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Guideline on the quality aspects of mRNA vaccines

Draft

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Guideline on the quality aspects of mRNA vaccines

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Executive summary

This guideline addresses the quality aspects of mRNA vaccines. It addresses specific aspects regarding the manufacturing process, characterisation, specifications and analytical control of mRNA vaccines, as well as the definition of starting materials, active substance and finished product for mRNA vaccines. Additional regulatory considerations are provided for changes in existing mRNA vaccine strains, bivalent and multivalent vaccines, self-amplifying mRNA vaccines, other delivery systems and use of platform technology/prior knowledge. The scope of this guideline is applicable to mRNA vaccines against infectious diseases. Other mRNA-based medicinal products are out of scope of this guideline, although relevant parts of this guideline may be applicable to those. It is not intended to address specific requirements for mRNA vaccines to be used in clinical trials, however the scientific principles described may also be applicable during pharmaceutical development.

1. Introduction

The number of clinical trial applications for human products and marketing authorisation applications for mRNA containing products significantly increased over the last few years and is expected to increase further in the future. Furthermore, significant experience with mRNA vaccines was gained during the COVID-19 pandemic.

mRNA vaccines against infectious disease have to follow the general guidelines for human vaccines, however this new technology is not specifically addressed in these guidelines. Therefore this guideline addresses those specific aspects regarding the manufacturing process, characterisation, specifications and analytical control as well as the definition of active substance and finished product for mRNA vaccines for the prevention of infectious disease.

2. Scope

The scope of this guideline is applicable to mRNA vaccines against infectious diseases (including self-amplifying mRNA). The guideline mainly addresses the requirements for development, manufacture and control which are specific to mRNA vaccines.

Other mRNA-based medicinal products are out of scope of this guideline, although relevant parts of this guideline may be applicable to those.

It is not intended to address specific requirements for mRNA vaccines to be used in clinical trials, however the scientific principles described may also be applicable during pharmaceutical development.

3. Legal basis

This guideline has to be read in conjunction with the introduction and general principles (4) and the Annex I to Directive 2001/83 (as amended). Applicants should also refer to other relevant European and ICH guidelines and European Pharmacopoeia monographs and chapters.

4. Active Substance

Only the mRNA itself is considered as active substance.

4.1. General information

The full mRNA sequence including the 5' cap, the 5' and 3' UTR regions and the poly(A)-tail needs to be provided as well as information on the encoded antigen(s) and specific features or alterations that might have been introduced. The functional features of the different elements of the molecular sequence should be described in detail and the amino acid sequence should be provided for the encoded antigen(s) and additional encoded proteins (if relevant). The 5' cap structure should be described in detail. Modifications/alterations to nucleosides should be indicated.

4.2. Manufacture

Control of materials

Starting materials are defined as a component, reagent or material used during the manufacture that is part of the final active substance. Therefore, components like nucleotides and the 5' cap or capping reagents are defined as starting materials for mRNA vaccines. Additionally, the linear DNA template is defined as a starting material (even though it is not part of the active substance itself because it defines the sequence of the mRNA).

Linear DNA templates can be produced by different manufacturing processes (for example, via a cell-based system or via enzymatic synthesis). As the linear DNA template is defined as a starting material, detailed information about the following aspects should be provided;

- The origin of the DNA template (including description of its manufacturing process and characterisation testing),
- The full sequence of the entire DNA template (including annotation of all functional and non-functional elements and justification of these elements),
- Information about the host cell line if applicable (including origin and modifications of the host strain, transformation, purification),
- Information on the cell bank system if applicable (describing manufacturing, qualification, release and stability testing. If justified, it could be acceptable if only a Master cell bank is established and no Working cell bank, for example in cases where the specific plasmid is only used for the manufacture of a seasonal vaccine).

The linear DNA template must be characterised, and the release testing should include parameters such as appearance, pH, DNA concentration, identity and sequence, integrity of the poly(A) tail coding region (if applicable), % linearised plasmid (if applicable), residual genomic DNA and RNA (if applicable), residual protein, bioburden and endotoxins.

