Lipid Nanoparticle – Points to Consider for Non Viral Delivery of RNA-based Therapeutics

CGTP Summit June 12, 2025

Mo Heidaran, Cellx, Inc. Consulting

Agenda

- Review of RNA-based Products
- Major Components of RNA-based Products
- Overall regulatory approach to use of materials
  - Applicable regulations (Raw Materials, Ancillary Materials, Critical Starting Materials, Excipients, Drug Substance and Drug Product)
- Example of Materials used for manufacturing and how to assess material quality
- Highlights of EMA Guidance Prophylactic mRNA Vaccines (Optional)
- Summary and recommendations

RNA-based Products (Overview)

- Examples:
  - Prophylactic vaccine
  - Genome editing products
  - Therapeutic vaccines, immune therapeutics
  - Personalized Medicine
  - In vivo CAR-T manufacturing
- Advantages:
  - Lack of genomic integration transient expression
  - Cost of Manufacturing
  - Speed of manufacturing

Products including lipid to formulate LNPs	Prophylactic Vaccine	Therapeutic cancer vaccines	Gene Modulation	Gene Correction
LNP/Cas9mRNA/gRNA (ex vivo and in vivo)				X
LNP/mRNA (in vivo)	X	X		
LNP/siRNA (in vivo)			X	
LNP/oligonucleotides (ASO) (in vivo)			X	

Product Specific Considerations

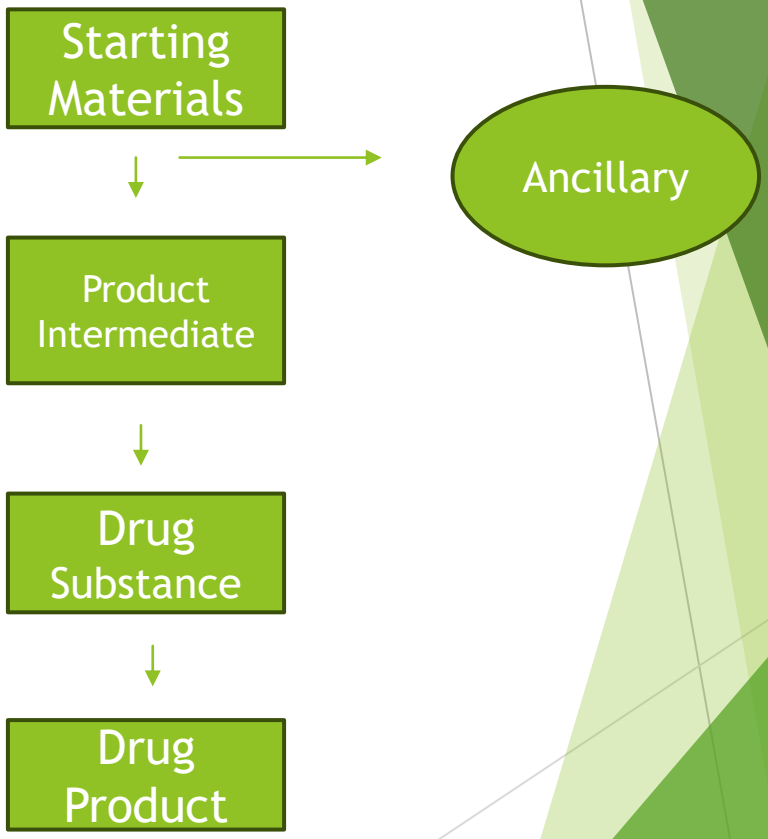
Product and jurisdictional centers and offices at FDA	Examples	MOA/Target Cells	Route of administration	Dose and frequency of administration	Target Population
LNP/mRNA (Prophylactic) CBER/OVRR	SARS-CoV-2	Immune Response (Prevention of Infection) Immune Cells	IM	30-100 ug	Healthy Individuals
LNP/mRNA (Therapeutics) Cancer Vaccines CBER/OTP	Neoantigen cancer vaccines	Immune Response (Anti-Tumor Response) Immune Cells	IV	Repeated administration	Cancer Patients
LNP/Cas9/gRNA (Therapeutics) CBER/OTP	In vivo genome editing	Gene Editing (Correction of gene in vivo) Hepatocytes	IV	One time administration 0.3, 0.45, 0.6 mg/kg.	Patients with Genetic Diseases
LNP/siRNA CDER	Alnylam Pharmaceuticals vutrisiran (Amvuttra)	Gene Silencing Hepatocytes	SC	25 mg dose every 3 months	Patient with hereditary variant transthyretin amyloidosis
LNP/siRNA CDER	Alnylum patisiran (Onpattro)	Gene silencing Hepatocytes	IV	0.3 mg/kg once every 3 weeks for under 100 kg or 30 mg/kg every 3 weeks for over 100 kg	Patient with hereditary variant transthyretin amyloidosis

