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IPAC-RS
International Pharmaceutical Aerosol Consortium on Regulation & Science

IPAC-RS Workshop:
Inhaled Biologics: Preparing for a Future Beyond Small Molecules

September 4-5, 2024

DNA MEDICINE TESTING FOR INHALED AND NASAL OLIGONUCLEOTIDE AND MRNA PRODUCTS .

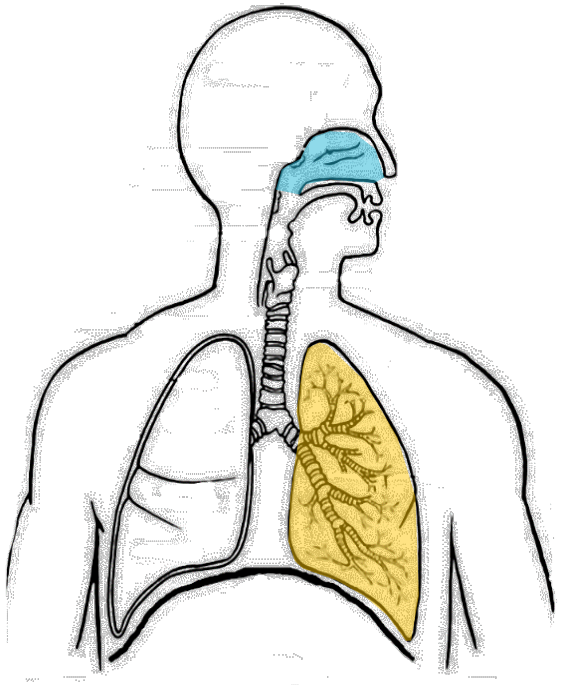
ASHLEIGH WAKE

UK and Switzerland Pharma Business Development Director

WHY INHALED AND NASAL DELIVERY OF OLIGONUCLEOTIDES AND MRNA?

For several years the potential of oligonucleotide and DNA based medicines has been evident, given their diversity of application and potential therapeutic effect. Currently 14 marketed oligonucleotide products, majority for injection or infusion.

Biggest obstacle potentially remains in identifying safe and effective delivery channels.



NASAL

INHALED

ICONOVO
Breathtaking innovation

ICONOVO AND ISR DEVELOP NEW COVID-19 INHALATION VACCINE

BIOSTOCK, NEWS

Intravacc.
Innovating vaccines

Publications Events News Career Partnering
About us Services Platforms Products

Intravacc announces positive pre-clinical data for its SARS-CoV-2 nose spray vaccine

Bilthoven, the Netherlands, 7 April 2021 – Intravacc, a global leader in translational research and development of viral and bacterial vaccines, today announced that it has obtained positive pre-clinical results for its SARS-CoV-2 Outer Membrane Vesicle (OMV) based recombinant Spike protein (rSp) candidate nose spray vaccine.

For the pre-clinical study four groups of mice and four groups of hamsters received two intranasal immunizations on day one and day 21. One group of mice and hamsters received a vaccine based on

UNIVERSITY OF OXFORD

Home

University of Oxford to study nasal administration of COVID-19 vaccine

PUBLISHED
25 MAR 2021

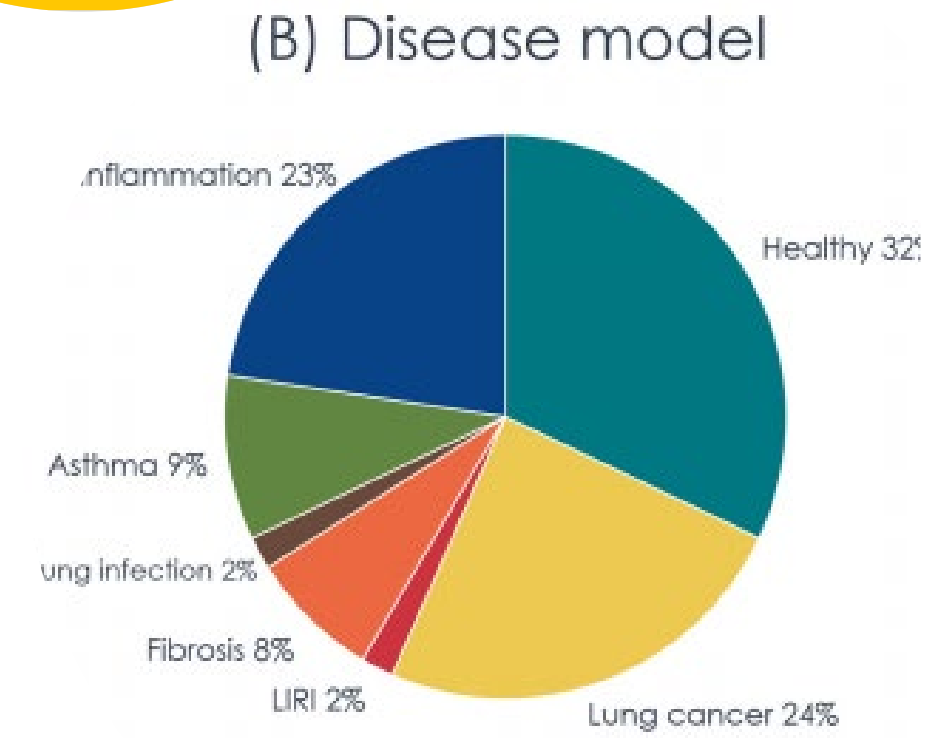
RESEARCH CORONAVIRUS COVID-19 VACCINE

SHARE THIS

The University of Oxford is launching a study investigating the delivery of the

PULMONARY DISEASES

- The first siRNA candidate to reach the clinic was ALN-RSV01 in 2008 which was, designed to treat respiratory syncytial virus (RSV) infection.
- Since then, several clinical studies have been performed , although no product has been approved.
- Several studies which report the potential for RNA treating a range of lung diseases, such as asthma, cystic fibrosis and lung cancer.
- Through this aerosol inhalation has been shown to be an effective delivery route as it maximizes local delivery whilst controlling systemic exposure.



Trends in Pharmacological Sciences, Inhaled RNA Therapy: From Promise to Reality, Oct 2020

AGENDA

1. Why Consider Inhaled or Nasal Delivery
2. DNA / RNA Intro
3. Development Process
4. Analytics.
5. Conclusions and Questions

DNA/MRNA INTRODUCTION

SO MANY NAMES!