The specifications need to be properly justified and also reflect process capability, and, if applicable, information on the stability of the linearized DNA template needs to be provided. Reference is made to Ph. Eur. general chapter *5.40 DNA templates for the preparation of mRNA substances*.

For other starting materials, such as nucleotides and 5' cap (and for capping reagents), the suppliers and specifications (including tests for, at least, identity and purity) should be stated and certificates of analysis should be provided.

In case starting or raw materials of biological origin are used they should comply with the "Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMA/410/01)" and appropriate EMA, Ph. Eur. and ICH documents on viral safety.

4.3. Characterisation

Characterisation studies should be conducted throughout the development process, taking into account individual components (including starting materials, intermediates, active substance and finished product) where relevant.

Batches used for characterisation studies should be clearly identified (development, pilot, full scale). Batches used in clinical studies should also be included in characterisation studies, since clinical experience is highly relevant for justification of specifications.

Structure, physicochemical properties

The structure, physicochemical and biological characteristics should be studied using a variety of state-of-the-art analytical methods applicable to mRNAs. The methods used should be described.

The integrity of the primary structure should be confirmed for the coding and non-coding regions including the 5' cap and 3' poly-A tail, since these elements are relevant for appropriate translation and functionality of the mRNA. Relevant methods include – but are not limited to – reverse transcription/sequence analysis, high throughput sequencing (also known as next generation sequencing, NGS) or other related methods, oligonucleotide mapping, liquid chromatography, spectroscopy, capillary electrophoresis etc. PolyA tail length has been shown to directly impact translation efficiency and hence, should be thoroughly characterized and controlled.

The higher order mRNA structure should be elucidated using methods such as circular dichroism (CD) spectroscopy, differential scanning calorimetry (DSC) or differential scanning fluorimetry (DSF).

Stability-indicating parameters should be identified, for example by forced degradation studies, to further inform specifications setting.

mRNA translation

The ability of the mRNA to be translated into the correct protein should be studied using suitable assays such as cell free translation systems or in vitro translation following transfection of suitable cell lines. The correct size and identity of the translated protein should be confirmed, for example by Western Blot analysis.

These studies should be complemented by studies of the functionality at finished product level, for example with cell-based in vitro expression assay to demonstrate uptake and antigen translation of the mRNA-LNP.

Impurities

Product-related impurities should be thoroughly characterized and controlled since these may impact translation efficiency or translation of the correct protein and hence vaccine efficacy. Product-related impurities include incomplete or differently sized RNA resulting from premature termination of transcription or degradation, high-molecular-weight impurities arising from transcriptional read-through or formation of multimers, point mutations, insertions/deletions, 5' cap- or 3'-polyA tail variants and 5'cap and/or tailless mRNA.

Furthermore, double-stranded (ds) RNA can potentially be formed during in vitro transcription. Since dsRNA can be recognized by receptors of the innate immune system, their formation may lead to unwanted release of immune-stimulatory cytokines. Incorporation of *N1*-methylpseudouridine may lead to +1ribosomal frameshifting at ribosome slippery sequences which should be considered during characterisation.

Knowledge is continuously evolving and hence, additional product-related aspects may become relevant in future and these should be thoroughly considered for characterisation studies.

Process-related impurities include residual DNA template and host cell DNA, residual protein (e.g. enzymes, host-cell derived), any chemical that might be used in the process for purification or stabilisation, and unincorporated nucleotides. Characterisation should be performed using suitable and sufficiently sensitive methods. The risk arising from these potential impurities should be thoroughly assessed. Orthogonal test methods should be used to quantify and characterise residual DNA. Analysis of the fragment size of residual DNA is expected to be performed to demonstrate the effectiveness of the enzymatic reaction and purification process.

