REAGAN-UDALL FOUNDATION FOR THE FDA

THE MEETING WILL BEGIN SOON

Advancing Drug Development by Reducing Reliance on Animal Testing

Case Example: Pre-Clinical Animal Models in Lung Toxicology

Hybrid Public Meeting
February 26, 2026 | 10am-4pm (eastern)

Aer Therapeutics, Avalyn Pharma, Inc., Biotechnology Innovation Organization, Charles River Laboratories, Endeavor BioMedicines, Ionis Pharmaceuticals, and VIDA provided funding for this meeting.

REAGAN-UDALL FOUNDATION FOR THE FDA

Welcome

Susan C. Winckler, RPh, Esq.

Chief Executive Officer
Reagan-Udall Foundation for the FDA

Housekeeping

REAGAN-UDALL FOUNDATION FOR THE FDA

- Due to the meeting size, your microphone and video will remain off during the meeting
- Please share your questions using the Zoom Q&A function
- This public meeting is being recorded.
- The slides, transcript, and video will be available at www.ReaganUdall.org

Agenda

REAGAN-UDALL FOUNDATION FOR THE FDA

10am	Welcome & Opening Remarks
10:15am	Use of Animal Models in Pre-Clinical Lung Toxicology Safety Studies: Current Expectations and Limitations
11am	Industry Experience in Current Environment
11:45am	Panel Discussion: Impact of Current Environment on Product Development and Patients
12:30pm	**Lunch**
1:15pm	Innovations in Lung Toxicology Safety Studies: New Approaches in Pre-Clinical Models and Clinical Monitoring
2:45pm	Panel Discussion: What the Future Might Look Like *Opportunities to Improve Product Development and Global Alignment*
3:55pm	Closing Remarks
4pm	Adjourn

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Opening Remarks

Steven Kozlowski, MD
Chief Scientist - Office of the Chief Scientist, Office of the Commissioner
U.S. Food and Drug Administration

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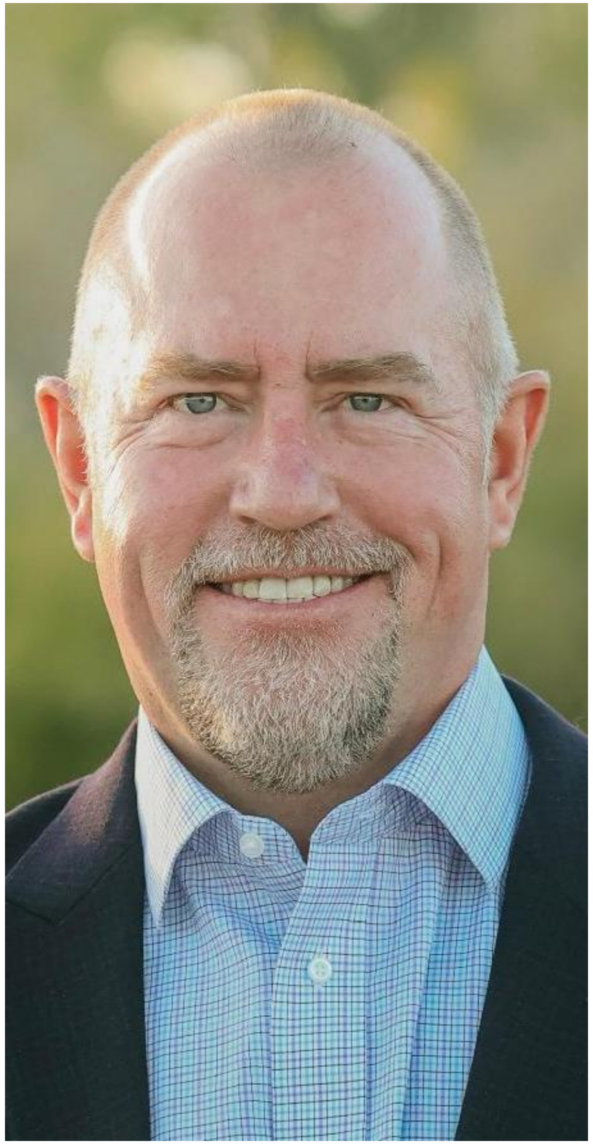
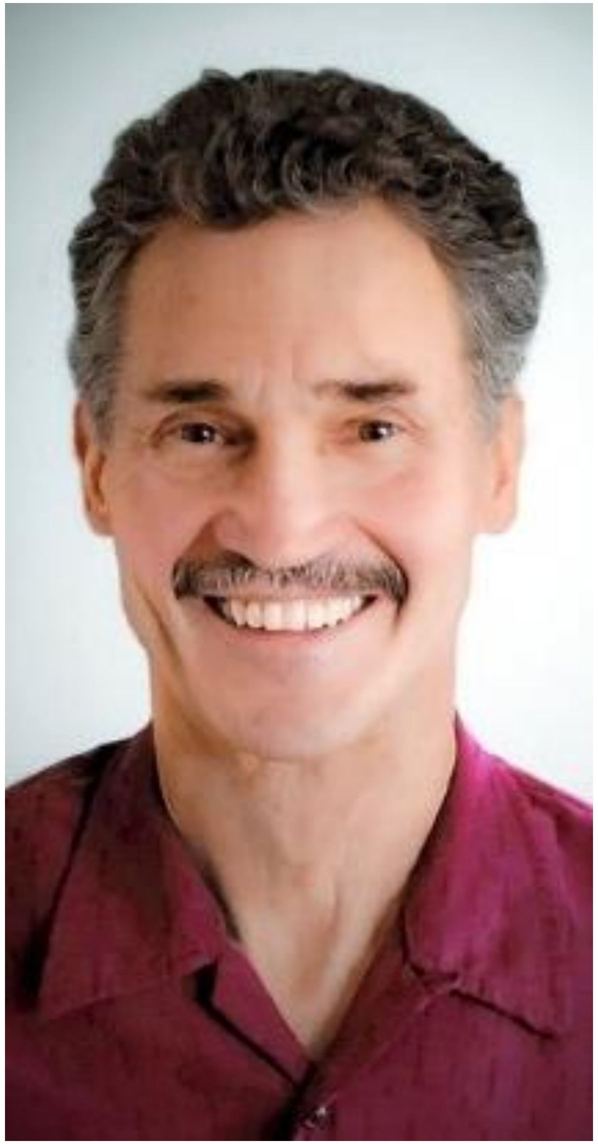
Use of Animal Models in Pre-Clinical Lung Toxicology Safety Studies:

Current Expectations and Limitations

Matt Reed, PhD, DABT
Principal, Coelus LLC

Jeff Tepper PhD, DABT, DSP
Consultant, Tepper Nonclinical Consulting





What are we doing here? Baselining Pulmonary Delivery and Dose Determination in Pulmonary Drug Development

Thursday, February 26, 2026

Matt Reed, PhD, DABT, F-ATS

Coelus LLC

10:15 AM ET Session

Use of Animal Models in Preclinical Lung Toxicology Safety Studies: Current Expectations and Limitations

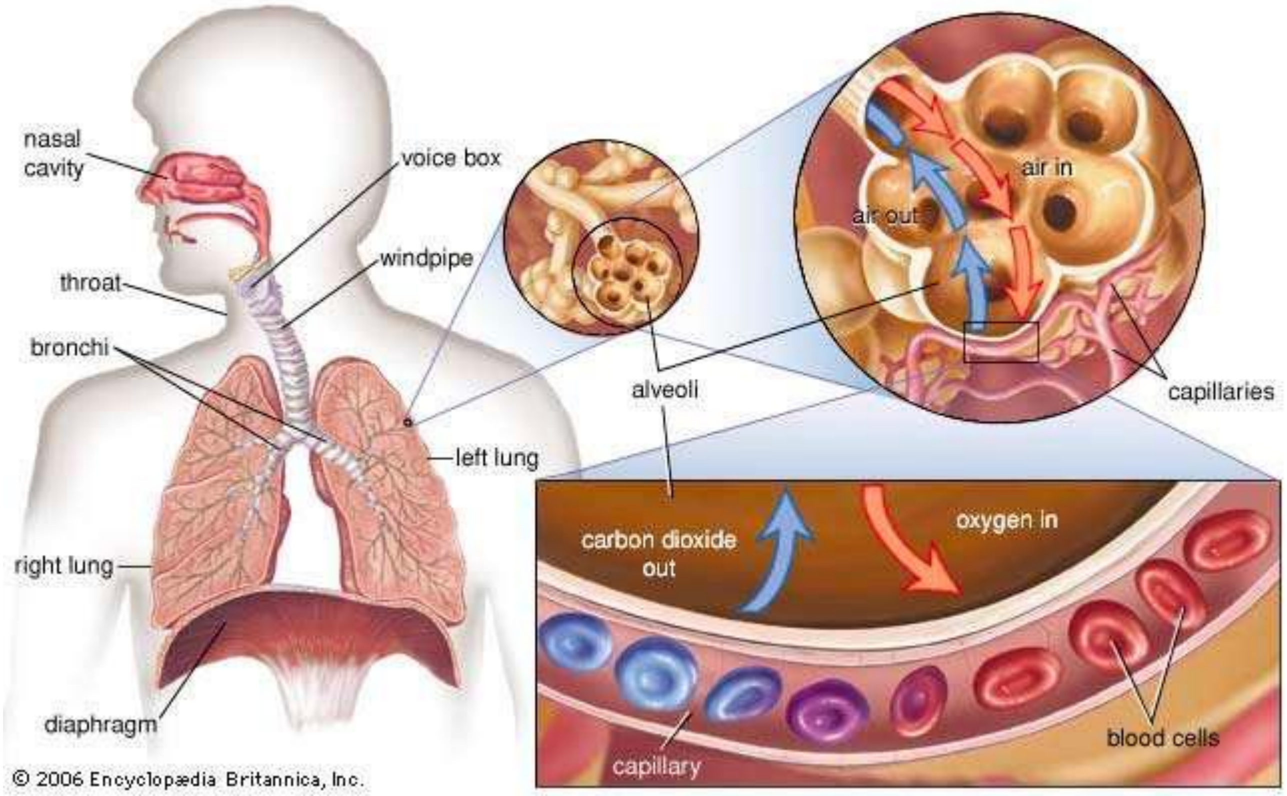
Outline

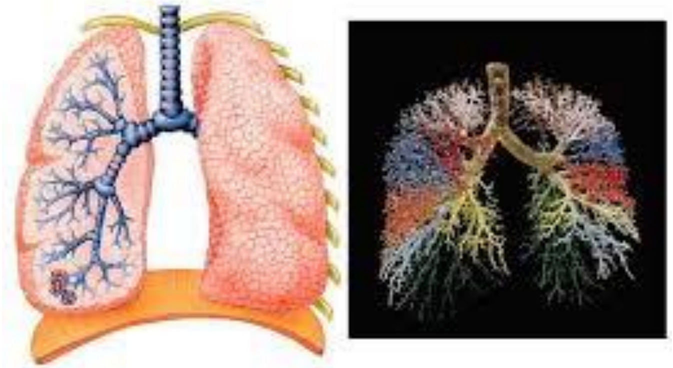
- Pulmonary delivery in humans (getting at a dose – this is hard, but doable)
- Pulmonary delivery in animals (getting at a dose - this is hard, but doable too)
- Regulatory convergence points and divergence (dose emphasis)
- A note on NAMS integration

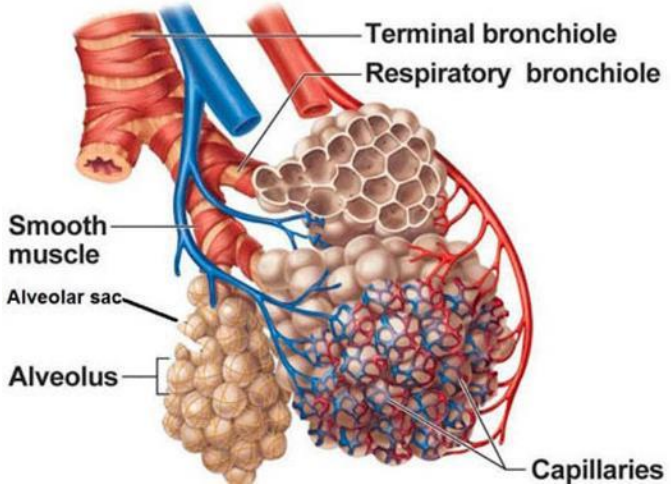
Note: Jeff gets the hard stuff

Disclaimer: I have no direct conflict. My business (I) support multiple clients seeking to move drugs utilizing pulmonary delivery to and through clinical development.

Delivering treatments to and across the respiratory tract. Noting disease can happen anywhere.







