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Classes of Inhaled and Nasal Biologics: Current Trends In Industry

IPAC-RS
International Pharmaceutical Aerosol Consortium on Regulation & Science

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HYBRID EVENT

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

Classes of Inhaled and Nasal Biologics: Current Trends In Industry

Outline of the presentation

- Introduction on Nasal and Orally Inhaled Biologics
- Nasal And Lung Delivery Definitions
- Biologics Definitions
- Market and Clinical Landscape
- Nasal and Orally Inhaled Therapeutic Proteins
- Nasal and Orally Inhaled Nucleic Acids
- Nasal and Orally Inhaled Vaccines
- Nasal and Orally Inhaled Other Classes of Biologics
- Final Remarks

*Martins, A.C.; Albericio, F.; de la Torre, B.G. FDA Approvals of Biologics in 2022. Biomedicines 2023, 11, 1434

Introduction

- Nowadays, biologics have become crucial therapeutic agents, accounting for over 40%* of FDA approvals in 2022, a record high. They are expected to overtake small molecules and dominate the pharmaceutical market in the coming years.

- The **oral route** is very challenging because biologics often have unfavourable physico-chemical properties (reduced stability, both chemically and enzymatic, and poor g.i. permeability).

- The **parenteral route** remains the preferred method of administration for biologics, even for lung-related diseases.

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HOWEVER

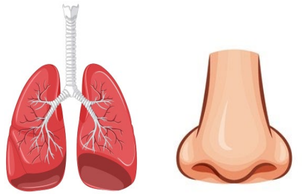
- Short half-life of protein and peptide in blood (often required frequent dosing)
- Invasive, Pain on administration
- Inconvenient (requirement of qualified personnel for administration)
- Sterility
- Poor patient compliance due to high proportion of people with needle phobia
- Requirement for cold chain
- Challenges in disposal of needles and syringes and other waste materials in particular in low resource settings

Parenteral delivery drawbacks

Alternative Route of Administration

Modern systems, such as autoinjectors, patch pumps, and advanced needles, can help overcome some of these challenges.

A possible alternative:..the nose and the lung.



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Among the non-invasive routes (oral, buccal, transdermal,…), **nasal and oral inhalation** represents one of the best alternative for local and systemic delivery of biologics with potential to treat various diseases for different reasons. Points of attention are not missing.

PROS

- **Directly target the airways** to maximize efficacy (site of action)
- Reduce risks of **needlestick injuries and blood-borne diseases**
- Don't require **specialized personnel**
- **Sterility:** Not required for non-aqueous products like DPIs and pMDIs.
- Intranasal and orally inhaled vaccines **trigger mucosal immune response**, offering better protection against respiratory infections.
- **High vascularization** of lungs and nasal cavity allows for **systemic delivery** of small proteins and peptides.
- **Well-established routes** for small drug molecules against respiratory diseases.
- Many **chemical structural modification** from parentererals are consolidated to address in particular stability (RNAs, Proteins)

CONS

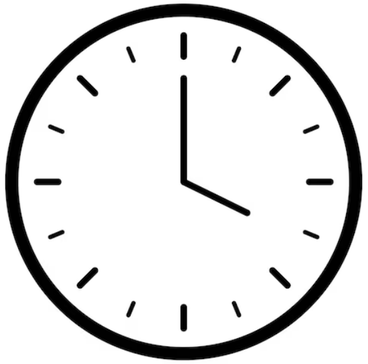
- **Complex structure and potential degradation** during inhalation delivery and mfg
- **Limited excipients approved** for stabilizing macromolecules for inhalation even through **particle engineering** techniques
- **Potential immunogenicity more pronounced in the lungs** compared to other routes
- Local lung treatment is more feasible, but **tissue penetration and systemic absorption are limited** by low stability, high MW, and hydrophilic properties
- PK/PD of biotherapuetics is difficult and particularly if the product is not intended for local delivery
- A **proper delivery** device is crucial (lesson learnt from Exubera®)
- **Higher costs**

Nasal and inhaled biologics has a great potential, testified by:

- Increased number of papers in the preclinical stage
- Increased number of clinical trials
- Increased number of dedicated events and conferences

While Market Authorization Applications for these products are not on par with those for parenterals, the resurgence of interest in this area suggests a promising future.

Nasal and Inhaled Biotherapeutics: hystorical launches



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1959	Synthocinon Nasal Spray – Synthetic Oxytocin Large Molecule – Not Biologic
1986	Colomycin Semi-Syntetic Colistimethate Sodium for Nebulization Large Molecule – Not Biologic
1993	Pulmozyme (Dornase Alfa) is the nebulized protein for inhalation launched on the market to treat Cystic Fibrosis First «true» Orally Inhaled Biologic
2003	Flumist – 1st live attenuated Nasal Vaccine First Nasal Vaccine
2006	Exubera (insulin for diabete) – first DPI containing a peptide as a Dry powder for inhalation - Launch and subsequent withdrawal (2007) First Biologic DPI
2014	Afrezza (insulin for diabete) – second DPI containing a peptide as a Dry powder for inhalation – Optimized Device still on the market
2020	Nasal Probiotics (Irrigation) – ProBioRinse First nasal Probiotic/Dietary Supplement
2021	Nasal Probiotics (Spray) - LiveSpo Navax Spray First nasal Probiotic/Medical Device
2022	Inhaled Viral Vector Vaccines for SARS-CoV2 (Convidecia Air in China) - First orally inhaled Vaccine

Drug Delivery and Translational Research (2022) 12:735–757

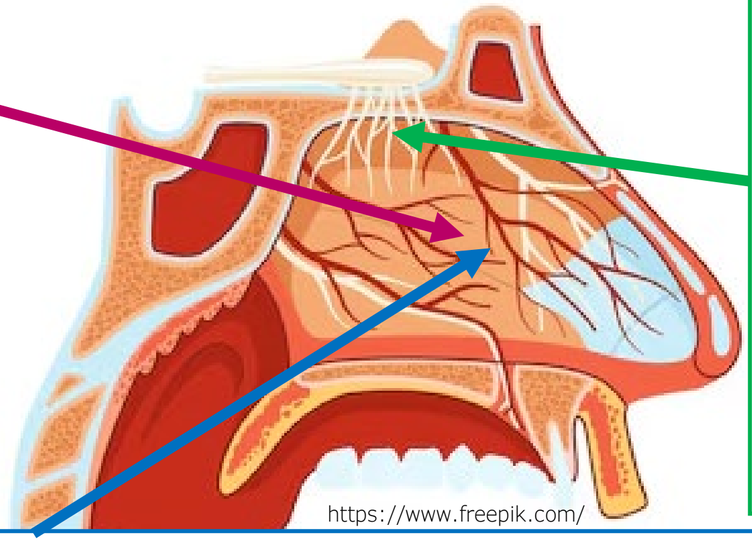
Nasal and Intranasal Drug Delivery

Nasal drug products are designed to deliver drugs into the nasal cavity.
These include various dosage forms such as nasal sprays, nasal drops, nasal aerosols, and nasal powders.

The nasal mucosa is easily accessible, abundant of blood vessels (for systemic drug absorption), and includes nerves which can be a direct passage to CNS.

Systemic

- Nasal mucosa **surface area > 150 cm² (highly vascularized)**
- **Rapid onset and higher bioavailability but** limited to low MW and lipophilic molecules
- Drugs can enter systemic circulation, **bypassing GI tract and first-pass** metabolism
- Currently marketed nasal peptides, for systemic use have MWs between 1.0-3.5 kDa.



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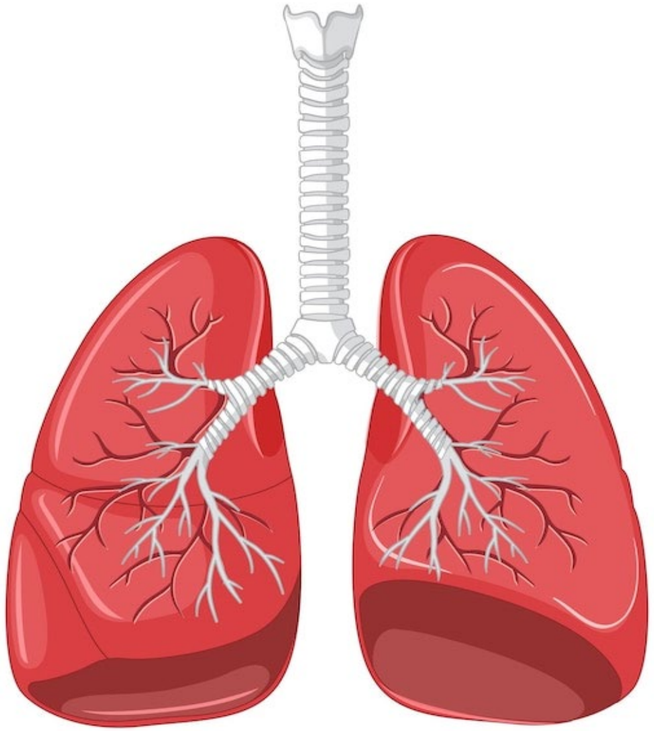
Nose to Brain Route (commonly referred to Intranasal)

- drugs inhaled into the nasal cavity and transported directly into the CNS.
- < 500 Da and lipophilic molecules cross the BBB (< 1% of macromolecules and only 2% of small molecules).
- Promising CNS diseases (e.g. Alzheimer, Parkinson)

Local

- Primarily for treating conditions in the nose/respiratory tract (rapid onset, reduced side effects)
- It gives mucosal immune response (vaccines)

Orally Inhaled Drug Delivery



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Pulmonary Delivery

- Orally inhaled drug products are typically constituted of aerosols (liquid droplets, solid particles) generated by systems such as DPIs, pMDIs, Nebulizers and Soft Mists.
- Particles of suitable size can reach different tracts of the airways up to the lung where traditionally the range is indicated between 1-5 µm.

Local Delivery to the lung

- Very attractive when the biological target is located in the lung (lung infections, asthma, COPD, …) to reduce the systemic exposure and side effects.
- Historically is the modality on which the most inhalable drugs on the market are delivered

Systemic Delivery through the lung

- Systemic Pulmonary drug delivery is a highly attractive offering unique advantages:
  - High bioavailability: The large surface area (70-100m^2) and high vascularization of the lung mucosa facilitate efficient drug absorption into the bloodstream (alternative to parenterals)
  - No first-pass effect: The drug bypasses the liver, avoiding initial metabolism.
  - Low proteolytic enzymes
  - PK/PD is more difficult to controlm for a biotherapeutic
  - Pain free and self administrable

Intratracheal Bolus Administration

- Direct instillation of the drugs directly into the trachea.
- It’s often used in research and clinical settings for direct delivery of medications to the lungs.
- Lung Surfactant replacement therapy uses this administration route in clinical setting.

*synthetic polypeptides <40aa are not considered biologics for FDA
**EMA considers mRNA as Gene Therapy
***Considered Biologics only if living organisms (often registered as medical device or Dietary Supplements)

Biologics, Macromolecules and ATMPs: Main Types

Small molecules

RNA/DNA Therapies (siRNA, mRNA**, …)

Peptides*

Proteins & enzymes

Lung Surfactants

Other Natural Extracts (e.g. Blood Derivatives)

Antibodies

Batteriophages

Viral Vector Vaccines

Microbiome Probiotics***

Somatic Cell therapies (SCTMP)

Gene Therapies (GTMP)

Tissue engineered product (TEP)

Xenotranplantation

Combined ATMP

Vaccines

Nucleic Acids

Therapeutic Proteins

Living Organisms (Virus, Bacteria)

Biologics

ATMPs

10^{-9} 10^{-8} 10^{-7} 10^{-6} 10^{-4} Size (meters)

1950s onward

Y2000 and onward

What is a biological product?

The United States and the European Union have distinct but overlapping schemes for the regulation of biologics, ranging from the definition of a biologic itself to the technical requirements for approval.