Factors Influencing Points to Consider for Manufacturing of RNA-based Products

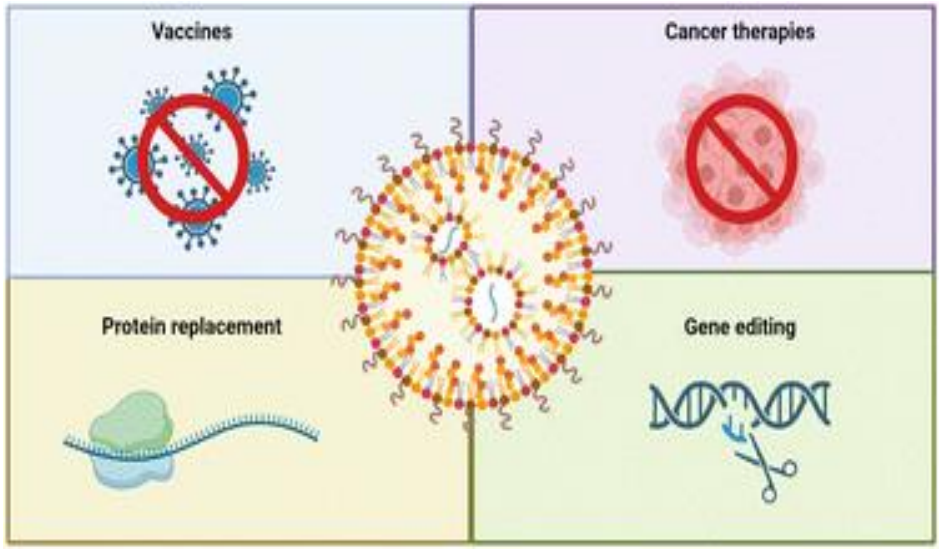
- Dosage and requirements for repeated administration
- Route of administration
- Target population (healthy versus patients)
- Off-the-shelf or personalized
- Time and cost factors
- Other Factors:
  - Lipid composition
  - mRNA quality/gRNA
  - LNP physiochemical characteristics (size distribution and nucleic acid content)

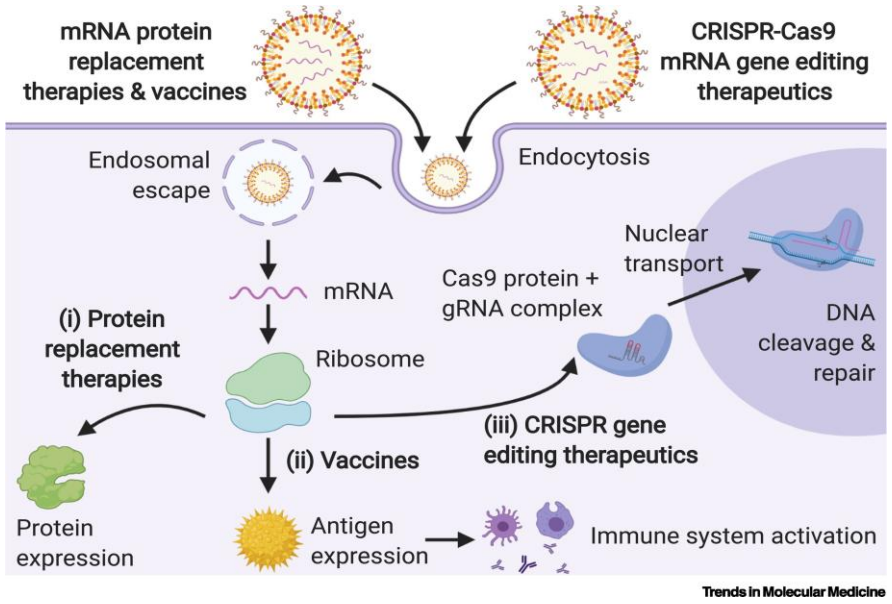
Regulatory Approach to Prevent Contamination and Quality Control

- Contamination Control
  - Application of Phase based CGMP approach
  - Control of materials, components
    - Risk and science-based approach
      - Products can be sterile filtered for microbiological controls, but sterile filtration is not adequate for endotoxin or viral reduction.
  - Control and classification of environment
  - Aseptic techniques and training
  - Testing and documentation
  - Risk based approach
  - <u>Sponsors are ultimately responsible for quality of all materials</u>
    - Material and vendor qualification
    - Cross referencing of DMF is not acceptable for DSI, DS and DP for BLA submission*



*https://www.fda.gov/about-fda/economic-impact-analyses-fda-regulations/biologics-license-applications-and-master-files-final-rule





Mechanism and major components of RNA-based Products (non viral delivery)