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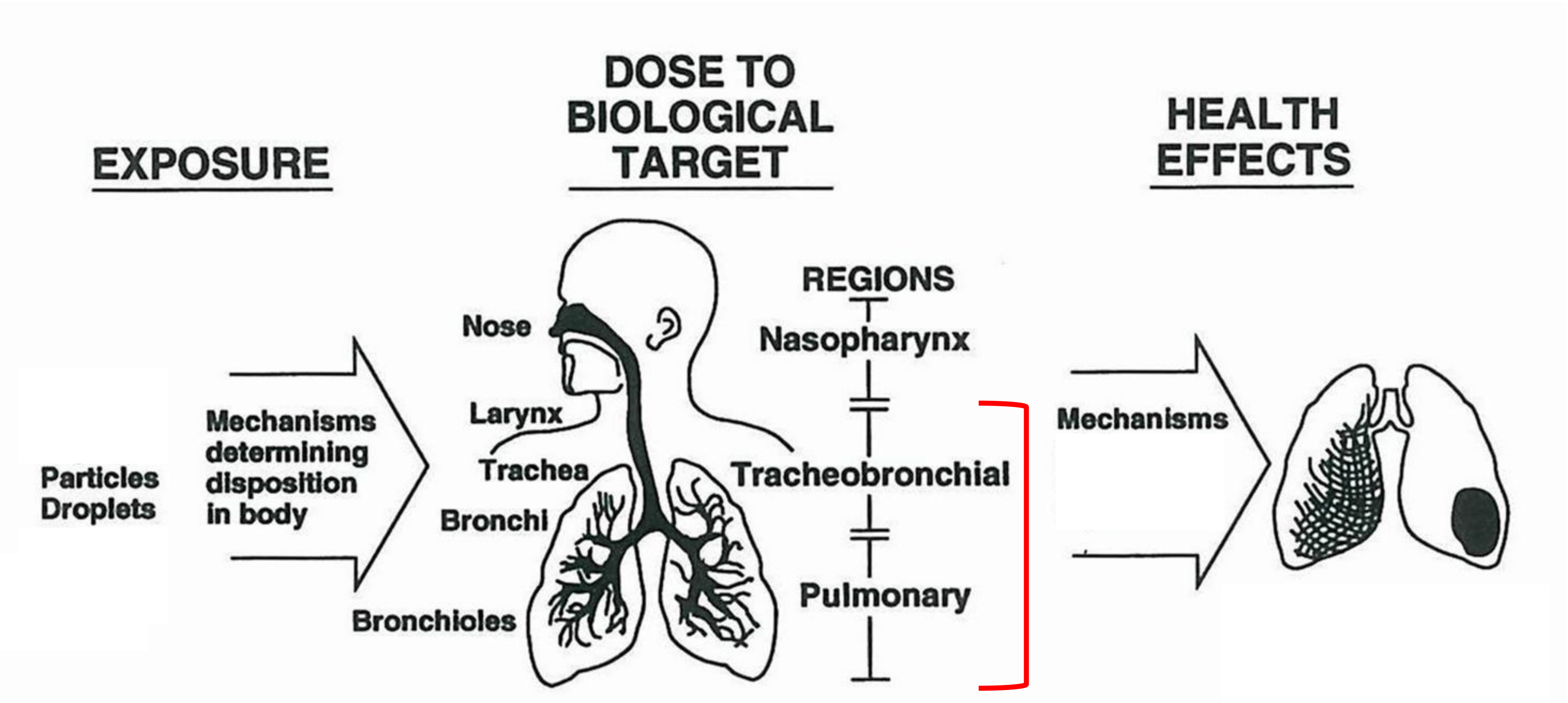
Building the paradigm of translational dose and health effect (positive or negative)