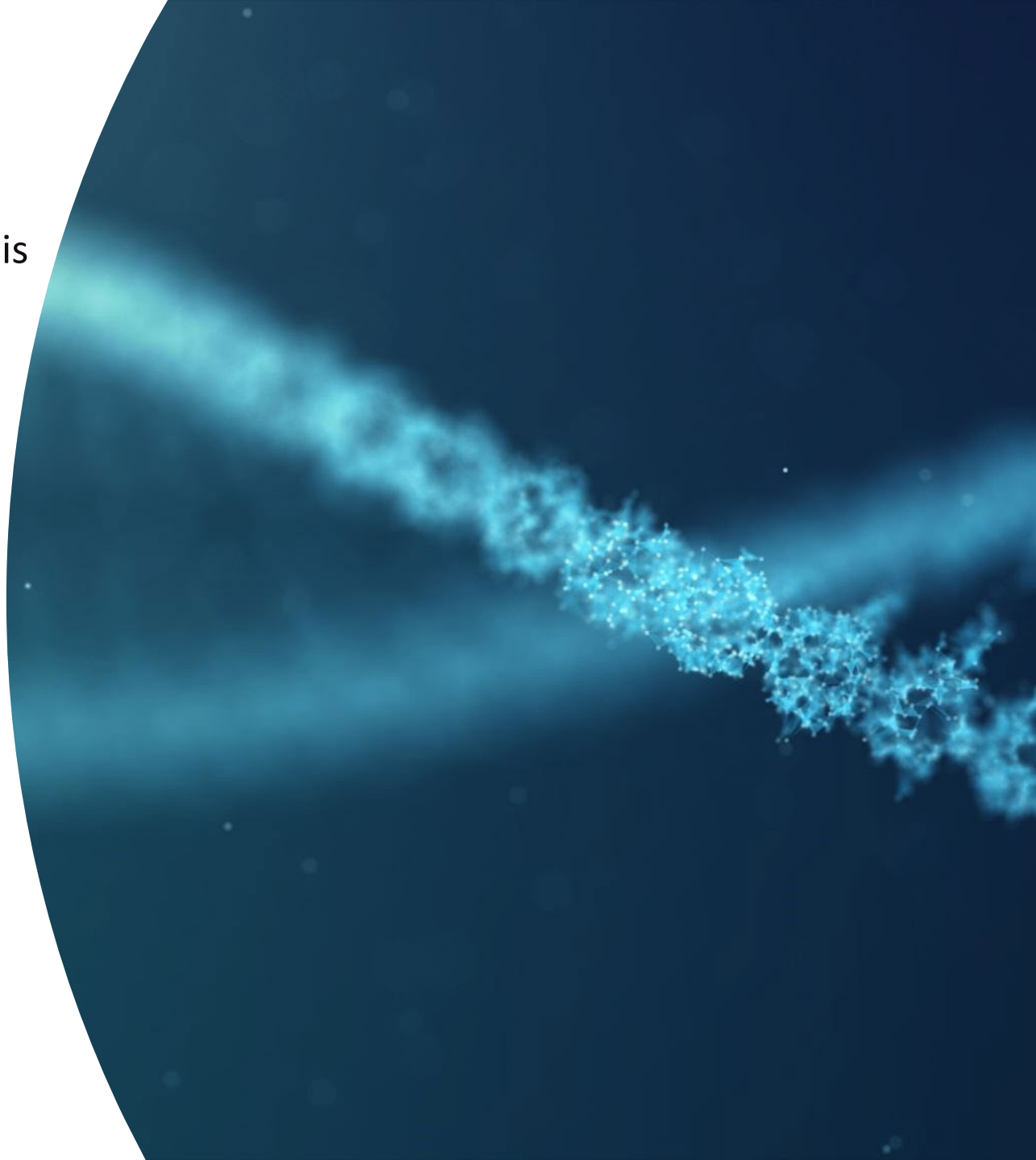
In simplest terms RNA is considered a messenger, in that it carries instructions from DNA to control synthesis of proteins.

RNA products can generally be divided into 3 classes:

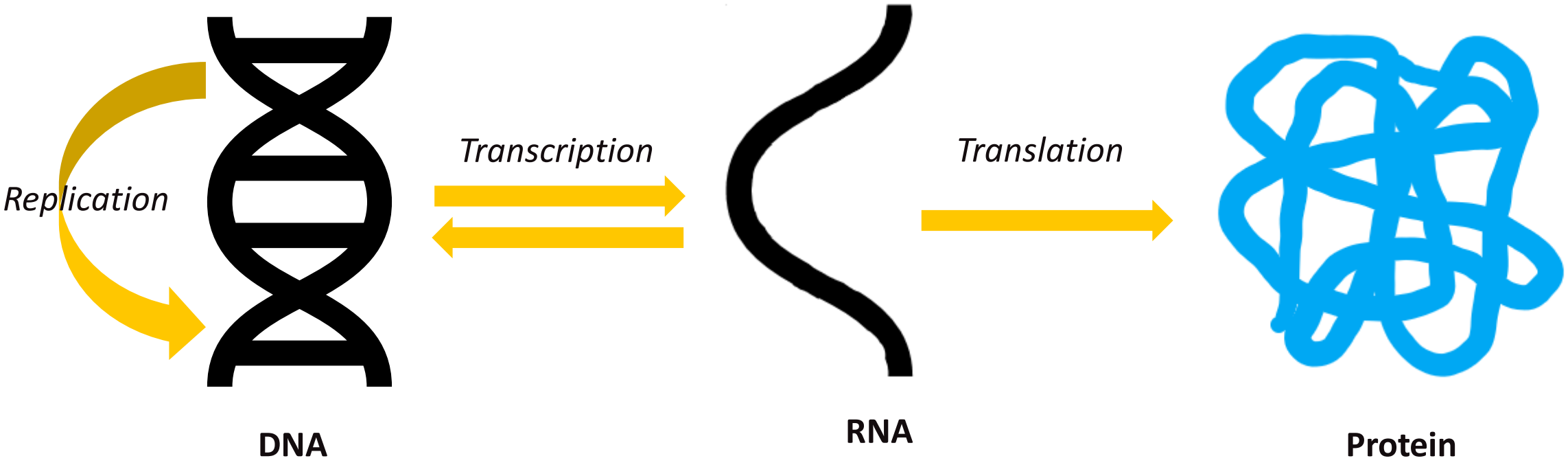
1: Protein Encoding - mRNA

2: Inhibition of Gene Expression - siRNA, antisense oligonucleotides, miRNA

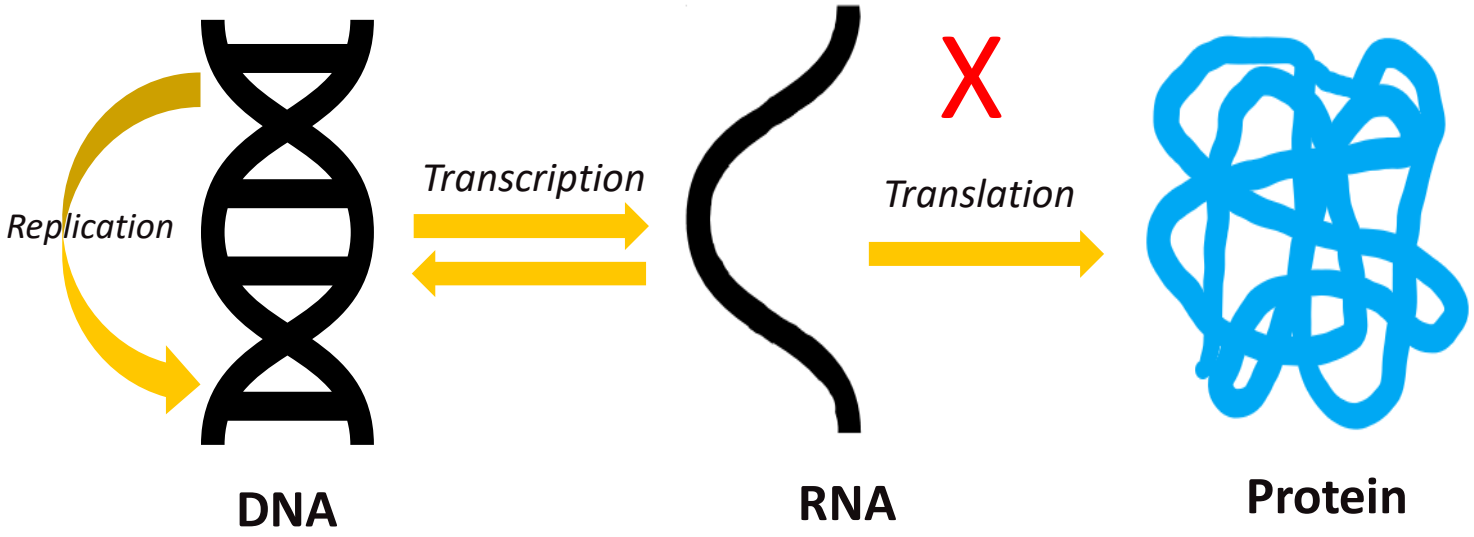
3: Protein Targeting – Aptamers



BIOCHEMISTRY 101 - CENTRAL DOGMA

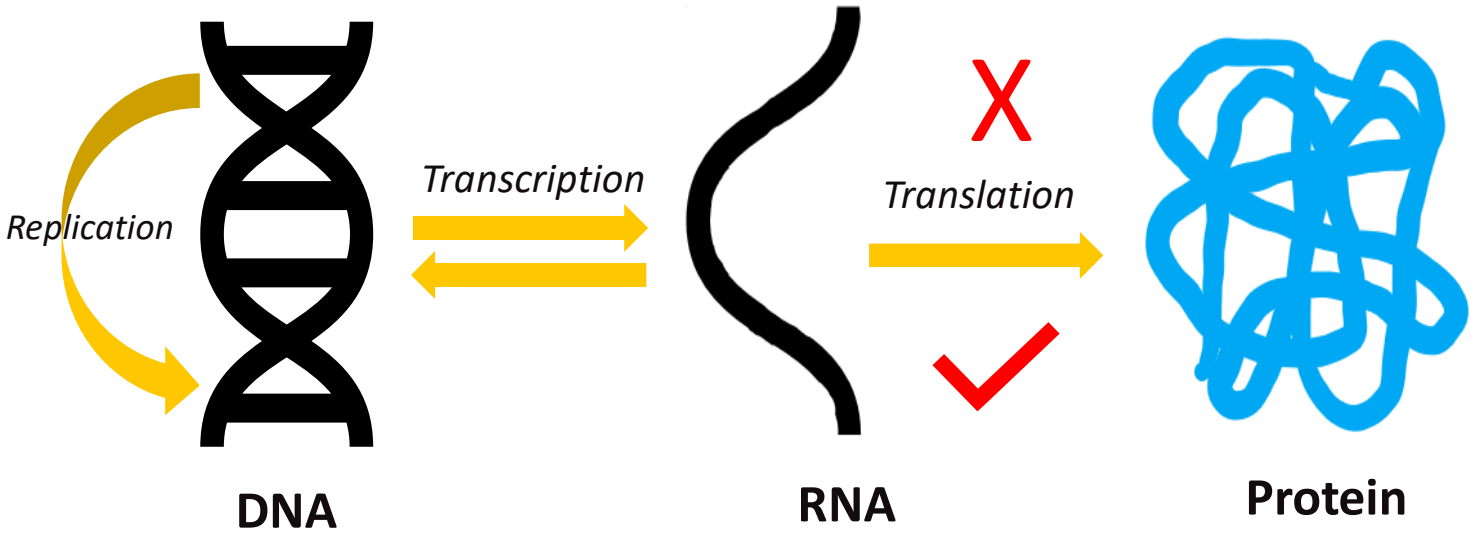


ANTISENSE OLGONUCLEOTIDES



- 8 ASO products currently marketed.
  1 additional withdrawn
  15 including siRNA and aptamers
- Approx 80 in Phase II/III trials with 102 different indications, ~2% via inhalation route.
- Short single stranded DNA, that bind to mRNA and modulate protein translation.
- 2 modes of actions, degradation of the RNA or inhibition/modulation of RNA.
- Modified chemistry give greater stability and affinity.

MRNA



- Single stranded, complementary to one of DNA strands of a gene.
- Transfers a portion of code to form proteins.
- Only for of RNA that can produce new protein.
- Unlike viral vectors or plasmids they do not enter the genome, therefore reduced risk of mutagenesis.

RNA :APPLICATIONS, ADVANTAGES AND DISADVANTAGES

APPLICATIONS (mRNA)
VACCINES
TUMOUR IMMUNOTHERAPY
INFECTIOUS DISEASE PREVENTION
ANTI-VIRALS
TISSUE REGENERATION
AND MORE

APPLICATIONS (ASOs)
GENETIC DISEASES
NEURODEGENATIVE DISEASE
INFLAMMETORY DISEASE
CANCERS

ADVANTAGES
Array of Indications
Rapid and cost-effective production
Rapid effect.

DISADVANTAGES
STABILITY - susceptible to nuclease attack.
LOW PERMEATION – over cellular membranes
IMMUNOGENICITY

THE FIGHT FOR STABILITY AND REACHING THEIR TARGET

Antisense Oligonucleotides

Chemistry – alterations to the chemistry of the oligo backbone, for example phosphortiorate which have longer half life and some resistance ro nuclease attack.

Complex encapsulation

mRNA

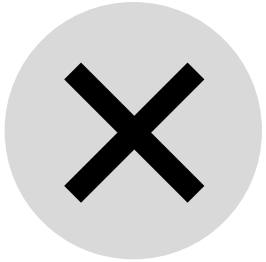
End Capping

Complex encapsulation

Achieving targeted Delivery and Stability tare the major hurdles for RNA therapies.

WHY INHALED AND NASAL DELIVERY?

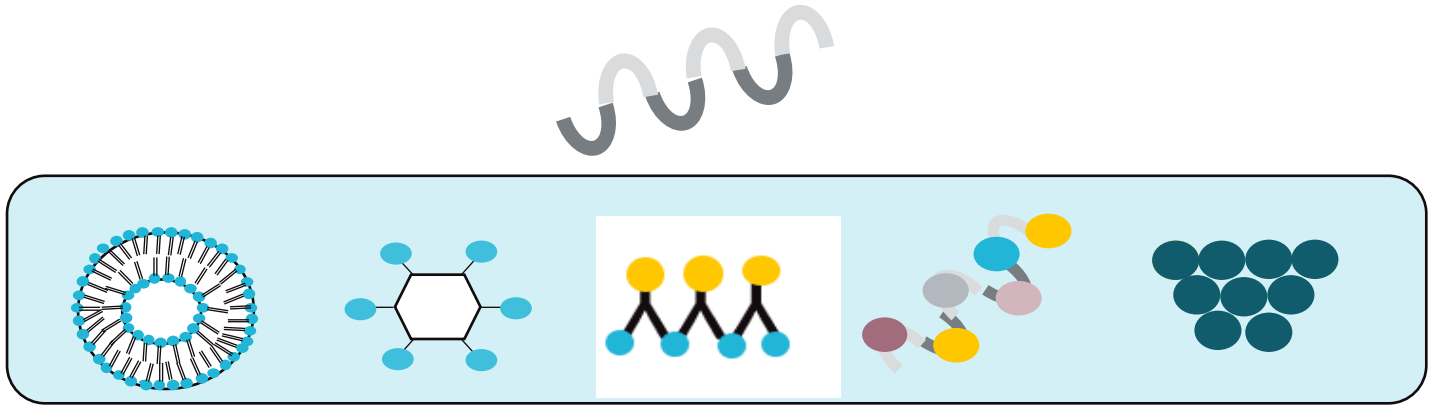
- Targeted Delivery
- Systemic Delivery With Different PK Profiles
- Improved Patient Experience
- Future Pipeline