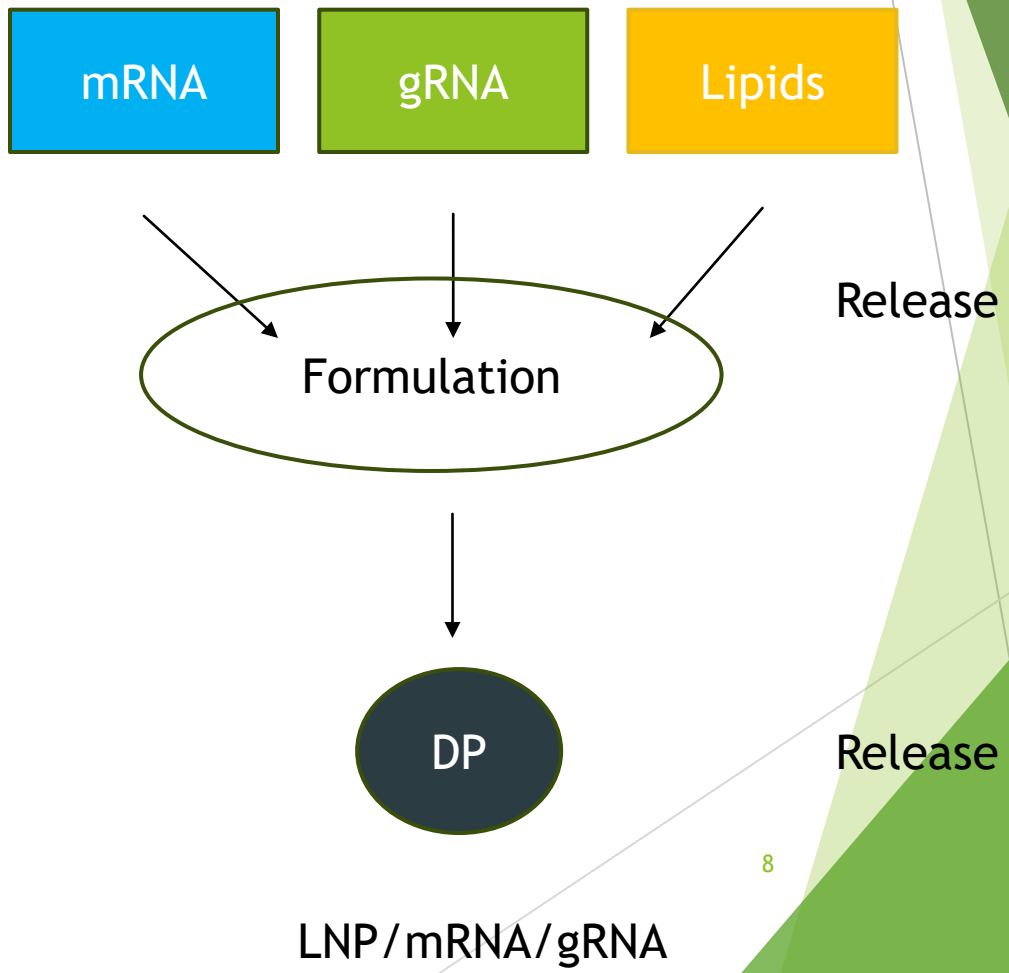
- Genome Editing
  - mRNA (Cas9)
  - gRNA (recombinant or synthetic
  - Lipid (synthetic or natural)
- Therapeutic Cancer Immunotherapies
  - mRNA (neoantigens)
  - Lipid

https://pubs.rsc.org/en/content/articlelanding/2024/nr/d4nr00019f

https://www.cell.com/trends/molecular-medicine/abstract/S1471-4914(21)00069-1

Generalizable Manufacturing Process

- Manufacturing Steps:
  - mRNA manufacturing
  - gRNA manufacturing
  - Lipid manufacturing
  - LNP/mRNA/gRNA Formulation



Regulatory Definition

- Components: Component means any ingredient intended for use in the manufacture of a drug product, including those that may not appear in such drug product. (21 CFR 210.3 (3))
- Drug product means a finished dosage form, for example, tablet, capsule, solution etc that contain active drug ingredient (21 CFR 210.3 (4)).
- Drug Substance (active ingredients) is any components that is intended to furnish pharmacological activity or other direct effect… The term includes those components that may undergo chemical changes in the manufacture of the drug product and presenting the drug product in a modified form intended to furnish the specified activity or effect (21 CFR 210.3 (7)).
- Ancillary Materials:
  - No FDA definition but FDA has adopted Health Canada definition which is any material used during manufacturing which are consumed but may remain in the final drug product as an impurity*
- Critical Starting Materials/Components:
  - No formal definition but refers to critical materials used for manufacturing of the DP. It is generally found in the DP*.
    - Examples (gRNA, RNP, plasmid DNA)
- Excipients:
  - Inactive component other than the drug substance – Any substance other than active pharmaceutical ingredient (API) in the dosage form. It consists of inactive components or ingredients which are intentionally added to the final drug product but are not intended to exert any therapeutics effects at the intended

*https://www.fda.gov/regulatory-information/search-fda-guidance-documents/chemistry-manufacturing-and-controls-changes-approved-application-certain-biological-products

Critical Starting Materials or components versus Ancillary Materials versus Excipients

- Critical starting materials or components require more complete information in the IND submission.
- Critical starting materials contribute to the DP active ingredient. Present in the final drug product!
  - Example: gRNA/Cas9RNP for ex vivo gene editing
- The material quality should be fully verified in house by testing the component extensively for *identity, safety, purity and potency*.