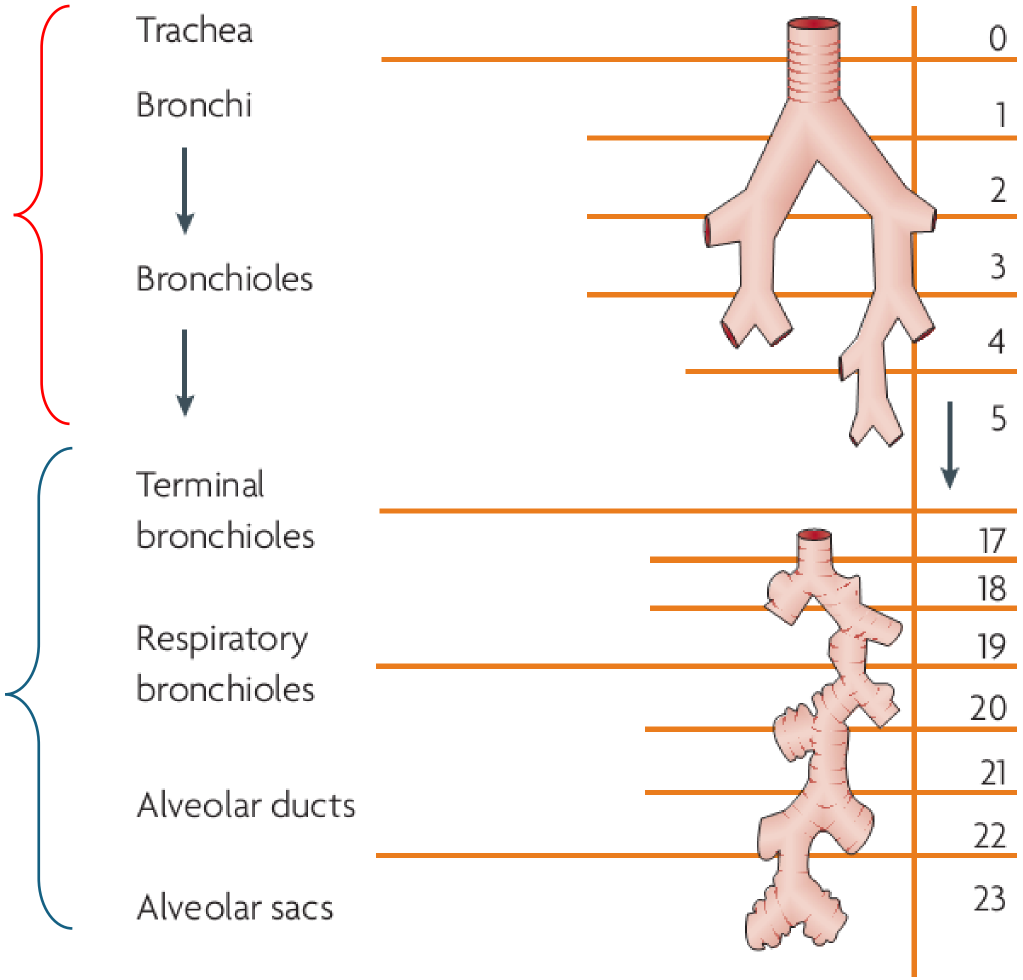


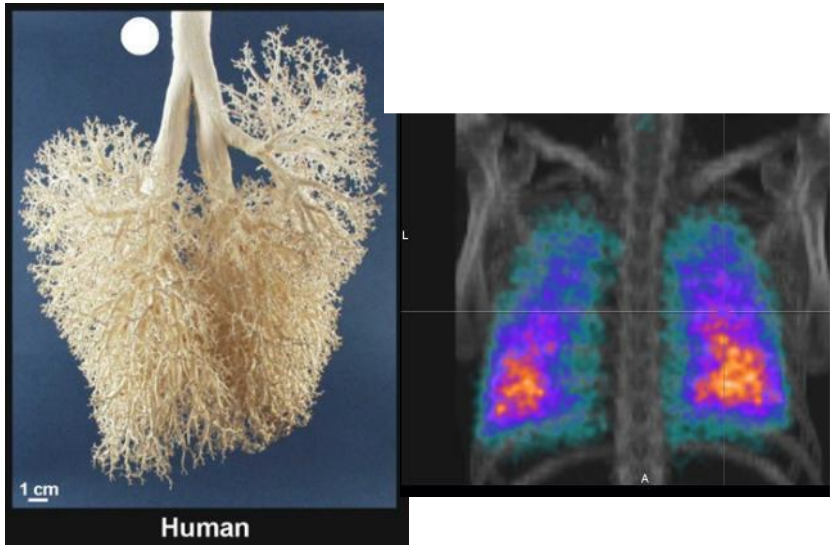
Schematic representation of the major elements of inhalation delivery oriented toward understanding the efficacy or toxicity of pharmaceutical aerosols

Pulmonary target areas?

TB (Tracheo-bronchial)

PUL (Pulmonary-Alveolar)

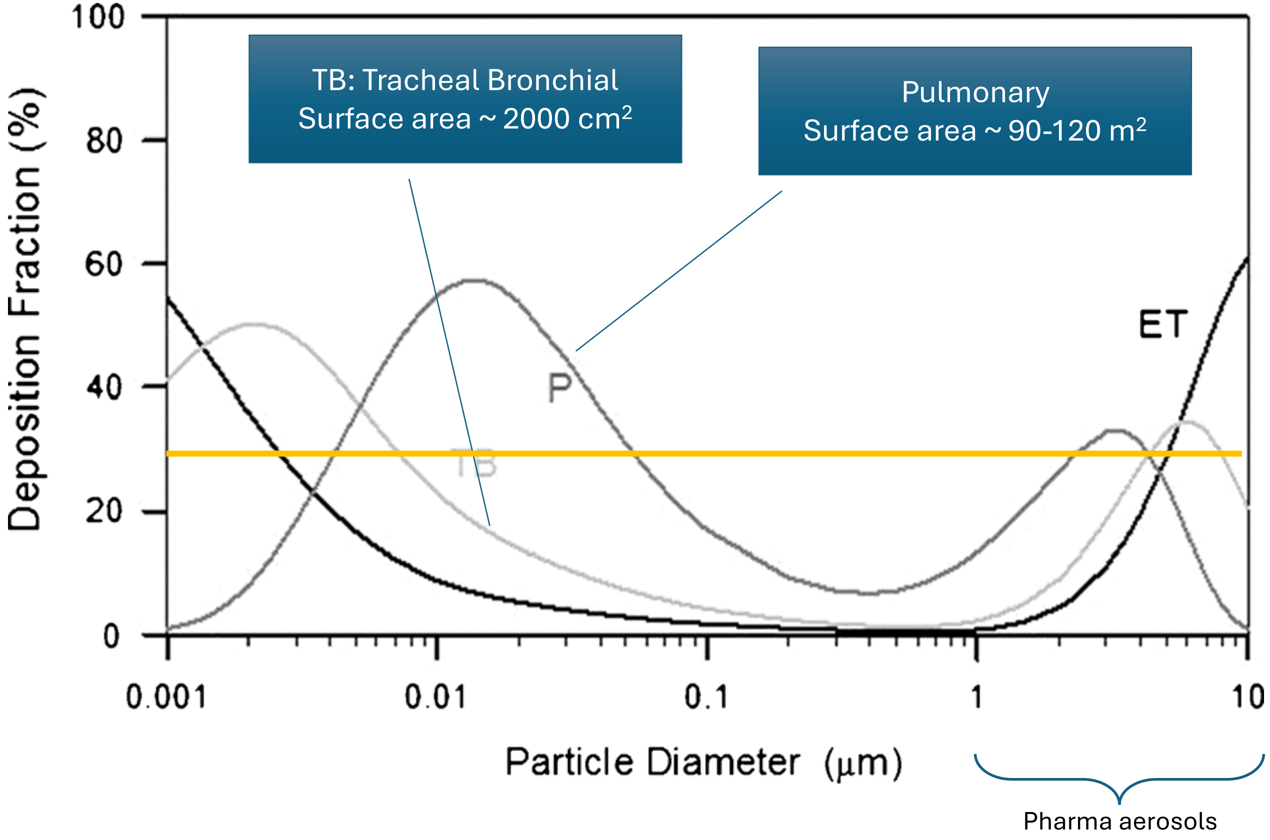




TB-Ciliated cells of the airways (tracheal-bronchial)-through generation 15

PUL- Unciliated terminal bronchioles (Alveolar-PUL)-past generation 15

Particle deposition in the human lung: Human oral tidal breathing (one example, e.g., nebulizer)



Distribution of particle deposition for different regions of the respiratory tract system for 100% mouth breathing. The data were calculated using the LUDEP software (NRPB, Oxon, UK) based on the ICRP model.

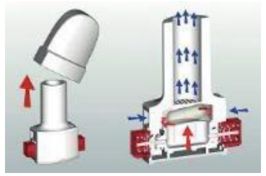
- Particle size and distribution of the aerosol (and airflow) defines where a particle goes in the respiratory tract
  - Deposition is primarily impaction and sedimentation driven (for pharma aerosols)
- Depending on mechanism of inhalation, we "lose" lung dose to the back of the throat and upper respiratory tract
- We "hit" the TB and PUL (pulmonary-distal airways and alveoli) with ~ equal efficiency in the range of delivery of "our" pharmaceutical aerosols generated
- But, there is a disparate level of surface area between the TB (centimetres) and pulmonary regions (meters)

Aerosol delivery in patients: understanding the feasibility of human delivery in a formulation and delivery platform/s

Examples respiratory Indications/ other

- <u>Asthma and COPD</u>
- <u>Cystic Fibrosis, Bronchiectasis</u>
- <u>Lung infection</u>
  - Viral (SARS-CoV-2, influenza, RSV)
  - Bacterial, fungal
- <u>Pulmonary Arterial Hypertension (PAH)</u>
- <u>Pulmonary fibrosis</u>
  - Idiopathic, cystic fibrosis, sarcoidosis
- <u>Systemic indications</u>
  - Migraine, diabetes

Delivery (how much aerosol can we deliver efficiently to impact disease)



Formulations?