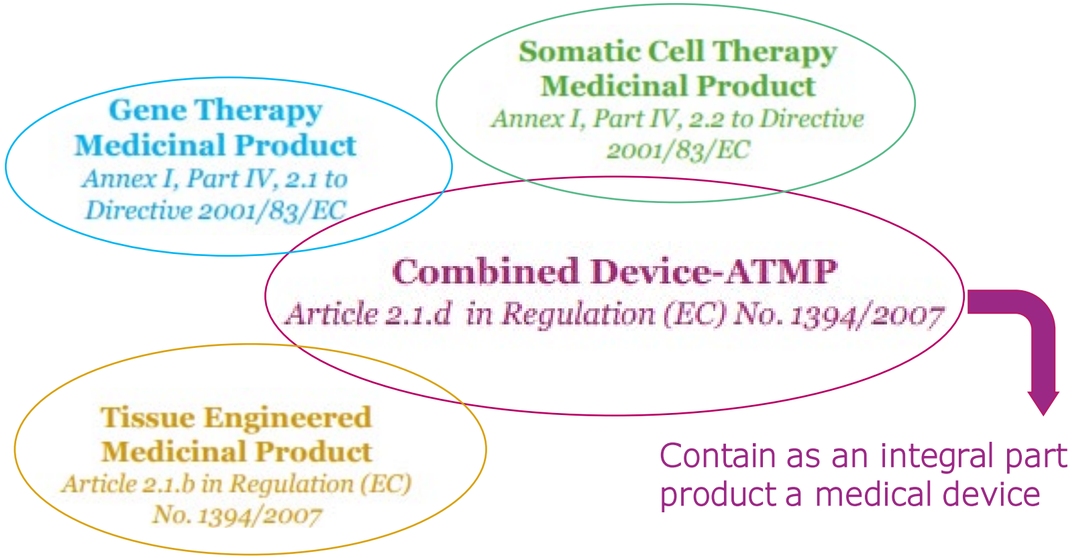
Definition by FDA

- Biological products are regulated by the Food and Drug Administration (FDA) and are used to diagnose, prevent, treat, and cure diseases and medical conditions
- Biological products are a diverse category of products and are generally large, complex molecules including a wide range of products such as vaccines, blood and blood components, allergenics, somatic cells, gene therapy, tissues, and recombinant proteins.
- Biologics can be composed of sugars, proteins, or nucleic acid or complex combinations of these substances, or may be living entities such as cells and tissues.
- Biologics are isolated from a variety of natural sources: human, animal, or microorganism, and may be produced by biotechnology methods and other cutting-edge technologies.

EMA definition is very similar but more high level:
"a medicine that contains one or more active substances made by or derived from a biological source"

Definition by EMA

ATMPs (Advanced Therapy Medicinal Products)



FDA has not a specific definition for ATMPs and the corrisponding products fall in the Biologics Category managed by CBER.

Landscaping Analysis

At the best of my possibilities I mapped around 217 products which fall in the definitions inhaled biologics or large molecules

Assumptions (inclusion and exclusion criteria):

✓ **Large Molecules (e.g. Synthetic Peptides or Proteins)**, although not always classified as biologics from a regulatory perspective, have been considered in the landscaping

✓ **Route of administration:** Nasal (Local, Systemic, Nose to Brain), Oral Inhaled Products (Local, Systemic)

✓ **Phase of development:** Marketed, Registered, Pre-registered, Clinical Development: Phase 3, Phase 2, Phase 1

☒ **Probiotics:** are not considered because their exploration is limited in clinics and are on the market as Dietary Supplement or Medical Device.

☒ **Lung surfactants** administered via endotracheal instillation are not included.

Cautionary Notes:

The products reviewed have been verified through ClinicalTrials.gov, the respective sponsor company websites, and with the available information on the web and im literature, to the best of my ability.

I apologize for any inaccuracies; the vast amount of information can sometimes be contradictory, incomplete, and changing over time, leading to potential errors, however, the intent is to create an archive, but to provide a general overview of current trends and offer a sense of scale for the discussion.

This snapshot captures the current landscape, which is subject to continuous evolution; what holds true today may change by tomorrow.

Market and Clinical Development Landscape: Drug Substances

TOTALS				
Class	Nasal		Lung	
	Counted Products	Percentage	Counted Products	Percentage
Vaccines	91	72.8%	10	10.9%
Phage Therapies	0	0.0%	6	6.5%
Gene Therapies, ATMPs	0	0.0%	5	5.4%
Nucleic Acids	1	0.8%	19	20.7%
Therapeutic Proteins	33	26.4%	52	56.5%
Total	**125**	**100.0%**	**92**	**100.0%**

At least half of the nasal vaccines that entered phase 1 and phase 2 did not progress to next phase.

15 RNA Therapies reached phase 2 but at least 6 have been halted:
ALN-RSV01
AROENac1001
Excellair
AIR645
Ionis ENAC 2.5Rx
TPU ASM8

Including products not progressed:

Aldesleukin, POL6014, EpoFc, VR942/UCB4144, GSK1995057

Including products discontinued:

Arginine-Vasopressin
Pralmorelin
PT141

Including products discontinued:

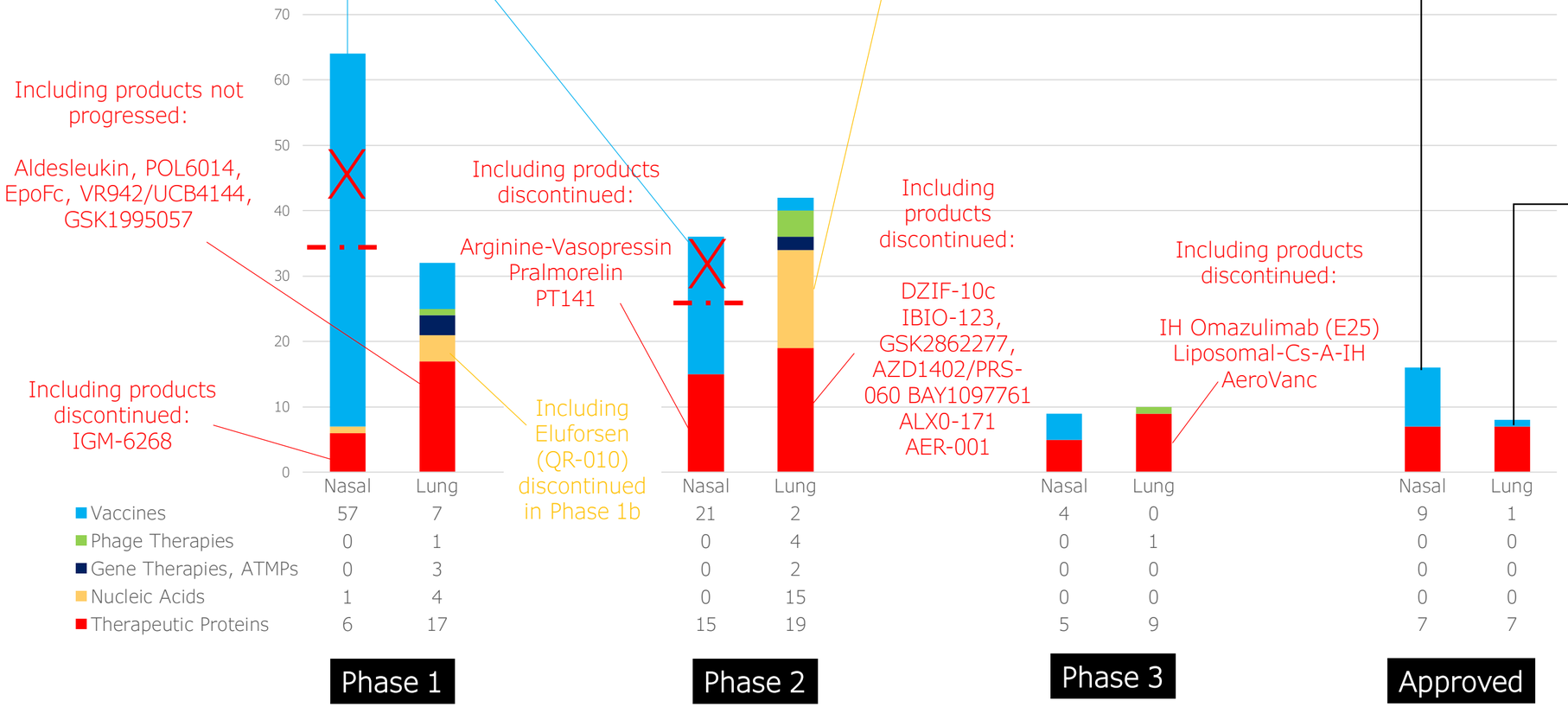
DZIF-10c
IBIO-123,
GSK2862277,
AZD1402/PRS-060 BAY1097761
ALX0-171
AER-001

Including products discontinued:

IH Omazulimab (E25)
Liposomal-Cs-A-IH
AeroVanc

Including products discontinued:
IGM-6268

Including Eluforsen (QR-010) discontinued in Phase 1b



NASAL PRODUCTS ON THE MARKET

- #5 **Influenza Vaccines** on the Market
- #1 **FluMist** withdrawn in 2016
- #1 **Pandemic Flu H5N1** approved by EMA for Preparedness
- **iNCOVACC** SARS-CoV-2 Vaccine (2022 India Area)
- **HeberNasvac** – Marketed i(2015 Cuba) - Chronic Hepatitis

- #7 Synhtetic Peptides* Not registered as biologic

ORALLY INHALED ON THE MARKET

- **Convidecia Air** - CanSinoBio (Viral Vector SARS-CoV-2 Vaccine) – Marketed in 2022 in China and other countries (No in EU and US)

- Insulin (**Afrezza** marketed in 2014 and **Exubera** marketed in 2006 and withdrawn in 2007)

- Dornase Alfa (**Pulmozime**) – rhDNAase (marketed in 1993)

- Sagramostim (**Sargmalin**, Marketed in 2024 – Only Japan)

- Colistimethate Sodium (**Colobreathe, Promixin**, …), Semisyntetic peptide * – Not registered as biologic

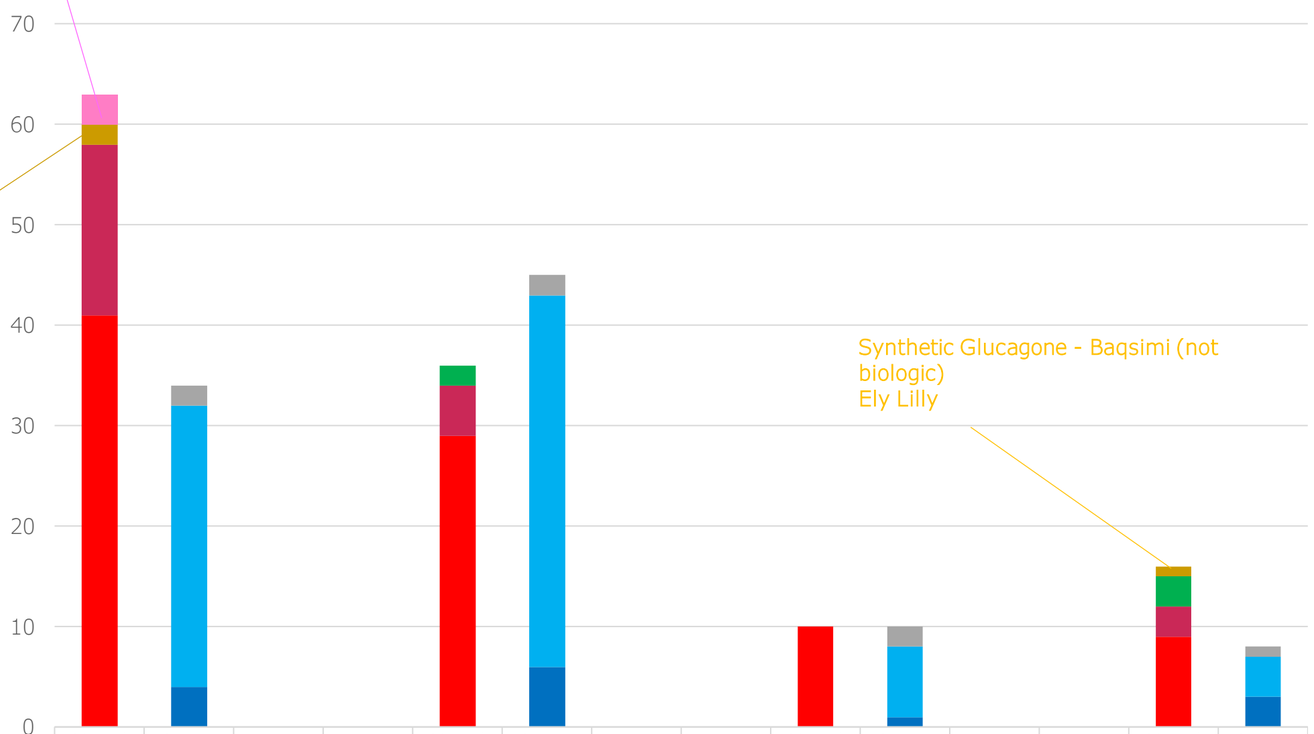
- Vancomycin – **Off Label Use** – Extracted glycopeptide* – Not considered as biologic

Out of 217 identified products, approximately 70 (42 nasal vaccines) have not progressed to the next phase. For some, there are clear statements indicating this, while for others, it can be inferred from the extended period of inactivity following the last reported clinical phase. These numbers should be considered indicative, as obtaining a reliable count is challenging.