	Test	Test Method	Acceptance Criteria	Note
Identity				
Purity				
Potency				
Impurities				

For ancillary materials it is possible to rely upon COA accompanied by selective tests to verify quality (sometime conduct critical test which is not performed by vendor)

- **Screen and/or test source of materials**
- **Test the final materials for relevant adventitious agents that could come from sources of materials and/or any other materials used that have direct or indirect contact with material**
- **Viral reduction or clearance may be required**

Excipients:

According to 21 CFR 210.3(b)(8), an inactive ingredient is any component of a drug product other than the active ingredient

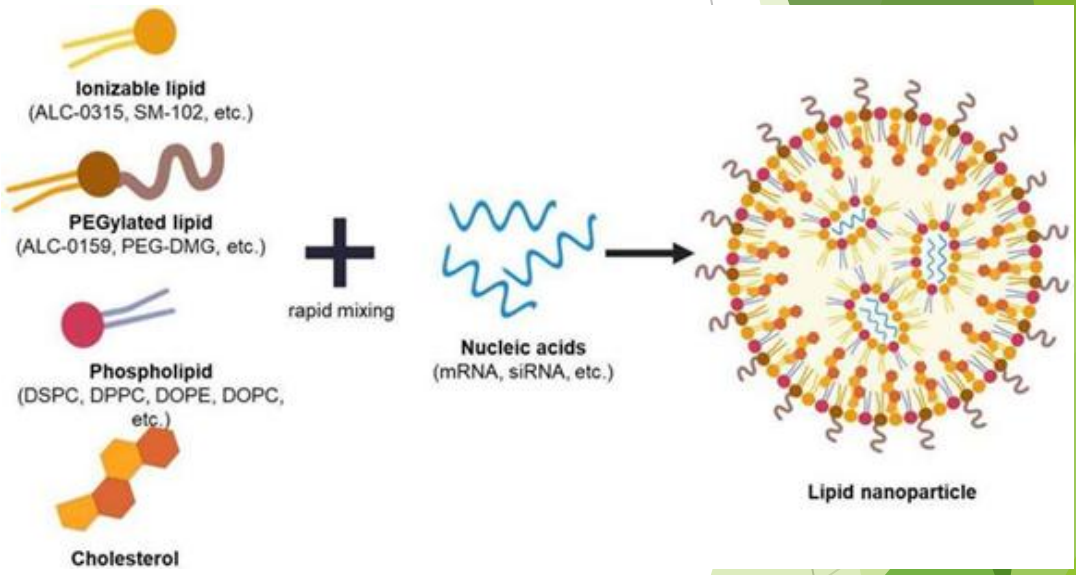
- **Excipients are not approved but qualified**

Guidance for Industry Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients (https://www.fda.gov/media/72260/download)

Pilot Program for the Review of Innovation and Modernization of Excipients (PRIME) https://www.fda.gov/drugs/development-approval-process-drugs/pilot-program-review-innovation-and-modernization-excipients-prime

Major Components of Lipids

- Lipids represent the major component of the drug product by weight.
- Lipids are complex mixture of lipids largely synthetic
  - The composition of lipids is not well documented by third party manufacturers and it appears to be variable
- Lipids quality is determined using minimal release criteria
- Regulatory approach to assess quality of lipid components is not consistent across different products regulated by FDA and EMA
  - **Is it critical component, starting materials or novel excipient?**



What are different components of RNA based Products from Regulatory Perspective

Product and jurisdictional center to regulate at FDA	Lipids	mRNA	gRNA
LNP/mRNA (Prophylactic) **CBER/OVRR**	Critical Components/Starting Materials	Critical Components/Starting Materials	Critical Components/Starting Materials
LNP/mRNA (Prophylactic) **EMA**	Excipient	Critical Components/Starting Materials	Critical Components/Starting Materials
LNP/mRNA **(Therapeutics)** **Cancer Vaccines** **CBER/OTP**	Critical Components/Starting Materials	Critical Components/Starting Materials	Critical Components/Starting Materials
LNP/Cas9/gRNA **(Therapeutics)** **CBER/OTP**	Critical Components/Starting Materials	Critical Components/Starting Materials	Critical Components/Starting Materials
LNP+/Cas9/gRNA (+ionizable lipid plus GalNAc) **CBER/OTP**	Critical Components/Starting Materials	Critical Components/Starting Materials	Critical Components/Starting Materials
LNP/siRNA **CDER**	Excipient	NA	NA
LNP/siRNA **CDER**	Excipient	NA	NA

Consideration for Quality of Lipids

- Lipids used are generally synthetic
- Regulatory Consideration:
  - Novel excipients, or critical starting materials
  - Critical Material Attributes
    - Impurities
    - Safety
    - Identity
    - Potency

- Specifications:

	Specification
Identity	
Purity	
Potency	
Safety	
Impurities	

SARS-COVID Vaccine Review Memo (EMA)

- Lipid SM-102 is a novel excipient, not previously used in an approved finished product within EU. 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) is a non-compendial excipient sufficiently controlled by an in-house specification.
- Cholesterol is controlled according to the *Ph. Eur.* monograph 0993 with additional tests for residual solvents and microbial contamination. However, the applicant is also referring to a non-compendial cholesterol. This should be clarified post-approval.
- **1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2000 DMG) is a novel excipient, not previously used in an approved finished product within EU**. The other excipients (tromethamol, acetic acid, sodium acetate trihydrate, sucrose and water for injections) are controlled according to respective *Ph. Eur.* monograph or by in-house specifications (tromethamine hydrochloride).
- a mRNA-1273 contains a single mRNA sequence that encodes for the full-length SARS-CoV-2 S-2P combined in a mixture of 4 lipids (SM-102, PEG2000-DMG, cholesterol, and DSPC) and formulated in 20 mM Tris, 87 mg/mL sucrose, 10.7 mM sodium acetate, pH 7.5.
- The applicant should provide evidence that the impurities and/or degradation products resulting from PEG2000-DMG, cholesterol and DSPC have been sufficiently investigated and do not result in the formation of lipid-RNA species by 31-01-2021.
-

SARS-COVID Vaccine CMC Review Memo (CBER/OVRR)

- **Product Type:** Human Coronavirus mRNA vaccine expressing SARS-CoV-2 Spike glycoprotein (Moderna UNII code number EPK39PL4R4) formulated with lipids SM-102, PEG2000-DMG, DSPC, and cholesterol to form RNA-encapsulating lipid nanoparticles (LNPs).