- Additional Challenges In Controlling Dose
- More Drug Is Required For Similar Systemic Delivery To Injection - Poor Absorption
- Further Formulation And Device Considerations And Restrictions
- Poor Cost / Benefit Profile For Some Indications

PRODUCT DEVELOPMENT

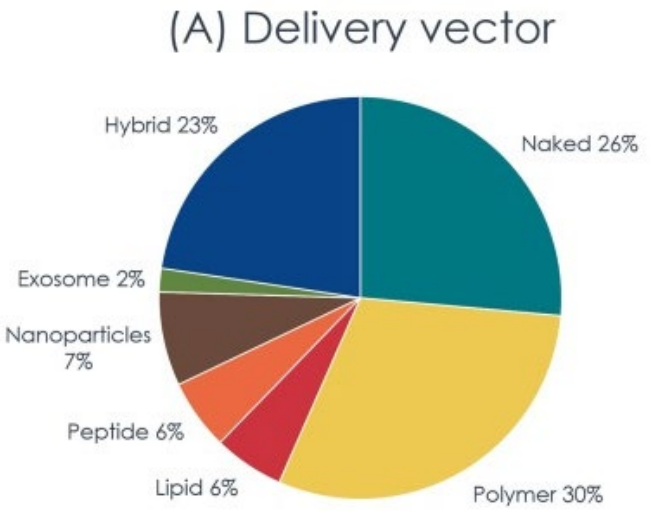
DEVELOPMENT OF AN INHALED OLIGO OR MRNA



Formulation Developmentt
Device Selection

OLIGO/mRNA Delivery System Device Performance

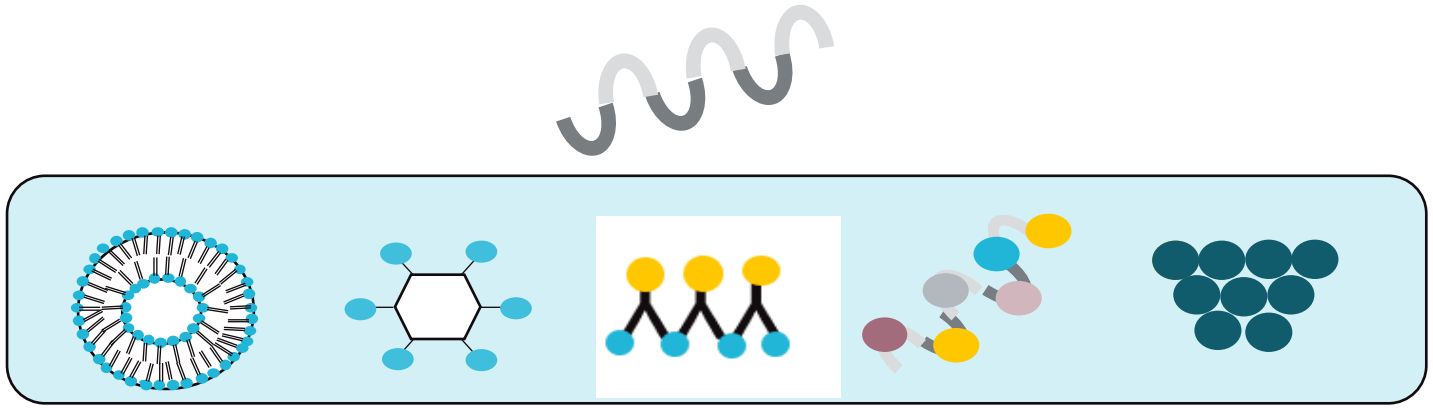
DELIVERY VECTOR

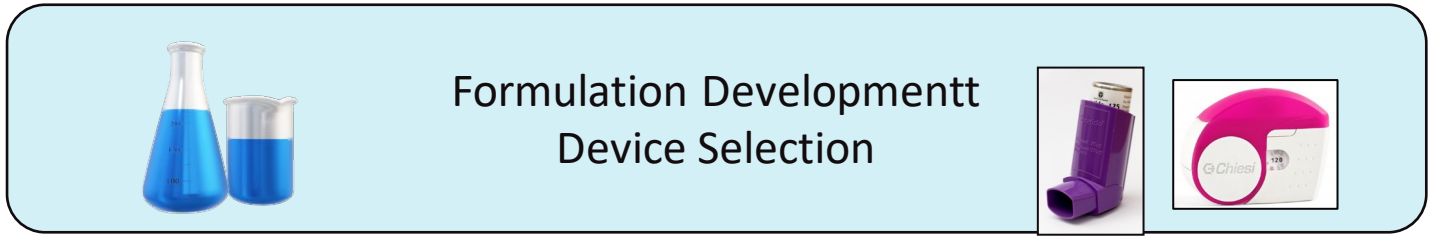


Trends in Pharmacological Sciences, Inhaled RNA Therapy From Promise to Reality, Oct 2020

Vector	Advantages	Disadvantages
Naked		Degradation by RNase
Polymer	**Natural:** Biocompatible/Safety Mucoadhesive/permeability. **Synthetic:** Targeting Aqueous Solubility	**Natural:** Poor physiological solubility Low transfection efficiency **Synthetic:** High Toxicity
Lipid	High Transfection Efficiency Toxicity	Poor Structural Stability
Exosome	High Biocompatibility Low toxicity/immunogenicity	
Peptide	Versatile Cell Entry Ability	Lack Cell Specificity Protease degradation
Nanoparticle	Ease of Synthesis Stability	No RNA-binding ability (functionalised on surface)
Hybrid	Designed to individually overcome disadvantages	

DEVELOPMENT OF AN INHALED OLIGO OR MRNA





OLIGO/mRNA Delivery System Device Performance

ANALYTICS FOR CHARACTERISATION AND QC

ANTISENSE OLIGONUCLEOTIDES CHARACTERISATION

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

15 September 2022
EMA/CHMP/QWP/735423/2022
Committee for Medicinal Products for Human Use (CHMP)
Committee for Veterinary Medicinal Products (CVMP)

Concept Paper on the Establishment of a Guideline on the Development and Manufacture of Synthetic Oligonucleotides

Agreed by Quality Working Party	29 June 2022
Adopted by CHMP for release for consultation	15 September 2022

Quality Attribute	Methodology
Identification (by molecular weight)	Mass spectrometry (MS); size-exclusion chromatography (SEC)
Structural characterization	Fourier-transform infrared (FTIR), nuclear magnetic resonance (NMR), MS/MS for sequence confirmation
Assay and impurities	High-performance liquid chromatography (HPLC): ion pair (IP), reversed-phase (RP), and ion-exchange (IEX) with ultraviolet (UV) and MS detection
Internucleoside linkages	Phosphorus-31 (^{31}P), NMR for assessment of phosphodiester, phosphorothioate, methylphosphonate, and other modified phosphates P=S/P=O ratio determined by combining ^{31}P NMR and SAX-HPLC
Aggregates	Size-exclusion chromatography (SEC)
Counter ions	Inductively coupled plasma (ICP)-MS or ICP-optical emission spectrometry (OES)
Chain length and distribution	Capillary gel electrophoresis (CGE)
Stereochemistry	Optical rotation and/or NMR
pH	pH Meter
Osmolality	Vapor pressure or freezing-point depression osmometry
Moisture content	Karl Fischer analysis
Hygroscopicity	Dynamic vapor sorption (DVS)

ASO IMPURITIES

Figure 6: Negative-ion electrospray mass spectrum obtained using standard ionization conditions for the 20-mer DNA sample; all impurities are labeled for this case study using a table of known impurities.

