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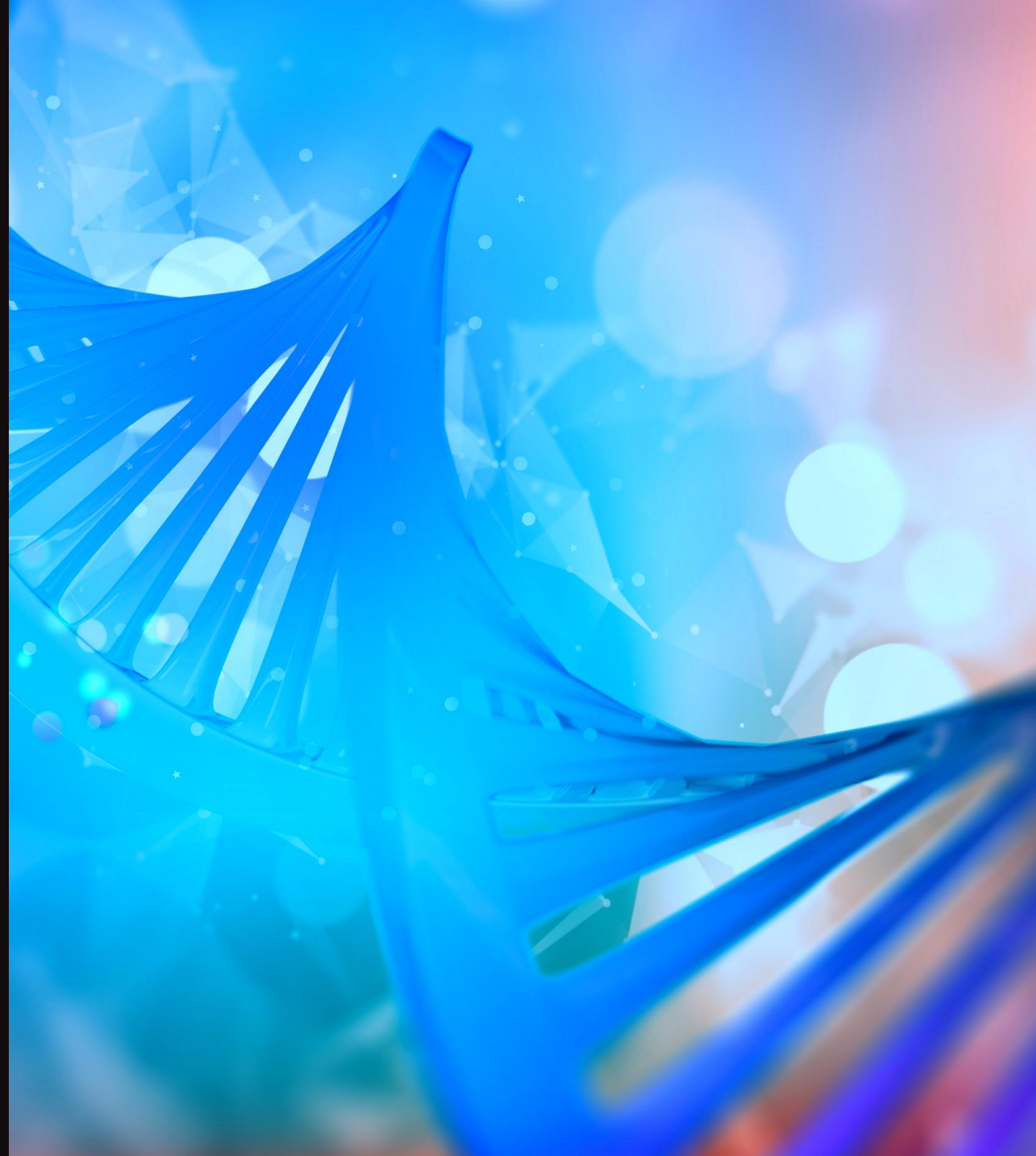
DEVELOPMENT STRATEGIES FOR INHALED AND NASAL OLIGONUCLEOTIDE AND MRNA PRODUCTS

ASHLEIGH WAKE

UK Business Development Director



WHY LISTEN TO US?

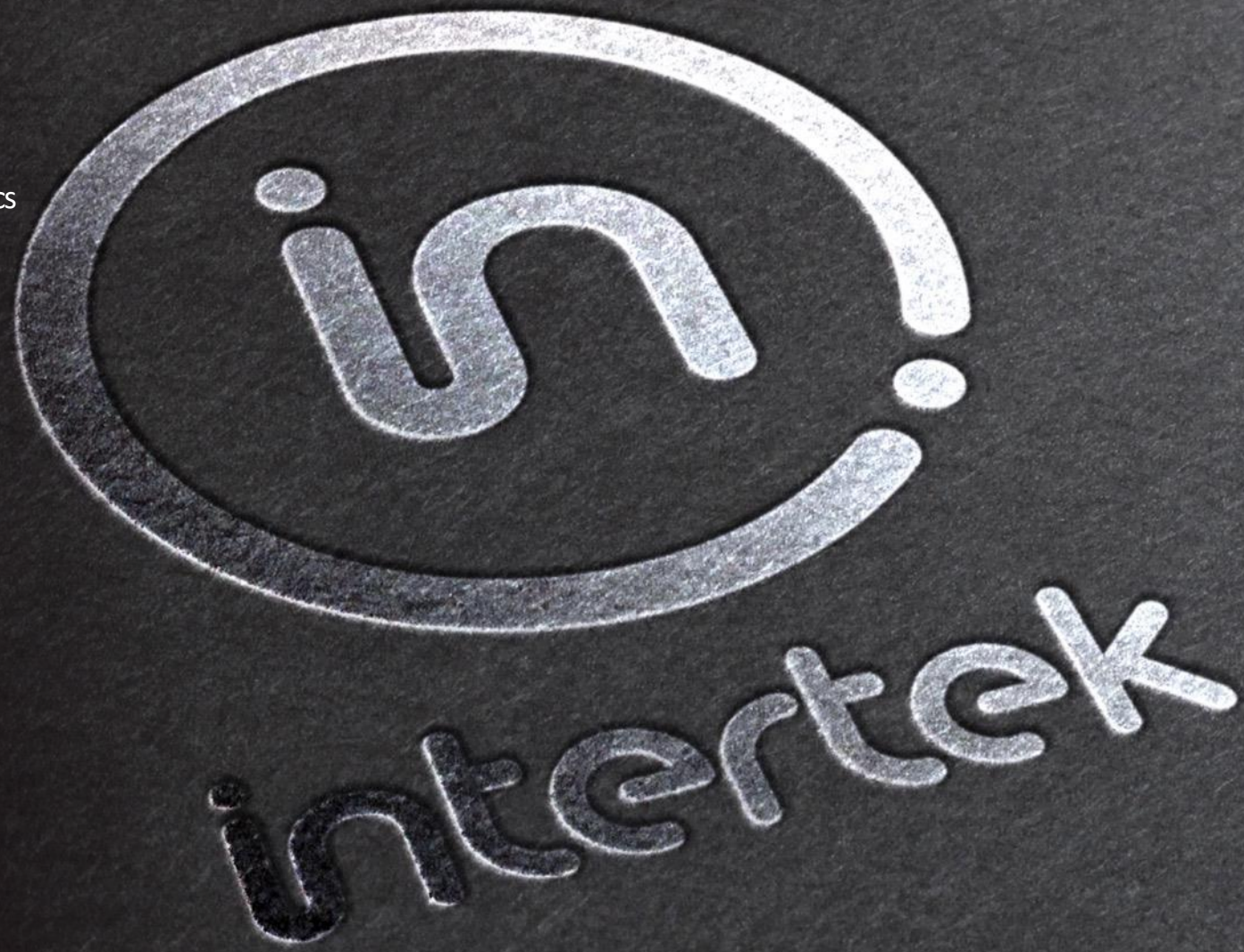
30+ years' experience providing regulatory-led analytical support for small molecule, vaccines, biologics and cell and gene therapies and inhaled product development