Market and Clinical Development Landscape: Drug Products & Device

- FGF (Zhittya Genesis Medicine, Inc). – ViaNase Electronic Atomizer
- UniFluVec (Pharmenterprises Biotech LLC) – Nasal Aerosol (not disclosed)
- IGM/6268 Ig/M for SARS-CoV2

- Norwalk Vaccine
- delNS1-H5N1 Vaccine



		Nasal	Lung			Nasal	Lung			Nasal	Lung			Nasal	Lung
Lung	Powder to be reconstituted for Nebulization		2				2				2				1
	Nebulization		28				37				7				4
	DPI		4				6				1				3
Nasal	Intranasal Atomizers/Nebulizers	3													
	Nasal Powders	2				0								1	
	Powders to be reconstituted	0				2								3	
	Nasal Drops	17				5								3	
	Nasal Spray	41				29				10				9	

Total Nasal		
Nasal Delivery System	**Counted**	**Percentage**
Nasal Spray	89	71.0%
Nasal Drops	25	20.2%
Powders to be reconstituted	5	4.0%
Nasal Powders	3	2.4%
Intranasal Atomizers/Nebulizers	3	2.4%

Liquid formulations dominate in few cases to be reconstituted.

Typical device is a mechanical spray such (e.g. BD Accuspray or Aptar Nasal Sprays).

Few examples of Nasal Powders and Atomizers/Nebulizers.

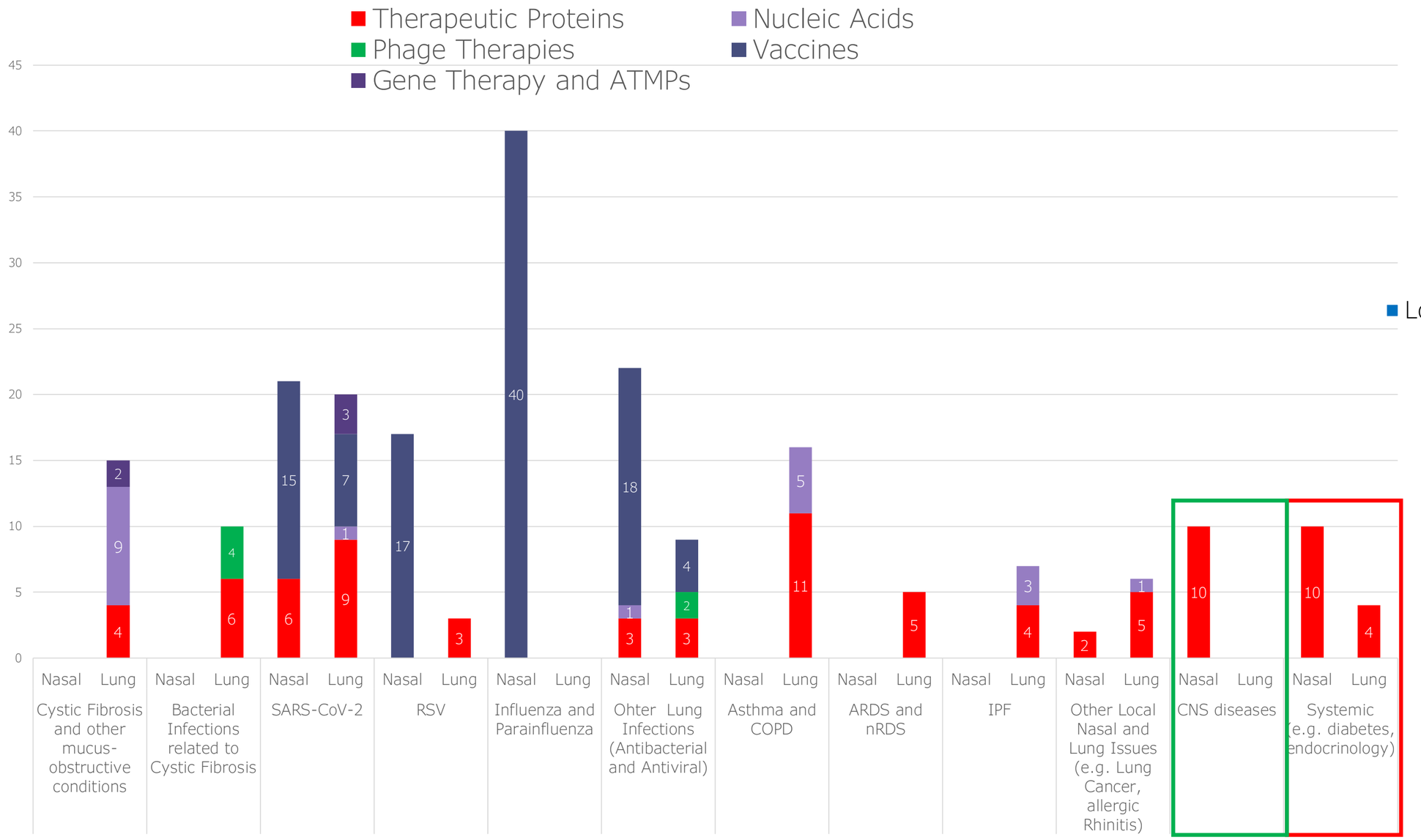
Total Lung		
Lung Delivery System	**Counted**	**Percentage**
DPI	14	14.4%
Nebulization (Liquids)	76	78.4%
Powder to be reconstituted for Nebulization	7	7.2%

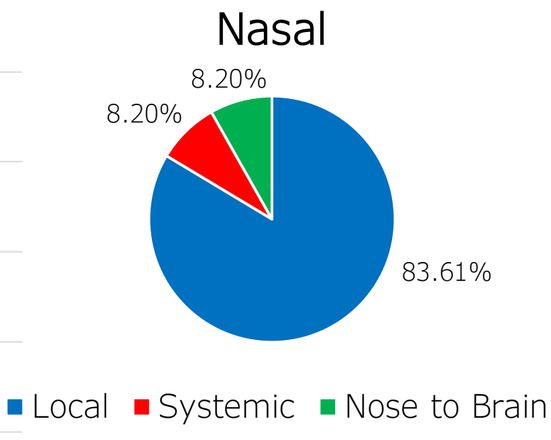
>80% of Biotherapeutics by nebulization
>94% vibrating mesh nebulizer (often customized and co-developed)
Most used Pari eFlow, Aerogen-Solo, I-neb AAD system.

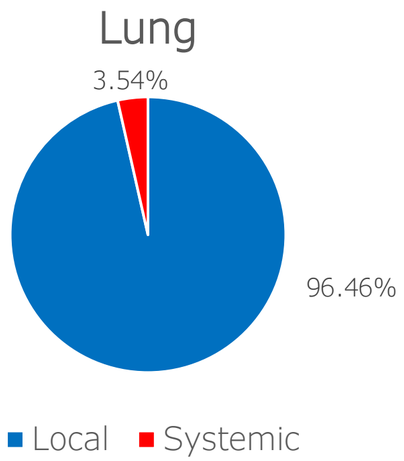
Single Unit Dose (Capsules, Blisters, Cartridges) preferred
Multi-Unit Dose explored only in 1 example (MMI-0100)
No Multidose Reservoir DPI Device Use

pMDI seems not to be a right choice for Biologics
Soft Mists promising but still in pre-clinical stage
New acoustic nebulizers are emerging but still at early stage.

Market and Clinical Development Landscape: Therapeutic Areas

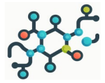
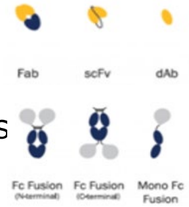


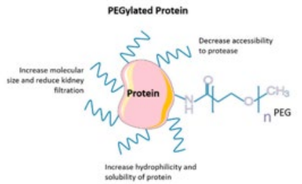




*May be not considered as Biologic

Classes of therapeutic proteins

Class	MW (kDa)	Description	Source	Nasal or Inhaled Examples
Peptides and Glicopeptides 	0.5-6	Typically 2-50 aa 3D structure varies but less defined than a large protein. Mainly Hormones.	Recombinant	Insulin (Afrezza) – DPI
			Synthetic*	Glucagon (Baqsimi) – Nasal Powder
			Extracted from natural sources	Inhaled Antimicrobial peptides (Vancomycin, Teicoplanin) – traditionally not considered biologics.
Miniproteins 	<20	Well defined 3D structure. They can be antibody mimetics such as affibodies, anticalins but also have different functions. Rarely extracted.	Recombinant	AZD1402/PRS-060: Phase 2 terminated in 2023
			Synthetic*	
Proteins and Glicoproteins 	5-100	Long chains of amino acids with complex secondary, tertiary and quarternary structure. Many functions (e.g. enzymes)	Recombinant	e.g. Dornase Alfa (Pulmozyme) - rhDNase Enzime
			Extracted from natural sources	e.g. Lactoferrin (glycoprotein used in ALX009 - Inhaled antibacterial in Phase 1 (NCT02598999) - Alaxia SAS - no news about P1 completion
Antibody Fragments 	sdAb (15 kDa) scFv (25-30kDa) Fab(50-60kDa) Minibodies (80kDa)	Parts of mAb, retaining the antigen binding specificity, but with smaller and simple structure and enhanced tissue penetration THs class includes: Nanobodies (sdAb), Single-Chain Variable Fragments (scFvs), Fragment Antigen Binding (Fab), Minibodies (Fc-Fusion)	Recombinant	fAb - AZD-8630 (AMG-104) in Phase 1 for Astma - Thymic stromal lymphopoietin inhibitors developed by AZ/Amgen as DPI
Antibody (mAb - IgG) 	Around 150	Large proteins that recognize specific targets in the body. Most often IgG, with a constant Fc region and a variable Fab region, which binds to a specific epitope or antigen. In some cases oligomers (IgM).	Recombinant	CT-P63 + CT-P66 (Celltrion) – Phase 3 (SARS-CoV-2)
		'Natural' polyclonal antibodies are produced by different lymphocytes in the immune system and recognize foreign substances in the body.	Extracted from natural sources	CSL-787 (CSL Behring) – Phase 1 for NCFB
Multispecific Antibody 	150-200	A multispecific antibody is a type of engineered antibody designed to recognize and bind to two or more different epitopes, which can be on the same or different targets.	Recombinant	Only 1 example found IGM-6268 (IGM Biosciences) failed in Phase 1 for SARS-CoV-2



