Preclinical Toxicology: Navigating the Respiratory Tract Barrier

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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

Overview

- Respiratory tract as a barrier
- Regulations for preclinical toxicology
  - Study design and endpoints
  - Aerosol characterization
- Considerations for inhaled biologics
  - Toxicokinetics and dose
  - Immunology
  - Safety pharmacology
  - Pathology

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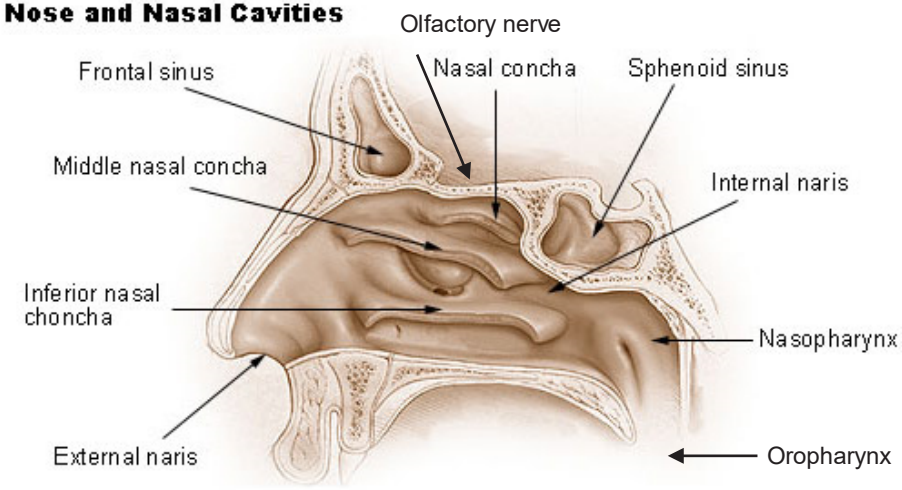
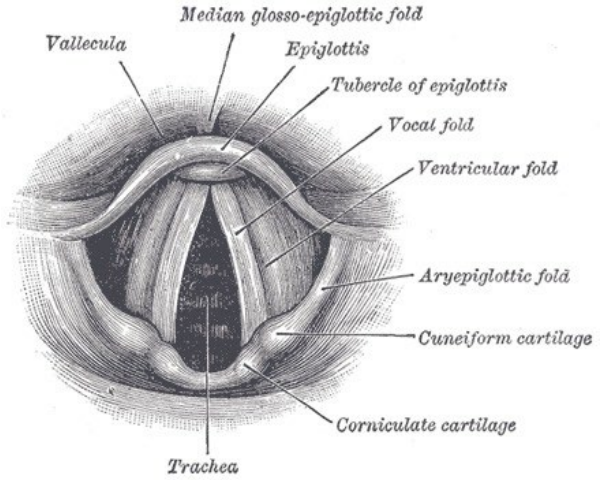
Anatomy provides barrier function

