRNA species (for example, in a combination or multivalent vaccine, or an sa-mRNA consisting of separate mRNAs), then the identity of each mRNA should be confirmed. Confirmation of mRNA 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). If the

vaccine contains more than one mRNA species (for example, in a combination or multivalent vaccine, or an sa-mRNA consisting of separate mRNAs), then the quantity of each mRNA should be measured and confirmation made that the ratio of each mRNA to the other is as intended and the total mRNA dose has not been exceeded.

6.8.4.4 Safety attributes

Each lot of final vaccine should be tested for bacterial and fungal sterility (including demonstration of lack of bactericidal or fungicidal activity of the test article). If the vaccine is to be administered by a non-parenteral route, then omission of the sterility test and inclusion of an appropriate alternative microbial 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). Animal testing should be avoided whenever alternative satisfactory testing is available and allowed. For scientific and ethical reasons, it is desirable 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 control 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 (*63*). This test should therefore not be required or requested.

6.8.4.5 Additional quality attributes

Other important quality attributes 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 attributes such as osmolality or viscosity may also be important. For the final vaccine (drug product), additional attributes should include lipid/polymer identification and content, nanoparticle size, mRNA–lipid ratio 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 attributes.

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 and correlated with clinical performance, as with all quality control tests.

When a new candidate vaccine against a new strain(s) is developed, consideration should be given to ensuring that the potency assay(s) used is valid for the strain change.

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. 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 and mRNA integrity) 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 an individual case-by-case basis. Therefore, discussing appropriate potency measures and reaching 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 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 against the same pathogen based on the same or similar technologies (for example, different COVID-19 mRNA vaccines).

6.8.4.8 Stability testing, storage and expiry date

The relevant guidance provided in WHO good manufacturing practices for biological products (*23*), WHO good manufacturing practices for sterile pharmaceutical products (*24*) and WHO Guidelines on stability evaluation of vaccines (*27*) 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 (*29*) 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 (*64, 65*).

6.8.4.8.1 *Stability*

Adequate stability studies form an essential part of vaccine development. To support commercial use, 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. Attributes 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 attributes 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. If long-term storage (for example, > 6 months) at temperatures above freezing is being considered, this may require additional analytical testing to assess potential lipid oxidation or other such changes and the resultant impact of these changes on potency.

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 (*32*).

During initial clinical development limited stability information would be expected. For example, some regulators accept 3 months of real-time stability of the lot of final vaccine to be used in the proposed clinical trial in the containers that will be used for the clinical trial, or one produced in the same manner in the same container type and size and meeting the same specifications, at the time of application for clinical trial authorization, but this should be agreed with the NRA. Initial clinical development may also be supported by including results from predictive stability modelling utilizing an accelerated stability assessment programme. Likewise, stability data on a platform technology can be supportive for new candidate vaccines based on that platform.

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 (*27*). 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 real-time 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 (*21*) 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 (*23*) 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 drug (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 (*23*) 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 (*28*). Shipments should be maintained within specified temperature ranges, as applicable, and packages should contain cold-chain monitors, if applicable (*29*).

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" (in the case of vaccines, 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 (*18*); and
- WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (*19*).

There are several potential concerns that need to be considered during the safety and proof-of-concept evaluations of mRNA vaccines. Because of the novelty of this product type, numerous issues are addressed below which may be relevant to any given mRNA vaccine. However, there may also be future additional concerns that come to light that would need to be taken into consideration when appropriate. Not all of these issues will necessarily be relevant to any given mRNA vaccine, depending on its design. However, it is incumbent upon the vaccine developer/manufacturer to provide evidence demonstrating the proof-of-concept (for example, immunogenicity and, if an appropriate animal model is available, 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 (*18, 19*), 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 (*18, 19*), 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, 66–71*). This potential 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 (*72, 73*). 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 indicators (*72, 73*) of 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 (*74–77*). 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. The choice of animal model will, as always, be critical, recognizing that anti-RNA innate immune responses in animal models are generally significantly milder than those observed in humans.

c. **Unexpected and serious toxicities from modified nucleosides**: some antivirals and anti-cancer drugs that contained specific unnatural nucleoside analogues with altered conformation 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 used. 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 that have not already been well characterized in other developed nucleic-acid-based products, 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 (*78–80*).

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 (*19*) and/or those for new chemical entities in the ICH guideline S2 (R1) (*62*).

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.

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 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 (*18*) and section D.2.3 of the WHO Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines (*19*) 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. As a result, data should be available at the time of filing for marketing authorization on populations that include women of childbearing potential, and such data should be evaluated prior to the intentional enrolment of pregnant women in clinical studies.

If clinical data from similar candidate vaccines based on the same platform technology are available, then agreement should be reached with the NRA on whether or not such data are scientifically sufficient to preclude the need for further nonclinical studies. If nonclinical safety data from similar candidate vaccines based on the same platform technology are available, agreement should also be reached with the NRA on whether or not such data are scientifically sufficient to preclude the need for further nonclinical safety studies. Likewise, nonclinical safety data (and if available, clinical data) from a monovalent drug product formulation can support the clinical development of a multivalent drug product formulation (for example, for different strains of the same disease antigen) or combination vaccine (different disease antigens) in cases where the same LNP with the same molar and mRNA–lipid ratios is used, and where the sum of all the mRNAs in the multivalent drug product formulation will be no more than the highest dose shown to be safe in the monovalent nonclinical safety study.

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 on the understanding that such nonclinical proof-of-concept studies are generally performed without full compliance to good laboratory practices (GLP). If safety information on veterinary vaccines expressing related antigens is available, 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. It should be noted that it may not be possible to investigate autoimmunity in nonclinical studies (*18*). 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 significantly 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 model or from an 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 (*25*) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*20*). Post-marketing pharmacovigilance is also discussed in the latter guidelines. Furthermore, these same guidelines provide considerations in evaluating dosing regimens, clinical development plans, boosting, 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 (*20*). 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 and with which regulators might be more familiar.

8.1 Safety and immunogenicity evaluation

Sufficient data should be obtained from preliminary clinical 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 (*81*). Such transient drops have

been observed for other vaccines and have resulted in no significant deleterious effect on the immune response (*82, 83*). 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 (*69, 84–86*). 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. Further general guidance on safety evaluation is provided in section 7 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*20*).

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 (*20*), 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, depending on what is known about the natural disease and the vaccine mode-of-action. To date, two clinical studies of COVID-19 mRNA vaccines have noted a Th1-type bias (*37, 43*). 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 (*20*) 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, epitope 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 a multivalent or combination vaccine, or an sa-mRNA vaccine

where separate mRNAs are used) and to the total dose of LNPs with regard to the maximally tolerable dose determined during the development of an mRNA vaccine. For platform technologies, a maximally tolerable dose for a given population may be suggested by the dose previously determined for vaccines (or candidate vaccines) produced using that platform.