<u>Nebulizer</u>
- Oral tidal breathing (e.g., jet, vibrating mesh, continuous output, on demand output., etc.)
- Face Mask naso-oral tidal breathing
- Soft Mist (Low flow - maneuver)

<u>Dry powder inhaler</u>
- Patient powered (forced inspiratory capacity-flow rate) - other

<u>Metered dose inhaler</u>
- Compressed gas-coordinated maneuver
- Oral or face mask (with spacer)

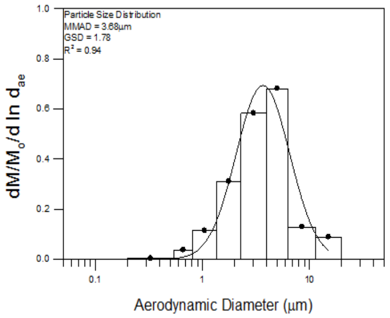
Know how a delivery platform’s design and use characteristics influence the dose

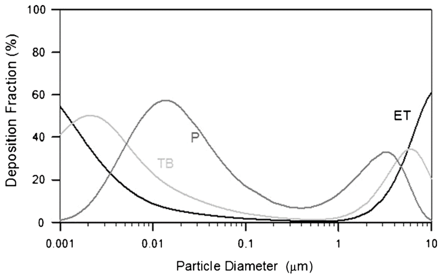
Platform delivery method

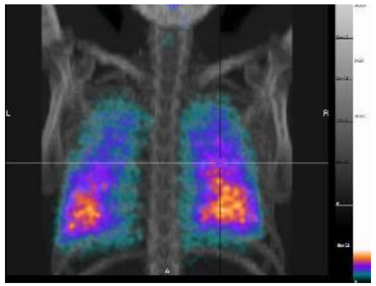
- Nebulizer
  - Tidal Breathing
- DPI
  - Forced maneuver
  - Forced inspiratory capacity/ flow
  - Breath hold
- MDI
  - Actuation
  - Coordination with forced inspiratory capacity/ flow
  - Breath hold

Determining inhaled dose

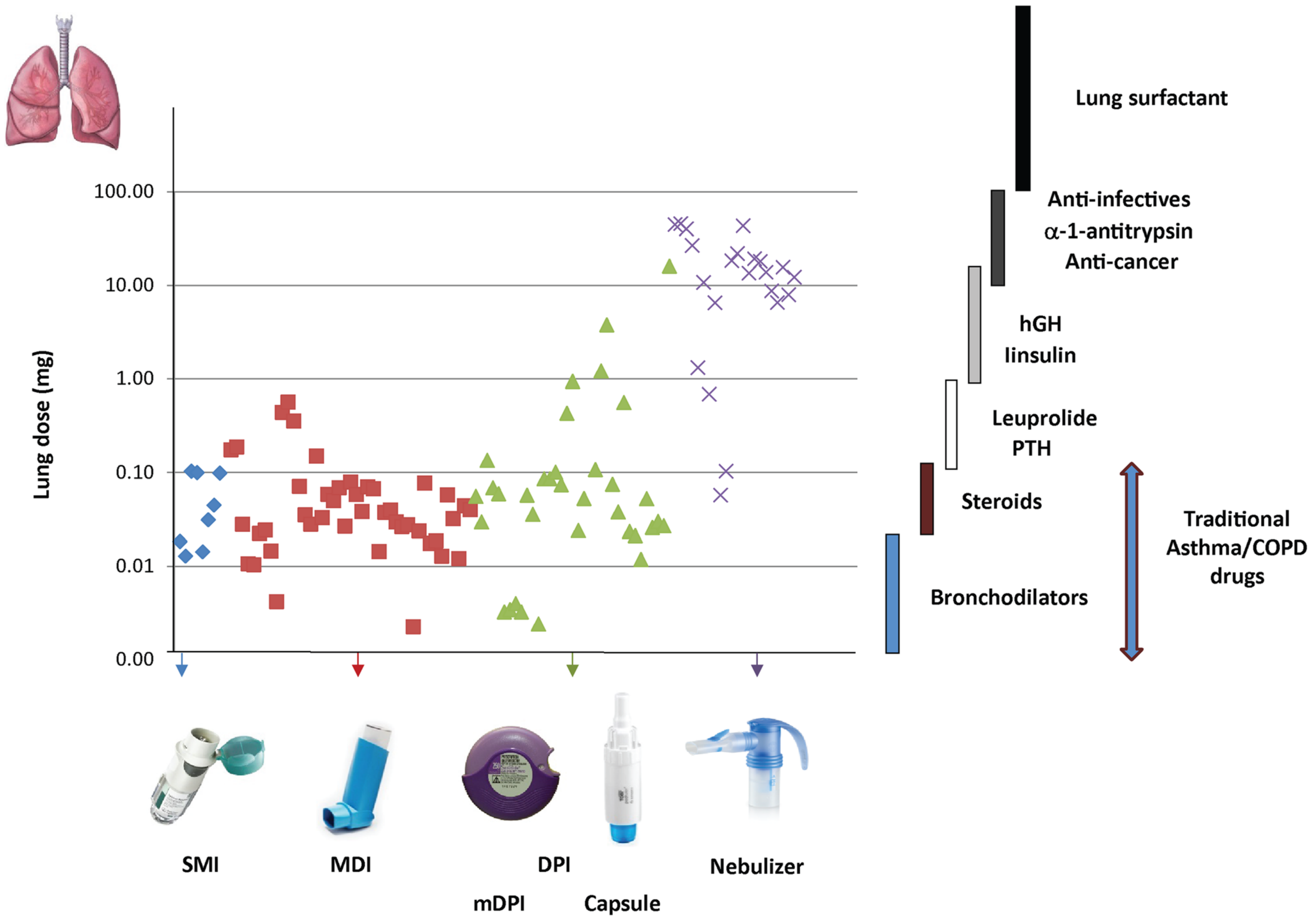
- Nominal dose (what goes in)
- In vitro characterization (USP)
  - MMAD/ GSD
  - Emitted Dose (ED) from device
  - Fine particle fraction (FPF)/Inhalable fraction (< 5 microns)
  - Breath simulation, etc.
- Modeling deposition
  - ICRP, MPPD, CFD
  - Contractors can help!
- Empirical data
  - Gamma scintigraphy (P/C)







Device-drug combinations deliver a wide range of inhaled treatment modalities over a multi-log range