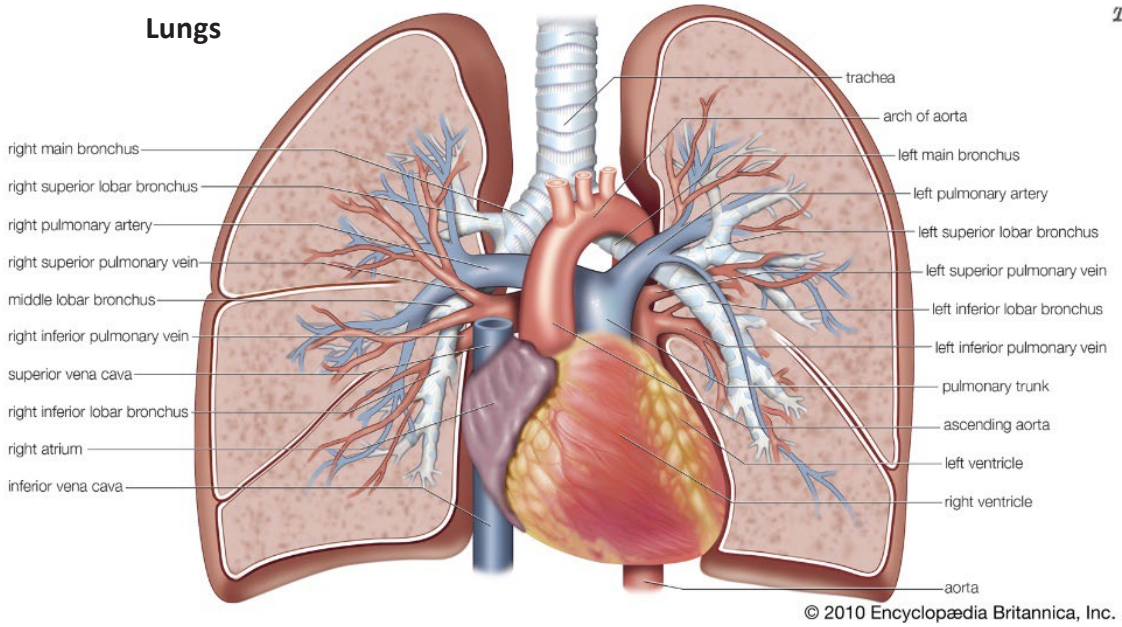
Nose and Nasal Cavities



Larynx



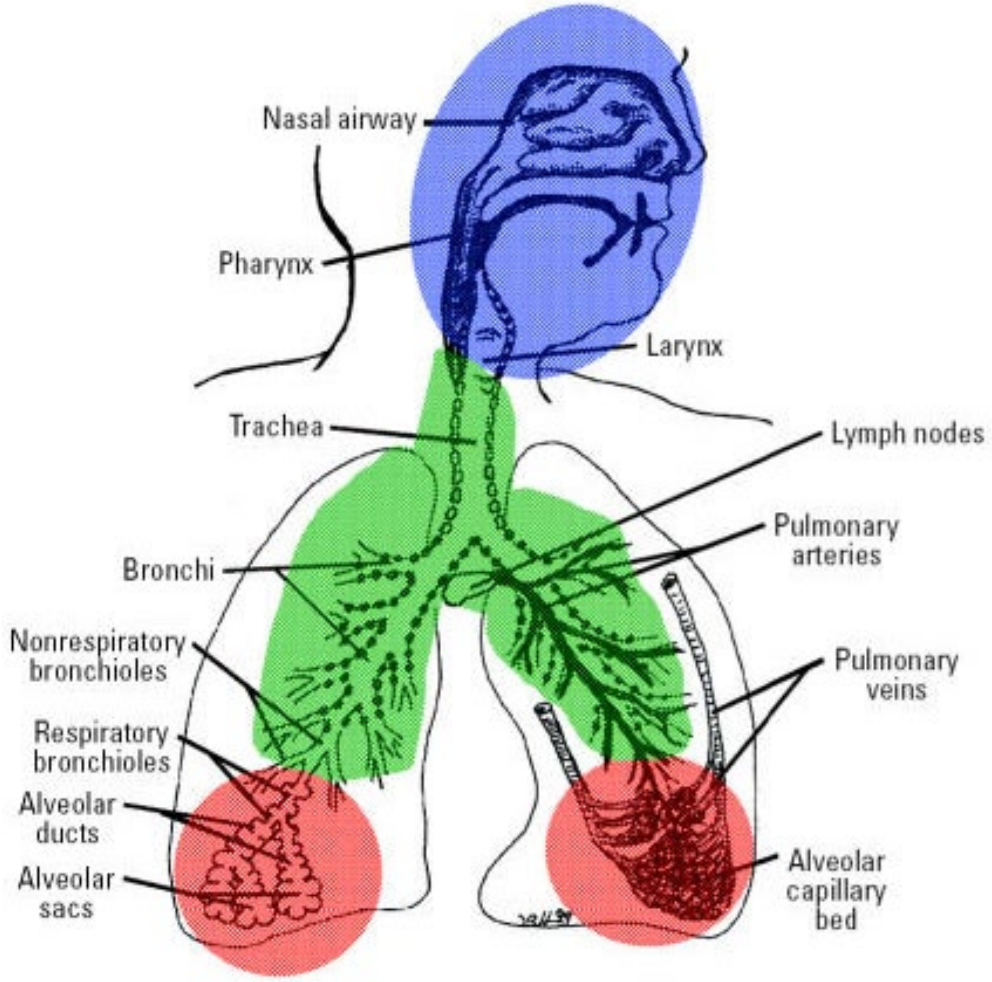
Lungs

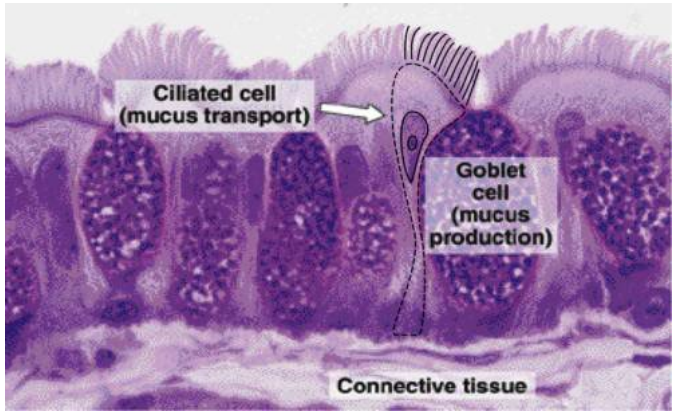


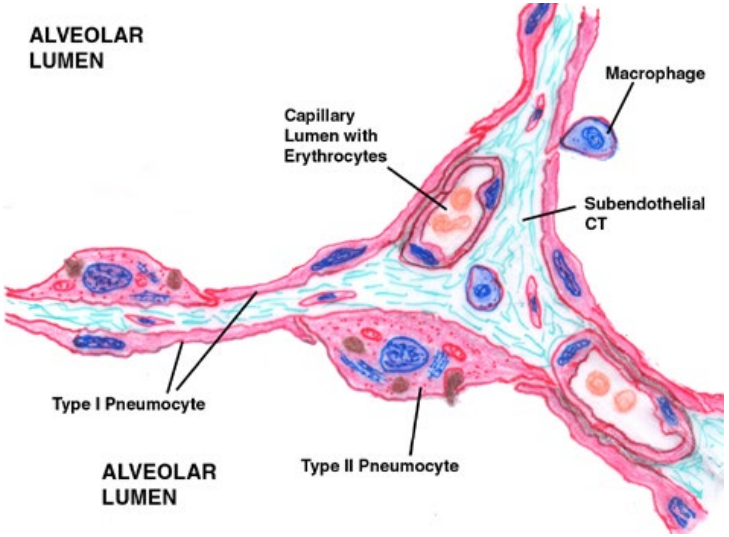
https://training.seer.cancer.gov/anatomy/respiratory/passages.html