We have the largest GMP facility in Europe for OINDP development support and biologics characterisation

Our methodology is built on our experience in supporting our clients' in over 5000 unique inhaled projects in the last 10 years

We are the only specialist OINDP Development CRO in EU to have extensive experience across both biologics and small molecule

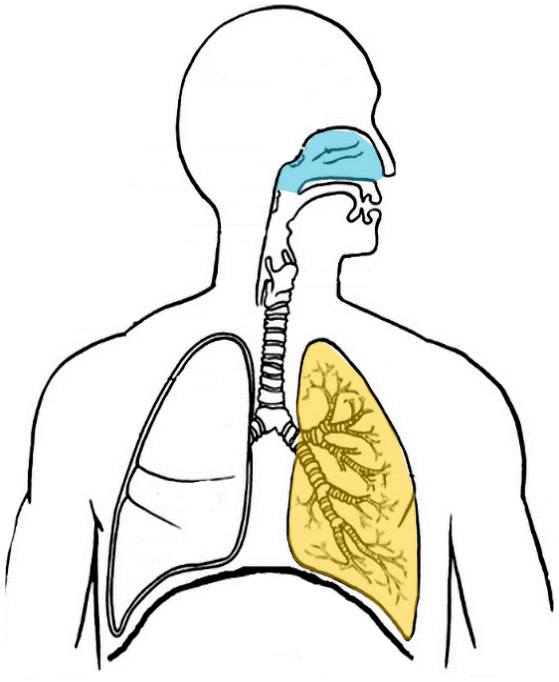
Scientists who have contributed to multiple major vaccine and therapeutic development over the last 20 years.



WHY INHALED AND NASAL DELIVERY OF OLIGONUCLEOTIDES AND MRNA?

For several years the potential of oligonucleotide and mRNA based medicines has continued to grow, given their diversity of application and potential therapeutic effect.

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

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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

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

PUBLISHED
25 MAR 2021

RESEARCH CORONAVIRUS COVID-19 VACCINE

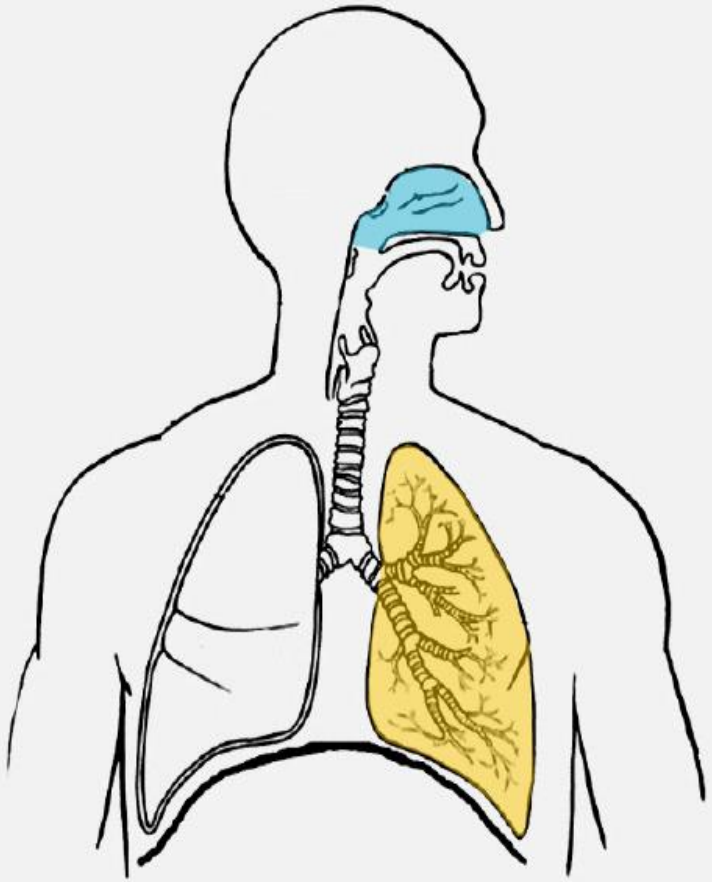
SHARE THIS

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

AGENDA

1. Why Consider Inhaled or Nasal Delivery
2. Development Process
3. Analytical Control, Inhaled Specific
4. Case Study
5. Conclusions and Questions

WHY INHALED AND NASAL ?

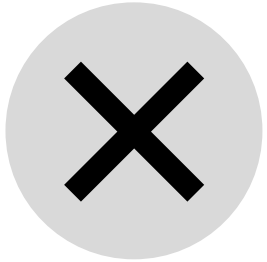


NASAL

INHALED

WHY INHALED AND NASAL DELIVERY OF OLIGONUCLEOTIDES?