While it is in general expected that the active substance is routinely tested for residual protein and residual DNA template at release, it may be acceptable to omit routine testing for other process-related impurities provided that characterisation studies and process validation demonstrate their consistent removal to acceptably low levels.

Characterisation studies should also include testing for general safety-relevant parameters, such as bacterial endotoxins and bioburden.

4.4. Control of the active substance

Specifications should take into account relevant quality attributes identified in characterisation studies. At least the following Critical Quality Attributes (CQA) should be controlled at release of mRNA Active Substance; identity, 5'-capping efficiency, Poly(A)-tail presence and length, mRNA content, mRNA integrity, product and process related impurities, functionality, appearance, pH, bioburden and endotoxin.

Specification limits should be clinically justified in line with the principles of ICH Q6B guideline.

Identity

The identity test should be highly specific for the mRNA and should be able to discriminate between different mRNAs that are manufactured at the same facility. The identity test is aimed at specific sequences within the mRNA, like the region encoding the antigen of interest, or another unique part of the sequence. Analytical procedures used may be based on sequencing or reverse-transcriptase PCR. Additional identity tests aimed at the length of the mRNA transcript (e.g. using electrophoresis techniques) are less specific than sequence-based methods but can be used as complementary to the sequence based methods.

5'-capping efficiency

The 5'-cap is attached to the mRNA during or after the in-vitro transcription reaction. The 5′ cap is essential for initiation of translation by the ribosome and also protects the mRNA from degradation and has a function in preventing undesirable immune responses. The relative presence of the 5'-cap (5'-capping efficiency) is determined by liquid chromatography techniques either with or without digestion of the mRNA.

Poly(A)-tail presence and length

The poly(A)-tail enhances RNA stability and translational efficiency. The poly(A)-tail is either encoded by the DNA-template and thus incorporated in the mRNA transcript during the in-vitro transcription reaction, or attached after the in vitro transcription reaction by an additional enzymatic reaction. The poly(A)-tail length is determined by suitable methods, for example liquid chromatography techniques. In case an additional enzymatic procedure is used to attach the poly(A)-tail, besides the length also the presence of the poly(A)-tail should be determined by suitable methods, for example liquid chromatography or reverse-transcriptase PCR.

RNA content

The RNA content is determined by UV absorption spectrophotometry or by quantitative reverse-transcriptase PCR.

mRNA integrity

The proportion of mRNA molecules with the intact full length is determined by a technique that has sufficient resolution to detect shorter or longer mRNAs, like degraded products, or shorter or longer transcripts. Suitable techniques to determine purity are capillary (gel) electrophoresis, agarose gel electrophoresis or liquid chromatography.

Product-related impurities

Longer or shorter transcripts, degradation products, aggregates

Product-related impurities could be controlled by the same test as used for control of purity (integrity). If the method for purity can detect the relevant product-related impurities there is no requirement to have an additional specification for product-related impurities. This needs to be justified during the characterisation testing.

dsRNA

During synthesis of mRNA by in-vitro transcription using T7 Polymerase, aberrant products including dsRNA can be produced. dsRNAs are known to cause immunogenic reactions and can cause protein synthesis inhibition. The level of dsRNA can be controlled by immunochemical methods like ELISA or immunoblot using antibodies specific for dsRNA and a reference standard with a known concentration of dsRNA.

Process-related impurities

Residual DNA

After the in vitro transcription reaction the DNA template is removed by DNase treatment and subsequent purification steps. Removal of the DNA template should be shown by a sensitive method, e.g. quantitative PCR.

Residual protein

Residual proteins (e.g. enzymes) are controlled by a sensitive method capable of detecting a wide range of protein impurities or ELISA specific for certain enzymes.

Residual solvents, reagents and starting materials

Residual solvents, enzymes, and free nucleotides or cap-analogs should be removed by the purification steps. Removal should be controlled at release or by in-process controls. It is acceptable to omit these controls provided that it has been shown by process validation that these process-related impurities are sufficiently removed by the purification steps, however they should be part of comparability assessments of future process changes and/or addition of new manufacturing sites.