https://www.ncbi.nlm.nih.gov/books/NBK535342

Tissue- to cellular-level anatomy







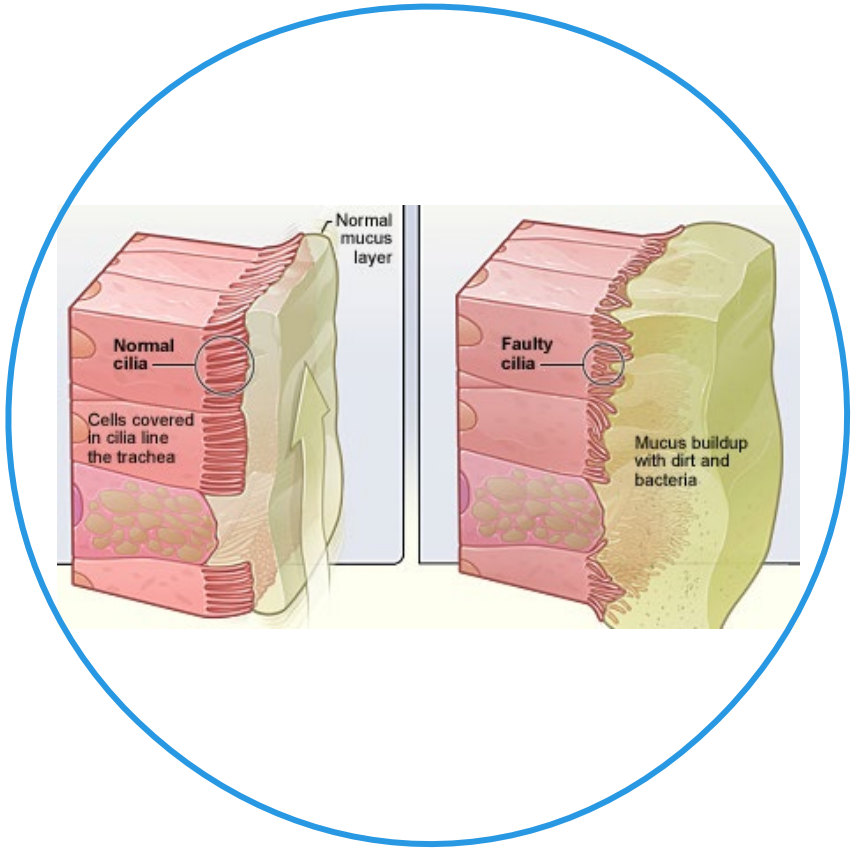
Oberdorster et al. *Environ Health Perspect*, (2005)
https://accessmedicine.mhmedical.com/content.aspx?bookid=3390&sectionid=281539645
beyondthedish.files.wordpress.com