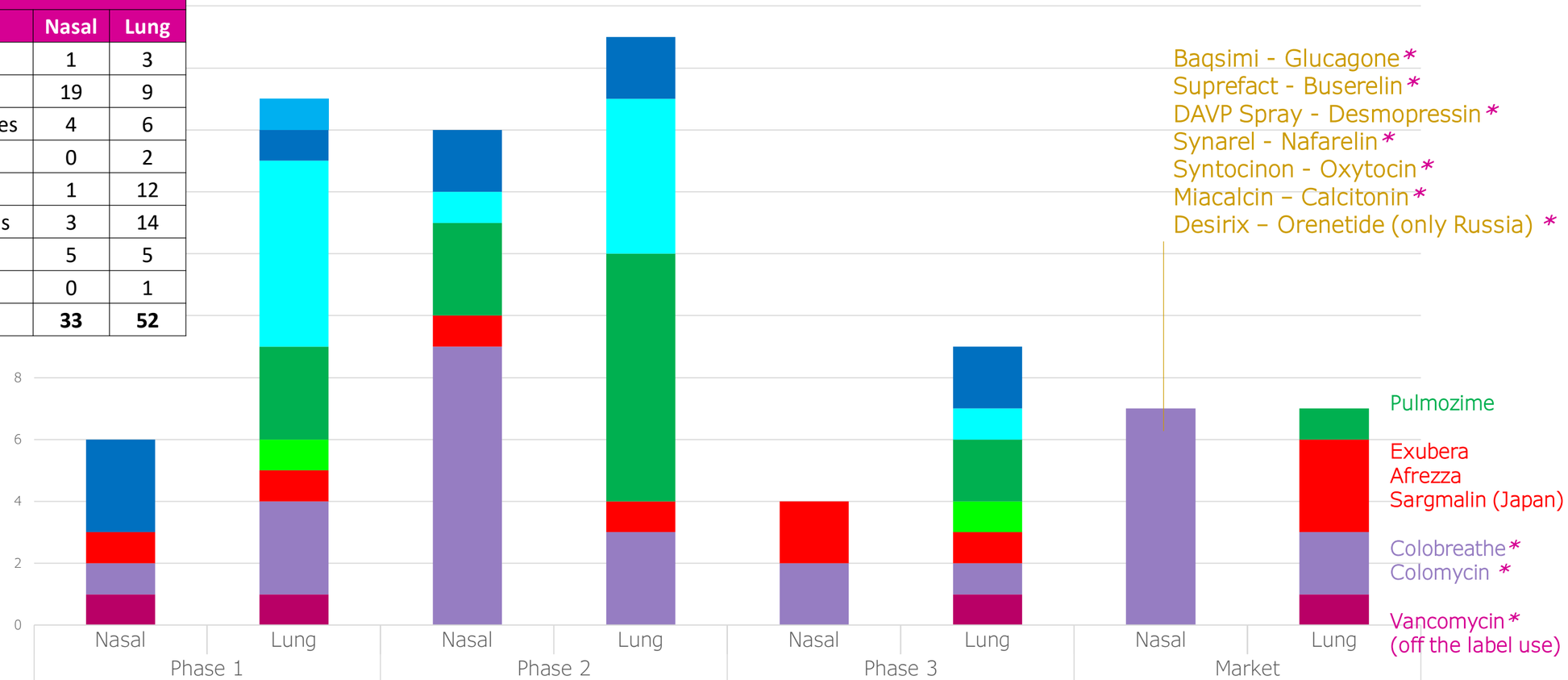
Protein Conjugation (e.g. PEG-ylation): longer duration of local effects, reduces dosing frequency, by mucoadhesion avoidance of alveolar macrophage uptake and decreases transepithelial transport to the bloodstream

e.g. BAY-1097761 (PEG-Adrenomedullin) – Lyo Powder for Nebulization - Terminated in Phase 2 for ARDS lack of efficacy

* *Not registered as biologic*

Marketed and Clinical Therapeutic Proteins: Drug Substances

Total		
Class of Therapeuric Proteins	**Nasal**	**Lung**
Extracted Peptides/Glycopeptides	1	3
Synthetic Peptides/Gkycopeptides	19	9
Recombinant Peptides/Glycopeptides	4	6
Extracted Proteins/Glycoproteins	0	2
Antibody Fragments	1	12
Recombinant Proteins/Glycoproteins	3	14
Recombinant mAb	5	5
Plasma Extracted mAb	0	1
Total	**33**	**52**



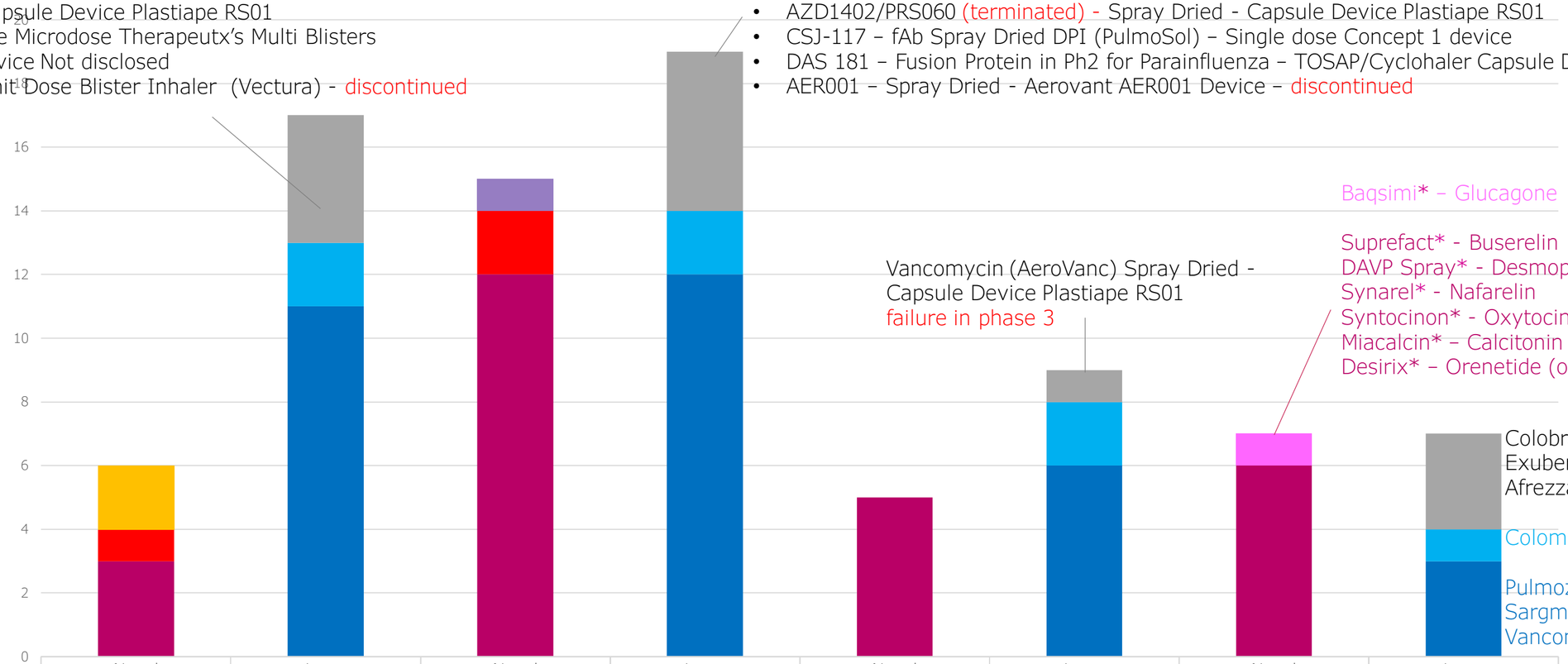
	Phase 1		Phase 2		Phase 3		Market	
	Nasal	Lung	Nasal	Lung	Nasal	Lung	Nasal	Lung
Plasma Extracted mAb		1						
Recombinant mAb	3	1	2	2		2		
Antibody Fragments		6	1	5		1		
Recombinant Proteins/Glycoproteins		3	3	8		2		1
Extracted Proteins/Glycoproteins		1				1		
Recombinant Peptides/Glycopeptides	1	1	1	1	2	1		3
Synthetic Peptides/Glycopeptides	1	3	9	3	2	1	7	2
Extracted Peptides/Glycopeptides	1	1				1		1

* *Not registered as biologic or may not be considered as biologic*

*TOSAP: Temperature-controlled organic assisted precipitation
**Colistimethate Sodium: Old semi-synthetic peptide (not considered biologic)

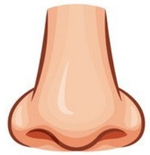
Marketed and Clinical Therapeutic Proteins: Drug Products

- LTI-03 – Pure API jet milled – Capsule Device Plastiape RS01
- MMI-0100 – Spray Dried – Maybe Microdose Therapeutx's Multi Blisters
- AZD-8630 – fAb Formulation/Device Not disclosed
- VR942- fAb Spray Dried - F1P Unit Dose Blister Inhaler (Vectura) - discontinued
- Oxytocin (Monash/GSK) -Spray Dried – Rotahaler DPI capsules
- AZD1402/PRS060 (terminated) - Spray Dried - Capsule Device Plastiape RS01
- CSJ-117 – fAb Spray Dried DPI (PulmoSol) – Single dose Concept 1 device
- DAS 181 – Fusion Protein in Ph2 for Parainfluenza – TOSAP/Cyclohaler Capsule Device
- AER001 – Spray Dried - Aerovant AER001 Device – discontinued



	Phase 1		Phase 2		Phase 3		Market		
	Nasal	Lung	Nasal	Lung	Nasal	Lung	Nasal	Lung	
DPI		4		5		1		3	Lung
Powder to be reconstituted for Nebulization		2		2		2		1	Lung
Nebulization		11		12		6		3	Lung
Intranasal Aerosol	2								Nasal
Nasal Dry Powders							1		Nasal
Powders to be reconstituted			1						Nasal
Nasal Drops	1		2						Nasal
Nasal Spray	3		12		5		6		Nasal

Therapeutic Proteins: Formulation



- Nasal Spray (simple liquid formulations) is the most common, sometimes reconstituted from lyo-powders.
- More sophisticated formulations (e.g. LNPs, Liposomes) reported for few cases (e.g. STI-2099 Covidrops mAb – Sorrento Therapeutics in Phase 1 ,Leukin-Enkephalin – Peptides in Phase 1 for Chronic Pain -Nanomerics Virpax Pharmaceuticals).
- 1 Dry Powder on the market (Baqsimi – Ely Lilly) - formulated with beta-cyclodextrin and dried by TFF (Thin Film Freezing)

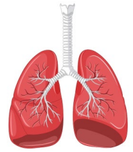
Market Clinical

- Nebulized products (simple liquid formulations) are the most common, sometimes reconstituted from lyo-powders
- Mesh Nebulizers are the preferred option in almost all the cases
- Few cases of liposomial formulations,
- Spray Drying is the most common technique to manufacture solid particles for inhalation, however only few cases of DPI in clinical trials, even though 2 insulin based products reached the market.

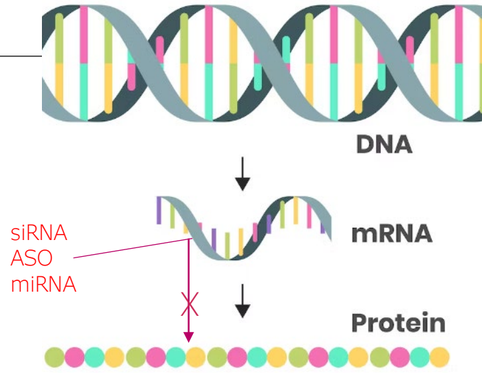
Preclinical & Lab Stage

Nano-carrier based nasal delivery for neurotherapeutic delivery (Solid LNPs, nanostructured lipid carriers, lipid-drug conjugates, …)
Mucoadhesive Formulations or In situ Gels: Prolonging residence time and sustaining release for a bettere bioavailability
Peptide and Protein Stability: Dry Powders
Advanced Delivery Device: e.g. Precision Spray Pumps for accurate dosing and efficient delivery of proteins

DPIs: Spray Drying, Spray Freeze Drying, Thin Film Freezing, SCF crystallization to get more stable and patient preferrable option.
Soft Mists: Great potential, even though limited to highly potent/low dosage proteins
Advanced Nebulizers: to minimize protein denaturation and degradation.
Nanoparticles: e.g. liposomes, polymeric nanoparticles, to enhance stability and bioavailability.
Protein Engineering: to improve stability and reduce immunogenicity.