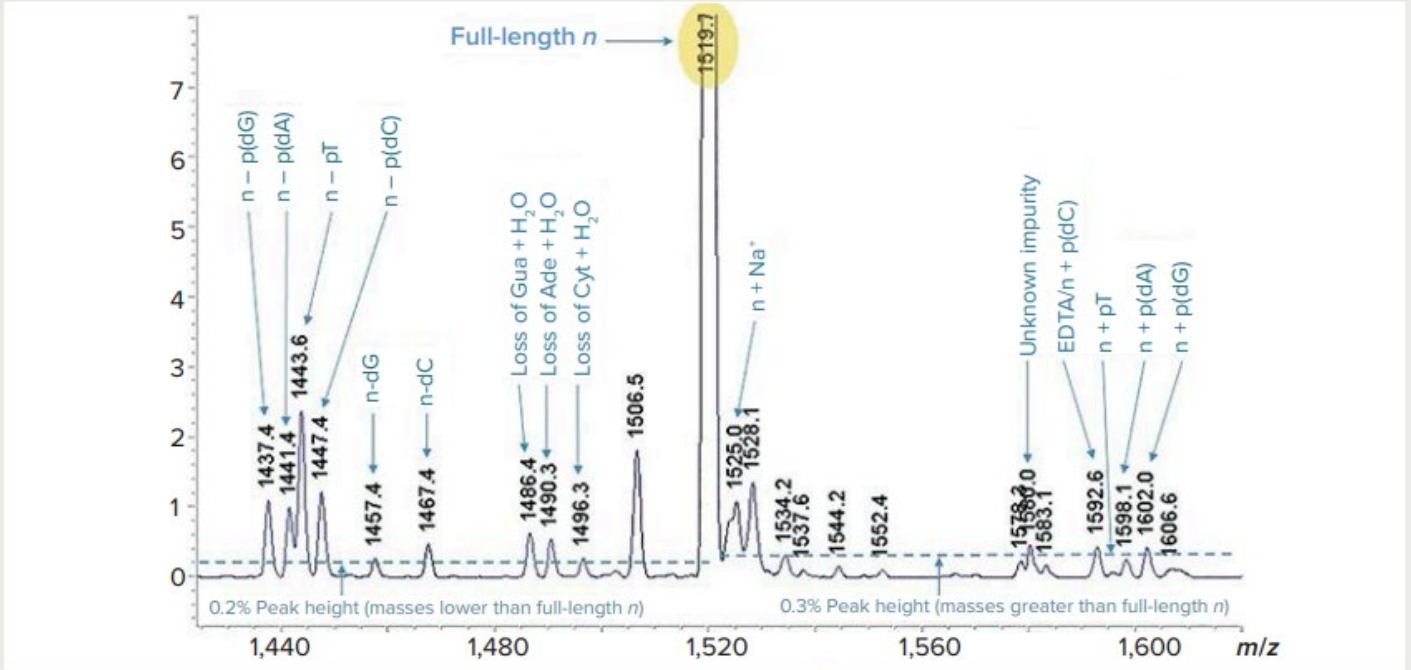


Figure 8: EICs were obtained under harsh (blue trace) and standard (red trace) ionization conditions. The EDTA adduct dissociates under harsh ionization conditions, which aids peak assignment and integration of the n + p(dC) impurity.

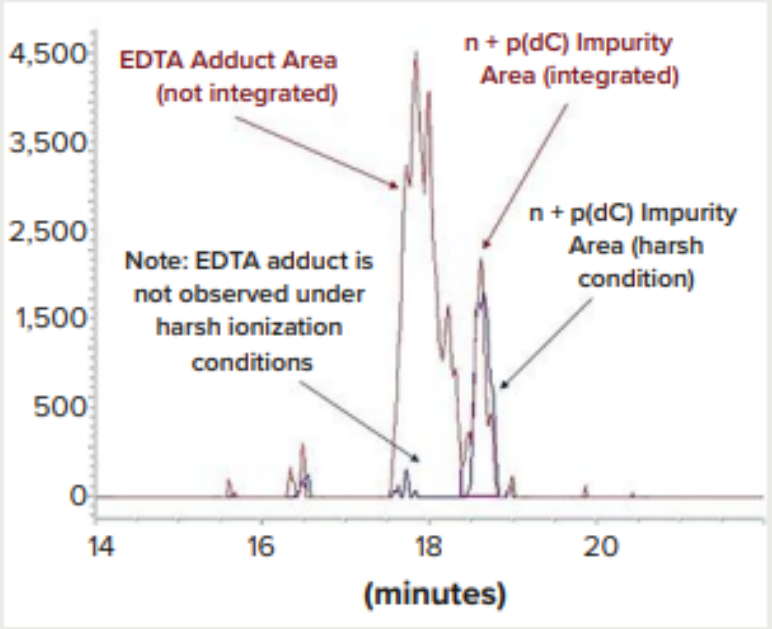
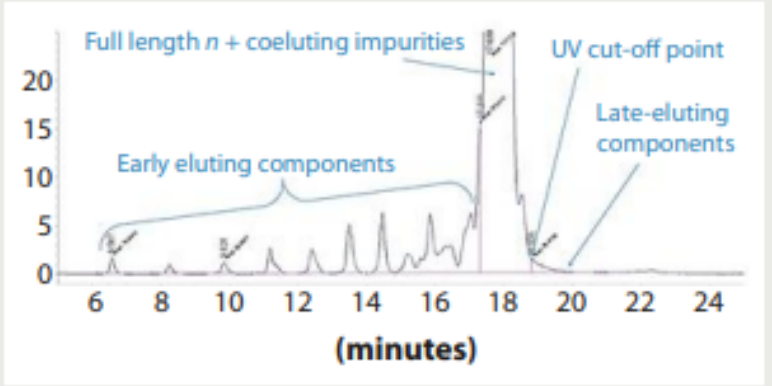


Figure 9: Integrated UV chromatogram of the 20-mer DNA sample with UV cut-off applied showing presence of early and late-eluting components



mRNA REGULATORY GUIDANCE

There are currently no dedicated regulatory guidance's, several in progress.

mRNA is considered a gene therapy product by the FDA

In the EU, mRNA based therapeutics have been classified as a Gene Therapy Medicinal Products

Seek scientific advice from the relevant local regulator.

Analytical method regulatory guidance

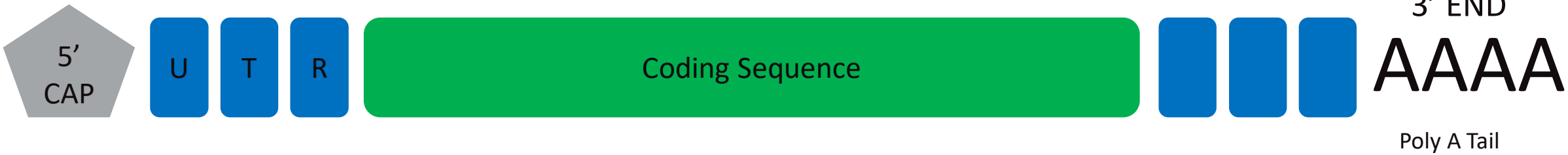
- Currently there is no specific ICH or FDA guidance for mRNA based therapeutics
- Some existing guidance can be applied;
  - 21 CFR, part 312.23 (a) (7) and 21 CFR, part 314.50 (d) (1); Chemistry, Manufacturing and Controls information
  - ICH Q7; cGMP
  - ICH Q2 (R1); validation
  - ICH Q3D; elemental impurities
  - ICH Q3C (R7); residual solvents
  - ICH Q6B; setting specifications
  - CMC Information for Human Gene Therapy INDs, FDA, 2018

mRNA ANALYTICS

Specification tests for batch release and stability study testing of mRNA drug substances and drug products

Compendial Methods	General Methods	mRNA Specific Methods
Appearance	Identification	Integrity
pH	Assay	Sequence
Osmolality	Content Uniformity	Potency
Particulate Matter	Residual Solvents	Capping efficiency
Container Closure Integrity	Elemental Impurities	Poly A Tail
Sterility		Residual dsRNA
Bacterial Endotoxins		Residual DNA Template

CRITICAL QUALITY ATTRIBUTES – 1ST UNDERSTAND STRUCTURE.