Barriers due to disease condition

Respiratory diseases

- Cystic fibrosis
- Primary ciliary dyskinesia
- Chronic obstructive pulmonary disease
- Cancer
- Alpha-1 antitrypsin deficiency (AAT)
- Bronchopulmonary dysplasia
- Pulmonary fibrosis

- Overcome by
  - Gene vector surface coating, nanoparticle coating/charge, size
  - Targeting ligands – increase (or decrease) uptake
  - Osmotically active excipients

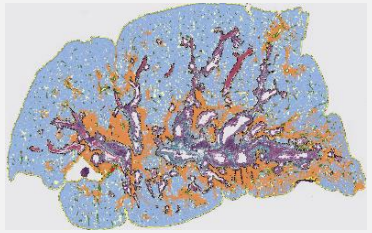


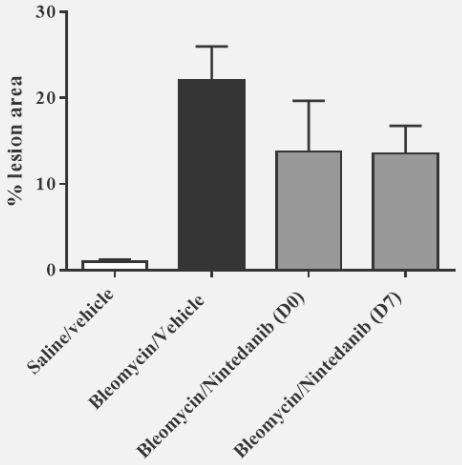
https://www.nhlbi.nih.gov/health/primary-ciliary-dyskinesia/causes

Inhaled drug development continuum

Nonclinical PoC

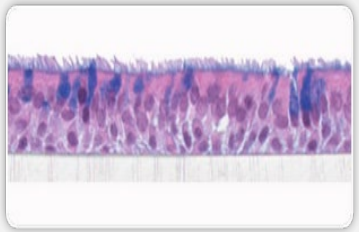
Rodent models of respiratory diseases, e.g., asthma, ARDS, IPF





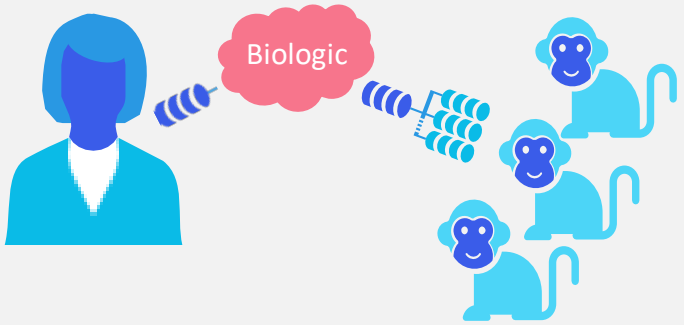
Early safety screening

High-throughput in vitro cell-based models, e.g., A549, EpiAirway™, MucilAir™ EpiOral™



FiH enabling

Safety assessment, toxicology studies, pharmacology, analytics



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Differences between subcutaneous, IV and inhalation studies

	Subcutaneous	Intravenous	Inhalation
Dosing methodology	A few minutes per animal (individual)	Bolus: a few min per animal Infusion: hours per animal	30 min - 6 hrs per animal (simultaneous)
Dosing equipment requirements	Minimal	Bolus: minimal Infusion: moderate	Generation, sampling, filtration
Analytical support	Weekly preparation and occasional analysis	Weekly preparation and occasional analysis	Daily analytical support
Data recording	Simple	Moderate	Extensive
Other study design considerations	Injection site reactions	Infusion [site] reactions	System characterization Respiratory tract pathology
TA requirements	mL/kg x animal body weight (kg) x overage factor	mL/kg x animal body weight (kg) x overage factor	Complex
Animal room requirements	Standard animal room requirements	Bolus: standard Infusion: larger footprint	Larger footprint and additional engineering
Safety considerations	Routine	Routine	Enhanced facility requirements