Classes of Inhaled Nucleic Acids



- Single strand DNA, and RNA, works without the need to be integrated (gene therapy) or modifying (gene editing) the host genome.
- They are poorly permeable (charged) and vulnerable to degradation (RNAse substrates) and requires ofted chemical modifications and sometimes vectors (viral or non-viral).
- The main classes involved in nasal and orally inhaled product:

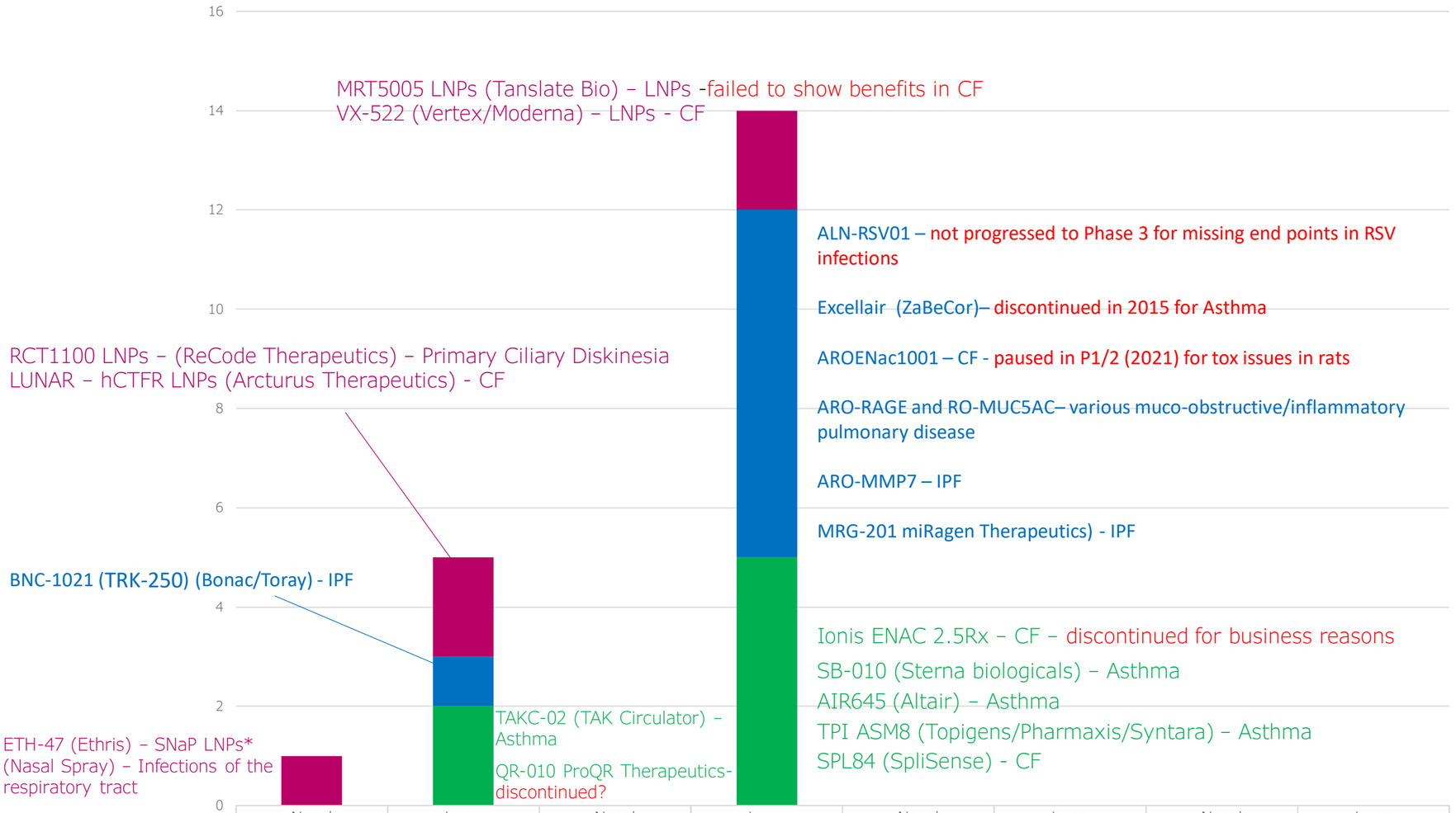
Type	Description	Size Range (nucleotides)	Mechanism of Action
DNA single strand - ASOs	Short single-stranded DNA or RNA that binds to a target mRNA through complementary base pairing, activating RNase H that leads to degradation of mRNA or modifying splicing and thereby preventing the translation of mRNA into protein.	13-25	RNAi: inhibition of gene expression
RNA - ASOs		13-25	RNAi: inhibition of gene expression
siRNA (Small Interfering RNA)	Short double-stranded RNA with two 3'-overhang nucleotides) in which the antisense strand binds to the target mRNA through complementary base pairing, preventing the translation of mRNA into protein via RNAi	21-23	RNAi: inhibition of gene expression
miRNA (Micro RNA)	Short RNA that is partially complementary to multiple messenger RNA (mRNA), preventing the translation ofmRNA into protein through the RNA interference mechanism. Similar to siRNA, miRNA also acts via the intracellular RNAi pathway but differs with regards to nonspecificity of mRNA pairing	18-24	RNAi: inhibition of gene expression
mRNA	mRNA that encodes proteins (e.g. antigen to elicit immune responses in the body for vaccines)	1k-15k	Protein Encoding

Gene dowregulation

Protein expression

*SNaP=Stabilized Nanoparticle Technology

Nucleic Acids in Clinical Development



ASOs/siRNAs/miRNAs are generally chemically modified with non-native nucleotides (often no need nanovectors)

mRNA are formulated in LNPs to be delivered both nasally and to the lung.

All the products are nebulized (except 1 nasal spray) and Vibrating Mesh Nebulizer (e.g. Pari eFlow) is the most commonly used aerosolization system.

Apparently at the moment no DPIs in clinical development for this class

None reached the market.

Intranasal and Inhaled Vaccines

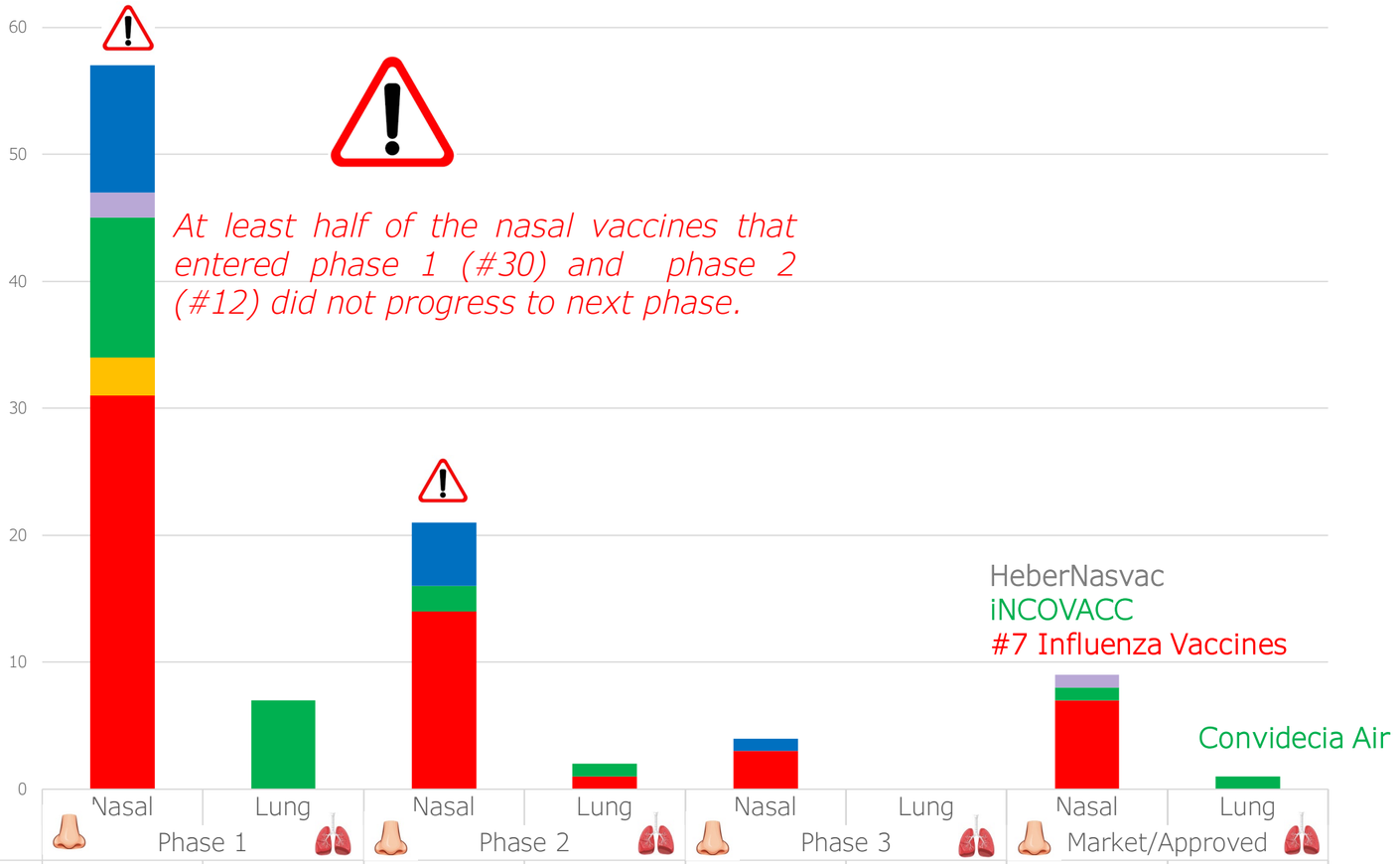
	Vaccine Platform	Description	Pros	Cons
Proteins	Virus-Like Particles 	Structural Proteins of the virus with antigenic power	Efficiency to penetrate cells No genetic material and risk of infections	Adjuvants often required to enhance the immune response.
Proteins	Subunit 	Viral antigens, typically proteins	No risk of infections	Adjuvants often required to enhance the immune response.
Nucleic Acids	DNA plasmid 	Transcribes and translates to the antigenic proteins	Adaptable to express different antigens No risk of infections	Requires a delivery vector Entry to the cells and nucleous could be challenging
Nucleic Acids	mRNA 	translates to the antigenic proteins	Adaptable to express different antigens No risk of infections	Requires a delivery vector Entry to the cells could be challenging
Virus	Viral Vector (e.g. adenovirus) 	Low pathogenic viruses with modified genome encoding antigenic proteins	Replicating or non-replicating Efficiency to penetrate cells	Immunogenicity can be compromised if a prior exposure to the viral vector
Bacteria	Live attenuated* 	Virus able to replicate but modified to be at reduced pathogenicity.	Strong Immunogenicity Longer Immune Response	No for people with compromised immunity Risk of reactivation
Bacteria	Inactivated* 	Virus particles «killed» by chemicals, heat or radiation.	Strong Immunogenicity	Adjuvants maybe required to enhance the immune response.

- Traditionally vaccines are injectables (potent systemic immunity).
- Nasal and Orally Inhaled vaccines more efficient in eliciting immune responses at the respiratory mucosa resembling the natural infection.
- Different classes of vaccines, requires specific delivery systems and in analogy to the specific chemical/biological structure.
- Virus Based Vaccines are usually formulated in solution similarly to the injecatable one.

Research Trends:

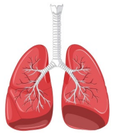
- Explore other vaccine types via inhalation (Subunit, Virus Particles, mRNA, DNA plasmid)
- Develop mRNA vaccine platforms for nasal and inhaled delivery
- Increase Immunity Activation (Adjuvants)
- Improve stability and Shelf-Life
- Innovative Delivery Systems (Polymer NPs of Chitosan or PLGA, Polysaccharide NPs (Maltodextrins), LNPs Liposomes,
- Spray Drying and Spray Freeze Drying for Nasal Dry Powders and DPI

Nasal and Inhaled Vaccines: Market and Clinical Development Landscape



	Phase 1		Phase 2		Phase 3		Market/Approved	
	Nasal	Lung	Nasal	Lung	Nasal	Lung	Nasal	Lung
Virus Subunit Vaccine	10		5	0	1			
Virus-Like Particles Vaccine	2						1	
Virus Vector Vaccine	11	7	2	1			1	1
Inactivated Virus Vaccine	3							
Live Attenuated Virus Vaccine	31		14	1	3		7	

- Nasal route is more explored than oral inhalation
- More than 80% are nasal spray, 20% Nasal Drops
- A couple of cases of powders (Freeze Dried) and 1 nebulized are reported

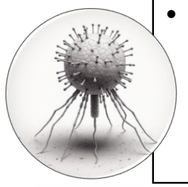


- Mesh Nebulizers are the preferred option to deliver orally inhaled vaccine.
- Only 1 DPI retrieved in Phase 2 as DPI (Measles Vaccine -Puffhaler/Solovent)

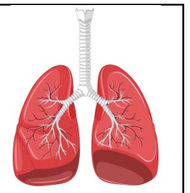
Other Classes of Biologics: Nasal and Orally Inhaled

Probiotics (Bacteria, Fungi and Virus): products that contain living microorganisms that, when administered at the correct quantity, benefit the host's health (WHO).