If boosting following a primary dose or series is being considered due to waning effectiveness, careful evaluation of any increased frequency or severity of local or systemic reactions should be performed. As with all vaccines, it is recommended that a careful exploration of dose, timing and number of immunizations (primary series and boosters if needed), and kinetics and durability of immune responses be performed in preliminary clinical trials to guide the design of the efficacy trial(s). Discussion of these issues can be found in section 5.5 of the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*20*). In certain situations, a determination that booster doses are needed might not be made until post-marketing data have been collected (for example, indicating waning immunity or protection). The above Guidelines (*20*) also discuss the boosting of licensed vaccines in section 5.6.1.2.3, which addresses the situation in which an alternative posology to the licensed product may need to be developed for the booster. Waning protection and the necessity for booster doses is then discussed in section 6.3.8. (*20*). Differences between the vaccine and circulating strains, including the potential need to add or replace strains, are briefly discussed in section 5.3.3 and other sections that discuss influenza vaccines, for which strain changes are frequently made.

It should be noted that during clinical trials or widespread use of COVID-19 mRNA vaccines, immunologically relevant adverse events of particular note (such as anaphylaxis or anaphylactoid reactions) have been observed (*87, 88*). 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 (*89–92*). Myocarditis and pericarditis have also been observed during COVID-19 mRNA vaccine pharmacovigilance and appear to be associated – though the biological mechanism and associated vaccine component have not yet been identified (*93, 94*). It should further be noted that recent publications by several regulatory authorities provide useful relevant information, including publications by the European Medicines Agency (*71, 95*), the Medicines and Healthcare products Regulatory Agency (*89, 96*) and the US Food and Drug Administration (*92, 97*).

In line with the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (*20*), the establishment and implementation of active pharmacovigilance plans are 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. As with any new vaccine, all adverse events potentially associated with COVID-19 vaccines are currently being further assessed 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 widespread use of COVID-19 mRNA vaccines, and the still unknown long-term safety impacts of mRNAs formulated with LNPs 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 at-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 (*20*).

It should be noted that in countries in which COVID-19 mRNA vaccines are currently being widely used, the use of placebo controls in trials requires special consideration. 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 (*98, 99*). Trial design issues (including the selection of appropriate comparators) are discussed in the above WHO Guidelines (*20*). Further guidance is also provided in the outcome document of a WHO Expert consultation on the use of placebos in vaccine trials (*100*). 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 benefit–risk context of the country in which regulatory authorization is being sought (*101, 102*). 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 (*15*).

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 (*20*) it may be feasible to consider immunobridging between the manufacturer's original candidate vaccine (or subsequently marketed 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 (or marketed) 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 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 an individual case-by-case basis.

In the specific case of COVID-19 vaccines, consideration may be given to the guidance provided by WHO (*103, 104*), the European Medicines Agency (*71, 95*), the Medicines and Healthcare products Regulatory Agency (*89, 96*), the US Food and Drug Administration (*92, 97*) and other regulatory authorities (*105–107*).

Currently, mRNA vaccines against influenza viruses are in development 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 (*108*) and WHO Recommendations for the production and control of influenza vaccine (inactivated) (*109*) should be consulted.

Authors and acknowledgements

The preliminary draft of this WHO document was prepared by Dr M.A. Liu, consultant, ProTherImmune, the USA; and Dr H-N. Kang, World Health Organization, Switzerland. This preliminary draft was then subject to expert working group review during the period 1–15 October 2020 and comments

received from: Dr. E. Grabski and Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr. K. Karikó, Dr A. Kuhn, Dr U. Blaschke, Dr C. Blume and Dr J. Diekmann, BioNTech, Germany; Dr D. Kaslow, PATH Vaccine Development Global Program, the USA; Professor S. Lu, University of Massachusetts Medical School, the USA; Professor E.E. Ooi, Duke-NUS Medical School, Singapore; Dr K. Peden, United States Food and Drug Administration, the USA; Professor P. Roy, London School of Hygiene & Tropical Medicine, the United Kingdom; Dr S. Sankarankutty, Health Sciences Authority, Singapore; Dr J. Ulmer, GSK (formerly); Dr F. Verdier, Sanofi Pasteur, France; Dr M. Watson and Dr C. Vinals, Moderna, the USA; and Dr T.Q. Zhou, World Health Organization, Switzerland.

The first draft was then prepared by a WHO drafting group comprising Dr R. Sheets, consultant, the USA; Dr M.A. 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.G. Balocco, 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. Brusselmans and Dr W. van Molle, Sciensano, Belgium; Dr J. Ulmer, independent consultant, the USA; Dr L. Viviani, Humane Society International, Italy; Dr A.L. Waddell, WHO temporary advisor, the United Kingdom; Dr M. Li and Dr W. Wei, Center for Drug Evaluation, National Medical Products Administration, China; Dr K. Weisser, Paul-Ehrlich-Institut, Germany; and Dr G. Zenhäusern, Swiss Agency for Therapeutic Products, Switzerland. Dr P. Barbosa, International Federation of Pharmaceutical Manufacturers & Associations (IFPMA) provided the consolidated comments of subject matter experts from: GSK Vaccines, Belgium; Pfizer Vaccines, the USA; and Sanofi Pasteur, France.

Taking into consideration the comments received, the second draft document was prepared by Dr R. Sheets, consultant, the USA; Dr M.A. 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; Dr K-W. Wan, Medicines and Healthcare products Regulatory Agency, the United Kingdom; and Dr T.Q. Zhou, World Health Organization, Switzerland. The second draft was then reviewed at a WHO informal consultation on regulatory considerations for evaluation of the quality, safety and efficacy of RNA-based prophylactic vaccines for infectious diseases, held virtually on 20–22 April 2021 and attended by: Dr I.G. Al Gayadh and Dr R.A. Hafiz, Saudi Food & Drug Authority, Saudi Arabia; Dr P. Aprea, Administración Nacional de Medicamentos, Alimentos y Tecnología Medica, Argentina; Dr M. Ayiro, Pharmacy and Poisons Board, Kenya; Dr C. Bae, Ministry of Food and Drug Safety, Republic of Korea; Dr K. Bloom, University of the Witwatersrand and South African Medical Research Council, South Africa; Dr K. Bok and Dr B.S. Graham, National Institutes of Health, the USA; Dr R. Bose, Central Drugs Standard Control Organization, India; Dr J. Fernandes, Health Canada, Canada; Dr E. Grabski and Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr S. Alireza Hosseini, Food and Drug Administration, the Islamic Republic of Iran; Dr N. de Jesus Huertas Mendez, Instituto Nacional de Vigilancia de Medicamentos y Alimentos, Colombia; Mrs T. Jivapaisarnpong, King Mongkut's University of Technology Thonburi, Thailand; Professor F. Krammer, Icahn School of Medicine at Mount Sinai, the USA; Dr M.A. Liu, consultant, ProTherImmune, the USA; Dr J. Lu, National Medical Products Administration, China; Professor S. Lu, University of Massachusetts Medical School, the USA; Dr A. Marti and Dr T. Schochat, Swiss Agency for Therapeutic Products,