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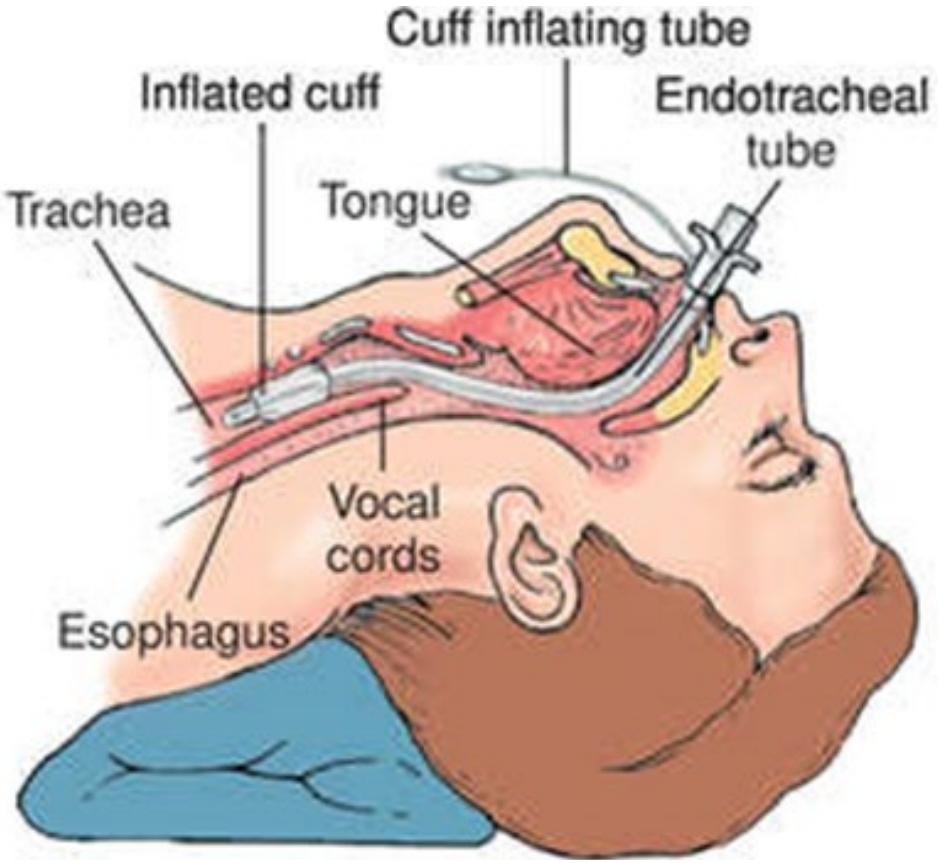
Emerging technologies

- Adeno-associated virus (AAV) and other gene therapies provide new approaches to addressing unmet medical needs
- AAV toxicity is related to doses used, local delivery ideal
- Single administration, literature examples by nebulisation
- Consider ensuring the infectivity of the gene therapy is unaffected by intratracheal microspray or nebulisation, etc.
- Suite of bioanalytical (qPCR, RT-qPCR) and immunological techniques required



https://speciality.medicaldialogues.in/wp-content/uploads/2017/06/Endocheal-tube-e1496910669333.jpg

Study setup considerations

- General study setup and protocol development
  - Same timelines across routes
  - Animal acclimation and habituation to dosing procedures
  - Characterization of the dosing atmosphere
- Species selection – same across route
  - Emphasis on pharmacology

> Safety evaluation programs should normally include two relevant species. However, in certain justified cases one relevant species may suffice (e.g., when only one relevant species can be identified or where the biological activity of the biopharmaceutical is well understood). In addition even where two species may be necessary to characterise toxicity in short term studies, it may be possible to justify the use of only one species for subsequent long term toxicity studies (e.g., if the toxicity profile in the two species is comparable in the short term).

- Study endpoints – same across route
  - Local and systemic exposure, immune activation, pharmacodynamics

Guidance

- ICH
  - M3 (R2) Guidance on Nonclinical Safety Studies
  - **S6 (R1) and Addendum – Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals**
  - S3A (toxicokinetics), S7A (safety pharmacology), S8 (immunotoxicity), S9 (anticancer), S12 (biodistribution for gene therapies)
- Regulatory agencies have guidance for biologics (general or specific classes)
  - FDA
  - EMA
  - Many others
    - Rahalkar H, Sheppard A, Salek S. Comparison of BRICS-TM Countries' Biosimilar Regulatory Frameworks With Australia, Canada and Switzerland: Benchmarking Best Practices. Front Pharmacol. 2021 Aug 9;12:711361. doi: 10.3389/fphar.2021.711361. PMID: 34434109; PMCID: PMC8381275.