- 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

RESPIRATORY /PULMONARY DISEASE -OLIGOS

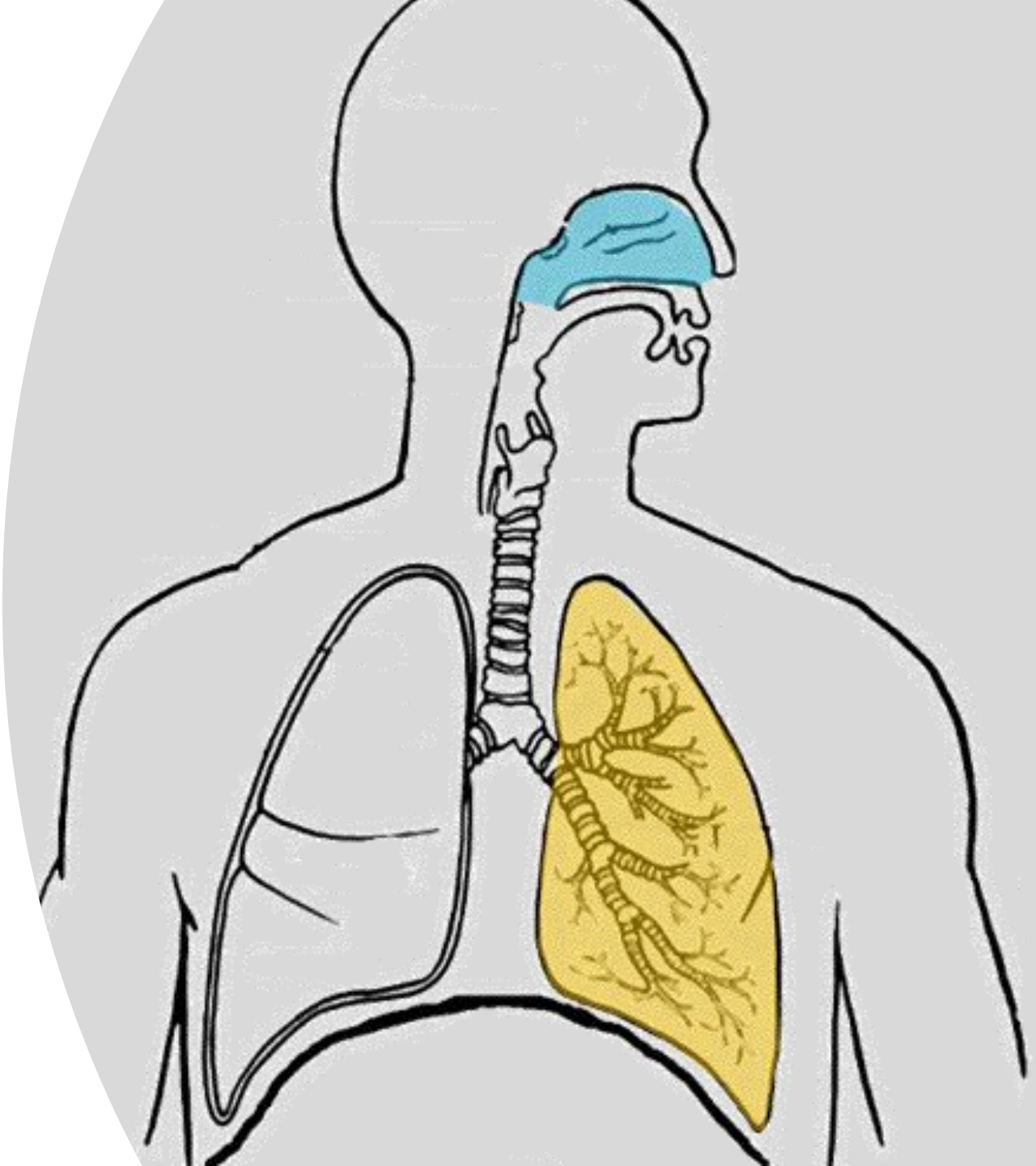
Lung is a powerful target for oligonucleotide delivery.

A bolus dose can be delivered by inhalation direct to the lung.

Surfactants in the lung enhance uptake and distribution.(the pH of the lung driving cationic properties which are often added to promote uptake in general systems)

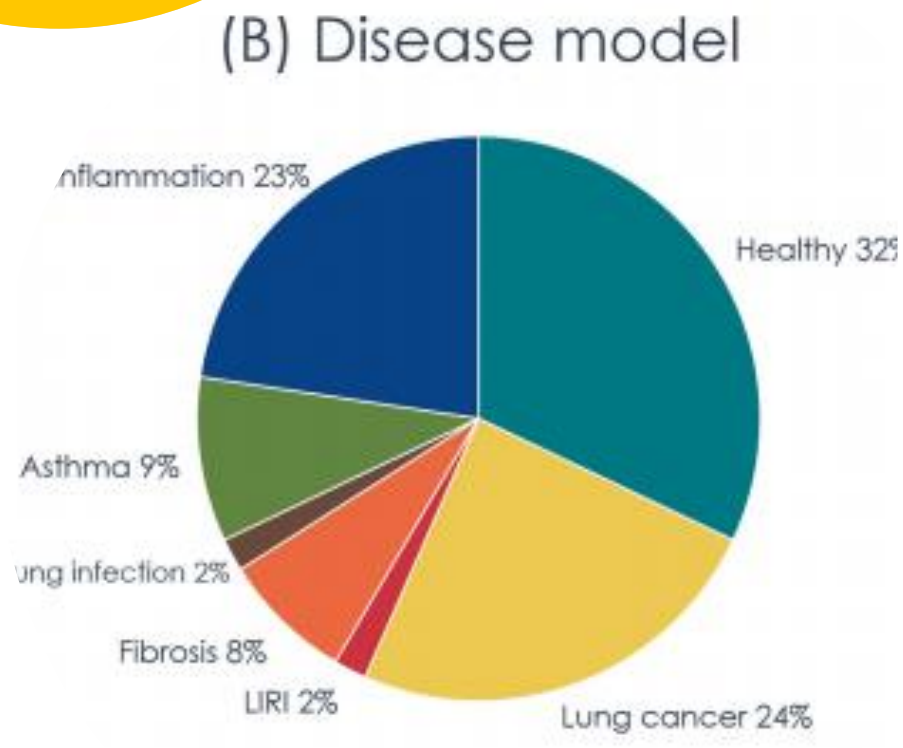
Historic issues with respiratory drugs is their associated systemic toxicity, an added benefit that different chemistries can be incorporated which modulate nuclease sensitivity such that the oligo is active in the lung but then degraded before they become systemically available and hence induce toxicity