- considered Biologic only if living organisms
- generally registered as Dietary Supplements or Medical Device
- many probiotics delivered orally (gut-lung axys) even though delivered for respiratory conditions.
- nasal route (Sprays, Irrigations)) has been relatively underexplored in clinical research



- **Bacteriophages (Phages)** are highly specialised in targeting bacteria and kill them.
  - Promising against MDR
  - Local treatment may be better than systemic or oral delivery (more explored for Phage Therapy)
  - Only 6 clinical studies found. All of them nebulized into the lung.
  - Concern about uncontrolled surge in endotoxin release (pro-inflammation) up to cytokine storm and immune response.

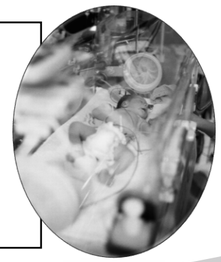


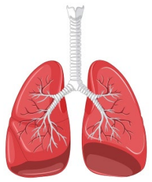
- ATMPs

ClinicalTrials.gov reference	Drug	Company/Sponsor	Development Phase	Target Disease	Chemical Class
NCT05248230	4D-710	4D Molecular Therapeutics	Phase 1/2	Cystic Fibrosis	Gene Therapy
NCT05504837	KB-407	Krystal Biotech, Inc.	Phase 1	Cystic Fibrosis	Gene Therapy
NCT04276987	Exosomes derived from Stem Cells	Ruijin Hospital	Phase 1	Novel CoronaVirus Pneumonia	Exosomes
NCT04389385	Exosomes from Allogenic COVID-19 T-Cell	TC Erciyes University	Phase 1	SARS-CoV2	Exosomes
NCT04473170	Autologous Non Hematopoietic Peripheral Blood Stem Cells	Abu Dhabi Stem Cells Center	Phase 1/2	SARS-CoV2	Stem Cells

- Combination product development is considered a complex undertaking, but a prudent approach to the development of ATMP/device combinations paying particular attention to the selection of the aerosol generator devices from early in the program.

- Lung Surfactants
  - Surfactant Replacement Therapy (SRT), especially preterm babies, who suffer from respiratory distress syndrome (RDS).
  - Natural Lung Surfactants are extracted from animal lungs (Porcine, Bovine) and fall in the biologics definition (at least 6 marketed)
  - Synthetic version are present in the market and are not considerable as biologics.
  - Generally administered as endotracheal instillation of bolus through catheters (ETT, LISA, INSURE) – e.g. Curosurf, Infasurf, Survanta
  - Aerosolization explored in Trials.





*Lung Surfactants and probiotics are not included in the list of 217

Final Remarks (1/2) – Drug Substances

- Among the different classes of Biologics, including large molecules not strictly classified by Regulatory agencies as biologics, around 217 products (market and clinics) have been identified that can be delivered trough the nose or the lung*.
- The classification is complex and not always easy, and the term ‘inhaled biologics,’ which has become very popular in recent days, encompasses a wide range of products that are very different from each other.
- Therapeutic Peptides and Proteins are more explored and mature, with examples on the market, as well as Vaccines (Nasal in particular).
- Lung Surfactants directly instilled into the trachea (neonatal RDS) are a niche class with several examples available on the market.
- All the other classes (Nucleic Acids, Bacteriophages, ATMPs) have not yet reached the market, with RNA therapies more explored in clinical setting and very promising for the future.
- Systemic Delivery (and nose to brain for nasal) is possible and successful but is seems limited to the simpler classes such as peptides and may require some innovative delivery system.
- Nasal Probiotics are available on the market but often not registered as drug/biologics.
- Costs, Time and Risks associated to development of an inhaled Biotherapeutic is in general significantly higher than an inhaled small molecule.

*For vaccines, the induction of an immune response is obviously the desired effect

Final Remarks (2/2) – Drug Products

- Degradation (stability), resistance to mechanical and thermal stress, and induction of immune response* complicate nasal and pulmonary delivery of biologics and are the main common challenges in this field for almost all the classes.

- Liquid Formulations are the main delivery systems both for nasal (sprays) and for lung (nebulization) with a large predominance of Mesh Nebulizers (often customized/co-developed).

- Indeed nebulization is often the quickest approach in the early stage of clinical development (P1 and P2a) even though, often not the preferred commercial presentation.

- Making a dry powder could improve the product shelf-life and it is often the preferred option for the market, for patient acceptance, but it requires challenging drying steps to isolate solid inhalable particles, and often biotherapeutics degradate during the mfg process.

- Spray Drying is the most explored technique, but other are utilized (e.g. Tecnospheres, Thin Film Freezing, Spray Freeze Drying,…)

- DPI Unit Devices (e.g. Capsules, Blisters) seems to be more protective for a powder and easy to develop (no carrier based formulations).

- Nanoparticles could help in improving stability and bioavailability of many Biotherapeutics, but with the exception of few cases (e.g. mRNA), the most of the work is in preclinical stage.

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BACK UP

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**Probiotics has a complex classification and depend on the claims that are made for the product.*
Not Living probiotics are in general registered as Dietary Supplements (US)/(Food Supplements (EU). Depending on the classification, development requirements vary.

Biologics, Macromolecules and ATMPs: Main Types

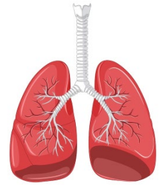
	EMA	FDA
Synthetic Peptides	Not a biologic if structure, mechanism and manufacturing are simple.- (CHMP) Biologics if structure, mechanism and manufacturing are not simple.- (CHMP)	Not a Biologic (CDER)
Animal Origin or Recombinant Peptides (e.g. Insulin)	Biologic (CHMP)	Biologic (CDER)
Synthetic Proteins	Not a biologic if structure, mechanism and manufacturing are simple.- (CHMP) Biologics if structure, mechanism and manufacturing are not simple.- (CHMP)	Not a Biologic (CDER)
Animal Origin or Recombinant Proteins	Biologic (CHMP)	Biologic (CDER)
Recombinant mAb	Biologic (CHMP)	Bioloigc (CDER)
Natural Extract (e.g. Lung Surfactant)	Biologic (CHMP)	Biologic (CBER)
siRNA	Biologic (CHMP)	Not a Biologic (CDER)
miRNA	Biologic (CHMP)	Not a Biologic (CDER)
ASO	Biologic (CHMP)	Not a Biologic (CDER)
mRNA	Biologic (CHMP)	Biologic (CBER) and considered as Gene Therapy
Microbiome (Live Probiotics)*	Food Supplement (Directorate-General for Health and Food Safety (DG SANTE) Medical Device – EMA Medical Devices Division Medicinal Product/Biologic(CHMP)	Dietary Supplements (Office of Dietary Supplement Programs – ODSP) Medical Device, (Centre for Devices and Radiological Health (CDRH) Drugs (CDER)/Biologic (CBER)*
Bacteriophages	Biologic (CHMP)	Biologic (CBER)
Vaccines	Biologic (CHMP)	Biologic (CBER)
Gene Therapy Medicines	ATMP (CAT)	Biologic (CBER)
Somatic-cell therapy medicines	ATMP (CAT)	Biologic (CBER)
Tissue-engineered medicines	ATMP (CAT)	Biologic (CBER)
Xenotransplantation	Not specifically mentioned, but fall in ATMP (CAT)	Biologic (CBER)
Combined ATMPs	ATMP (CAT)	Combination Products (OCP: Office of Combination products and CBER)

MARKETED PRODUCTS: Nasal (1/2)

Drug Name	Trade Name	Company	Chemical Class	Launch Date and Country	Delivery Route/Formulation/Device	Therapeutic Indication
Trivalent Influenza Vaccine	FluMist	MedImmune, LLC/Astra Zeneca	Live Attenuated Virus Vaccine	(2003, withdrawn in 2016	Refrigerated Solutions - Single-dose pre-filled intranasal syringe sprayer (Becton Dickinson (BD) Accuspray™ delivery device)	Influenza Vaccine
Quadrivalent Influenza Vaccine	FluMist Quadrivalent	MedImmune, LLC/Astra Zeneca	Live Attenuated Virus Vaccine	2013 (US)	Refrigerated Solutions - Single-dose pre-filled intranasal syringe sprayer (Becton Dickinson (BD) Accuspray™ delivery device)	Influenza Vaccine
Quadrivalent Influenza Vaccine	Fluenz Tetra	MedImmune, LLC/Astra Zeneca	Live Attenuated Virus Vaccine	2013 (EU)	Refrigerated Solutions - Single-dose pre-filled intranasal syringe sprayer (Becton Dickinson (BD) Accuspray™ delivery device)	Influenza Vaccine
H5N1 Influenza Vaccine	Pandemic Influenza Vaccine	MedImmune, LLC/Astra Zeneca	Live Attenuated Virus Vaccine	2016 approved for preparedness by EMA (EU)	Single-dose pre-filled intranasal sprayer (Becton Dickinson (BD) Accuspray™ delivery device)	Influenza Vaccine
trivalent inactivated influenza vaccine	Influenza Vaccine, Live, Nasal, Freeze-dried	Changchun BCHT Biotechnology Co.	Live Attenuated Virus Vaccine	2021 (China)	Nasal Spray: Nasal Lyophilized Powder to be reconstituted	Influenza Vaccine
	Nasovac-S	Serum Institute of India Pvt. Ltd/MyLab Discovery Solutions Pvt. Ltd	Live Attenuated Influenza Vaccine	2013 (India area)	Nasal Spray (Vial and Syringe to spray) - lyophilized form able to be reconstituted with sterile water in a homogeneous suspension	Influenza Vaccine
	Nasovac-S4	Serum Institute of India Pvt. Ltd/MyLab Discovery Solutions Pvt. Ltd	Live Attenuated Influenza Vaccine	2021 (India area)	Nasal Spray (Vial and Syringe to spray) - Refrigerated Liquid Vial to be extraxted and sprayed by a syringe.	Influenza Vaccine
	HeberNasvac	Center for Genetic Engineering and Biotechnology (CIGB) in Cuba	Virus-Like Particles Vaccine	2015 (Cuba)	Nasal Drops	Chronic Hepatitis Vaccine
	iNCOVACC	Bharat Biotech	Viral Vector Vaccine	2022 (India)	SARS-CoV-2 Vaccine	SARS-CoV-2 Vaccine

MARKETED PRODUCTS: Nasal (2/2) NOT REGISTERED AS BIOLOGICS

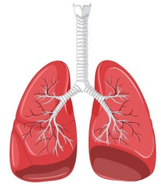
Drug Name	Trade Name	Company	Chemical Class	Launch Date and Country	Delivery Route/Formulation/Device	Therapeutic Indication
Buserelin Acetate	SuprefactTM (solution/ spray), Suprecur® (solution/ spray)	Sanofi-Aventis Hoechst Roussel	Synthetic Peptide	Market (1st launch 1988)	Nasal Solution or Spray	Endometriosis, prostate cancer, Prostate cancer, breast cancer, endometriosis, Uterine fibroids
Desmopressin Acetate	DAVP® Spray, Minirin, Desmospray	Ferring Pharmaceuticals Pharmaceuticals LLC	Synthetic Peptide	Market (1st Launch 1978)	Nasal Spray: Aptar V3 Multidose Spray Pump	Diabetes Insipidus, Haemophilia A, Nocturia, central cranial diabetes insipidus
Nafarelin Acetate	Synarel	Pfizer G.D. Searle LLC Synthex Inc.	Synthetic Peptide	Market (1990)	Nasal Spray: Aptar V3 Multidose Spray Pump	Fertility, endometriosis, Central precocious, puberty, Endometriosis
Oxytocin	Syntocinon, Oxitocina Hikma	viatris, Hikima, Orifarm	Synthetic Peptide	Market (1959 CH)	Nasal Spray: Aptar V3 Multidose Spray Pump	Start/Streengthrn uterinie contraction duringn labour
Salmon Calcitonin	Miacalcin, Fortical, Calcimar	Novartis, Upsher-Smith Laboratories, Mylan	Synthetic Peptide	Market (1986)	Nasal Spray: Aptar V3 Multidose Spray Pump	Osteoporosis, Paget's disease,
Glucagon	Baqsimi	Eli Lilly & Co. Ltd.	Synthetic Peptide	Market (launch 2019)	beta-ciclodestrina (E459); dodecil-fosfocolina (agente surfattante) - Nasal Dry Powder (single-use, pre-filled)	Severe Hypoglycaemia
BP-101 Orenetide	Desirix	Ovoca Bio / Ivix	Synthetic Peptide	2022 (Russia)	Nasal Spray	Sexual dysfunction, female