- Since mPEG2000-DMG and SM-102 custom lipids are classified as **starting materials** for the manufacture, the related CMC information is discussed in section 3.2.S.2.3 *Control of Materials* of the current review memo

Patisiran Review Memo (CDER)

- Patisiran is a synthetic small interfering ribonucleic acid (siRNA) formulated as lipid nanoparticle (LNPs) containing 2 mg/mL patisiran and lipid excipients in phosphate buffered saline for slow IV infusion.

- Note: The chemistry reviewer requested that DMA look at the excipients to determine if the ML and endotoxin limits are appropriate. **There a 5 excipients, two are novel, one is not novel but does not have a USP monograph and two have a USP monograph; non are sterile**.
- The novel excipients are DLin-MC3-DMA and PEG2000-C-DMG. The DLin-MC3-DMA is a lipid and the release specifications include bioburden (NMT cfu/gr for both TAMC and TYMC), specified microorganism (absence of *Salmonella, E. coli, S. aureus* and *P. aeruginosa*) and has an endotoxin limit of NMT EU/mg. The PEG2000-C-DMG release specifications includes bioburden (NMT cfu/g for both TAMC and TYMC) with an endotoxin limit of NMT EU/mg.
- A third excipient which is not novel is 1, 2-Distearoyl-sn-glucero-3-phosphocholine (DSPC). The release specifications for this excipient include bioburden (NMT cfu/g for TAMC, NMT cfu/g for TYMC, and absence of E. coli) with an endotoxin limit of NMT EU/g.

Lipid Components of Drug Product

- Lipids constitute a major components of drug product
- Lipid quality is not reviewed consistently across different centers at FDA and or by EMA
- Lipid quality assessment is not generally adequate
- Recommend consistent approach to quality assessment
  - Lipids components should be fully characterized for identity purity safety and potency at minimum.
  - Reference materials or monographs for lipids may be useful
  - Guidance for industry is generally lacking

Consideration for Quality of mRNA

- mRNA is commonly manufactured by scale out process of in vitro transcription
  - In vitro transcription: Plasmid Template, RNA Polymerase
  - Followed by capping and poly adenylation
  - Purification Step
- Regulatory Consideration:
  - Drug substance or Critical starting materials
  - Critical Material Attributes
  - Impurities, safety

- Specifications:

	Specification
Identity	
Purity	
Potency	
Safety	
Impurities	

mRNA Release Specification

Release Test	Purpose	Analytical Method
Identity Testing	Confirms the mRNA sequence to ensure identity and distinguish them from unrelated mRNA	Next-Generation Sequencing (NGS), Sanger Sequencing, PCR-based Methods
Purity and Integrity	Ensure full-length mRNA without degradation or truncated products	Capillary Electrophoresis (CE), High-Performance Liquid Chromatography (HPLC), LC-MS
5' Capping Efficiency	Verifies the presence and efficiency of the 5â€™ cap for stability and translation	LC-MS, Immunoassay
Poly(A) Tail Length and Integrity	Confirms proper poly(A) tail length, ensuring mRNA stability and efficient translation	LC-MS, Electrophoresis, Enzymatic Digestion Assays
Residual DNA Contamination	Detects and quantifies any residual plasmid DNA from the manufacturing process	qPCR, Droplet Digital PCR (ddPCR)
Double-Stranded RNA (dsRNA) Analysis	Identifies the presence of dsRNA contaminants, which can trigger immune responses	ELISA, HPLC, Immunoassays
In Vitro Translation Assay	Assesses the ability of mRNA to produce the intended protein in vitro	Cell-Based Assay, Western Blot, ELISA
Endotoxin Testing	Measures endotoxin levels to ensure compliance with safety standards	Limulus Amebocyte Lysate (LAL) Assay
Sterility Testing	Confirms the absence of microbial contamination to meet regulatory requirements	USP Sterility Test
Mycoplasma Testing	Detects mycoplasma contamination that could compromise the product's safety	qPCR, Cell Culture Assay