Or can be engineered to promote systemic delivery



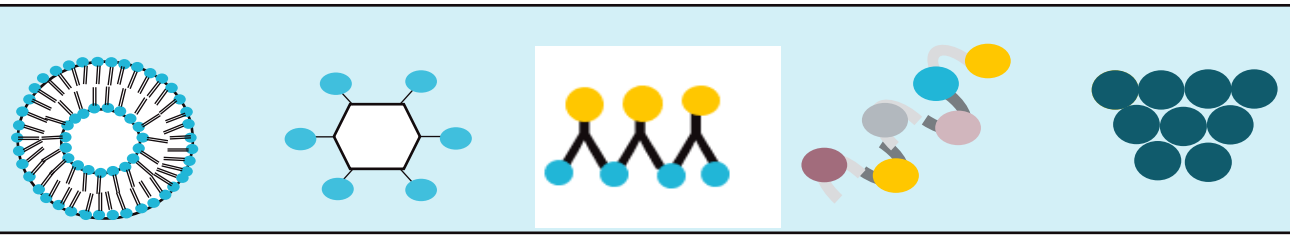
PULMONARY DISEASES -MRNA

- 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

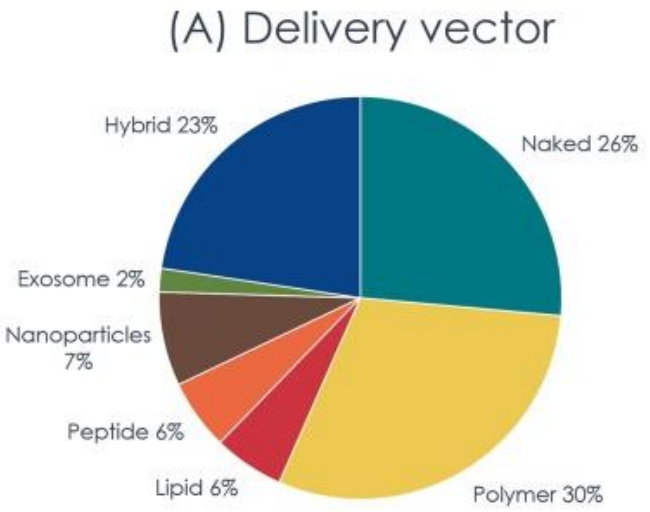
DEVELOPMENT OF AN INHALED OLIGO OR MRNA



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	

FORMULATIION OPTIONS - SYSTEMIC DELIVERY

Increase Residency Time

Bio-adhesives and viscosity adjusters

- Chitosan
- CMC, HPMC, HMC
- CMC/MCC –Avicel® RC591
- Polyethylene Glycol (PEG)
- Polyvinylpyrrolidone (PVP)
- Glycerol

Enhance Absorption Rate

Permeability enhancer sand solubilisers

- NeurelisIntravail®
- Polysorbate 20/80
- Cyclodextrins
- Lecithin
- HPMC
- Oleic Acid
- Propylene Glycol
- Ethanol

STRATEGIC FORMULATION REQUIREMENTS - NASAL

Agent	Action	Examples
Permeation Enhancing Agents	Helps to increase the transport of proteins and peptides across the nasal membrane by several modes of action	n-dodecyl beta-D-maltoside (Neurelis's Intravail), Surfactants e.g. polysorbates and lecithin
Mucolytic Agents	Enhance the nasal absorption	N-acetyl-L-cysteine (NAC)
Mucoadhesive / Bio adhesive Agents	Enable prolonged retention at the site of application, providing a controlled rate of drug release for improved therapeutic outcome	HPMC, carbopol 934 and sodium alginate
In Situ Gelling Agents	Fluids which are non-Newtonian fluid that is free flowing when being mixed or sprayed but then forms a thick gel following actuation.	Avicel RC591 (DuPont)
Drug Carrier technologies	Agents that enhance their absorption through encapsulation or surface modification	Liposomes, emulsions, nanoemulsions, nano/micro particles

DEVICE SELECTION

Aqueous Multi Dose

Aqueous Mono Dose

Dry Powder Mono Dose

Nebulised

pMDI Multi Dose

pMDI Nasal Through Mouth

TESTING REQUIREMENTS

- **Chemical**
  - Assay (API)
  - Assay (Excipients)
  - Degradation Products
- **Physical**
  - Appearance
  - Moisture Content
  - Particle Size
  - Leak Rate
  - Device Function
  - Foreign Particulates
- **Performance**
  - Delivered Dose
  - Aerodynamic Particle/Droplet Size
  - Spray Pattern
  - Plume Geometry
  - Through Life Performance
- **Microbiological**
  - Microbial Assay
  - Sterility