Region	Function
5’ CAP	The efficiency of capping and the cap structure effect protein production and immunogenicity.
UTR	Translational efficiency is influenced by their length
CODING SEQUENCE	Codon optimization can improve expression
3’ Poly A Tail	Length is important for translation and protection of mRNA
Impurity Levels	Control of impurity level, promotes expression

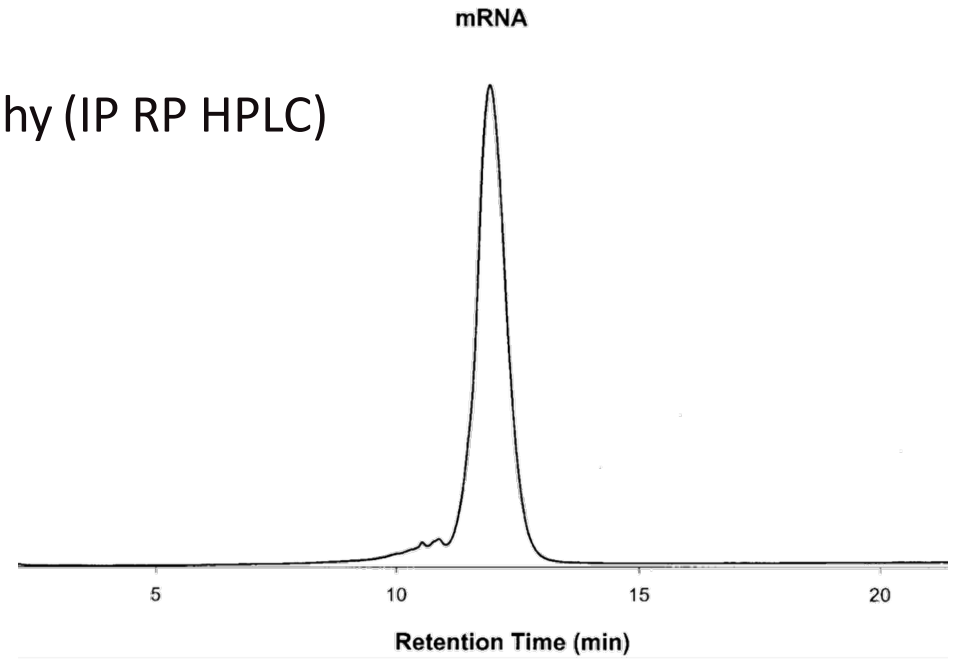
POTENTIAL IMPURITIES IN mRNA

Process Related Impurities	Product Related Impurities
DNA Template	Uncapped mRNA
Unreacted rNTPs	Short mRNA
Unreacted cap dinucleotide	dsRNA
Enzymes	

mRNA INTEGRITY TESTING

Chromatographic approaches to test intact mRNA

- Size exclusion chromatography (SEC)
- Ion-pair reversed-phase high performance liquid chromatography (IP RP HPLC)
- Capillary gel electrophoresis (CGE)



Stability indicating methods required for stability studies

A reduction in intact mRNA peak %area is observed under forced degradation conditions along with an increase in peak %area for degraded RNA

mRNA sample preparation must include precautions to prevent degradation

A. Azarani, K. H. Hecker, Nucleic Acids Research 2000, Vol. 29, No. 2, e7

POTENCY

- *21 CFR 600.3(s): The word **potency** is interpreted to mean. the specific ability or capacity of the product, as indicated. by appropriate laboratory tests or by adequately controlled. clinical data obtained through the administration of the. product in the manner intended, to effect a given.*
- *21 CFR 610.10: Tests for potency shall consist of wither in vitro on in vivo tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in 600.3(s) of this chapter.*

Why Needed:

Provide information on practically all critical attributes: Identity, Purity, Potency, and Stability.

Challenges for mRNA based products *In Vitro*:

- Generally, have a complex mode of action, which may take time to understand and may have multiple effects which might require multiple endpoints or steps. This might mean multiple assays or a multi step assay which is complex to mimic.
- Require a fully characterised reference material.
- Need to be shown to be specific/selective, able to demonstrate product issues.
- Choice of “marker” difficult as it needs to be something that is truly specific to the mode of action.

In vitro translation followed by analysis of the protein produced

THE POTENCY ASSAY

Infectivity assay - to demonstrate that the mRNA (and delivery system) is capable of reaching target cell population.

Assays needed to be designed with the critical understanding that they must facilitate delivery of the mRNA to the target cells and are capable of expressing a marker protein.

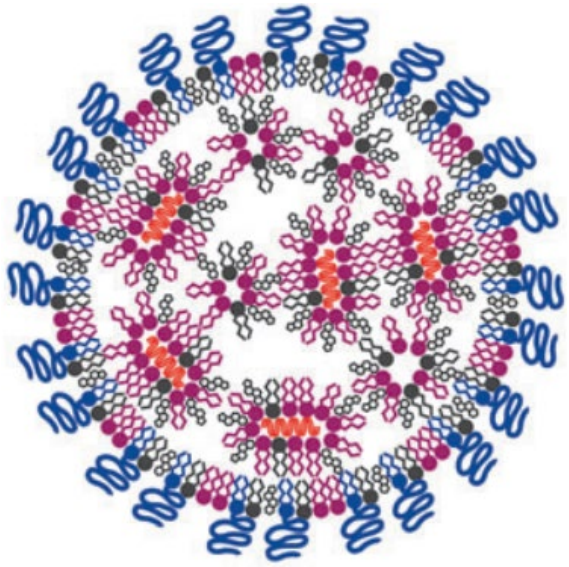
- *In vitro* translation followed by analysis of a marker protein produced.
- Transfection of mRNA into cells can often be the major challenge, especially for drug substance which do not contain the “delivery system” inherent to facilitate uptake in the drug product.
- Both physical and chemical means of transfection can be used.
- Ideally inclusion of a transfection control (GFP etc) and fluorescence microscopy to evaluate.
- Inclusion of a positive control, elicit a measurable, reproducible response
- Inclusion of a negative control (can look at cell viability)