Switzerland; Dr T. Matano, National Institute of Infectious Diseases, Japan; Dr C. Milne, European Directorate for the Quality of Medicines & HealthCare, France; Dr P. Minor, consultant, the United Kingdom; Dr W. van Molle and Dr L. Tesolin, Sciensano, Belgium; Professor E.E. Ooi, Duke-NUS Medical School, Singapore; Dr M. Page, Dr N. Rose and Dr S. Schepelmann, National Institute for Biological Standards and Control, the United Kingdom; Dr K. Peden, United States Food and Drug Administration, the USA; Dr S. Pumiamorn, Ministry of Public Health, Thailand; Dr J.S. Robertson, independent expert (*representative of the International Nonproprietary Names Programme*), the United Kingdom; Professor P. Roy, London School of Hygiene & Tropical Medicine, the United Kingdom; Professor K. Ruxrungtham, Chulalongkorn University, Thailand; Dr S. Sankarankutty, Health Sciences Authority, Singapore; Dr Y. Shelar, Central Drugs Standard Control Organization (HQ), India; Dr R. Sheets, consultant, the USA; Dr R. Shivji, European Medicines Agency, Netherlands; Ms G.S. Silveira, Agência Nacional de Vigilância Sanitária, Brazil; Dr V.G. Somani, Central Drugs Standard Control Organization, India; Dr K-W. Wan, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr J. Wang and Dr Y. Wang, National Institutes for Food and Drug Control, China; Dr W. Wei, Center for Drug Evaluation, National Medical Products Administration, China; and Professor D. Weissman, Penn Center for AIDS Research, the USA. *Representatives of other organizations*: Dr S.K. Acharya, United States Pharmacopeia, the USA; Dr N. Jackson, Coalition for Epidemic Preparedness Innovations, the United Kingdom; Dr D. Kaslow, PATH Vaccine Development Global Program, the USA; and Dr C. Vinals, the Biotechnology Innovation Organization, the USA. *Representatives of the Developing Countries Vaccine Manufacturers Network (DCVMN)*: Dr M.M. Ahasan, Incepta Vaccine, Bangladesh; Dr D.T. Dat, Vabiotech, Viet Nam; Dr C. Roy, Bharat Biotech, India; Dr W. Wijagkanalan, BioNet-Asia Co., Ltd, Thailand; and Mr A. Wong, Walvax Biotechnology, China. *Representatives of the IFPMA*: Dr D. Boyce, Pfizer, Ambler, PA, the USA; Dr S. Chiyukula and Dr S. Simons, Sanofi, Cambridge, MA, the USA; Dr R. Forrat, Sanofi, Lyons, France; Dr S. Gregory and Dr G. Maruggi, GSK, Rockville, MD, the USA; Dr S. Lockhart, Pfizer, New York, NY, the USA; and Dr F. Takeshita, Daiichi Sankyo, Japan. *Individual manufacturers/other industries*: Dr T. Class, Translate Bio, the USA; Dr S. Gould, Charles River Laboratories, Lyons, France; Dr A. Kuhn (20 and 21 April), Dr R. Rizzi (20 and 21 April), Dr C. Lindemann (22 April) and Dr E. Lagkadinou (22 April), BioNTech SE, Germany; Dr K. Lindert, Arcturus Therapeutics, the USA; Dr J. Maslow, GeneOne Life Science Inc., the USA; Dr J.M. Miller and Dr D. Parsons, Moderna, Inc., the USA ; Dr F. Neske and Dr L. Oostvogels, CureVac AG, Germany; Mr Y. Park, GeneOne Life Science Inc., Republic of Korea; and Dr B. Ying, Suzhou Abogen Biosciences Co., Ltd. P.R., China. *WHO staff*: Dr C. Ondari, Dr I. Knezevic, Dr T.Q. Zhou, Dr M. Friede, Dr B. Giersing, Ms

E. Sparrow, Dr U. Loizides, Dr R.G. Balocco and Dr D. Wood, World Health Organization, Switzerland; and Dr S. Escalante and Dr J. Shin, WHO Regional Office for the Western Pacific, Philippines.

Based on the outcomes of the above informal consultation, the document WHO/BS/2021.2402 was prepared by Dr R. Sheets, consultant, the USA; Dr M.A. 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; Dr K-W. Wan, Medicines and Healthcare products Regulatory Agency, the United Kingdom; and Dr T.Q. Zhou, World Health Organization, Switzerland. Document WHO/BS/2021.2402 was then posted on the WHO Biologicals website for a second round of public consultation from July to September 2021 and written comments received from: Dr S. Acharya and Dr F. Atouf, United States Pharmacopeia, the USA; Dr L. Bisset, Medicines and Healthcare products Regulatory Agency, the United Kingdom; Dr E. Cocuzzi, Health Canada, Canada; Dr T. Class, Translate Bio, the USA; Dr S. Dubey, Dr J. Nussbaum, Dr L. Plitnick, Dr J. Prescott, Dr J. Wei and Dr Y. Zhang, Merck Vaccines, the USA; Dr M. Li and Dr S. Jin, Center for Drug Evaluation, National Medical Products Administration, China; Dr S. Gould, Charles River Laboratories, France; Dr Z. Guo, Chinese Pharmacopoeia Commission, China; Dr A. Gudjonsson, Dr G. Abrahamsen and Dr T.K. Andersen, Norwegian Medicines Agency, Norway; Dr F. Neske, CureVac, Germany; A.A. Zuniga, A.M. Santana Puentes, Instituto Nacional de Vigilancia de Medicamentos y Alimentos, Colombia; Dr S. Muchakayala, Touchlight Genetics Ltd, the United Kingdom; Dr S. Wendel, Hospital Sírio-Libanês Blood Bank, Brazil; Dr G. Sanyal, Dr P-A. Gilbert and Dr D. Robinson, Bill & Melinda Gates Foundation, the USA; Dr J. Wang, National Institutes for Food and Drug Control, China; Dr I. Zadezensky, Moderna, the USA; and A. Kuhn, U. Blaschke, K. Karikó, D. Theisen, B. Vallazza, C. Lindemann and E. Lagkadinou, BioNTech SE, Germany. Dr P. Barbosa, IFPMA, provided the consolidated comments of subject matter industry experts.