Functionality

The functionality of the mRNA can be confirmed by a cell-free translation system to measure the translation into the correct protein. Translation into the correct protein can be confirmed by ELISA or Western Blot. As functionality should also be confirmed at the finished product level it is acceptable to omit testing of functionality on active substance, provided that translation into the correct protein has been shown in characterisation studies, and that the correct sequence of the mRNA, and integrity of the mRNA transcript is adequately controlled.

General tests

Appropriate tests for appearance, pH, bioburden and endotoxin should be included.

4.5. Reference standards or materials

Where needed, reference standards or materials should be established in line with the ICH Q2(R2) guideline. For example, for the control of identity, purity (RNA integrity) standards are used to confirm the correct length of the mRNA transcript. For the control of dsRNA, a reference standard is used of known concentration. If available, international standards should be used.

4.6. Container Closure System

No additional requirements

4.7. Stability

For the shelf life claim, supportive data from development or clinical batches can be used, however these batches should be representative of commercial material and should therefore be manufactured using the same (or a highly similar) process. They should be formulated with the same buffer, have the same RNA concentration and should be stored in the same container closure systems as the commercial batches, to be considered representative. Stability indicating parameters will follow from the characterisation testing but should generally include purity (mRNA integrity), concentration and poly(A)-tail.

Shelf life is supported with consecutive stability studies reflecting all intermediate storage conditions (i.e. temperatures) that are proposed for storage. A study subjecting the batches to the consecutive storage as proposed for the shelf life should be conducted, at least as part of post-approval and annual stability studies, unless otherwise justified.

5. Finished Product

5.1. Description and composition of the Finished Product

The complete qualitative and quantitative composition of the finished product should be specified. The information provided should include:

- a short description and tabulation of the dosage form
- composition, i.e. a list of all components of the finished product (for example, the mRNA active substance(s), the various types of lipids and other excipients), the rationale for and function of the components, and a reference to their quality standards
- the information on amount per dose should be defined for the mRNA active substance(s), the various types of lipids and the other excipients included in the composition.
- an outline of the type of container and closure used for the finished product. A complete description should be provided in section P.7.

5.2. Pharmaceutical development

Pharmaceutical development studies are conducted to demonstrate that formulation, pharmaceutical form, manufacturing process, container closure system and microbiological attributes are appropriate and consistently produce a product of acceptable quality.

A Quality Target Product Profile (QTPP) should be defined. Typical critical quality attributes (CQAs) for mRNA vaccines include LNP size, LNP polydispersity, potency (RNA content, mRNA integrity, mRNA encapsulation), functionality, individual lipids content, 5´-cap and Poly(A) tail content.

The mRNA active substance and the selected excipients in the formulation, including the lipids used for the LNP-formation, should be described and defined. The formulation development studies should be sufficiently described to justify the selection of suitable lipids and other excipients for a robust LNP formulation of the mRNA vaccine finished product. The lipid based nano delivery system of the mRNA vaccine (i.e. LNP) needs to be sufficiently characterised.

The physicochemical and biological properties of the mRNA vaccine finished product relevant to the safety, performance and manufacturability should be identified and appropriately characterised and controlled.

Typical quality attributes studied during these development studies can include LNP size and shape, LNP polydispersity, LNP surface properties (for example, charge, zeta potential, topology, surface PEG), mRNA integrity, mRNA encapsulation, RNA content, 5´-cap content, Poly (A) tail content, Poly (A) length and distribution, functionality, mRNA/lipid ratio, product and process related impurities (mRNA- and lipid by-products and RNA-lipid adducts).

Potential product and process related impurities in the finished product should be discussed. Product related impurities may arise during manufacture or storage, and should be characterised. Of special concern for mRNA-LNP vaccines are RNA-lipid adducts. These adducts form when RNA, in either intact mRNA or truncated RNA species, interacts with impurities in the lipid components. The formation of lipid adducts containing intact mRNA will negatively affect its ability to be translated. RNA-lipid adducts are hydrophobic by nature and may be characterised and controlled using suitable chromatography and mass spectrometry methods. The effect of product related impurities on functionality may be characterised using a suitable cell-based translation assay.