Clark et al., 2015 reproduced by Hastedt et al 2016

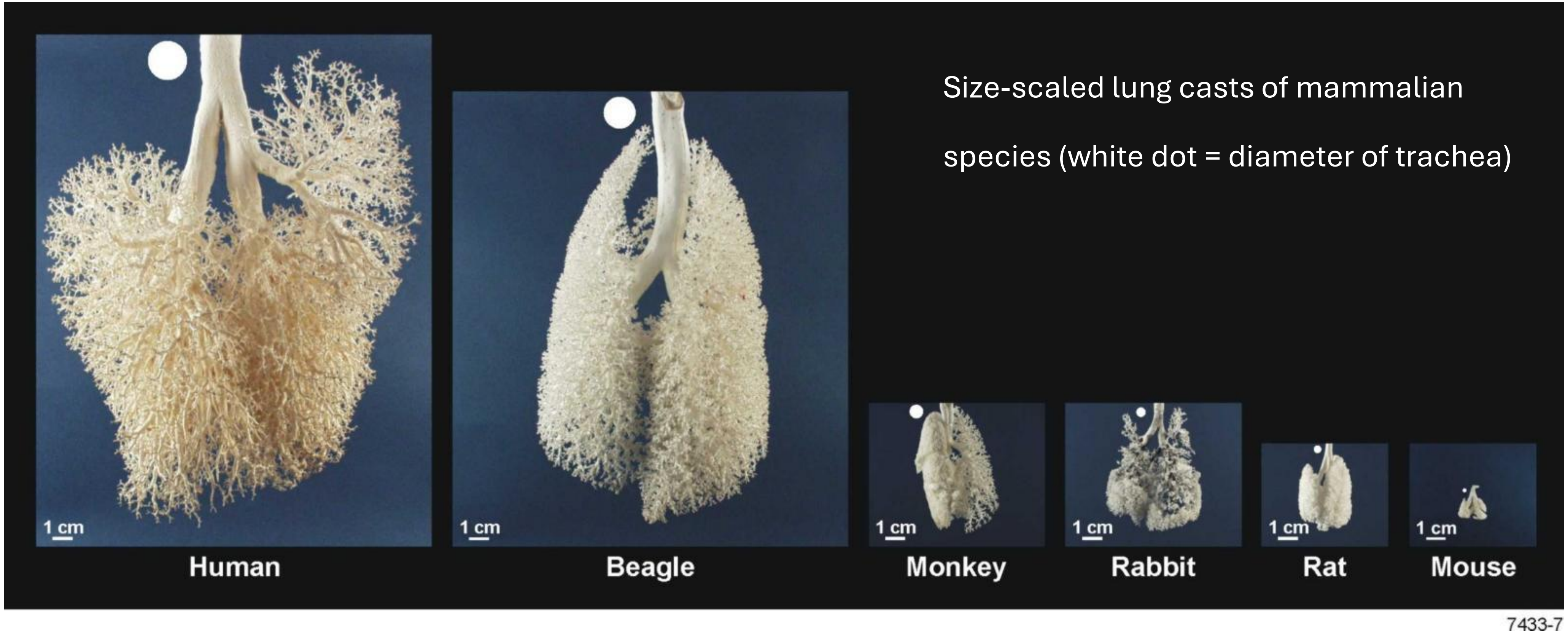
<u>Take home points</u>:

- Wide range of therapeutic modalities with different therapeutic indexes used to treat a breadth of pulmonary and systemic diseases
- Wide range of doses and devices used to deliver these modalities
- **Accurate estimation of inhaled human dose is feasible across a wide range of modalities and devices**
- **Toxicology? Understanding clinical dose potential (exposure) is critical to inhaled hazard assessment and development of safety margins**

Current state of the art: Determining inhaled dose in nonclinical safety studies

- 70+ years of using nonclinical species for hazard assessment, general toxicology, risk assessment across disciplines (e.g., pharma, chemicals, pesticides, environmental, etc.)
- Regardless of species of interest, nonclinical inhalation delivery systems use most of the same principles that are used for human drug delivery (e.g., aerosol concentration/ dose presentation, particle size, particle deposition principles, flow, etc.)
- Almost exclusively, nonclinical inhalation delivery systems make use of tidal breathing (e.g., nasal and/or oro-nasal)

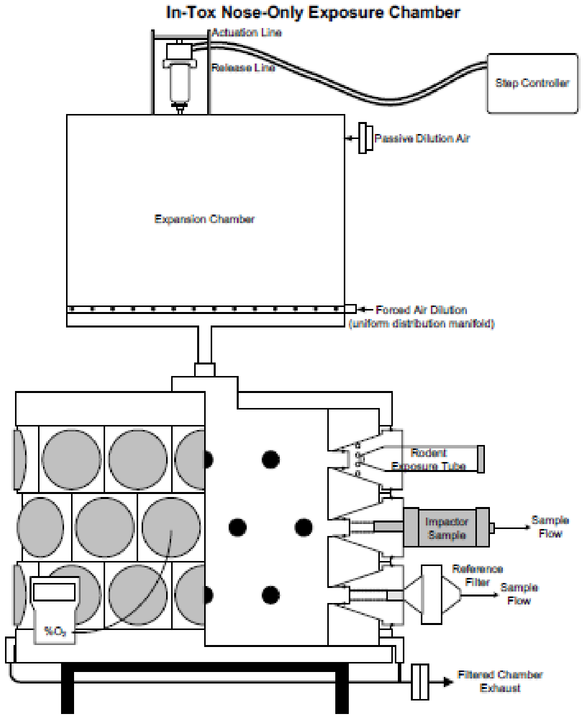
Particles flying in a lung?



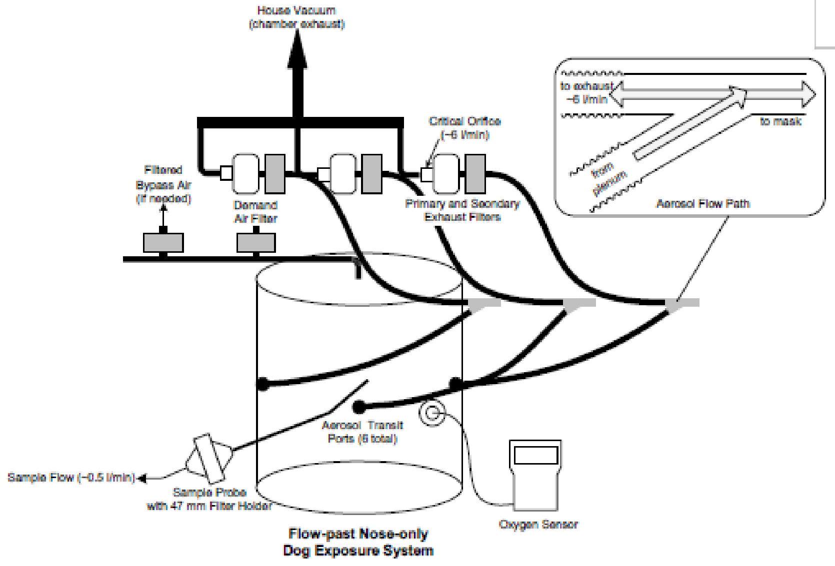
Lung is a lung right????? Not really. Size matters and it is mostly the "plumbing sizes" that drives differential deposition (vs humans) in lungs for pharmaceutical aerosols among species.