Further changes were made to document WHO/BS/2021.2402 by the WHO Expert Committee on Biological Standardization.

References

1. Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics – developing a new class of drugs. Nat Rev Drug Discov. 2014;13(10):759–80 (https://www.nature.com/articles/nrd4278?fbclid=IwAR0B8wG6qI3QCvTOPYSOAdlU6GiiM7FQFjD25u1V69BjcdmzhwWbufKOgGs, accessed 22 September 2021).
2. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A et al. Direct gene transfer into mouse muscle in vivo. Science. 1990;247(4949 Pt 1):1465–8 (https://www.researchgate.net/publication/21464693_Direct_Gene_Transfer_into_Mouse_Muscle_in_Vivo, accessed 22 September 2021).

3. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines – a new era in vaccinology. Nat Rev Drug Discov. 2018;17(4):261–79 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5906799/, accessed 22 September 2021).
4. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S et al. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther. 2008;16(11):1833–40 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2775451/, accessed 22 September 2021).
5. Thess A, Grund S, Mui BL, Hope MJ, Baumhof P, Fotin-Mleczek M et al. Sequence-engineered mRNA without chemical nucleoside modifications enables an effective protein therapy in large animals. Mol Ther. 2015;23(9):1456–64 (https://www.sciencedirect.com/science/article/pii/S1525001616302738, accessed 22 September 2021).
6. Rauch S, Lutz J, Kowalczyk A, Schlake T, Heidenreich R. RNActive® technology: generation and testing of stable and immunogenic mRNA vaccines. Methods Mol Biol. 2017;1499:89–107 (abstract: https://pubmed.ncbi.nlm.nih.gov/27987144/, accessed 22 September 2021).
7. Hinz T, Kallen K, Britten CM, Flamion B, Granzer U, Hoos A et al. The European regulatory environment of RNA-based vaccines. Methods Mol Biol. 2017;1499:203–22 (abstract: https://pubmed.ncbi.nlm.nih.gov/27987152/3, accessed 22 September 2021).
8. Jackson NAC, Kester KE, Casimiro D, Gurunathan S, DeRosa F. The promise of mRNA vaccines: a biotech and industrial perspective. NPJ Vaccines. 2020;5(1):11 (https://www.nature.com/articles/s41541-020-0159-8, accessed 22 September 2021).
9. Naik R, Peden K. Regulatory considerations on the development of mRNA vaccines. Curr Top Microbiol Immunol. 2020 (abstract: https://link.springer.com/chapter/10.1007%2F82_2020_220, accessed 22 September 2021).
10. Schmid A. Considerations for producing mRNA vaccines for clinical trials. Methods Mol Biol. 2017;1499:237–51 (abstract: https://pubmed.ncbi.nlm.nih.gov/27987154/, accessed 22 September 2021).
11. Hager G. Nonclinical safety testing of RNA vaccines. Methods Mol Biol. 2017;1499:253–72 (abstract: https://pubmed.ncbi.nlm.nih.gov/27987155/, accessed 22 September 2021).
12. Guideline on the chemistry, manufacture and control (CMC) of prophylactic COVID 19 mRNA vaccines (current version). Center for Drug Evaluation. Beijing: National Medical Products Administration; 2020 (https://www.ccfdie.org/en/gzdt/webinfo/2021/09/1632893004599121.htm, accessed 9 November 2021).
13. Knezevic I, Liu MA, Peden K, Zhou T, Kang H-N. Development of mRNA vaccines: scientific and regulatory issues. Vaccines. 2021;9(2):81 (https://www.mdpi.com/2076-393X/9/2/81/htm, accessed 5 March 2022).
14. Liu MA, Zhou T, Sheets RL, Meyer H, Knezevic I. WHO informal consultation on regulatory considerations for evaluation of the quality, safety and efficacy of RNA-based prophylactic vaccines for infectious diseases, 20–22 April 2021. Emerg Microbes Infect. 2022;11(1):384–91 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8812800/, accessed 5 March 2022).
15. Health product and policy standards. Vaccine standardization [website]. Geneva: World Health Organization (https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/vaccine-standardization, accessed 9 November 2021).
16. Main outcomes of the meeting of the WHO Expert Committee on Biological Standardization held from 24 to 28 August 2020. Geneva: World Health Organization; 2020 (https://www.who.int/publications/m/item/WHO-ECBS-aug-2020-executive_summary, accessed 22 September 2021).

17. Main outcomes of the meeting of the WHO Expert Committee on Biological Standardization held from 9 to 10 December 2020. Geneva: World Health Organization; 2020 (https://www.who.int/publications/m/item/ECBS-Executive-Summary.IF.IK.TW-15_Dec_2020, accessed 22 September 2021).

18. WHO guidelines on nonclinical evaluation of vaccines. In: WHO Expert Committee on Biological Standardization: fifty-fourth report. Geneva: World Health Organization; 2005: Annex 1 (WHO Technical Report Series, No. 927; http://www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/ANNEX%201Nonclinical.P31-63.pdf?ua=1, accessed 22 September 2021).

19. Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines. In: WHO Expert Committee on Biological Standardization: sixty-fourth report. Geneva: World Health Organization; 2014: Annex 2 (WHO Technical Report Series, No. 987; https://www.who.int/biologicals/areas/vaccines/TRS_987_Annex2.pdf?ua=1, accessed 22 September 2021).

20. Guidelines on clinical evaluation of vaccines: regulatory expectations. In: WHO Expert Committee on Biological Standardization: sixty-seventh report. Geneva: World Health Organization; 2017: Annex 9 (WHO Technical Report Series, No. 1004; http://www.who.int/biologicals/expert_committee/WHO_TRS_1004_web_Annex_9.pdf?ua=1, accessed 22 September 2021).

21. WHO good manufacturing practices for pharmaceutical products: main principles. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-eighth report. Geneva: World Health Organization; 2014: Annex 2 (WHO Technical Report Series, No. 986; http://www.who.int/medicines/areas/quality_safety/quality_assurance/TRS986annex2.pdf?ua=1, accessed 22 September 2021).

22. Good manufacturing practices: supplementary guidelines for the manufacture of pharmaceutical excipients. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: thirty-fifth report. Geneva: World Health Organization; 1999: Annex 5 (WHO Technical Report Series, No. 885; https://www.who.int/medicines/areas/quality_safety/quality_assurance/SupplementaryGMPPharmaceuticalExcipientsTRS885Annex5.pdf?ua=1, accessed 22 September 2021).