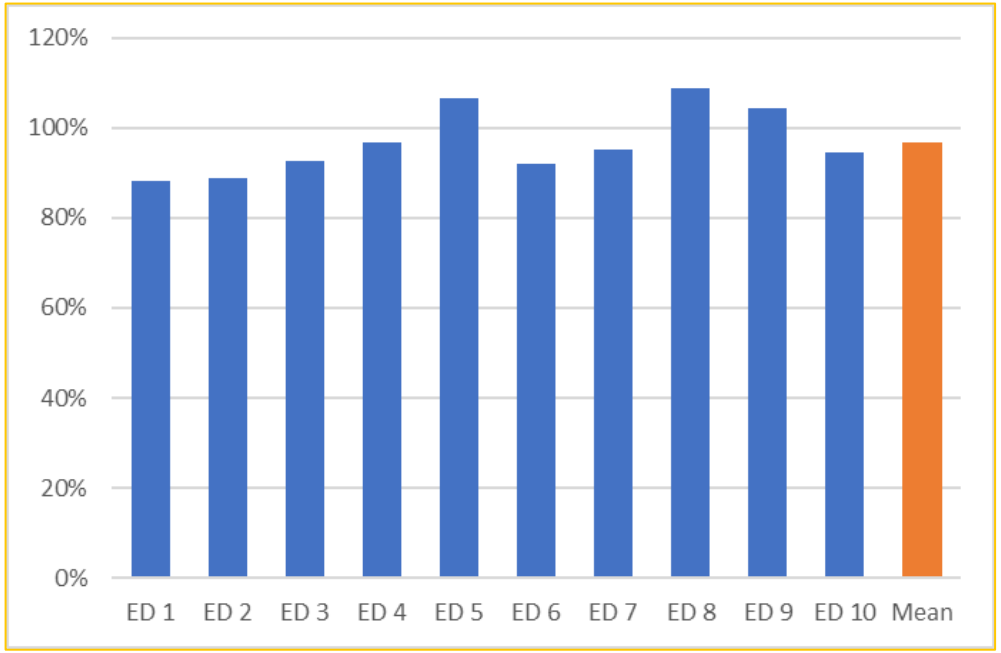
INHALER SPECIFIC TESTING - DEVICE

Delivered Dose

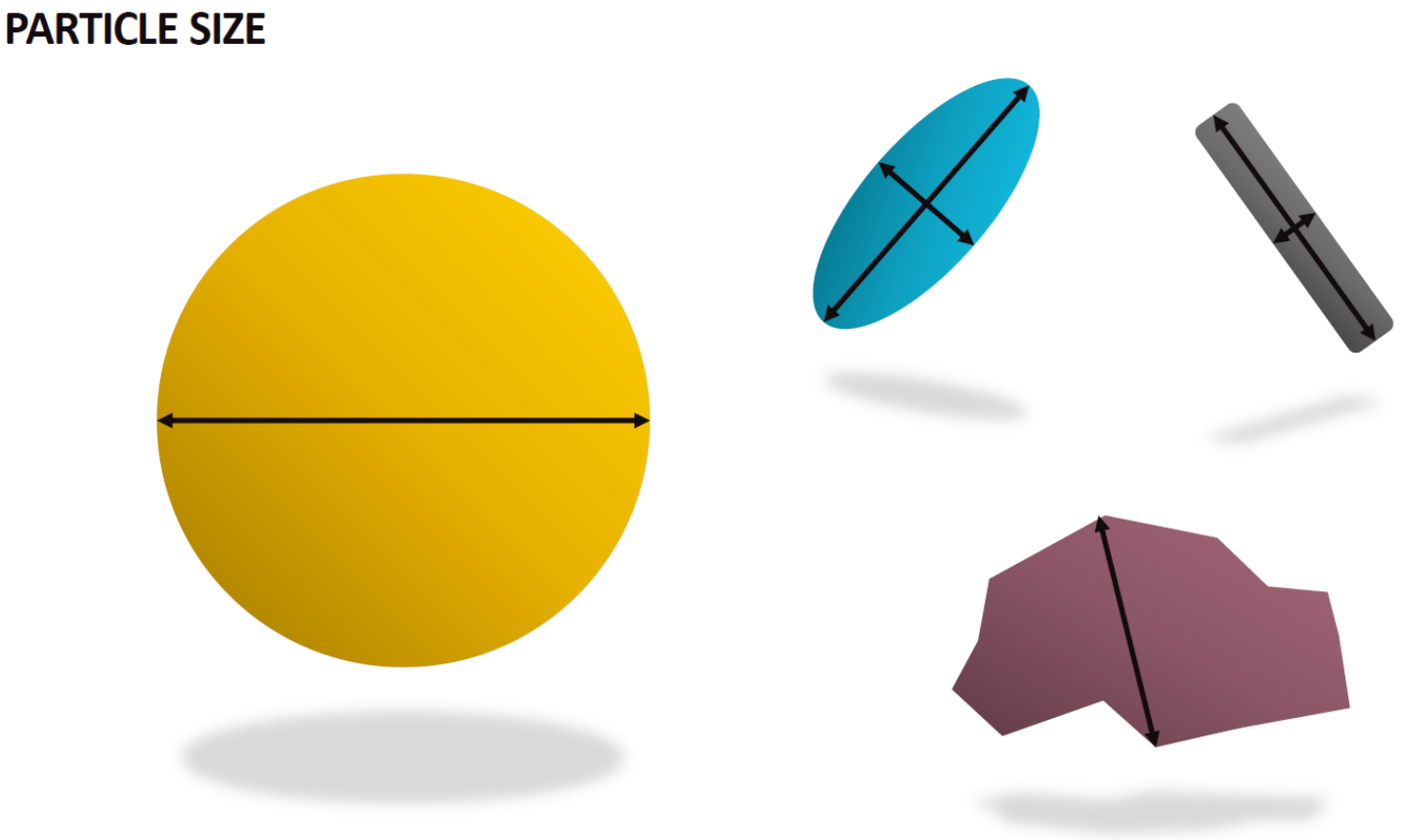
Aerodynamic Particle/Droplet size

Spray Pattern / Plume Geometry

Performance through life / Device Operation



PARTICLE SIZE



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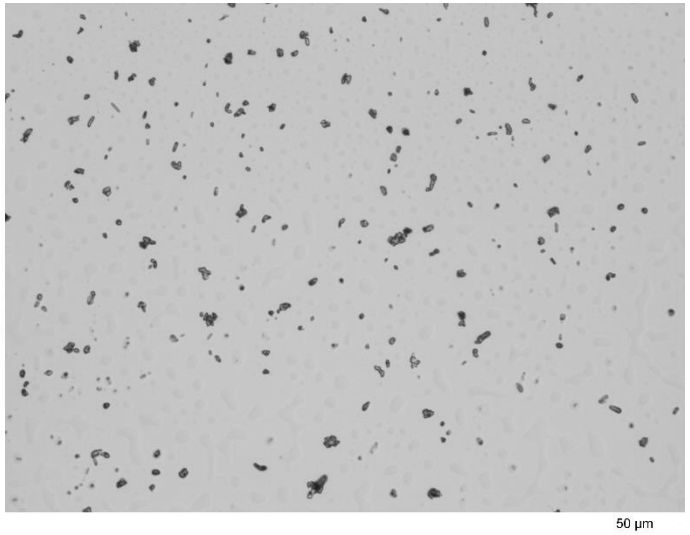
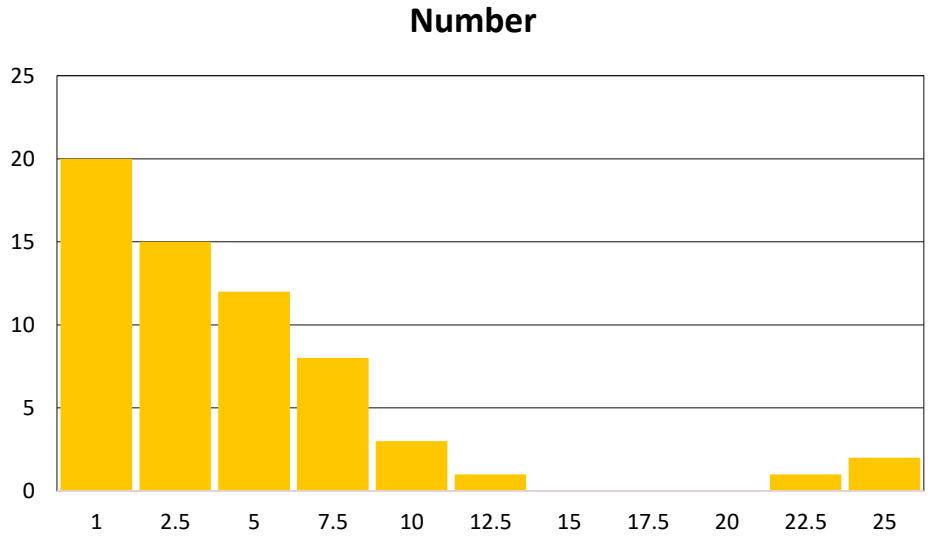
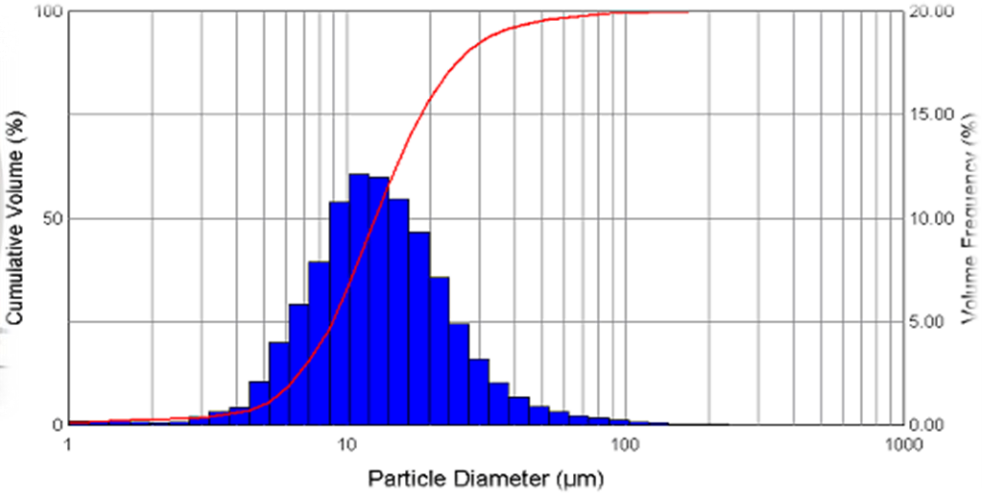
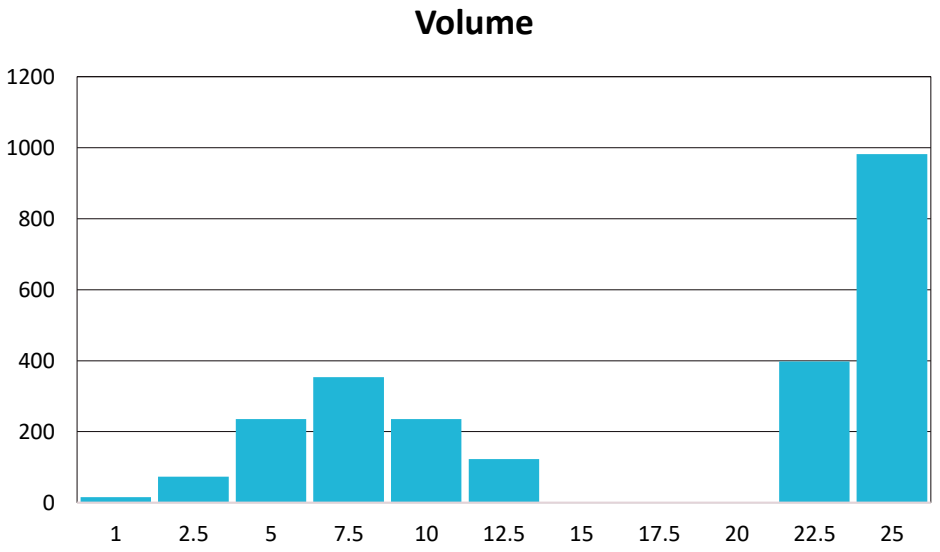