Manuscript in preparation: Lipid Nanoparticle – Points to Consider in Assessing Materials Quality for RNA-based Therapeutics. Sita Somara and Mo Heidaran

mRNA Characterization

Characterization Test	Purpose	Analytical Method
mRNA Secondary Structure Analysis	Examines RNA folding patterns and secondary structures that may affect translation	Circular Dichroism (CD), NMR, SHAPE-MaP
Codon Usage and Optimization	Assesses codon frequency to optimize translation efficiency in the target host	Bioinformatics Analysis, Mass Spectrometry
Thermal Stability Assessment	Determines mRNA stability under different temperature conditions	Differential Scanning Calorimetry (DSC), Thermal Shift Assay
Aggregation and Degradation Studies	Monitors mRNA aggregation and degradation pathways	Dynamic Light Scattering (DLS), Gel Electrophoresis, LC-MS
Translation Kinetics and Efficiency	Measures translation speed and efficiency in various cell lines	Cell-Free Translation Assays, Luciferase Reporter Assays
Ribosome Profiling	Analyzes ribosome binding sites and efficiency of translation initiation	Ribosome Foot printing, RNA-Seq
Immunogenicity Assessment	Evaluates the immune response triggered by mRNA or its components	ELISA, Cytokine Release Assay, In Vivo Models
mRNA-LNP Interaction Studies	Investigates how mRNA interacts with lipid nanoparticles for efficient delivery	Fluorescence Spectroscopy, Cryo-EM
Oxidative and Hydrolytic Stability	Tests the chemical stability of mRNA under oxidative and hydrolytic stress	LC-MS, HPLC, Forced Degradation Studies
Long-Term Storage Stability	Determines long-term storage stability under varying conditions	Stability-Indicating Assays, Real-Time and Accelerated Stability Studies
Biodistribution and Pharmacokinetics	Tracks mRNA distribution and clearance in vivo	qPCR, RNA-Seq, PET Imaging, Mass Spectrometry

Manuscript in preparation: Lipid Nanoparticle – Points to Consider in Assessing Materials Quality for RNA-based Therapeutics. Sita Somara and Mo Heidaran

Consideration for Quality of gRNA

- gRNA is commonly manufactured by synthetic means, followed by purification Step
- Regulatory Consideration:
  - Drug substance or critical starting materials
- Critical Material Attributes
  - **Impurities**
  - Safety
  - Potency
  - Identity

- **Specifications:**

	Specification
Identity	
Purity	
Potency	
Safety	
Impurities	

gRNA Release Specification

Test Category	Test Name	Purpose
Identity	Mass Spectrometry (MS)	Confirms molecular weight and sequence integrity
Identity	Capillary Electrophoresis (CE) / PAGE	Assesses RNA integrity and verifies expected length
Purity & Impurities	High-Performance Liquid Chromatography (HPLC)	Quantifies RNA purity and detects truncated sequences
Purity & Impurities	Capillary Electrophoresis (CE)	Separates full-length gRNA from smaller byproducts
Purity & Impurities	UV-Vis Spectroscopy (A260/A280 Ratio)	Determines RNA purity and potential protein contamination
Purity & Impurities	Residual DNA Analysis (qPCR or ddPCR)	Ensures no residual plasmid DNA or unwanted sequences
Potency	In vitro Cleavage Assay	Verifies the efficiency of gRNA-directed cleavage
Potency	Cas9 Binding Assay	Confirms the ability of gRNA to bind Cas9
Safety	Endotoxin Testing (LAL Assay)	Detects endotoxins that can induce immune responses
Safety	Microbial Contamination (Bioburden & Sterility)	Ensures sterility and absence of microbial contamination
Safety	dsRNA Contamination Assay	Detects immunogenic double-stranded RNA contaminants
Stability	Accelerated Stability Testing	Evaluates RNA stability under stress conditions

gRNA heterogeneity should be minimized during manufacturing

gRNA Characterization

Structural Characterization	Circular Dichroism (CD) Spectroscopy	Analyzes secondary structure integrity and folding properties
	Thermal Shift Assay (Tm Determination)	Determines thermal stability of gRNA
	Nuclear Magnetic Resonance (NMR) Spectroscopy	Provides detailed structural information on RNA conformation
Purity & Impurity Analysis	High-Performance Liquid Chromatography (HPLC)	Identifies RNA purity and detects truncated sequences
	Polyacrylamide Gel Electrophoresis (PAGE)	Assesses RNA integrity and potential degradation products
Functional Assays	Cas9 RNP Assembly Assay	Confirms gRNA incorporation into the Cas9 (RNP) complex
	In vitro Cleavage Assay	Evaluates cleavage efficiency of the gRNA-Cas9 complex
	Cell-Based Functional Assay	Validates gRNA activity in cellular systems
Safety & Stability	Endotoxin Testing (LAL Assay)	Detects endotoxins that may trigger immune responses
	dsRNA Contamination Assay	Identifies immunogenic double-stranded RNA contaminants
	Long-Term Stability Testing	Assesses gRNA stability under long-term storage conditions

Consideration for Quality of Drug Product

- Manufacturing Process
- Formulation of LNP/mRNA
- Purification Step and filtration
- Regulatory Consideration:
  - Drug substance or Critical starting materials
- Critical Material Attributes
- Impurities
- Safety
- Identity
- Potency

- Specifications:

	Specification
Identity	
Purity	
Potency	
Safety	
Impurities	

Key Quality Attributed for Final Drug Product consistent of LNP/mRNA/gRNA complexes