23. WHO good manufacturing practices for biological products. In: WHO Expert Committee on Biological Standardization: sixty-sixth report. Geneva: World Health Organization; 2016: Annex 2 (WHO Technical Report Series, No. 999; http://www.who.int/biologicals/areas/vaccines/Annex_2_WHO_Good_manufacturing_practices_for_biological_products.pdf?ua=1, accessed 22 September 2021).

24. WHO good manufacturing practices for sterile pharmaceutical products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-fifth report. Geneva: World Health Organization; 2011: Annex 6 (WHO Technical Report Series, No. 961 (https://www.who.int/docs/default-source/medicines/norms-and-standards/guidelines/production/trs961-annex6-gmp-sterile-pharmaceutical-products.pdf?sfvrsn=61682f0c_0, accessed 22 September 2021).

25. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products. In: WHO Expert Committee on the Use of Essential Drugs: sixth report. Geneva: World Health Organization; 1995: Annex 3 (WHO Technical Report Series, No. 850; https://apps.who.int/iris/bitstream/handle/10665/37340/WHO_TRS_850.pdf?sequence=1, accessed 9 November 2021).

26. WHO guidelines on transmissible spongiform encephalopathies in relation to biological and pharmaceutical products. Geneva: World Health Organization; 2003 (https://apps.who.int/iris/handle/10665/68932, accessed 9 November 2021).

27. Guidelines on stability evaluation of vaccines. In: WHO Expert Committee on Biological Standardization: fifty-seventh report. Geneva: World Health Organization; 2011: Annex 3 (WHO Technical Report Series, No. 962; http://www.who.int/biologicals/vaccines/Annex_3_WHO_TRS_962-3.pdf?ua=1, accessed 22 September 2021).
28. Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-fifth report. Geneva: World Health Organization; 2011: Annex 9 (WHO Technical Report Series, No. 961; http://www.who.int/medicines/areas/quality_safety/quality_assurance/ModelGuidanceForStorageTransportTRS961Annex9.pdf, accessed 22 September 2021).
29. Guidelines on the stability evaluation of vaccines for use under extended controlled temperature conditions. In: WHO Expert Committee on Biological Standardization: sixty-sixth report. Geneva: World Health Organization; 2016: Annex 5 (WHO Technical Report Series, No. 999; http://www.who.int/biologicals/areas/vaccines/Annex_5_Guidelines_on_Stability_evaluation_vaccines_ECTC.pdf?ua=1, accessed 22 September 2021).
30. Guidelines for independent lot release of vaccines by regulatory authorities. In: WHO Expert Committee on Biological Standardization: sixty-first report. Geneva: World Health Organization; 2013: Annex 2 (WHO Technical Report Series, No. 978; http://www.who.int/biologicals/TRS_978_Annex_2.pdf?ua=1, accessed 22 September 2021).
31. Guidelines on procedures and data requirements for changes to approved vaccines. In: WHO Expert Committee on Biological Standardization: sixty-fifth report. Geneva: World Health Organization; 2015: Annex 4 (WHO Technical Report Series, 993; http://www.who.int/biologicals/vaccines/Annex4_Guidelines_changes_to_approved_vaccines_eng.pdf?ua=1, accessed 22 September 2021).
32. WHO policy statement: multi-dose vial policy (MDVP). Handling of multi-dose vaccine vials after opening. Revision 2014. Geneva: World Health Organization; 2014 (Document WHO/IVB/14.07; http://apps.who.int/iris/bitstream/10665/135972/1/WHO_IVB_14.07_eng.pdf, accessed 22 September 2021).
33. Krammer F. SARS-CoV-2 vaccines in development. Nature. 2020;586(7830):516–27 (https://www.nature.com/articles/s41586-020-2798-3, accessed 23 September 2021).
34. Zhong Z, Mc Cafferty S, Combes F, Huysmans H, De Temmerman J, Gitsels A et al. mRNA therapeutics deliver a hopeful message. Nano Today. 2018;23:16–39 (abstract: https://www.sciencedirect.com/science/article/pii/S1748013218301099, accessed 23 September 2021).
35. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020;383(27):2603–15 (https://doi.org/10.1056/NEJMoa2034577, accessed 23 September 2021).
36. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021;384(5):403–16 (https://www.nejm.org/doi/full/10.1056/NEJMoa2035389, accessed 23 September 2021).
37. Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature. 2020;586(7830):594–9 (https://www.nature.com/articles/s41586-020-2814-7.pdf, accessed 23 September 2021).
38. Lundstrom K. Nanoparticle-based delivery of self-amplifying RNA. Gene Ther. 2020;27(5):183–5 (https://www.nature.com/articles/s41434-020-0132-1, accessed 23 September 2021).
39. Bertrand N, Leroux J-C. The journey of a drug-carrier in the body: an anatomo-physiological perspective. J Control Release. 2012;161(2):152–63 (https://www.researchgate.net/publication/51723559_The_journey_of_a_drug-carrier_in_the_body_An_anatomo-

physiological_perspective, accessed 23 September 2021).