Study designs

Examples

- Mouse
  - Dose on Days 1, 8, 15, 22, 29 (~4 weeks)
  - Clinical observations, body weight, food consumption, clinical pathology, anatomic pathology, toxicokinetics, immunotoxicology
  - Necropsy on Day 32; recovery phase necropsy after 2 weeks

		Dose Level	Number of Animals	
Groupa	Subgroup	(mg/kg/day)	Males	Females
1 (Control)	1 (Toxicity)	0	15	15
	2 (Toxicokinetic)	0	3	3
	3 (Cytokine/IPT)	0	5	5
2 (Low)	1 (Toxicity)		10	10
	2 (Toxicokinetic)		18	18
	3 (Cytokine/IPT)		5	5
3 (Intermediate)	1 (Toxicity)		10	10
	2 (Toxicokinetic)		18	18
	3 (Cytokine/IPT)		5	5
4 (High)	1 (Toxicity)		15	15
	2 (Toxicokinetic)		18	18
	3 (Cytokine/IPT)		5	5

IPT = Immunophenotyping.

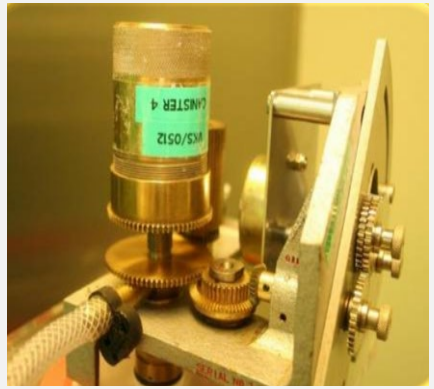
- Nonhuman primate
  - Dose on Days 1, 29, 57, 85 (~13 weeks)
  - Clinical observations, body weight, food consumption, safety pharmacology, clinical pathology, anatomic pathology, toxicokinetics, immunotoxicology
  - Necropsy on Day 92; recovery phase necropsy after 4 weeks

	Dose Level	Number of Animals	
Groupa	(mg/kg/day)	Males	Females
1 (Control)	0	5	5
2 (Low)		3	3
3 (Intermediate)		3	3
4 (High)		5	5

Aerosol generation

Powder

- Good stability for NCEs
- Challenging for biologics
  - Some oligos
- Wright dust feed
- Rotating brush generator

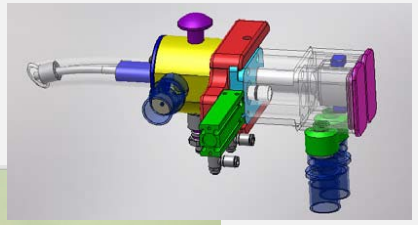


Liquid

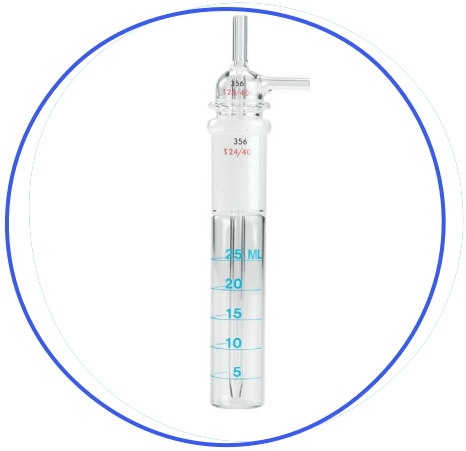
- Dose form for most biologics
- Vibrating mesh nebulizer
  - Preferred for biologics
- Pneumatic/air jet nebulizer
  - Protein aggregation and unfolding

Clinical device

- If no other option
- pMDIs
- Capsules

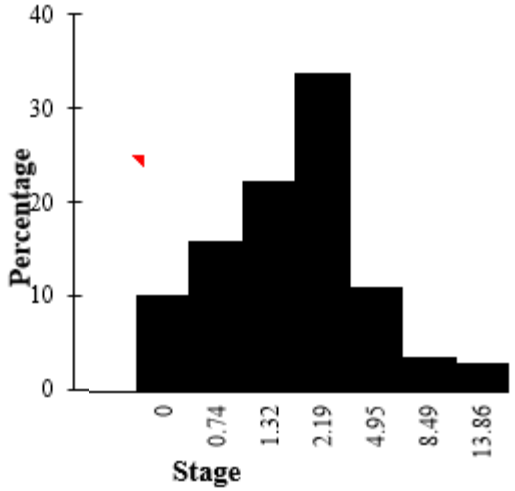


Aerosol characterization



Concentration

- Snapshot representative of full dose
- Expect ≤25% of target aerosol concentration (device dependent)
- HPLC-MS, A280



Aerodynamic behavior

- Particle size distribution (PSD) – by mass (cascade impactor)
- Aggregation (HPLC-SEC, gel electrophoresis, microscopy)