MARKETED PRODUCTS: LUNG

Drug Name	Trade Name	Company	Chemical Class	Launch Date and Country	Delivery Route/Formulation/Device	Therapeutic Indication
Recombinant adenovirus type-5 vectored vaccine	Convidecia® air	CanSinoBio	Viral Vector Vaccine	2022 (china)	Nebulization (Trachea)	SARS-CoV-2 Vaccine
Dornase alfa	Dornase alfa	Genentech/Roche	Recombinant Protein	1993	Jet nebuliser/air compressor combinations and eRapid™ nebuliser systema and AKITA2 APIXNEB®	Cystic Fibrosis
Colistimethate* sodium	Tadim, Colomycin, Colifin, Promixin, Colfinair		Semi-Synthetic Peptide	1986	Lyo-powder to be reconstituted	MDR bacterial infections in CF
Colistimethate* sodium	Colobreathe®	TEVA UK Ltd	Semi-Synthetic Peptide	2012	Jet milled API	MDR bacterial infections in CF
Insulin	Afrezza	Sanofi/MannKind	Recombinant Peptide	2006 (withdrawn 2007)	Technosphere DPI - Dreamboat® inhaler	Diabetes mellitus
Insulin	Exubera	Pfizer/Nektar Therapeutics	Recombinant Peptide	2014	Spray Dried DPI	Diabetes mellitus
GM-CSF (Sargramostim)	Sargmalin	Nobelpharma Co., Ltd	Recombinant GlycoPeptide	2024 (Japan)	Mesh Nebulizer	aPAP Autoimmune Pulmonary Alveolar Proteinosis
Vancomycin*	Parenteral Injection Vancocin, Vancoled, Firvanq.	Stony Brook University and Other Hospital/Academia	Extracted Glycopeptide	Off Label Use	jet nebulizer or a vibrating mesh nebulizer: Pari LC Plus and eFlow rapid nebulisers	Treatment of MRSA infections in CF patients

*Not registered as Biologics



EXAMPLES OF DPI PRODUCTS IN CLINICAL DEVELOPMENT

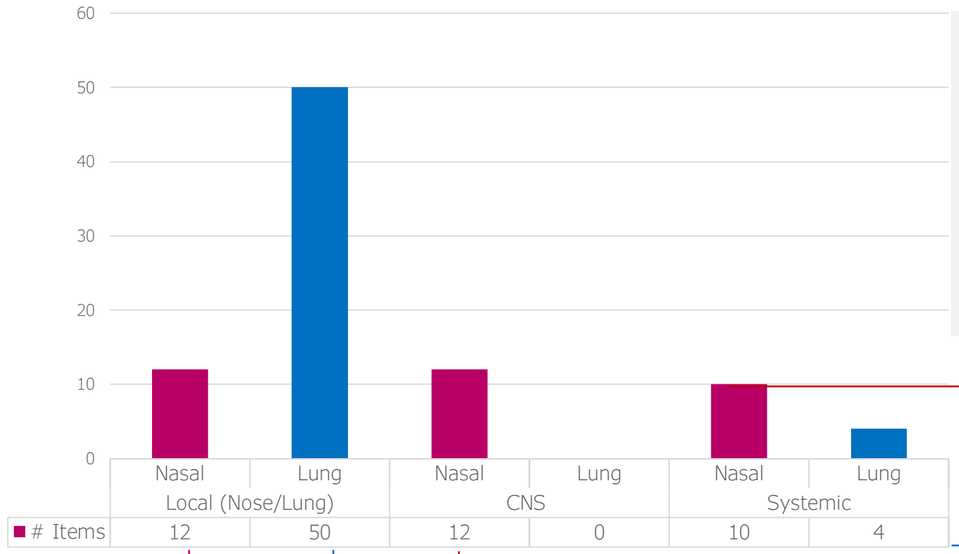
Drug Name	Clinical Phase	Reference NCT	Company	Chemical Class	Delivery Route/Formulation/Device	Therapeutic Indication
LTI-03(CSP-7)*	Phase 1b	NCT05954988, NCT04233814	Lung Therapeutics, Inc	Synthetic Peptide	Pure API jet milled – Capsule Device Plastiape RS01	IPF
MMI-0100*	Phase 1a	NCT02515396	Moerae Matrix, Inc.	Synthetic Peptide	Spray Dried – Microdose Therapeutx's MultiUnit Blisters Device	IPF
Oxytocin*	Phase 1/2	NCT02999100	Monash/GlaxoSmithKline	Synthetic Peptide	Spray Dried – Rotahaler DPI capsules	Hemorragiae Post Partum
AZD1402/PRS-060 Elarekibep	Phase 2a (terminated in 2023 for tox issues)	Pieris and AZ communication on website	AstraZeneca & Pieris Pharmaceuticals	Recombinant Protein	Spray Dried - Capsule Device Plastiape RS01	Asthma
Pitrakinra (AER-001)	Phase 2b (discontinued)	NCT00801853	Aerovance, Inc.	Recombinant Peptide	Powder Not disclosed - Aerovant AER001 Device	Asthma
DAS181 Fludase®	Phase 2	NCT01924793	Ansun BioPharma	Recombinant Fusion Protein	TOSAP Temperature-controlled organic assisted precipitation – Cyclohaler Capsule Device	Parainfluenza virus infection
AZD-8630 (AMG-104)	Phase 1	NCT05110976	AstraZeneca; Amgen	Recombinant fAb	Powder Not disclosed – Monodose Inhaler not disclosed	Asthma
CSJ117 Ecleralimab	Phase 2b	NCT04410523, NCT04882124, NCT04946318	Novartis/Morphosys	Recombinant fAb	Spray Dried DPI (PulmoSol) – Single dose Concept 1 device	COPD (discontinued for Asthma)
VR942/UCB4144/CDP7766 abrezekimab	Phase 1 (Phase 2 not conducted)	NCT02473939	UCB Pharma/Vectura	Recombinant fAb	Spray Dried - F1P Unit Dose Blister Inhaler (Vectura)	Asthma
Measles Vaccination	Phase 2	NCT01557699	Serum Institute of India Pvt. Ltd.WHO/Bill&Melinda Gates Foundation/NIH	Live Attenuated Virus Vaccine	Bubble Dryer® supercritical fluid drying - Puffhaler® or a Solvent™ DPI Inhaler	Vaccine
Vancomycin (AeroVanc)	Phase 3 (failure to meet endpoint)	NCT03181932	Savara Inc.	Extracted Glycopeptide	Spray Dried - Capsule Device Plastiape RS01	Methycillin-resistant Staphylococcus Aureus (MRSA) in CF

*Likely not considered as Biologics

EXAMPLES OF CLINICAL NASAL POWDERS and NASAL PRODUCT ATOMIZED/NEBULIZED

Drug Name	Clinical Phase	Reference NCT	Company	Chemical Class	Delivery Route/Formulation/Device	Therapeutic Indication
Norwalk VLP vaccine	Phase 1	NCT00806962, NCT00973284	Takeda (LigoCyte Pharmaceuticals, Inc.)	Virus-Like Particles Vaccine	Monophosphoryl lipid A (MPL): An adjuvant that enhances the immune response. Chitosan: A mucoadhesive agent that helps the vaccine adhere to the mucosal surfaces. Drying obtained by TFF - Thin Film Freezing - Nasal Bespak Dry Powder Delivery Device	Norovirus infections. Norwalk virus is a common cause of acute gastroenteritis.
delNS1-H5N1	Phase 1	NCT01258062	Resilience Government Services, Inc./Ology Bioservices	Inactivated Virus Vaccine	GelVac™ nasal powder H5N1 influenza vaccine. GelVacTM nasal powder H5N1 influenza vaccine, evaluation of frequency and severity of the adverse effects	Influenza type A (H5N1)
IGM-6268	Phase 1 (discontinued)	NCT05184218	IGM Biosciences	Recombinant mAb (IgM)	Teleflex Mucosal Atomization Device Nasal™	SARS-CoV-2
UniFluVec	Phase 1	NCT04650971	Pharmenterprises Biotech LLC	Live Attenuated Virus Vaccine	Suspension of virus not disclosed - Nasal aerosol	Influenza Vaccine
FGF - Fibroblast Growth Factor	Phase 1	NCT05493462	Zhittya Genesis Medicine, Inc.	Recombinant Peptide	ViaNase® Electronic Atomizer	Parkinson's (Compassionate Use)

Therapeutic Proteins: MW and Administration Route



- Lung Delivery of therapeutic proteins is mostly directed toward local treatment of lung related diseases.
- Few examples of delivery for systemic Disease, but possible and in some cases successful.
- Nasal delivery investigation is balanced between local, systemic and nose to brain.
- General Indication: r**ange of suitability for inhalation** is from 1-2 kDa to 40-45 kDa, even though it's possible to find examples of larger proteins in development.

All of them are synthetic peptides (1-4 kDa)
Some of them are marketed

Few examples of larger proteins

e.g.
IGM-6268 (IGM Biosciences) – dicontinued after Phase 1 for SARS-CoV-2

SA55 (Sinova Biotech Ltd) in Phase 2 for SARS CoV 2.

Mostly Investigated for Peptides (1-4kDa)
1 example is FGF (17-18 kDa) developed by Zhittya Genesis Medicine, Inc. in Phase 1 for Parkinson and Foralumab (150 kDa) developed by Tiziana Life Sciences/Merck in Phase 2 for Multiple sclerosis, Alzheimer and Dementia

Around 30% of the investigated drugs are peptides <6 kDa (only 3 discontinuation reported)
Arounf 70% are 10<MW<80 kDa: at least 9 of them discontinued in Phase 1 or 2
6 mAbs (4 in development for SARS-CoV2 Infection – 2 of them discontinued in Phase 2). CSL-787 (CSL Behring) is in Phase for NCFB.
Omaluzimab was tested in Phase 3 as nebulized product but unsuccessfully.

2 Insulins (5.7 kDa) are Afrezza and Exubera that reached the Market.

EpoFC (60 kDa) developed in Phase 1 by Synthonix Pharmaceuticals was discontinued after the acquisitio of Biogen.

Monash University is developing an inhaled Oxytocin for Haemorragia Post Partum

RNA Therapies: Pros and Cons

Recombinant proteins have limitations as drugs, particularly due to size and stability issues (Antosova et al., 2009; Lam et al.,2015). Furthermore, they must be properly folded and often require post-translational modifications (Li et al., 2015) that complicate the synthetic process.

RNA therapies can be considered a disruptive groups of therapeutic technologies (a new revolution after recombinant proteins 50 years ago), allowing small biotech startups, as well as academic groups, to rapidly develop new and personalized constructs.

RNA Therapies can be manufactured in vitro and delivered without the use of living organisms such as viruses or cells.

Pros	Cons
RNAs Therapies don't need to penetrate the nuclear membrane as DNA therapeutics and hence there is no risk of chromosomal integration.	Rapid degradation of exogenous RNA by RNases that are ubiquitous in the environment and tissues.
Ability to act on targets that are otherwise "undruggable" for a small molecule or a protein.	Delivery of negatively charged RNA across hydrophobic cytoplasmic membrane could be difficult.
Rapid and cost effective development, by comparison to that of small molecules or recombinant proteins.	Strong immunogenicity of exogenous RNA that caused cell toxicity and impaired translation into therapeutic proteins.
Ability to rapidly alter the sequence of the RNA construct for personalized treatments or to adapt to an evolving pathogen.	