40. Sedic M, Senn JJ, Lynn A, Laska M, Smith M, Platz SJ et al. Safety evaluation of lipid nanoparticle-formulated modified mRNA in the Sprague-Dawley rat and cynomolgus monkey. Vet Pathol. 2018;55(2):341–54 (https://journals.sagepub.com/doi/10.1177/0300985817738095, accessed 23 September 2021).
41. Li S-D, Huang L. Pharmacokinetics and biodistribution of nanoparticles. Mol Pharm. 2008;5(4):496–504 (abstract: https://pubs.acs.org/doi/10.1021/mp800049w, accessed 23 September 2021).
42. Mui BL, Tam YK, Jayaraman M, Ansell SM, Du X, Tam YYC et al. Influence of polyethylene glycol lipid desorption rates on pharmacokinetics and pharmacodynamics of siRNA lipid nanoparticles. Mol Ther Nucleic Acids. 2013;2:e139 (https://www.cell.com/action/showPdf?pii=S2162-2531%2816%2930197-4, accessed 23 September 2021).
43. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN et al. An mRNA vaccine against SARS-CoV-2 – Preliminary report. N Engl J Med. 2020;383(20):1920–31 (https://www.nejm.org/doi/10.1056/NEJMoa2022483, accessed 23 September 2021).
44. Ghasemiyeh P, Mohammadi-Samani S. Solid lipid nanoparticles and nanostructured lipid carriers as novel drug delivery systems: applications, advantages and disadvantages. Res Pharm Sci. 2018;13(4):288–303 (https://www.researchgate.net/publication/328281162_Solid_lipid_nanoparticles_and_nanostructured_lipid_carriers_as_novel_drug_delivery_systems_Applications_advantages_and_disadvantages, accessed 2 October 2021).
45. Guevara ML, Persano F, Persano S. Advances in lipid nanoparticles for mRNA-based cancer immunotherapy. Front Chem. 2020;8:589959 (https://www.frontiersin.org/articles/10.3389/fchem.2020.589959/full, accessed 2 October 2021).
46. Hassett KJ, Benenato KE, Jacquinet E, Lee A, Woods A, Yuzhakov O et al. Optimization of lipid nanoparticles for intramuscular administration of mRNA vaccines. Mol Ther Nucleic Acids. 2019;15:1–11 (https://www.sciencedirect.com/science/article/pii/S2162253119300174, accessed 2 October 2021).
47. Buschmann MD, Carrasco MJ, Alishetty S, Paige M, Alameh MG, Weissman D. Nanomaterial delivery systems for mRNA vaccines. Vaccines (Basel). 2021;9(1):65 (https://www.mdpi.com/2076-393X/9/1/65/htm, accessed 2 October 2021).
48. Alfagih IM, Aldosari B, AlQuadeib B, Almurshedi A, Alfagih MM. Nanoparticles as adjuvants and nanodelivery systems for mRNA-based vaccines. Pharmaceutics. 2021;13(1):45 (https://www.mdpi.com/1999-4923/13/1/45/htm, accessed 2 October 2021).
49. Kedmi R, Ben-Arie N, Peer D. The systemic toxicity of positively charged lipid nanoparticles and the role of Toll-like receptor 4 in immune activation. Biomaterials. 2010;31(26):6867–75 (abstract: https://pubmed.ncbi.nlm.nih.gov/20541799/, accessed 2 October 2021).
50. Pizzuto M, Bigey P, Lachagès A-M, Hoffmann C, Ruysschaert J-M, Escriou V et al. Cationic lipids as one-component vaccine adjuvants: a promising alternative to alum. J Control Release Off J Control Release. 2018;287:67–77 (abstract: https://pubmed.ncbi.nlm.nih.gov/30110615/, accessed 2 October 2021).
51. Bloom K, van den Berg F, Arbuthnot P. Self-amplifying RNA vaccines for infectious diseases. Gene Ther. 2021;28:117–29 (https://www.nature.com/articles/s41434-020-00204-y, accessed 2 October 2021).
52. Technical and regulatory considerations for pharmaceutical product lifecycle management – Q12. ICH Harmonised Guideline. Final version: 20 November 2019. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; 2019 (https://

database.ich.org/sites/default/files/Q12_Guideline_Step4_2019_1119.pdf, accessed 9 November 2021).

53. Guidance on the use of International Nonproprietary Names (INNs) for pharmaceutical substances. Geneva: World Health Organization; 2017 (https://www.who.int/medicines/services/inn/FINAL_WHO_PHARM_S_NOM_1570_web.pdf?ua=1, accessed 2 October 2021).
54. Robertson JS, Chui W-K, Genazzani AA, Malan SF, de la Rica Manjavacas AL, Mignot G et al. The INN global nomenclature of biological medicines: a continuous challenge. Biologicals. 2019;60:15–23 (https://www.sciencedirect.com/science/article/pii/S104510561930048X, accessed 2 October 2021).
55. Arbuthnot P, Ely A, Bloom K. A convenient method to generate and maintain poly(A)-encoding DNA sequences required for in vitro transcription of mRNA. Biotechniques. 2019;66(1):37–9 (https://www.researchgate.net/publication/330389042_A_convenient_method_to_generate_and_maintain_polyA-encoding_DNA_sequences_required_for_in_vitro_transcription_of_mRNA, accessed 3 October 2021).
56. Trepotec Z, Geiger J, Plank C, Aneja MK, Rudolph C. Segmented poly(A) tails significantly reduce recombination of plasmid DNA without affecting mRNA translation efficiency or half-life. RNA. 2019;25:507–18 (https://rnajournal.cshlp.org/content/25/4/507.full, accessed 3 October 2021).
57. Crommelin DJA, Anchordoquy TJ, Volkin DB, Jiskoot W, Mastrobattista E. Addressing the cold reality of mRNA vaccine stability. J Pharm Sci. 2021;110(3):997–1001 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7834447/, accessed 3 October 2021).
58. Muralidhara BK, Baid R, Bishop SM, Huang M, Wang W, Nema S. Critical considerations for developing nucleic acid macromolecule based drug products. Drug Discov Today. 2016;21(3):430–44 (http://csmres.co.uk/cs.public.upd/article-downloads/Critical-considerations-for-developing-nucleic-acid-macromolecule-based-drug-products.pdf, accessed 3 October 2021).
59. Poveda C, Biter AB, Bottazzi ME, Strych U. Establishing preferred product characterization for the evaluation of RNA vaccine antigens. Vaccines (Basel). 2019;7(4):131 (https://europepmc.org/article/PMC/PMC6963847, accessed 3 October 2021).
60. Impurities: guideline for residual solvents – Q3C(R6). ICH Harmonised Guideline. Final version: 20 October 2016. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; 2016 (https://database.ich.org/sites/default/files/Q3C-R6_Guideline_ErrorCorrection_2019_0410_0.pdf, accessed 3 October 2021).
61. Guideline for elemental impurities – Q3D(R1). ICH Harmonised Guideline. Final version: 22 March 2019. International Conference for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; 2019 (https://database.ich.org/sites/default/files/Q3D-R1EWG_Document_Step4_Guideline_2019_0322.pdf, accessed 3 October 2021).
62. ICH guideline S2 (R1) on genotoxicity testing and data interpretation for pharmaceuticals intended for human use. Step 5. London: European Medicines Agency; 2012 (Document EMA/CHMP/ICH/126642/2008; https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-s2-r1-genotoxicity-testing-data-interpretation-pharmaceuticals-intended-human-use-step_en.pdf, accessed 3 October 2021).
63. Lei D, Schmidt H, Knezevic I, Zhou T, Kang H-N, Kopp S. Removal of the innocuity test from The International Pharmacopoeia and WHO recommendations for vaccines and biological products. Biologicals. 2020;66:17–20 (https://www.sciencedirect.com/science/article/pii/S1045105620300610, accessed 3 October 2021).

64. Getting started with vaccine vial monitors. Geneva: World Health Organization; 2002 (Document WHO/V&B/02.35; https://apps.who.int/iris/bitstream/handle/10665/67806/WHO_V-B_02.35_eng.pdf;sequence=1, accessed 2 October 2021).

65. Bar-codes, QR codes and Vaccine Vial Monitors in the context of COVID-19 vaccines. Geneva: World Health Organization; 2020 (https://www.who.int/publications/m/item/bar-codes-qr-codes-and-vaccine-vial-monitors-in-the-context-of-covid-19-vaccines, accessed 3 October 2021).