Activity/integrity

- Nanoparticle integrity (nucleotide encapsulation)
- Pharmacologic activity (antibody integrity, binding and activity, phage replication)

https://www.sensidyne.com

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  - Safety pharmacology
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Inhaled or delivered dose determination

$$\text{Delivered Dose} = \frac{C \times RMV \times D \times IF}{BW}$$

	Delivered dose (mg/kg)
C	= Aerosol concentration of TA in air (mg/L)
RMV	= Respiratory minute volume (L/min)
D	= Duration (min)
IF	= Proportion by weight of particles that are inhalable (assumed as 1 if >90% of particles are <7 µm)
BW	= Bodyweight (kg)

Inhaled or delivered dose determination

Deposited Dose = Deposition Fraction x Delivered Dose (mg/kg or mg/g)

Assumed lung dose

- 100% deposition in humans, from device emission
- 10% in rats and mice, from breathing zone
- 25% in dogs, minipigs and nonhuman primates, from breathing zone

Intranasal dose

- Constant mass or normalization for surface area

Why?

- Oronasal deposition
  - Ingestion, exhalation
- Multiple animals dosed simultaneously, each with different breathing patterns
- >> Include local dose quantification when appropriate

Regulatory safety margins

- Based on species (5- to 10-fold PDD)
- Appropriate for biologics with high target engagement and low off-target effects or systemic exposure?
  - What about biologic therapeutic units and scalability (genome copies, phage CFU)?

Tepper, J. S., Kuehl, P. J., Cracknell, S., Nikula, K. J., Pei, L., & Blanchard, J. D. (2016). Symposium Summary. *International Journal of Toxicology*, 35(4), 376–392. doi: 10.1177/1091581815624080.

Exposure and sampling considerations

- Aerosol characterization helps understand TK results
- Generally, as size of molecule increases the level of systemic exposure decreases
  - Tight junctions or transcellular passage
  - Macrophage uptake
- Plasma pharmacokinetics might not reflect lung exposure
- Determining lung concentrations can be done [e.g., bronchoalveolar lavage (BAL)] but:
  - Small animals BAL is a terminal endpoint
  - Large animals will require anaesthesia and there will be limited number of occasions
  - Dose will be diluted/removed due to the procedure
  - Development of immunoassays for BAL can prove challenging

Stability and immunogenicity in the lung

- Endogenous protease and nuclease activity designed to degrade pathogens
- Immune components (e.g., macrophages) patrol respiratory tract looking to degrade and present foreign proteins
- Safety assessment matrices
  - Systemic – plasma, serum, whole blood
  - Local – bronchoalveolar lavage fluid (BALF), tissue (frozen or fixed)
- Safety assessment endpoints
  - Cytokines and complement proteins to look for acute immunostimulation after dosing
  - Immune cell recruitment into airways
  - IgG production in response to the drug (anti-drug antibody assay)
  - If a vaccine, measure humoral and cellular responses

Safety pharmacology and exaggerated pharmacology

- Vital organs and systems (cardiovascular, respiratory, central nervous system) with exposure to the therapeutic range or above, defining dose-response
  - Core battery, follow-up, supplemental
- Uses clinical route of administration unless anticipated difference in local/systemic exposure and effects, and a single dose or repeat dose
  - *In vitro* studies for concentration-effect relationship

Study design implications:

- Consider the mechanism of action of the inhaled biologic and known pathology (epithelial remodeling, immune activation, fibroblast activation)
- Immune activation can have secondary effect on CV, RESP parameters

Histopathology

Class effects in the respiratory tract

- Antibodies – perivascular/peribronchiolar mononuclear cell infiltrates, eosinophilic deposits, increased alveolar macrophages, draining lymph node hyperplasia and hypercellularity
- Oligonucleotides – ASO (vascular injury) or siRNA (increased alveolar macrophages and tracheobronchial macrophages or hypercellularity)
- Lipid nanoparticles – (inflammation, vacuolation, foamy macrophages)
- Bacteriophages – increased cellularity and germinal center development in lymphoid tissues
- Viral vectors – still novel in terms of regulated safety assessment

Use weight of evidence in the context of the study for adversity consideration

Hypothetical example of a preliminary study

Antibody that neutralizes a pro-inflammatory cytokine

- Species selection – *in vitro* assay supports cynomolgus monkey as most/only relevant
- Aerosol characterization – concentration and PSD by A280, aggregation by HPLC-UV/SEC [post-nebulization cell culture assay for potency]
- Added evaluations to include body temperature, cytokine analysis, complement analysis, immunophenotyping, and safety pharmacology – predose and postdose
- Blood samples for TK, BAL from accessory lobe of lung for ADA and cytokines (PD)