PARTICLE SIZE – AERODYNAMIC SIZE

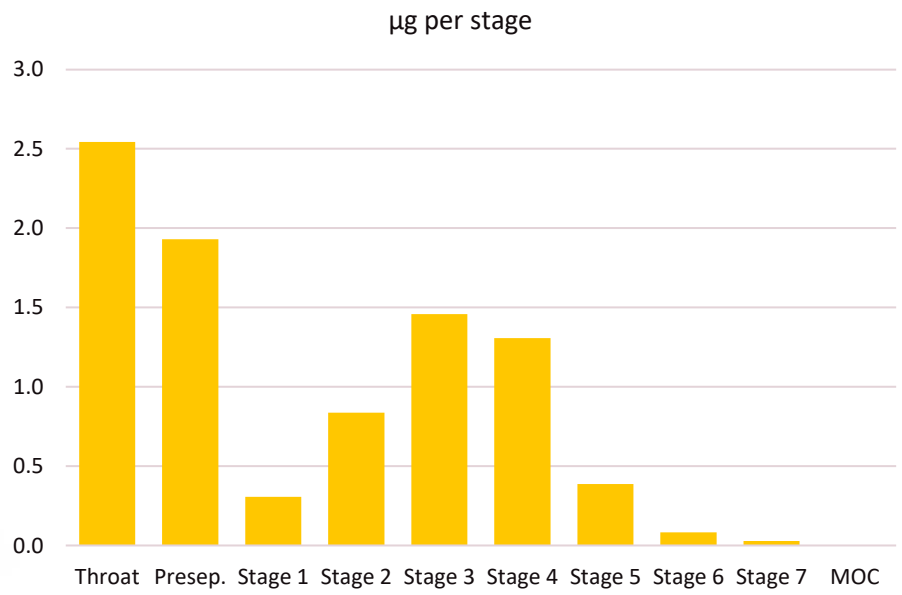
INHALER SPECIFIC TESTING - DEVICE

Delivered Dose

Aerodynamic Particle/Droplet size

Spray Pattern / Plume Geometry

Performance through life / Device Operation



UNDERSTANDING APSD DATA, SETTING SPECIFICATION

FPD - Fine Particle Dose

Total Dose (µg or %) that is under a specified size (commonly 5µm)

MMAD - Mass Median Aerodynamic Diameter

The size at which 50% of the total dose is smaller and 50% is larger

GSD – Geometric Standard Deviation

A measure of the width of the size distribution ($GSD = (d_{84}/d_{16})^{1/2}$)

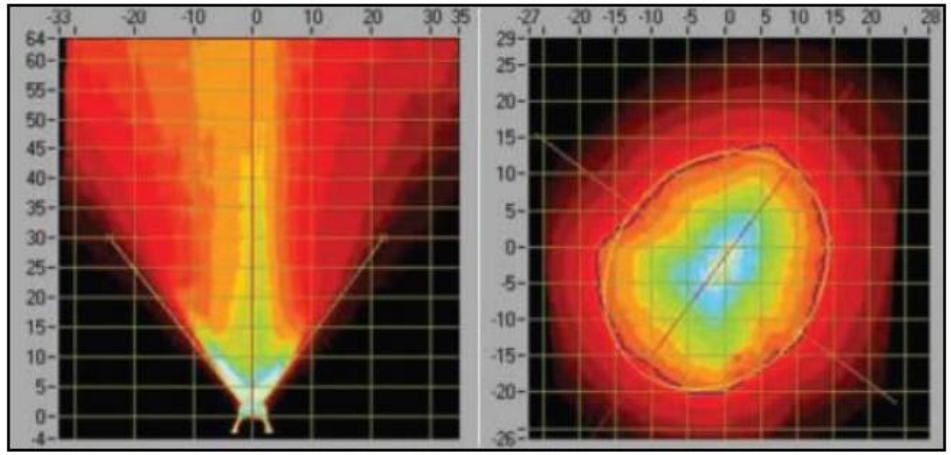
INHALER SPECIFIC TESTING DEVICE

Delivered Dose

Aerodynamic Particle/Droplet size

Spray Pattern / Plume Geometry

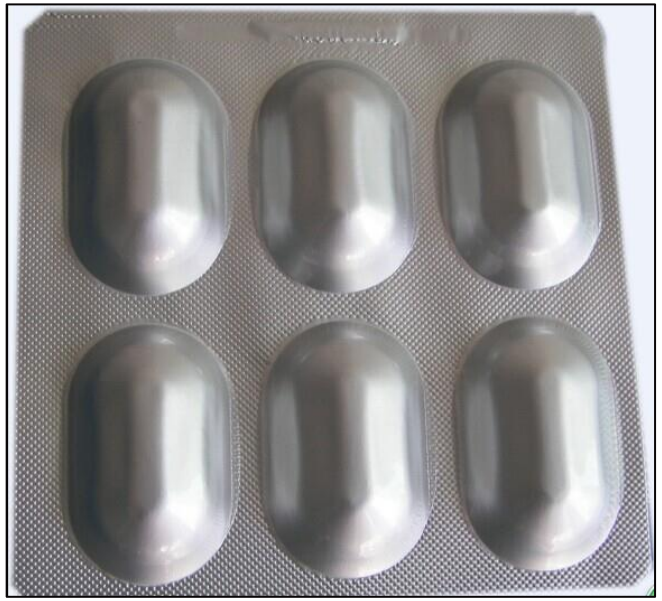
Performance through life / Device Operation