Test Category	Test Description	Method Used	Acceptance Criteria
Identity	Size Distribution	Dynamic Light Scattering	100-200 nM
Purity	Percent mRNA/gRNA incorporated homogeneously	Gel Electrophoresis	MT 80% w/w
Impurity	Free mRNA/gRNA	ddPCR	NMT 10 ng/mL
Potency	Ability to express proteins in appropriate cell line	Western Blot	Expression of Protein
Safety	Sterility	USP 71 culture-based assay	No Growth
Safety	Endotoxin	Chromogenic Assay	less than 5 EU/kg/hour
Safety	Mycoplasma	Mycoplasma detection PCR	Not Detected

Summary and Recommendations

- RNA-based Products are diverse and highly complex (multi components)
- The Lipid component represents the major “inactive ingredient” of these products are reviewed inconsistently across different FDA centers and international authorities
- DNA Impurities in RNA-based products should be minimized and controlled in view of the product and its intended use
- Heterogeneity of gRNA should be minimized during production
- There is unmet need for regulatory harmonizations between FDA centers and globally
  - Guidance documents and white papers
  - Quality standards and monographs

Optional

Draft Guidance for mRNA Vaccines

27 March 2025

EMA/CHMP/BWP/82416/20252

Committee for Medicinal Products for Human Use (CHMP)

Guideline on the quality aspects of mRNA vaccines

Characterization of mRNA Active Substance (EMA)

Clinical batch inclusion	Batches used in clinical and development stages should support specification setting		Not specified in the guidance
Primary structure integrity	Includes 5' cap, coding and non-coding regions, poly(A) tail		Reverse transcription/sequence analysis, NGS, oligonucleotide mapping, LC, spectroscopy, capillary electrophoresis, etc.
Poly(A) tail	Presence and length		Not specified in the guidance
Higher-order structure	Structural conformation relevant to translation		CD, DSC, DSF
Stability-indicating parameters	To inform and justify specification		Forced degradation studies
mRNA translation ability	Demonstrate correct protein synthesis		Cell-free systems; cell-based assays only at finished product level
Product-related impurities	Incomplete or differently sized RNA, HMW, point mutations, insertions. deletions, 5'cap- or 3'-poly(A) tail variants and 5'cap and/or tailless mRNA		Not specified in the guidance
	Frameshifting due to N1-methylpseudouridine; dsRNA as innate immune trigger		
Process-related impurities	Residual DNA template	Quantify	Orthogonal methods
		Fragment size analysis	N/A
	HCD, residual protein, chemicals		Not specified in the guidance

https://www.advancingrna.com/doc/ema-issues-draft-guideline-on-quality-aspects-of-mrna-vaccines-0001?utm_source=linkedin&utm_medium=social&utm_campaign=ARW+linkedin+posts&utm_id=ARW

Release Specifications for mRNA active substance recommended by EMA

Identity		Sequencing or RT-PCR targeting specific sequence	Must distinguish between different mRNAs made at same site
5'-capping efficiency		Liquid chromatography (with/without digestion)	Not specified in the guidance
Poly(A) tail presence and length		LC or RT-PCR	Length and presence must be assessed if enzymatically added
RNA content		UV spectrophotometry or qRT-PCR	Not specified in the guidance
mRNA integrity		Capillary or agarose gel electrophoresis, LC	Should detect shorter/longer transcripts; also covers some product-related impurities
Product-related impurities	Longer or shorter transcripts, degradation products, aggregates	Same as purity/integrity test	No additional specification if justified in characterization
	dsRNA	ELISA or immunoblot	Using antibodies specific for dsRNA and a RS with known concentration
Process-related impurities	Residual DNA	qPCR	Should be a sensitive method
	Residual protein	ELISA	Should be a sensitive method for a wide range of protein impurities
	Residual solvents, reagents, and starting materials	Not specified in the guidance	May be omitted at release if clearance validated; still needed for comparability/change evaluation
Functionality		Cell-free translation + Western blot or ELISA	May be omitted at active substance level if confirmed at finished product level
General tests		Appearance, pH, bioburden, endotoxins	Standard release parameters

Finished Product CQA Considerations

Attribute	Included in CQA	Included in Typical QA for Characterization	Additional Impurity Characterization
LNP size	Yes	Yes	–
LNP polydispersity	Yes	Yes	–
RNA content	Yes	Yes	–
mRNA integrity	Yes	Yes	–
mRNA encapsulation efficiency	Yes	Yes	–
Functionality	Yes	Yes	–
Individual lipids content	Yes	Yes	–
5'-cap content	Yes	Yes	–
Poly(A) tail content	Yes	Yes	–
mRNA/lipid ratio	–	Yes	–
mRNA- and lipid by-products	–	Yes	–
RNA-lipid adducts[1]	–	Yes	–
Unencapsulated/fragmented mRNAs	–	–	Yes
Empty LNPs	–	–	Yes
Lipid impurities (e.g., degradation of the LNPs)	–	–	Yes