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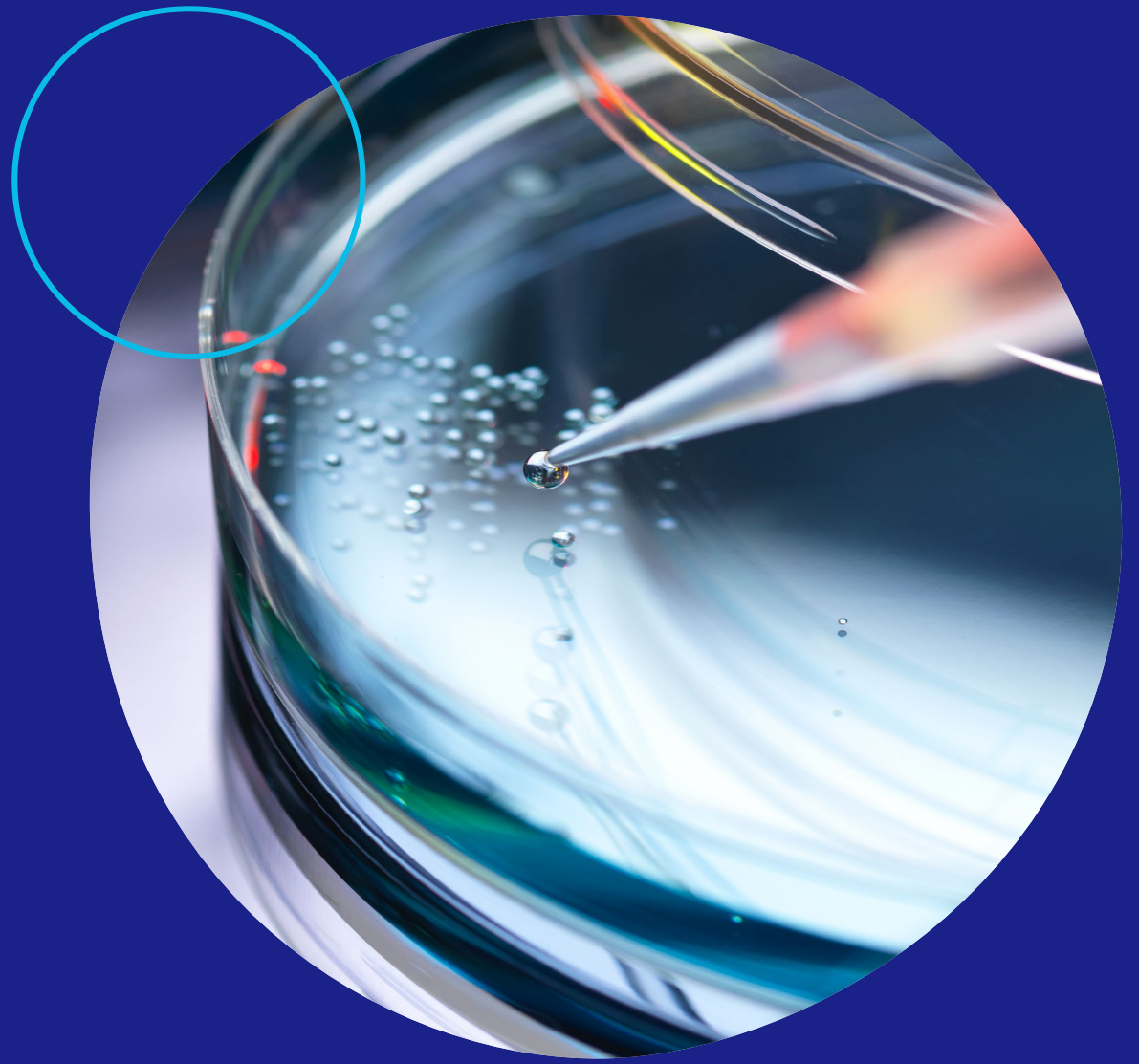
Summary

- Respiratory tract is an innate barrier, keeping foreign material from entering the body
- Understanding pharmacology will inform study design including species selection
- Scrutinize results based on [aerosol characterization] methodology
- Balance regulatory guidance with scientifically sound dose/exposure justification
- Interpret immunological changes in the context of the therapeutic
- Keep improving health to improve lives!



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Thank you

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