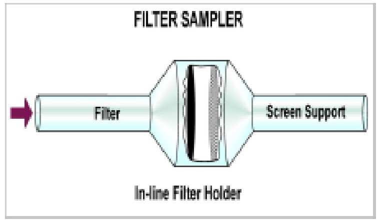
Coupling aerosol generators to exposure chambers

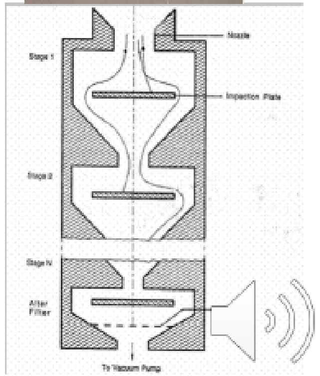
Rodent System



Large Animal Exposure System







Courtesy Lovelace Biomedical

- Select devices are used to generate liquid and dry powder aerosols (some are clinical).
- Animals breathe tidally/ passively as the aerosol flows past the animal's nose/ snout (minute volume measured or estimated) .
- The aerosol concentration is determined by collection under known flow/ time and monitored by chemistry.
- Aerosol size is measured to understand "inhalability" and respiratory deposition.

<u>Key metrics of lung dose</u>: time of exposure, aerosol concentration, tidal volume (measured or estimated), deposition determination. Jeff to layer into safety assessment practice.

Regulatory convergence and divergence in nonclinical pulmonary drug development

- What's generally consistent with the Agency and Internationally (there is always a caveat)?
  - Regardless of geographic region or Agency Division, ICH Guidance drive nonclinical performance standards and general study design (examples............)
    - M3/R2 small molecules: 2 species
    - S6 for biologics: appropriate species (NHPs or surrogates)
    - Conditions for "other" like oligonucleotides
    - Conditions for "gene" therapy
- What's not (there is always a caveat)?
  - Clinical lung dose estimates (US Pulmonary vs ROW).
    - Nominal approach (i.e., what goes into the device) vs what is estimated to be deposited in the respiratory tract
  - Nonclinical lung dose estimates (See Jeff's talk)
  - Use of NAMS for regulatory purposes

Matt’s word on NAMS……..

- Use models to provide confidence in the “weight of evidence” approach
    - Methods that build a case for or against translation for critical observations
        - E.g., is this animal-based histopathology finding relevant to humans?
        - Do I need to to do more or less NAM-based or *in vivo* work to inform the science or protect patient safety?
- Models for pharm/tox screening or dose translation (many currently in use, being refined, or near qualification status)
    - Lung on chip
    - ALI-airway; ALI-alveolar
    - Organoids
    - Other???????
- *In silico, In vitro*, literature [use AI, but don’t forget to read (and write)!], other?
    - Combinations of methods and data sets that build evidence for or against the use of animal testing
- Question the need for an animal study even if precedent dictates (e.g., guidance). Build evidence for or against testing by using multiple methods to make rational decisions regarding risk to the patient and the need for animal studies.

Disease State

TI

cm²

NOAEL

IC50

ELF

MRSD

PK

mg/ml

Recommendations

- We have the hard science and the capability to predict human dose with near any inhalation device and formulation. Let’s do it!
- Similarly, we understand inhalation exposures and metrics and have multiple tools at our disposal to use the best science to determine nonclinical doses. Let’s use them!
- Let’s incorporate NAMS on case-by-case basis to facilitate our use of weight of evidence approaches and rational determinations for informing the need for animal testing

IRA Demystified

Thursday, February 26, 2026

Jeff Tepper, PhD, DABT, DSP
Tepper Nonclinical Consulting

10:35 AM ET Session

Use of Animal Models in Preclinical Lung Toxicology Safety Studies: Current Expectations and Limitations

IRA: What is it?

Irish Republican Army

IRA: Inhalation Risk Assessment

Outline

2005 FDA Guidance

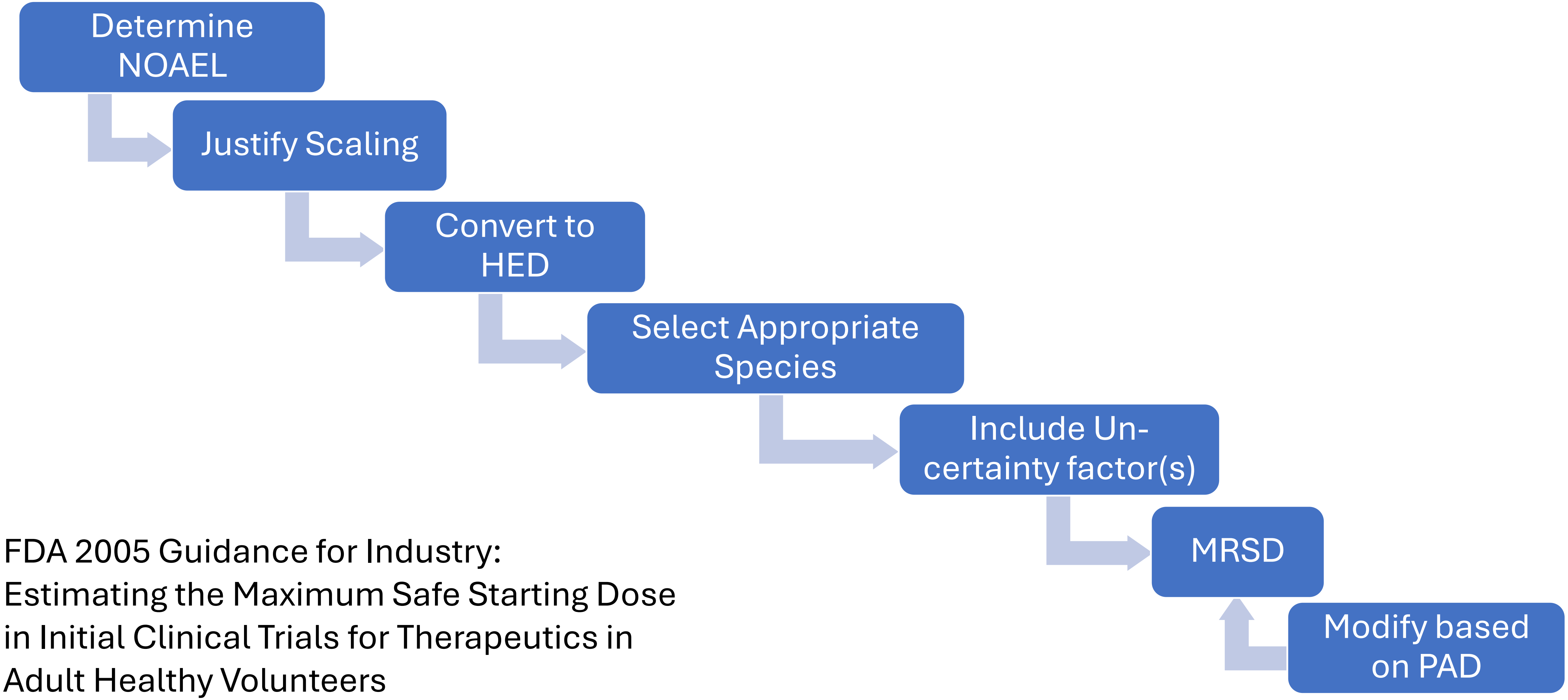
Determination of Starting Dose for Inhaled Drugs