Control of Finished Drug Product

Test		Example Methods	Notes
mRNA identity		RT-PCR, agarose gel electrophoresis, reverse transcription, and Sanger sequencing	Not specified in the guidance
RNA content		UV, RiboGreen assay, AEX-HPLC, SE-HPLC	Not specified in the guidance
Purity and impurities	mRNA integrity	CGE, agarose gel electrophoresis, IP-RP-HPLC	Not specified in the guidance
	Encapsulation efficiency	RiboGreen assay, absorption spectrophotometry	Not specified in the guidance
	Product-related impurities	Ion-pair reverse-phase HPLC and HPLC-CAD	Detect degraded RNA, lipid degradation, RNA-lipid adducts
	Process-related impurities	Risk-based control strategy	Evaluate solvents, residual reagents if not cleared by validation
LNP attributes	Lipid identity	HPLC-CAD	
	Lipid content		
	Particle size	DLS	
	Polydispersity index		
Potency		Not specified in the guidance	Combined from RNA content, integrity, and encapsulation
Functionality		Antigen-specific cell-based assay (can be semiquantitative)	Confirm LNP uptake, mRNA escape, and protein/antigen translation
General Tests		Opalescence, coloration, visible particles, subvisible particles, extractable volume, pH, and osmolality	Ph. Eur.
Safety Tests		Sterility, endotoxins, container closure integrity	

Guidance for mRNA Vaccines by EMA

Control of Excipients

- Excipients should be compendial when available, with additional quality attributes tested where needed to ensure suitability. If water is a component of the finished drug product, it must meet the quality minimums for water for injection (Ph. Eur.). Excipients of human or animal origin should comply with ICH Q5A and Q11. **For novel excipients, full manufacturing, characterization, and control information with appropriate cross-references is expected.**

Questions

- For more information:
- Mo Heidaran Moheidaran@cellx-genex.com
- Visit our Regulatory portal WWW.cellxgenex.com

References

Guideline on the quality aspects of mRNA vaccines

https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-quality-aspects-mrna-vaccines_en.pdf

Guidance for Industry Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients (https://www.fda.gov/media/72260/download)

Gene therapy and genome editing guidance document (https://www.fda.gov/media/156894/download)

Human Gene Therapy Products Incorporating Human Genome Editing Guidance for Industry (https://www.fda.gov/media/113760/download)

Immunogenicity of lipid nanoparticles and its impact on the efficacy of mRNA vaccines and therapeutics (https://www.nature.com/articles/s12276-023-01086-x.pdf)

Assessment report on the claim of new active substance (NAS) status of 5'capped mRNA encoding full length SRAS-CoV-2 Spike protein contained in COVID-19 mRNA Vaccine BioNTech. EMEA/H/C/005735/RR (https://voorwaarheid.nl/wp-content/uploads/2022/12/Rapporteurs-Rolling-Review-Report-New-Active-Substance-Status-COVID-19-mRNA-Vaccine-BioNTec.pdf) and BLA Clinical Review Memorandum, SPIKEVAX STN: 125752 Proper Name: COVID-19 Vaccine, mRNA, Tradename: SPIKEVAX, Manufacturer: Moderna Tx Inc.

(https://www.fda.gov/media/156342/download), (https://www.fda.gov/vaccines-blood-biologics/spikevax).

USP Cholesterol Monograph: (https://doi.usp.org/USPNF/USPNF_M17220_03_01.html), DSPC Monograph: https://doi.usp.org/USPNF/USPNF_M16917_10101_01.html

Drug Products, Including Biological Products, that Contain Nanomaterials Guidance for Industry (file:///C:/Users/mohei/Downloads/FDA%202022%20April_Drug%20Products,%20Including%20Biological%20Products,%20that%20Contain%20Nanomaterials%20Guidance%20for%20Industry.pdf)

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A perspective of lipid nanoparticles for RNA delivery (https://onlinelibrary.wiley.com/doi/full/10.1002/EXP.20230147)

typically consist of four different components: DLin-MC3-DMA (ONPATTRO)/SM-102 (Moderna)/ALC-0315 (BioNTech/Pfizer) as the ionizable lipid, DSPC as the phospholipid and PEG-2000-C-DMG (ONPATTRO)/DMG-PEG-2000 (Moderna)/ALC-0159 (BioNTech/Pfizer) as the PEG-lipid and cholesterol).

mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8032477/pdf/main.pdf. Center for Drug Evaluation and Research Application Number:210922Orig1s000.

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Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) https://www.fda.gov/regulatory-information/search-fda-guidance-documents/chemistry-manufacturing-and-control-cmc-information-human-gene-therapy-investigational-new-drug

Applicable regulations for Lipids as critical starting materials or as a novel excipient

- Critical Starting Material:

1) According to 21 CFR 210.3(b)(7), an active ingredient is any component of a drug product intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of humans or other animals.
    1) No formal definition but critical starting components can directly contribute to the active ingredient of the product and these components are generally can be detected in the product.
        1) Gene therapy and genome editing guidance document (https://www.fda.gov/media/156894/download)
        2) Human Gene Therapy Products Incorporating Human Genome Editing Guidance for Industry (https://www.fda.gov/media/113760/download)

- Excipients:
- According to 21 CFR 210.3(b)(8), an inactive ingredient is any component of a drug product other than the active ingredient- This includes the excipients
    - Excipients are not approved but qualified
- Guidance for Industry Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients (https://www.fda.gov/media/72260/download)
- Pilot Program for the Review of Innovation and Modernization of Excipients (PRIME) https://www.fda.gov/drugs/development-approval-process-drugs/pilot-program-review-innovation-and-modernization-excipients-prime