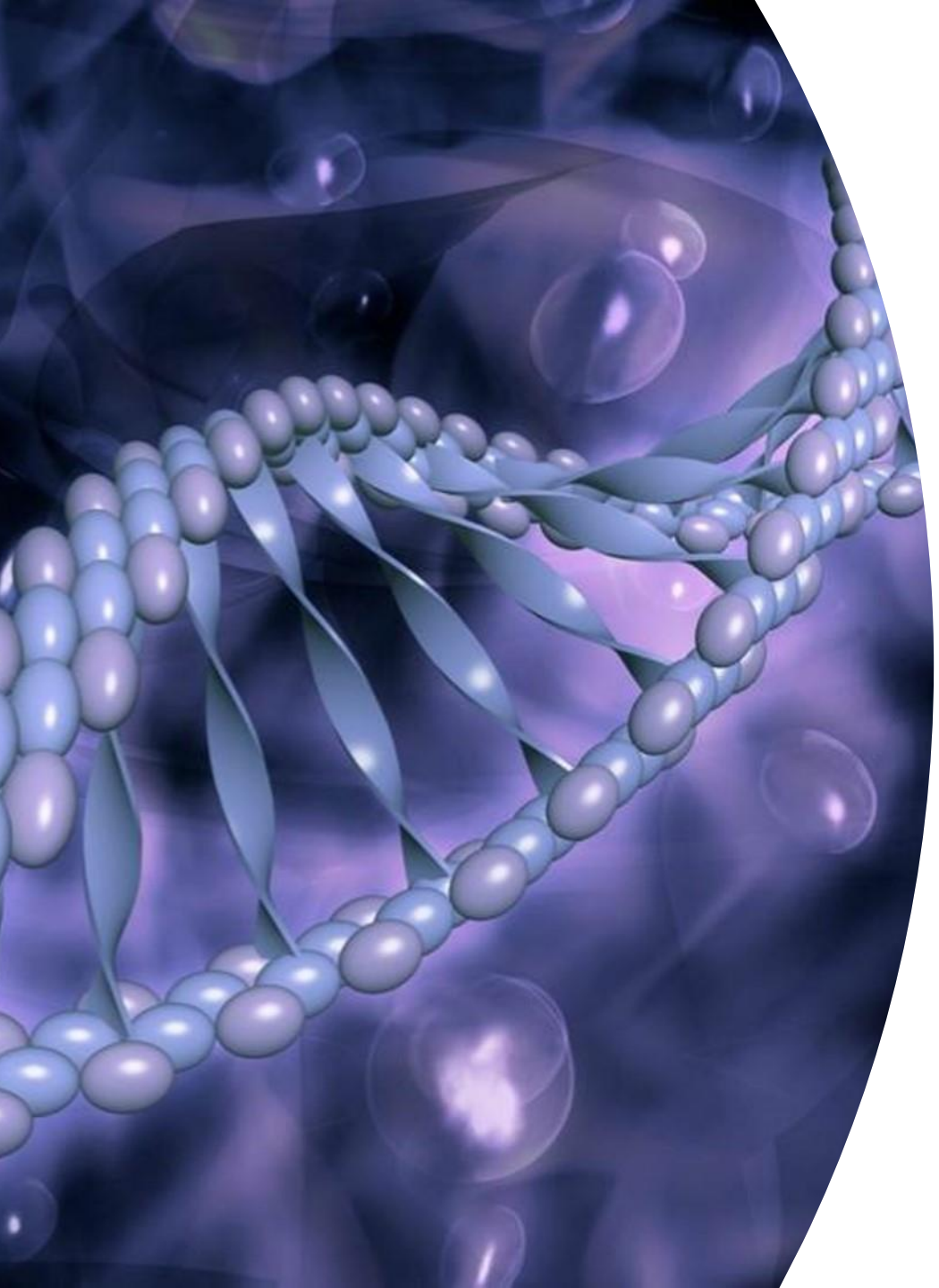


INHALER SPECIFIC TESTING – CONTAINER / PACKAGING

Leak Rate

Moisture





CASE STUDY

Formulation of a 20mer phosphorothioate oligonucleotide (GCCATCTTAGGACTTGATTC) for inhalation delivery was developed.

Three different formulations were selected and stability in aqueous solution assessed during and after nebulisation

The commercially available Philips Innospire Go nebuliser using vibrating mesh technology was considered to offer a less aggressive mode of nebulisation, this was considered in preference to a Jet nebuliser (Sprint) as these constantly circulates droplets of formulation (creating an increased surface area) the effect of which was unknown.

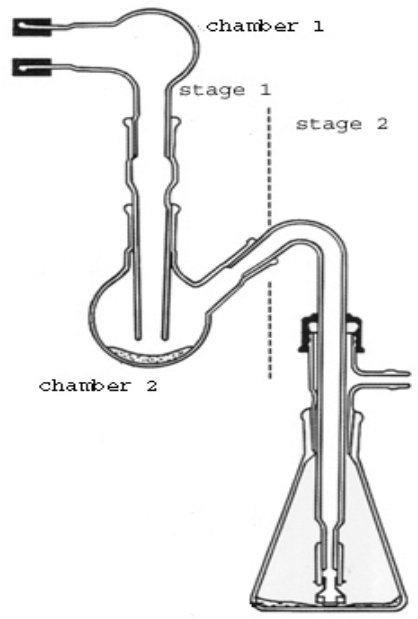
The formulated product aerosolisation performance was assessed in terms of nebulised assay, aerodynamic droplet size distribution (ADSD) by Next Generation Impactor (NGI) and delivered dose. The post-nebulisation stability of the model oligonucleotide was analysed by RP-HPLC.