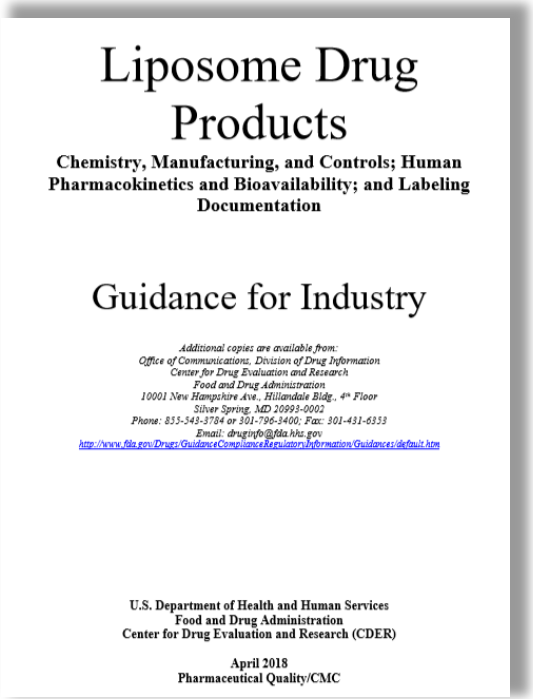
mRNA DELIVERY SYSTEMS; LIPID NANOPARTICLES (LNPs)

Encapsulation of mRNA inside LNPs provides stability, delivery and safety advantages

LNPs for nucleic acid-based therapeutics typically consist of;

- Ionisable lipid
- Structural lipid
- PEG-lipid
- Cholesterol





Liposome Drug Products

Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation

Guidance for Industry

Additional copies are available from:
Office of Communications, Division of Drug Information
Center for Drug Evaluation and Research
Food and Drug Administration
10001 New Hampshire Ave., Hillandale Bldg., 4th Floor
Silver Spring, MD 20993-0002
Phone: 855-543-3784 or 301-796-3400; Fax: 301-431-6353
Email: druginfo@fda.hhs.gov
http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

April 2018
Pharmaceutical Quality/CMC

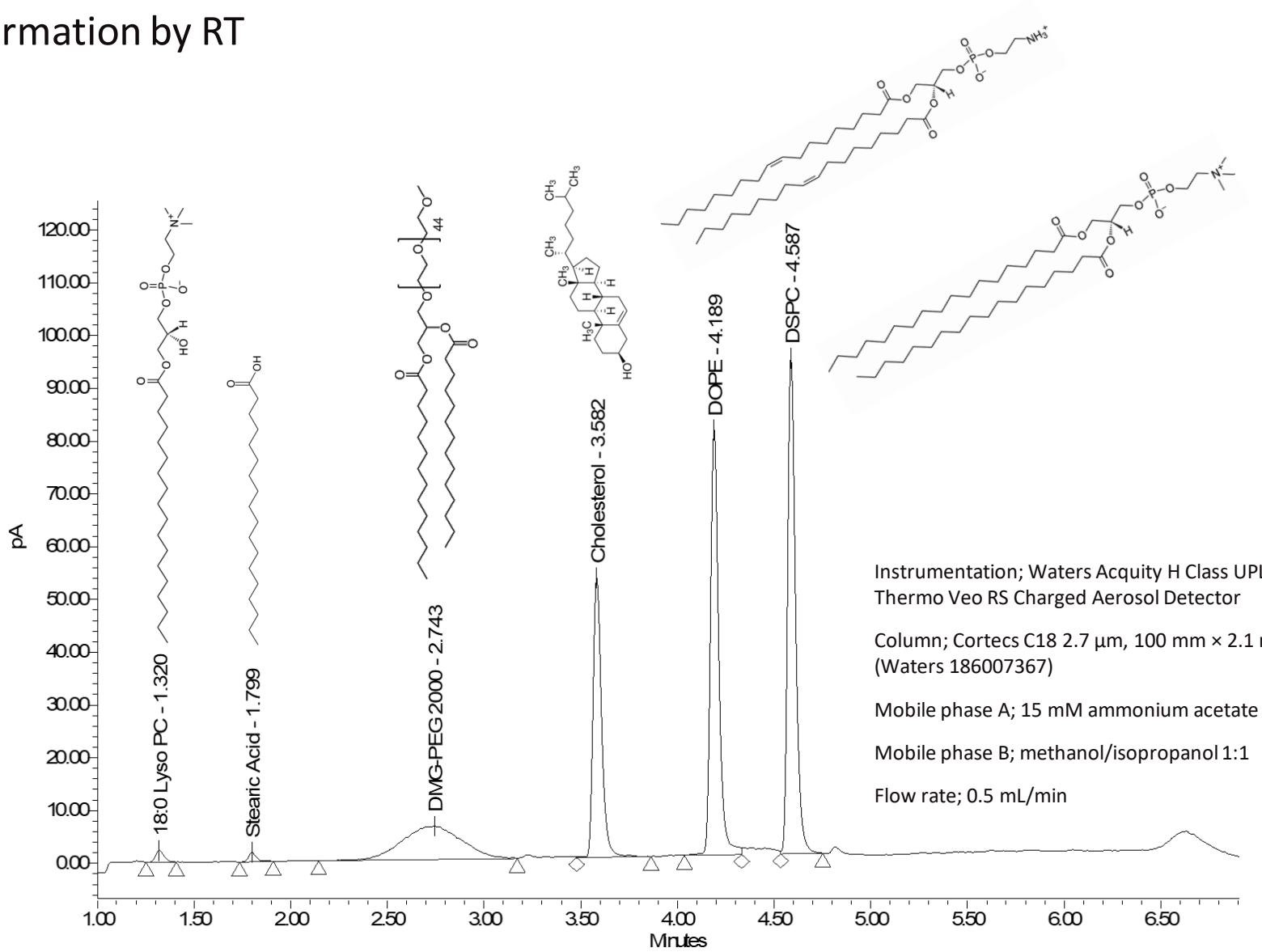
Lipid components and relative ratios are chosen to provide optimal encapsulation efficiency, stability, cellular uptake and endosomal release of the drug substance

J. A. Kulkarni et al., Nucleic Acid therapeutics 2018, Vol. 28, No. 3, 146

UPLC-CAD ANALYSIS OF LIPID NANOPARTICLES (LNPs)

Analysis of the lipid components of the LNPs can be performed using UPLC-CAD

- Lipid component identity confirmation by RT
- Lipid quantification
- Related impurity monitoring



Instrumentation; Waters Acquity H Class UPLC with Thermo Veo RS Charged Aerosol Detector

Column; Cortecs C18 2.7 µm, 100 mm × 2.1 mm (Waters 186007367)