The impurity characterisation strategy should also address the presence of un-encapsulated and fragmented mRNAs, empty LNPs, as well as lipid impurities originating from degradation of the LNPs. As these types of impurities may result from impurities present in excipients a sound characterisation and control of the excipient impurity profile is essential. Residual ethanol is a process related impurity of particular relevance for the manufacture of LNPs, which should be characterised and controlled.

5.3. Manufacture

Depending on the manufacturing process, there may be one or more intermediate bulk steps prior to final formulation of the mRNA active substance and lipids into the finished product. These intermediates are defined as finished product intermediates and may, for example, consist of lipid mixtures, mRNA mixtures or lipid-mRNA mixtures.

Suitable specifications should be set for finished product intermediates, and when designing the overall control strategy, it is important to consider that some tests may only be meaningful or feasible to perform on the finished product intermediates and not the finished product.

Where a finished product intermediate is held or stored for a period of time, the holding/storage times and conditions should be qualified with process validation/stability data.

5.4. Control of excipients

The excipient specifications, related analytical procedures and summary of their validation should be described in the dossier. Where compendial (e.g. Ph. Eur.) grade excipients are used, a cross-reference to the relevant monograph is sufficient to cover this information.

For compendial lipid excipients, additional quality attributes may be required to be tested to ensure that the quality of the material is appropriate to support the formation of stable LNPs.

The water used in manufacture of finished product should be Water for Injections (WFI) Ph. Eur..

While mRNA vaccines are not formally in scope of ICH Q5A, the principles of ICH Q5A on viral safety aspects are applicable to raw materials of human or animal origin. If excipients of human and animal origin are used, the principles of ICH Q5A and Q11 guidelines should be applied.

A novel excipient is an excipient which is being used for the first time in a finished product, or by a new route of administration. It may be a new chemical entity or a well-established one which has not yet been used for human administration and /or for a particular human administration pathway in the EU and/or outside the EU. Full details of manufacture, characterisation and controls with cross references to supporting safety data should be provided for novel excipients, according to the active substance format, in line with the *EMA guideline on excipients in the dossier for application for marketing authorisation of a medicinal product*.

5.5. Control of finished product

Quality control tests should be performed at the finished product level. Tests on attributes which are specific to the formulated product in its final container and quality attributes which may have been impacted by the formulation steps should be included in the release testing.

Specifications should be proposed for finished product release and shelf-life.

Specifications of the finished product should include parameters to test all vaccine components, including the mRNA and the LNP.

The release specifications for each batch of mRNA vaccine finished products are expected to include testing of mRNA identity, mRNA content, purity and impurities, LNP attributes, potency), functionality, general tests and safety tests.

General tests should include appearance and physicochemical properties specific to the finished product, such as pH, osmolality, visible and subvisible particles, particulate matter and extractable volume.

Safety tests should include sterility endotoxins and container closure integrity.

Assays for critical excipients, such as the lipid nanoparticle components, should be applied, particularly where these ensure the expected bioactivity and/or maintain the stability of the final formulated product.

mRNA Identity

The identity of the sequence encoded by the mRNA molecule should be confirmed. Appropriate methods might include separation of fragments by size RT-PCR & agarose gel electrophoresis, reverse transcription and Sanger sequencing.

RNA Content (quantity)

The total RNA content should be determined by a suitable analytical procedure such as UV absorption spectrophotometry, fluorescence measurement after incubation with fluorescent dyes (RiboGreen assay), AEX-HPLC) or SE-HPLC. Preparation and calibration of the RNA calibration standard used for RNA content, if applicable, should be described.