ASOs that reached Clinical Development

NCT	Drug Name	Company (Date of Launch)	Chemical Class	Formulation	Clinical Phase	Delivery Route/Device	Therapeutic Indication
NCT05018533	TAKC-02 (LNA-anti-Mex-3B)	University of Tokyo; Tak-Circulator	ASO	Naked chemically modified ASO, simple buffered solution	Phase 1	Pari eFlow Nebulizer	Asthma
NCT02532764	Eluforsen (QR-010)	ProQR Therapeutics	ASO	Naked chemically modified ASO, simple buffered solution	Phase 1b (discontinued?)	Pari eFlow Nebulizer	Cystic Fibrosis /F508 del)
NCT03647228	Ionis ENAC 2.5Rx	Ionis Pharmaceuticals	ASO	Naked chemically modified ASO, simple buffered solution	Phase 1/Phase 2 (discontinued for business reasons)	Pari eFlow Nebulizer	Cystic Fibrosis
NCT01743768	SB-010	Sterna Biologicals	ASO	Naked chemically modified ASO, simple buffered solution	Phase 2	ActiVero FAVORITE Nebulizer	Asthma
NCT00941577	AIR645	Altair Pharmaceutics	ASO	Naked chemically modified ASO, simple buffered solution	Phase 2	Not disclosed Nebulizer	Asthma
NCT00822861	TPI ASM8	Pharmaxis/Syntara	ASO	Naked chemically modified ASO, simple buffered solution	Phase 2	Pari eFlow Nebulizer	Asthma
NCT06429176	SPL84	SpliSense Ltd.	ASO	Naked chemically modified ASO, simple buffered solution	Phase 2	Pari eFlow Nebulizer	Cystic Fibrosis

ASO are delivered in clinics as nebulized product to the Lung. No Nasal delivery examples found in literature.
No DPI products found in clinics.
All the products this list are nebulized by using vibrating mesh nebulizers.
Chemically modified ASOs apparently don’ t require nanovectors, they are delivered naked, in simple solutions with buffers, stabilizers, …

siRNA and miRNA that reached Clinical Development

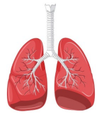
NCT	Drug Name	Company (Date of Launch)	Chemical Class	Formulation	Clinical Phase	Delivery Route/Device	Therapeutic Indication
NCT01065935	ALN-RSV01	Alnylan Pharmaceutics	siRNA	Naked siRNA, chemically modified	Phase 2b (not progressed to Phase 3 because end points missed)	investigational eFlow(R) Nebulizer System (PARI Pharma).	Respiratory syncytial virus (RSV) infection
Trends in Pharmacological Sciences, October 2020, Vol. 41, No. 10 https://doi.org/10.1016/j.tips.2020.08.002	Excellair	ZaBeCor Pharmaceuticals	siRNA	Naked siRNA, chemically modified	Phase 2 (discontinued in 2015)	Pari eFlow	asthma
NCT03727802	BNC-1021 (TRK-250)	Bonac Corp; Toray Industries	siRNA	Naked siRNA, chemically modified	Phase 1	Mesh Nebulizer	IPF
NCT05292950	ARO-MUC5AC	Arrowhead Pharmaceuticals	siRNA	Arrowhead's proprietary Targeted RNAi Molecule (TRiM™) modified siRNA conjugated to targeting ligands	Phase 1/2a	Mesh Nebulizer	Various muco-obstructive and inflammatory pulmonary diseases.
NCT04375514	AROENaC1001	Arrowhead Pharmaceuticals	siRNA	Arrowhead's proprietary Targeted RNAi Molecule (TRiM™) modified siRNA conjugated to targeting ligands	Phase 1/2 (paused in 2021 for inflammation in chronic tox in rats)	Mesh Nebulizer	Cistyc Fibrosis
NCT05276570	ARO-RAGE	Arrowhead Pharmaceuticals	siRNA	Arrowhead's proprietary Targeted RNAi Molecule (TRiM™) modified siRNA conjugated to targeting ligands	Phase 1/2	Mesh Nebulizer	Various muco-obstructive and inflammatory pulmonary diseases.
NCT05537025	ARO-MMP7	Arrowhead Pharmaceuticals	siRNA	Arrowhead's proprietary Targeted RNAi Molecule (TRiM™) modified siRNA conjugated to targeting ligands	Phase 1/2	Mesh Nebulizer	IPF
NCT03601052	MRG-201	miRagen Therapeutics	synthetic miRNA mimic	Naked siRNA, chemically modified	Phase 2	Mesh Nebulizer	IPF

Similar considerations as ASOs.

To be noticed Arrowhead's TRiM™ platform: modified siRNA conjugated to targeting ligands, ensuring precise tissue-specific gene silencing. This enhances therapeutic effects while minimizing off-target impacts.

mRNA that reached Clinical Development

NCT	Drug Name	Company (Date of Launch)	Chemical Class	Formulation	Clinical Phase	Delivery Route/Device	Therapeutic Indication
NCT05737485	RCT1100	ReCode Therapeutics	mRNA	LNPs	Phase 1	Pari eFlow Nebulizer	Primary Ciliary Dyskinesia
https://arcturusrx.com/mrna-medicines-pipeline/	LUNAR®-hCFTR	Arcturus Therapeutics	mRNA	LNPs	Phase 1	Mesh Nebulizer	Cystic Fibrosis
https://www.ethris.com/news/eth47-first-in-human-dosing/	ETH-47	Ethris	mRNA	SNaP LNP technology	Phase 1	NASAL Spray	Infection, respiratory tract
NCT03375047	MRT5005	Translate Bio (Sanofi)	mRNA	LNPs	Phase 1/Phase 2 (Fast Track Deignation Failed to show benefits)	Pari eFlow Nebulizer	Cystic Fibrosis
NCT05668741	VX-522	Vertex/Moderna	mRNA	LNPs	Phase 1/Phase 2	Mesh Nebulizer	Cystic Fibrosis





Among 5 products found, 1 is a nasal spray.

Despite siRNA and ASOs, mRNA are formulated in LNPs to be delivered both nasally and to the lung.

For nebulized product Mesh Nebulizer seems the most commonly used as ell as siRNA and

No DPIs in clinical decelopment also for this class.

Probiotics

Probiotics (Bacteria, Fungi and Virus): are defined as quantifiable live bacteria with evidence for health benefits at either a strain or group level, according to the International Scientific Association for Probiotics and Prebiotics.

Probiotics are generally registered as Dietary Supplements and, therefore, do not require Clinical Trials. Occasionally, Clinical Trials are conducted either to register them as drugs with proven efficacy or for marketing purposes.

Probiotics are considered Biologics only if they are living organisms.

Many probiotics are delivered orally (gut-lung axys) even though they are delivered for respiratory conditions.

The nasal and inhaled delivery route is less explored, but being more direct it could exert a greater protective response against viral respiratory infections even though it is still not clear their efficacy and safety, when delivered into the airways.

Preclinical investigations on:

Intranasal administration of probiotics has resulted in positive outcomes in respiratory infections with animal models (*Lactobacillus rhamnosus, casei, pentosus and plantarum* - influenza virus (IFV).

Oral delivery of Probiotics has a certain effect also on respiratory tract (gut-lung axis), nasal and lung delivery seems to effective in mice.

Studies on respiratory delivery of probiotics in humans are limited and the translatability of animal data to humans requires investigation.

Dry powders could be in principle helpful to stabilize formulations and avoid cold chain (Spray Drying, Freeze Drying).however to maintain 90% viable probiotics, temperature shouldn't exceed 70°C.

Data in humans are still limited, and nasal administration more explored than lung delivery for allergic rhinitis and rhinoconjunctivitis.

Typical Formulations are (Nasal Sprays, Nasal Irrigations)

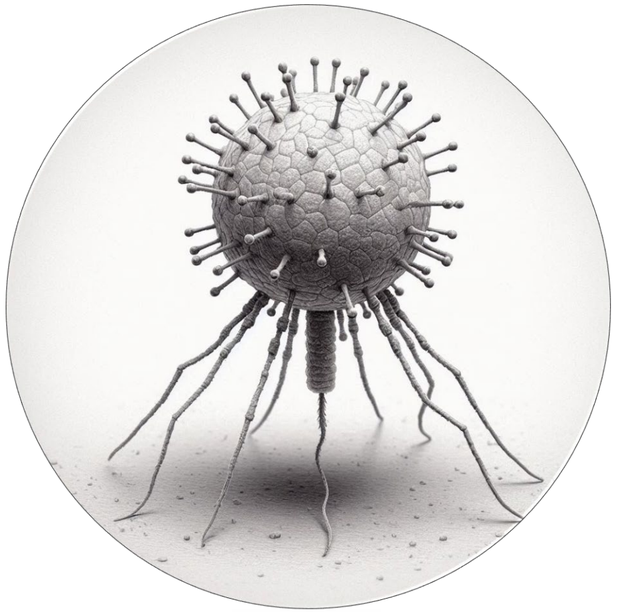
Due to difficulties in formulating probiotics for lung delivery, this delivery route seems still not explored in a clinical setting.

Symbioflor Nasal Spray - Enterococcus faecalis – SymbioPharm GmbH
Many registered as Dietary Supplements

No product in clinics and on the market

Bacteriophages

- Bacteriophages (phages) are highly specialised in targeting bacteria
- Rising concerns regarding antibiotic resistance have fueled research on using bacteriophages to combat this significant threat.
- Numerous in vitro and in vivo studies demonstrating the efficacy against MDR infections.
- Delivering bacteriophages directly to the site of infections (such as pneumonia) seems to be very promising (more effective and faster and non-invasive)) even though still less explored than other delivery routes (oral and intravenous)
- Main concern is they could cause an uncontrolled surge in endotoxin release (pro-inflammation) up to cytokine storm and immune response.



"obligate intracellular parasite of bacteria", model proposed in 1917 by the French-Canadian microbiologist, Félix d'Hérelle.

Most abundant life form, with about 10_{31} phage particles on Earth

Preclinical investigations on:

- Studies in synergy with antibiotics
- Storage Temperature is a critical point
- Freeze Drying proved to be suitable but not able to generate inhalable particles.
- Spray drying, on the other hand, is a one-step process that can produce inhalable phage powders with only a moderate titre loss.

Clinical Investigations

Success of phage therapy, especially against *P. aeruginosa,* was observed in humans, but only through intravenous administration.
Other delivery routes (oral and topical) have been investigated.
The inhalation route is less explored with only few studies reporting its efficacy.

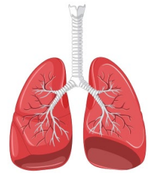
Even though explored in pre-clinical studies, nasal delivery apparently has not been investigated in a clinical setting.

All the Phages in clinics are nebulized as sterile liquids, even though formulations are not easily retrievable as well as the type of nebu

On the Market

Phages provide a promising treatment against AMR as some phages have synergy with antibiotics.
Synergistic use of Phages with Antibiotics could be even more advisable.
However at the moment, no phage therapies are approved on the market, including nasal and inhaled ones.

- In the West, the development was interrupted by WWII after the advent of antibiotics while in the East (Russia, Georgia, Poland) there is a longer history with phage therapy
- Specific guidelines to produce Phage Therapies in GMP are still missing.
- This could be one of the reasons for the hesitation in administering phages in humans and this is true also for nasal and inhaled ones.



Main Natural Lung Surfactants

Trade Name	Chemical Name	Company	Market/Stage of Development	Source	Delivery System
Survanta	Beractant	Abbvie Inc.	Market	Natural (Bovine)	ETT, INSURE, LISA
Curosurf	Poractant alfa	Chiesi Farmaceutici SpA	Market	Natural (Porcine)	ETT, INSURE, LISA
Infasurf	Calfactant	ONY Biotech Inc.	Market	Natural (Bovine)	ETT
Alveofact	Bovactant	Lyomark Pharma GmbH	Market	Natural (Bovine)	ETT, LISA
BLES	Bovine Lipid Extract Surfactant	BLES Biochemicals Inc.	Market	Natural (Bovine)	ETT, INSURE, LISA
KeLiSu	Calsurf	CR Double-Crane Pharmaceuticals Co., Ltd	Market	Natural (Bovine)	ETT, LISA
InfasurfAero	Calfactant	ONY Biotech Inc.	Phase 3	Natural (Bovine)	InfasurfAero nebulizer (modified Solarys nebulizer + pacifier)
AeroFact	Bovactant	Lyomark Pharma; Aerogen Pharma Limited	Phase 2b	Natural (Bovine)	Aerogen's PDAP™ delivery technology combined with a synchronization device (Grasby capsule)
Curoneb	Poractant alfa	Chiesi Farmaceutici SpA	Phase 2 (DISCONTINUED)	Natural (Porcine)	eFlow Neos

Other minor natural Lung Surfactant and Synthetic version are not reported in the table.