FORMULATIONS

Constituent	Final Concentration (mg/mL)
Oligonucleotide	0.1
Sodium Chloride	9.0
Hydrochloric Acid/Sodium Hydroxide	Adjusted to pH 8.0 ± 0.5
Water for Injection	To volume

Constituent	Final Concentration (mg/mL)
Oligonucleotide	0.1
Super Refined Polysorbate 80 (Tween 80)	0.5
Monosodium Phosphate Dihydrate	3.84
Disodium Phosphate Anhydrous	10.7
Sodium Chloride	8.6
Water for Injection	To volume

Constituent	Final Concentration (mg/mL)
Oligonucleotide	0.1
Tris-HCl Buffer	1.21
Ethylenediaminetetraacetic acid (EDTA)	0.292
Hydrochloric Acid/Sodium Hydroxide	Adjusted to pH 8.0 ± 0.5
Water for Injection	To volume



Schematic Diagram of Glass Twin Impinger

RESULTS

Results of nebulised assay using a twin impinger

Stage	Formulation Variant		
	1	2	3
Upper Chamber (µg)	18.626	28.170	29.122
Lower Chamber (µg)	120.420	142.098	133.822
Device (µg)	24.853	19.679	16.359
Sum (exc. Device) (µg)	139.046	170.268	162.944
Sum (inc. Device) (µg)	163.899	189.947	179.302
Recovery (exc. Device) (%)	69.7	85.3	81.7
Total Recovery (%)	82.1	95.2	89.9

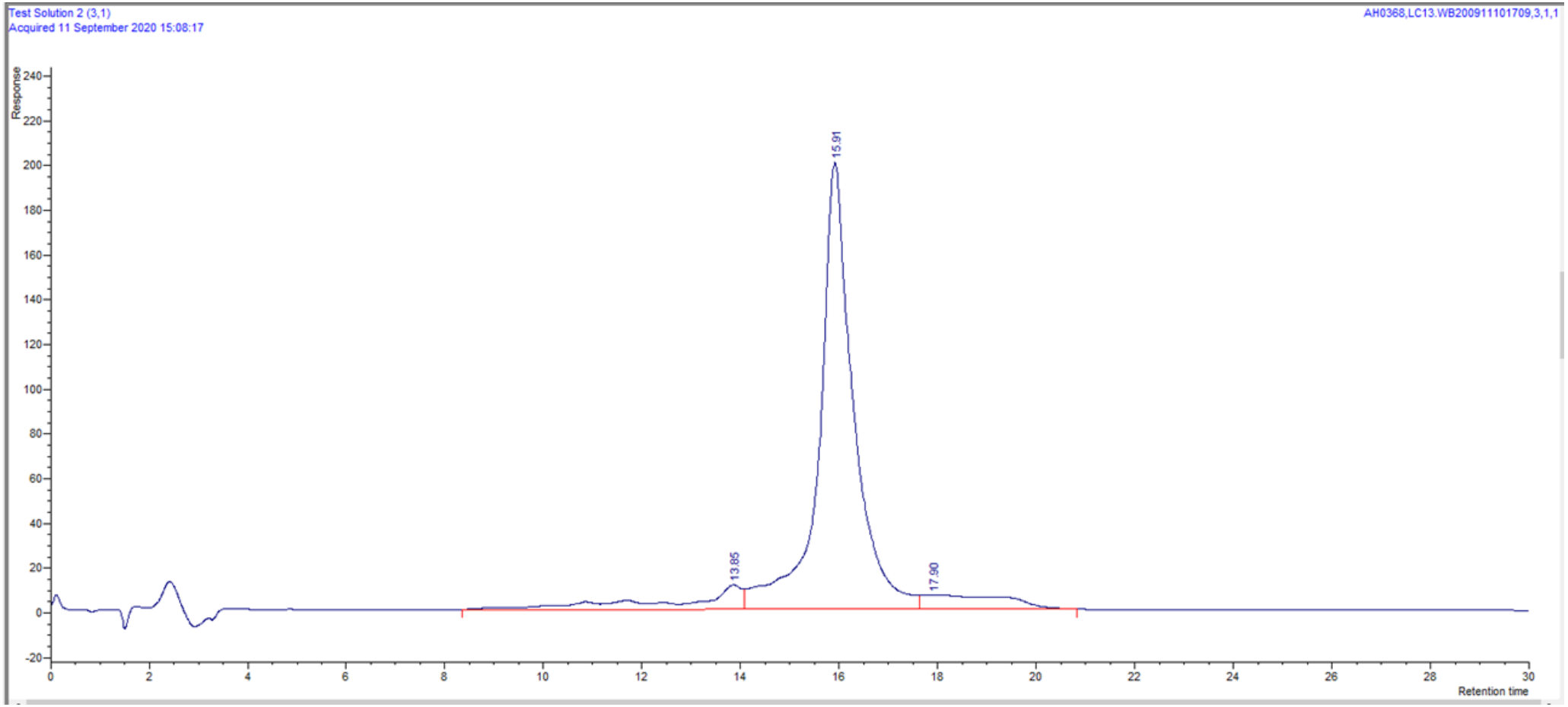
Results from the determination of Delivery Rate and Total Delivered Dose

Formulation Number	1	2	3
Delivered weight (g)	1.5664	1.6186	1.5193
Residual weight (g)	0.2989	0.3078	0.2977
Total Delivery Time (s)	189	192	176
Delivery Rate (over the first two minutes) (µg/min)	16.372	11.708	14.349
Mean Delivery Rate (over the first two minutes) (µg/min)	14.1		
* % Efficiency	37.99774	38.3875	32.33444
% Nominal Inhalation Delivered	29.760	31.067	24.563
Mean % Nominal Inhalation Delivered	28.5		

APSD

Stage	Formulation 1	Formultaion 2	Formulation 3	Mean
Throat & Mouth	2.23702	2.11368	1.03742	1.80
Stage 1	4.79607	5.28172	5.29410	5.12
Stage 2	10.72826	11.77988	10.21055	10.91
Stage 3	29.92908	34.11731	31.18155	31.74
Stage 4	40.88656	50.24979	47.70498	46.28
Stage 5	32.08488	33.10766	33.68233	32.96
Stage 6	10.54283	10.85859	10.50556	10.64
Stage 7	3.60194	3.91854	3.21432	3.58
MOC	1.11132	2.13909	5.08414	2.78
Sum	135.91796	153.56626	147.91495	145.80
Device	20.85000	10.86426	12.28825	14.67
Delivered Mass (g)	1.5618	1.7279	1.6086	1.63
FPD ≤ 5 µm* (µg)	82.19533	92.82839	93.27370	89.43
FPD/Delivered Mass (µg/g)	52.62859	53.72324	57.98440	54.78
FPF (FPD as % Total Dose) ≤ 5 µm	60.47422	60.44843	63.05901	61.33
GSD*	1.8	1.8	1.8	1.8
MMAD*(µm)	4.2	4.2	4.1	4.2

INTEGRITY


Test Solution 2 (3,1)
Acquired 11 September 2020 15:08:17
AH0368,LC13.WB200911101709,3,1,1
Response
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
15.91
13.85
17.90
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
Retention time

CONCLUSIONS

- The results showed the successful and stable nebulised delivery of oligonucleotide solutions for all formulations.

- Together, the generated data suggests that DNA sequences of approximately 20 nucleotides in size can successfully be formulated as nebulisable solutions and nebulised without significant degradation or loss of the oligonucleotide. All three formulation variants showed acceptable recovery and stability, with formulation variant 2 proven to be the most successful variant.

Thank You!

QUESTIONS?

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intertek.com/pharmaceutical/

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Total Quality. Assured.