Purity and impurities

- *mRNA integrity:* The proportion of intact full-length mRNA should be determined by an appropriate method (CGE, Agarose gel electrophoresis or IP-RP-HPLC).

- *Efficiency of mRNA encapsulation:* The % of mRNA encapsulated in the LNPs should be determined by a suitable analytical procedure, such as fluorescence measurement after incubation with fluorescent dyes (e.g. RiboGreen assay) or absorption spectrophotometry.

- *Product-related impurities:* Impurities such as degraded mRNA, lipid degradation products and RNA-lipid adducts, should be tested using suitable analytical procedures. Appropriate methods are ion-pair reverse-phase HPLC and HPLC-CAD.

- *Process-related impurities:* A control strategy, based on a risk assessment, should be in place for the determination of relevant residual solvents or other process-related impurities.

LNP Attributes

- *Lipid identity:* The identity of each individual lipid composing the LNP should be confirmed. Appropriate methods include HPLC-CAD.

- *Lipid content:* The content of each individual lipid composing the LNP should be confirmed. Appropriate methods include HPLC-CAD.

- *particle size and polydispersity index:* The particle size (diameter)and polydispersity index (PDI) of the mRNA-containing LNPs should be determined by a suitable analytical procedure, such as dynamic light scattering (DLS) analysis.

Potency

Potency of the finished product is determined by a combination of tests including evaluation of RNA content, mRNA integrity and efficiency of mRNA encapsulation.

Potency testing of the finished product should be performed at release and at shelf-life.

Functionality

An antigen-specific cell-based functionality assay should be developed to confirm the LNP uptake, mRNA escape from the endosome and translation of the mRNA into encoded protein/antigen. Due to the limitations of such methods, this assay can be semiquantitative.

Functionality of the finished product should be tested at release and at shelf-life.

General tests

- Appearance by visual inspection including examination of degree of opalescence (Ph Eur 2.2.1) and degree of coloration (Ph Eur 2.2.2) and visible particles (Ph Eur 2.9.20)

- particulate contamination (subvisible particles) (Ph Eur 2.9.19)

- extractable volume (Ph Eur 2.9.17)

- pH (Ph Eur 2.2.3)

- osmolality (Ph Eur 2.2.35)

Safety tests

- sterility (Ph Eur 2.6.1)

- bacterial endotoxin (Ph Eur 2.6.14 or 2.6.32)

- Container Closure Integrity

5.6. Reference standards or materials

Where needed, reference standards or materials should be established in line with ICH Q2(R2) guideline. For example, standards are used for the control of purity (integrity) to control the correct length of the mRNA transcript, content and for functionality testing to measure relative protein expression. For the control of lipids, standards are used to control identity and content. If available, international standards should be used.

5.7. Container closure system

No additional requirements.

5.8. Stability

Guidance stated in ICH Q5C 'Stability testing of biotechnological/Biological products' and also general guidance stated in ICH 'Stability Testing of New Drug Substances and Products' may, in general, apply to mRNA vaccines.

However, mRNA vaccines do have particular characteristics, such as a sensitivity of the mRNA component to storage temperature (i.e. deep-freeze) and temperature changes, to have in consideration to design a stability testing program.

In-use stability studies are of particular importance to ensure maintenance of the functionality during use and to study the effect of freeze/thaw cycles on integrity if the product is stored at different conditions after thawing. In that case, duration in refrigerator and/or at room temperature should be included in the stability study.

The stability program should include testing of identity and purity (mRNA integrity), including the quantitative detection of degradation. The evaluation of stability should include testing of functionality.

Primary data to support a requested storage period for finished products should always be based on long-term, real-time, real-condition stability studies. Also, the annual stability testing should reflect the end-to-end stability claim including, if applicable, all temperature conditions.

For the shelf life claim supportive data from development or clinical batches can be used, however these batches should be representative of the commercial material and should therefore be manufactured using the same (or a highly similar) process.

These general principles can also be applied for stability of finished product intermediates.