66. Stokes A, Pion J, Binazon O, Laffont B, Bigras M, Dubois G et al. Nonclinical safety assessment of repeated administration and biodistribution of a novel rabies self-amplifying mRNA vaccine in rats. Regul Toxicol Pharmacol. 2020;113:104648 (abstract: https://pubmed.ncbi.nlm.nih.gov/32240713/, accessed 3 October 2021).

67. Lou G, Anderluzzi G, Schmidt ST, Woods S, Gallorini S, Brazzoli M et al. Delivery of self-amplifying mRNA vaccines by cationic lipid nanoparticles: the impact of cationic lipid selection. J Control Release. 2020;325:370–9 (abstract: https://pubmed.ncbi.nlm.nih.gov/32619745/, accessed 3 October 2021).

68. Pardi N, Tuyishime S, Muramatsu H, Kariko K, Mui BL, Tam YK et al. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. J Control Release. 2015;217:345–51 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4624045/, accessed 3 October 2021).

69. Bahl K, Senn JJ, Yuzhakov O, Bulychev A, Brito LA, Hassett KJ et al. Preclinical and clinical demonstration of immunogenicity by mRNA vaccines against H10N8 and H7N9 influenza viruses. Mol Ther. 2017;25(6):1316–27 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5475249/#!po=0.505051, accessed 3 October 2021).

70. Liang F, Lindgren G, Lin A, Thompson EA, Ols S, Röhss J et al. Efficient targeting and activation of antigen-presenting cells in vivo after modified mRNA vaccine administration in rhesus macaques. Mol Ther. 2017;25(12):2635–47 (https://www.sciencedirect.com/science/article/pii/S1525001617303659, accessed 3 October 2021).

71. Assessment report. Comirnaty. Common name: COVID-19 mRNA vaccine (nucleoside-modified). Procedure no. EMEA/H/C/005735/0000. Amsterdam: European Medicines Agency; 2021 (Document EMA/707383/2020 Corr.1; pp. 46–47; https://www.ema.europa.eu/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf, accessed 3 October 2021).

72. Obeid MA, Tate RJ, Mullen AB, Ferro VA. Lipid-based nanoparticles for cancer treatment. In: Grumezescu AM, editor. Lipid nanocarriers for drug targeting. Norwich (NY): William Andrew Publishing; 2018;313–59 (abstract: https://www.sciencedirect.com/science/article/pii/B9780128136874000086, accessed 3 October 2021).

73. Marlowe JL, Akopian V, Karmali P, Kornbrust D, Lockridge J, Semple S. Recommendations of the Oligonucleotide Safety Working Group's Formulated Oligonucleotide Subcommittee for the safety assessment of formulated oligonucleotide-based therapeutics. Nucleic Acid Ther. 2017;27(4):183–96 (abstract: https://pubmed.ncbi.nlm.nih.gov/28609186/, accessed 3 October 2021).

74. CHMP Safety Working Party's response to the Paediatric Committee regarding the use of PEGylated drug products in the paediatric population. London: European Medicines Agency; 2012 (Document EMA/CHMP/SWP/647258/2012; https://www.ema.europa.eu/en/chmp-safety-working-partys-response-paediatric-committee-regarding-use-pegylated-drug-products, accessed 2 October 2021).

75. Turecek PL, Bossard MJ, Schoetens F, Ivens IA. PEGylation of biopharmaceuticals: a review of chemistry and nonclinical safety information of approved drugs. J Pharm Sci. 2016;105(2):460–75 (abstract: https://www.sciencedirect.com/science/article/abs/pii/S0022354915001306, accessed

3 October 2021).

76. Liu G, Li Y, Yang L, Wei Y, Wang X, Wang Z et al. Cytotoxicity study of polyethylene glycol derivatives. RSC Adv. 2017;7(30):18252–9 (https://pubs.rsc.org/en/content/articlelanding/2017/ra/c7ra00861a, accessed 9 November 2021).
77. Turecek PL, Siekmann J. 4 - PEG–protein conjugates: nonclinical and clinical toxicity considerations. In: Pasut G, Zalipsky S, editors. Polymer-protein conjugates. Elsevier; 2020;61–101 (abstract: https://www.sciencedirect.com/science/article/pii/B9780444640819000048, accessed 3 October 2021).
78. Johnson AA, Ray AS, Hanes J, Suo Z, Colacino JM, Anderson KS et al. Toxicity of antiviral nucleoside analogs and the human mitochondrial DNA polymerase. J Biol Chem. 2001;276(44):40847–57 (https://www.sciencedirect.com/science/article/pii/S0021925820779317, accessed 3 October 2021).
79. Moyle G. Toxicity of antiretroviral nucleoside and nucleotide analogues: is mitochondrial toxicity the only mechanism? Drug Saf. 2000;23(6):467–81 (abstract: https://pubmed.ncbi.nlm.nih.gov/11144657/, accessed 3 October 2021).
80. Nucleoside analogues. In: LiverTox: clinical and research information on drug-induced liver injury. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012 (Overview: http://www.ncbi.nlm.nih.gov/books/NBK548938/, accessed 3 October 2021).
81. Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature. 2020;586:589–93 (https://www.nature.com/articles/s41586-020-2639-4, accessed 9 November 2021).
82. Bovay A, Speiser DE, Marraco SAF. Early drop of circulating T cells negatively correlates with the protective immune response to Yellow Fever vaccination. Hum Vaccines Immunother. 2020;16(12):3103–10 (https://www.tandfonline.com/doi/full/10.1080/21645515.2020.1750249, accessed 2 October 2021).
83. Faguet GB. The effect of killed influenza virus vaccine on the kinetics of normal human lymphocytes. J Infect Dis. 1981;143(2):252–8 (abstract: https://pubmed.ncbi.nlm.nih.gov/7217721/, accessed 3 October 2021).
84. Alberer M, Gnad-Vogt U, Hong HS, Mehr KT, Backert L, Finak G et al. Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial. Lancet. 2017;390(10101):1511–20 (Summary: https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(17)31665-3/fulltext, accessed 3 October 2021).
85. Richner JM, Jagger BW, Shan C, Fontes CR, Dowd KA, Cao B et al. Vaccine mediated protection against Zika virus-induced congenital disease. Cell. 2017;170(2):273–283.e12 (https://www.sciencedirect.com/science/article/pii/S0092867417307596, accessed 3 October 2021).
86. Heidenreich R, Jasny E, Kowalczyk A, Lutz J, Probst J, Baumhof P et al. A novel RNA-based adjuvant combines strong immunostimulatory capacities with a favorable safety profile. Int J Cancer. 2015;137(2):372–84 (https://onlinelibrary.wiley.com/doi/full/10.1002/ijc.29402, accessed 3 October 2021).
87. Shimabukuro TT, Cole M, Su JR. Reports of anaphylaxis after receipt of mRNA COVID-19 vaccines in the US – December 14, 2020-January 18, 2021. JAMA. 2021;325(11):1101–2 (https://jamanetwork.com/journals/jama/fullarticle/2776557, accessed 3 October 2021).
88. Klein NP, Lewis N, Goddard K, Fireman B, Zerbo O, Hanson KE et al. Surveillance for adverse events after COVID-19 mRNA vaccination. JAMA. 2021;326(14):1390–9 (https://jamanetwork.com/journals/jama/fullarticle/2784015, accessed 9 November 2021).