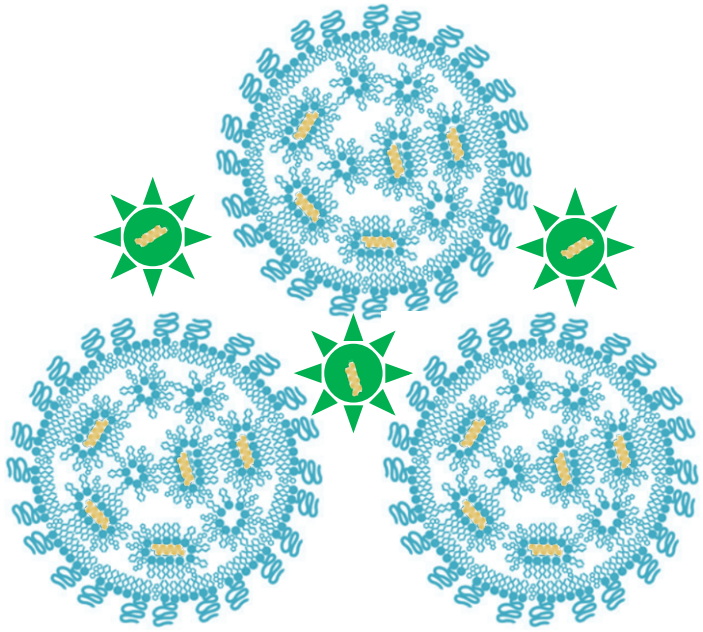
Mobile phase A; 15 mM ammonium acetate

Mobile phase B; methanol/isopropanol 1:1

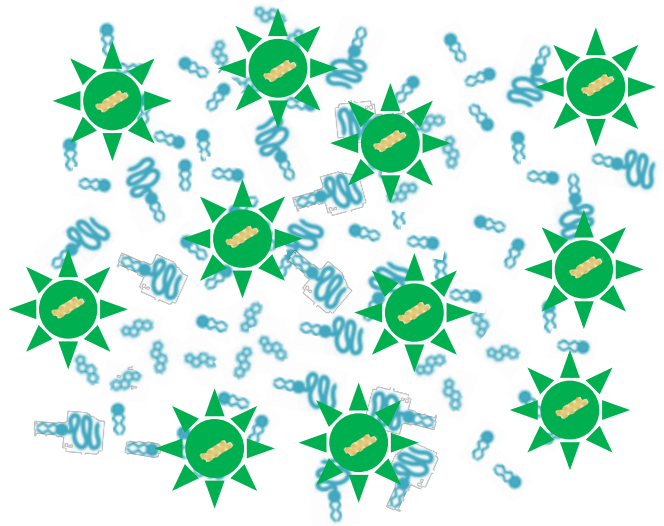
Flow rate; 0.5 mL/min

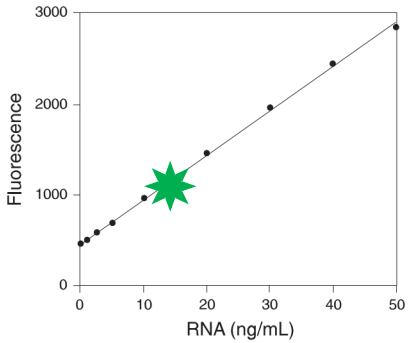
mRNA ENCAPSULATION EFFICIENCY

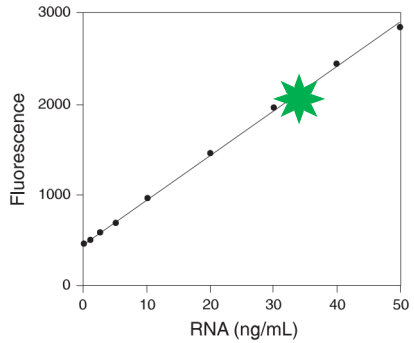
Analysis of encapsulation efficiency of the mRNA inside the LNP can be performed using a RiboGreen® assay



LNP disruption







NOW YOU FORMULATE /SELECT DEVICE

CRITERIA TO CHECK DURING DEVELOPMENT
(On top of standard device performance criteria)

1: Is Potency Lost Following Delivery?

2: Can you recover all the material from the device.?

3: Has the structural Integrity been affected, i.e. product related impurities increased.

4: Has the rate of release of the mRNA from the encapsulation system been affected.

5: Do you see degradation of the delivery system through delivery.

RELEASE /STABILITY

ASOs

Impurities/Purity

Delivered Dose

mRNA

Potency

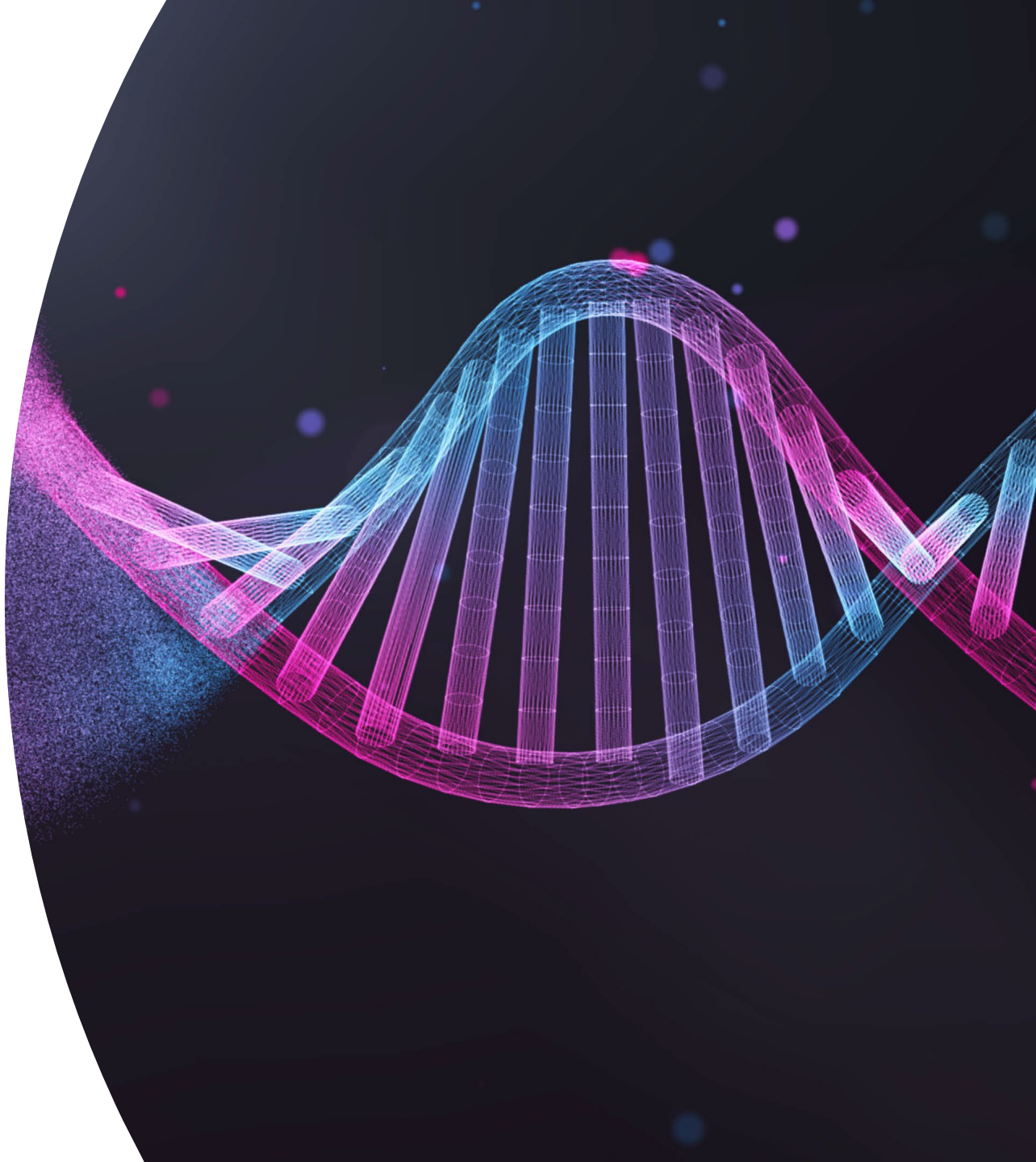
Integrity

Encapsulation System

Encapsulation Efficiency

System Breakdown

Release



CONCLUSIONS

- Inhaled /Nasal delivery has the potential to be of huge benefits for DNA/RNA medicine delivery. Overcoming one of the industries biggest potential challenges for lung/CNS delivery.
- Requires new approaches to analytics and a lot more !

INHALED

Thank You!

ANY QUESTIONS?

Ashleigh Wake

biopharma@intertek.com

intertek.com/pharmaceutical/

intertek

Total Quality. Assured.