When a manufacturing platform is robustly established, it could be considered to extrapolate the product shelf-life and storage conditions using stability data from different products manufactured by the same platform and in the same formulation, provided that the degradation profiles (under accelerated and stress conditions) and batch analyses data are comparable, as further discussed in section 6.5.

6. Regulatory Considerations

6.1. Changes in existing mRNA vaccine strains

Strain updates – quality part:

This part will not conclude on the potential need of non-clinical and/or clinical data to support a strain update procedure but only focus on quality expectations to support strain updates.

Only changes related to the new strains used may be introduced. Parallel variation procedures should not be submitted during a strain change variation procedure.

The following should be included in the dossier:

General expectations:

3.2.S.2	Manufacture
3.2.S.2.2	Description of Manufacturing Process and Process Controls In case of change(s) to manufacturing process, a justification e.g. by using a risk assessment is expected. The process flow chart should clearly indicate any change(s). If necessary additional information should be provided in sections 3.2.S.2.5 and 3.2.S.2.6.
3.2.S.2.3	Control of Materials - source, history and generation of MCB/WCB for manufacture of the plasmid DNA if applicable - confirmation of approved specification of starting material(s) including compliance with the approved levels of impurities
3.2.S.2.4	Control of Critical Steps and Intermediates (if changed)
3.2.S.2.5	Process validation and/or evaluation - validation of three active substance batches (PPQ)
3.2.S.2.6	Manufacturing Process Development - Comparability analysis between historical data (previous strain(s)) and the new strain - Detailed information and justification in case of changes to the process. In case of changes justification e.g. by using a risk assessment and if necessary, characterisation data should be submitted.
3.2.S.3	Characterisation - characterisation of the new strain e.g. secondary structure of mRNA, transgene expression compared to previous strain(s)
3.2.S.4.1	Specification (copy of approved specifications in a tabular format) - Clear justification in S.4.5 in case specifications are amended due to changes in the manufacturing process and/or control strategy, or due to different characteristics of the active substance
3.2.S.4.2	Analytical procedures - In case of method adaptations due to new strain full details of the updated analytical procedures
3.2.S.4.3	Validation of analytical procedures - validation of all strain specific methods
3.2.S.4.4	Batch analysis - results of three commercial active substance batches
3.2.S.7	Stability - Stability data from three active substance batches (real time and accelerated)
3.2.P.1	Composition
3.2.P.2.2.1	Pharmaceutical development - Detailed information and justification in case of changes to the formulation
3.2.P.3.2	Batch formula (actual formula)
3.2.P.3.5	Process validation and/or evaluation - validation of three finished product batches (PPQ)
3.2.P.3.6	Manufacturing Process Development - Comparability analysis between historical data (previous strain(s)) and the new strain - Detailed information and justification in case of changes to the process
3.2.P.5.1	Specifications (Copy of approved specifications and routine tests analytical methods in a tabular format) - Detailed justification in P.5.6 in case of changes to the specifications
3.2.P.5.3	Validation of analytical procedures - validation of all strain specific methods
3.2.P.8	Finished Product: Stability - Stability data from representative material to support shelf life claim, at least accelerated and stressed conditions to compare degradation profile - Stability commitment(s)

- Post-approval stability protocol for end-to end stability study including interim storage conditions if applicable

6.2. Bivalent and multivalent vaccines

For bi- or multivalent vaccines some specific aspects in the finished product section need to be considered in addition to the general guidance provided. The composition of the vaccine needs to be presented in tabular form and the content of each variant/strain/antigen needs to be specified. Depending on the manufacturing process (either mixing of mRNAs before encapsulation or mixing of encapsulated mRNAs) the pharmaceutical development needs to be described in detail also justifying the stages of testing for critical quality attributes like encapsulation, mRNA integrity, LNP characteristics (polydispersity, size) and in vitro functionality assay. Results from mixing studies and additional characterisation data related to the mixing of the different strains are expected to confirm homogeneity of the active substances/strains after mixing and in the finished product lots are expected.