89. Regulatory approval of Pfizer/BioNTech vaccine for COVID-19 [website]. London: Medicines and Healthcare products Regulatory Agency (https://www.gov.uk/government/publications/regulatory-approval-of-pfizer-biontech-vaccine-for-covid-19, accessed 9 November 2021).

90. Castells MC, Phillips EJ. Maintaining safety with SARS-CoV-2 vaccines. N Engl J Med. 2021;384(7):643–9 (https://www.nejm.org/doi/10.1056/NEJMra2035343, accessed 3 October 2021).

91. Sellaturay P, Nasser S, Ewan P. Polyethylene glycol-induced systemic allergic reactions (anaphylaxis). J Allergy Clin Immunol Pract. 2021;9(2):670–5 (abstract: https://www.sciencedirect.com/science/article/abs/pii/S2213219820310072, accessed 3 October 2021).

92. Moderna COVID-19 vaccine Emergency Use Authorization. Review Memorandum. US Food and Drug Administration; 2020 (https://www.fda.gov/media/144673/download, accessed 9 November 2021).

93. Gargano JW, Wallace M, Hadler SC, Langley G, Su JR, Oster ME et al. Use of mRNA COVID-19 vaccine after reports of myocarditis among vaccine recipients: update from the Advisory Committee on Immunization Practices – United States, June 2021. MMWR Morb Mortal Wkly Rep. 2021;70(27):977–82 (http://www.cdc.gov/mmwr/volumes/70/wr/mm7027e2.htm?s_cid=mm7027e2_w, accessed 10 November 2021).

94. Høeg TB, Krug A, Stevenson J, Mandrola J. SARS-CoV-2 mRNA vaccination-associated myocarditis in children ages 12–17: a stratified national database analysis (online preprint: https://www.medrxiv.org/content/10.1101/2021.08.30.21262866v1, accessed 10 November 2021).

95. Spikevax (previously COVID-19 Vaccine Moderna). European Medicines Agency; 2021 (https://www.ema.europa.eu/en/medicines/human/EPAR/spikevax, accessed 10 November 2021).

96. Regulatory approval of Spikevax (formerly COVID-19 Vaccine Moderna) [website]. London: Medicines and Healthcare products Regulatory Agency; 2021 (https://www.gov.uk/government/publications/regulatory-approval-of-covid-19-vaccine-moderna, accessed 10 November 2021).

97. Comirnaty [website]. US Food and Drug Administration; 2021 (https://www.fda.gov/vaccines-blood-biologics/comirnaty, accessed 10 November 2021).

98. Goodman S. Considerations for placebo-controlled trial design if an unlicensed vaccine becomes available. Presentation to the U.S. Food and Drug Administration Vaccines and Related Biological Products Advisory Committee, 10 December 2020 (https://www.fda.gov/media/144354/download, accessed 4 October 2021).

99. Goodman S. Considerations for placebo-controlled trial design if an unlicensed vaccine becomes available. Presentation to the U.S. Food and Drug Administration Vaccines and Related Biological Products Advisory Committee, 17 December 2020 (https://www.fda.gov/media/144582/download, accessed 4 October 2021).

100. Expert consultation on the use of placebos in vaccine trials. Meeting report. Geneva: World Health Organization; 2013 (http://apps.who.int/iris/bitstream/10665/94056/1/9789241506250_eng.pdf, accessed 4 October 2021).

101. Guideline on influenza vaccines – non-clinical and clinical module. London: European Medicines Agency; 2016 (Document EMA/CHMP/VWP/457259/2014; https://www.ema.europa.eu/en/documents/scientific-guideline/influenza-vaccines-non-clinical-clinical-module_en.pdf, accessed 4 October 2021).

102. Weir JP, Gruber MF. An overview of the regulation of influenza vaccines in the United States. Influenza Other Respir Viruses. 2016;10(5):354–60 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4947948/?_escaped_fragment_=po=0.925926, accessed 4 October 2021).

103. Coronavirus disease (COVID-19) [website]. Geneva: World Health Organization (https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/vaccine-standardization/coronavirus-disease-(covid-19), accessed 4 October 2021).

104. COVID-19 vaccines [website]. Geneva: World Health Organization (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines, accessed 10 November 2021).

105. Reflection paper on the regulatory requirements for vaccines intended to provide protection against variant strain(s) of SARS-CoV-2. Amsterdam: European Medicines Agency; 2021 (Document EMA/117973/2021; https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-regulatory-requirements-vaccines-intended-provide-protection-against-variant_en.pdf, accessed 4 October 2021).

106. Access Consortium's guidance on strain changes in authorised COVID-19 vaccines [website]. Singapore: Health Sciences Authority (https://www.hsa.gov.sg/therapeutic-products/international-collaboration/access/guidance-strain-changes-in-authorised-covid-19-vaccines, accessed 4 October 2021).

107. ICMRA COVID-19 Virus Variants Workshop [website]. International Coalition of Medicines Regulatory Authorities, 10 February 2021 (http://www.icmra.info/drupal/en/covid-19/10february2021, accessed 4 October 2021).

108. Recommendations to assure the quality, safety and efficacy of influenza vaccines (human, live attenuated) for intranasal administration. In: WHO Expert Committee on Biological Standardization: sixtieth report. Geneva: World Health Organization; 2013: Annex 4 (WHO Technical Report Series, No. 977; https://www.who.int/publications/m/item/influenza-attenuated-intranasal-administration-annex-4-trs-no-977, accessed 4 October 2021).

109. Recommendations for the production and control of influenza vaccine (inactivated). In: WHO Expert Committee on Biological Standardization: fifty-fourth report. Geneva: World Health Organization; 2005: Annex 3 (WHO Technical Report Series, No. 927; https://www.who.int/biologicals/publications/trs/areas/vaccines/influenza/ANNEX%203%20InfluenzaP99-134.pdf?ua=1, accessed 4 October 2021).