Furthermore, the identity and the cell-based functionality test need to be able to detect all variant/strain/antigens included in the vaccines and an additional test determining the ratio of the mRNAs needs to be conducted at release. Therefore, suitable methods for identity and ratio testing need to be implemented e.g. ddPCR.

6.3. Self-amplifying mRNA vaccines

Self-amplifying mRNAs (sa RNAs) are mostly derived from alphaviruses, such as the Venezuelan equine encephalitis virus (VEEV), Semliki Forest virus (SFV) or Sindbis virus. These saRNA encode the replicase of the alphavirus followed by the gene encoding the antigen. The replicase consists of four non-structural proteins. After translation of the replicase in the cytosol it replicates both the entire RNA strand as the RNA encoding the antigen. This will lead to an increase in the cytosol of the mRNA encoding the antigen. Consequently, lower doses of mRNA can be administered than with non-amplifying mRNA vaccines to reach the same efficacy.

Self-amplifying mRNAs have many structural similarities to non-amplifying mRNA: they contain a 5’ cap, 3’ poly(A)-tail and 5’ and 3’ UTRs. They differ from non-amplifying mRNA in their length; self-amplifying mRNAs are much larger as, besides the antigen of interest, they also encode a viral replicase. The minimal requirements for characterisation and control of the Active Substance and control of the Finished Product are essentially the same as for non-amplifying mRNAs. Additionally, it should be shown in characterisation studies that both the replicase and the antigen are correctly expressed and that expression of the replicase results in increased transcription and subsequent translation of the antigen.

6.4. Other delivery systems

Besides the LNPs, alternative systems might be used for mRNA delivery to the cells.

Recently used systems include:

1. Cationic nano emulsion (CNE)

CNE is a delivery system based on an oil-in-water emulsion. When a cationic lipid (such as 1,2-dioleoyl-3-trimethylammonium propane, DOTAP) is used in the oil phase to encapsulate the negatively charged mRNA, this system is known as cationic lipid nano-emulsion.

2. Cationic polymers

Cationic polymers condense negatively charged RNA into complexes called polyplexes that have various sizes and shapes.

Examples of cationic polymers include polyethylenimine (PEI) and polyamidoamine (PAMAM).

3. Cationic peptides

Cationic peptides contain multiple Arg and Lys. These positively charged residues can complex with negatively charged RNA molecules. Protamine is an example of cationic peptide.

Information regarding the components of the alternative delivery system should be provided in section P.4 Excipients. In the case of novel excipients, complete description and characterisation should be included in section A.3.

The components of the delivery system should be tested at finished product release.

6.5. Use of platform technology/prior knowledge approach for new targets

Prior knowledge gained from previous developments or "platforms" can be used to support a new marketing authorisation. Nevertheless, a product-specific dossier is still required, meaning the lead documents need to be product-specific. Prior knowledge can be used throughout the dossier to support for example reduced or focused process development and or validation activities, method validation, container closure usage or shelf-life claims.

It is the responsibility of the applicant to provide the supportive data and sound justifications for its relevance to allow for proper assessment of the proposed strategy. It should be noted that there are several factors that are considered important concerning the value of the supportive data. These include, but are not limited to, the number of licensed or developed products that can be considered representative, the in depth understanding of parameters that are product-dependent vs. product-independent, and conclusive risk assessments in case product-specific data are reduced or omitted due to prior knowledge. Therefore, it will be a case-by-case decision whether the proposed approach is considered acceptable.

7. References

1. Ph. Eur. general chapter 5.36 *mRNA vaccines for human use* (to be published in Ph. Eur. Supplement 12.1)
2. Ph. Eur. general chapter 5.39 *mRNA substances for the production of mRNA vaccines for human use* (to be published in Ph. Eur. Supplement 12.1)
3. Ph. Eur. general chapter 5.40 *DNA templates for the preparation of mRNA substances* (to be published in Ph. Eur. Supplement 12.1)