- Adversity
- Lung dose nomenclature
- Default values for deposition
- Scaling and Safety Margins

Summary/Recommendations

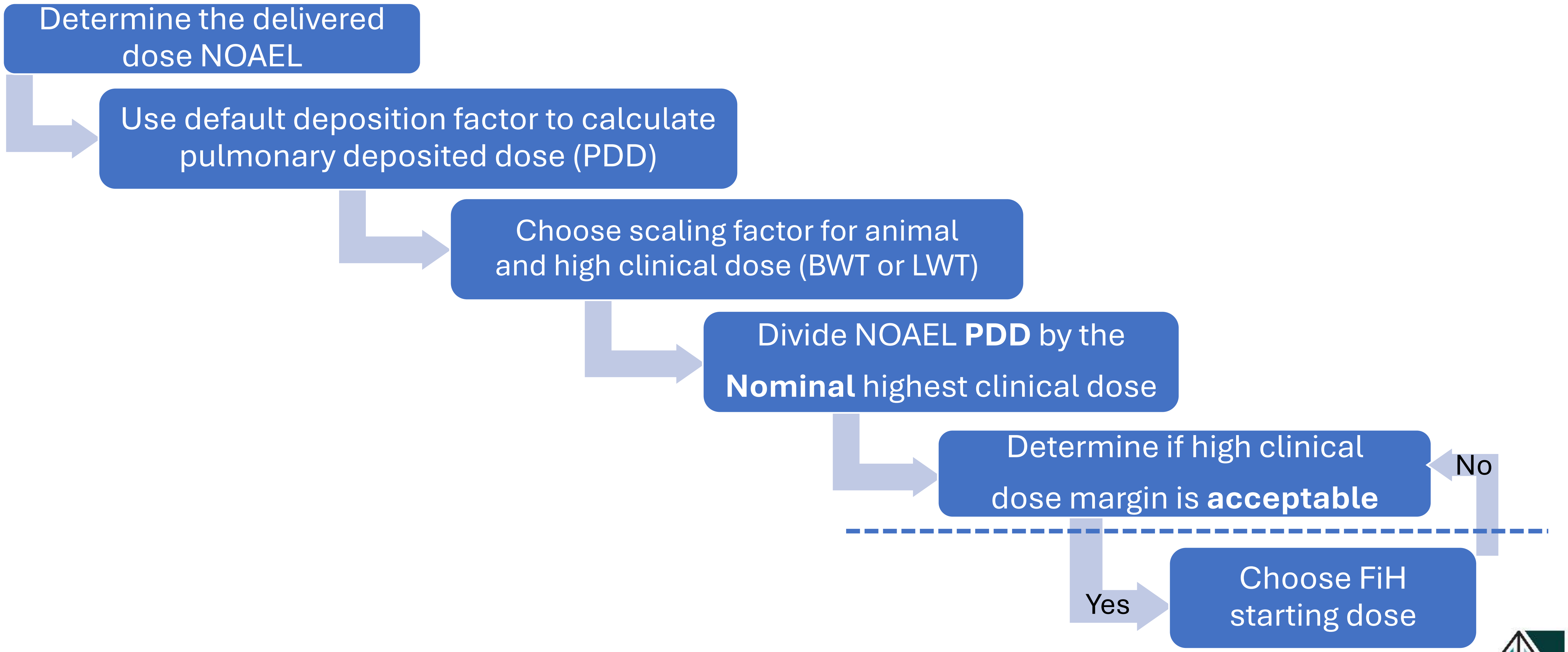
Disclaimer: These are mostly my thoughts. I have no conflict of interest.

2005 FDA Guidance **Maximum Recommended Safe Starting Clinical Dose** (MRSD) for FiH



FDA 2005 Guidance for Industry:
Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers

Pulmonary Division Algorithm to Determine **Maximum Clinical Dose** for FiH



Tepper et. al., 2016

NOAEL is the No Adverse Effect Level

The NOAEL dose is the primary output of GLP toxicology studies

The NOAEL is "the highest dose level that does not produce a significant increase in adverse effects in comparison to the control group'' (FDA, 2005 MRSD Guidance)

Adversity

Adversity is a fairly nebulous concept with no globally accepted definition

Recently, adversity has been defined as an effect that:

- "likely results in an impairment of functional capacity to maintain homeostasis and/or an impairment of the capacity to respond to an additional challenge." (Palazzi et al 2016)

or:

- "Adversity'' is a term indicating "harm'' to the test animal, within the constraints of the study design (dose, duration, etc.) (Kerlin et. al., 2016)

However, the NOAEL should not include speculation about the origin and/or pathogenesis of the effect, theoretical human/patient extrapolations or clinical non-monitorability. These concerns should be stated specifically and included as uncertainty factors.

NOAEL is a Dose but which Dose?

Nominal

Dose added to the device

Emitted/Presented

Dose that leaves the device

Delivered/Inhaled

Dose at breathing zone

Deposited

Pulmonary dose

Systemic

The pulmonary region most specifically refers to the area where gas exchange occurs

Calculation of Lung Dose

Lung Dose (mg/kg) = (C x T x MV x Df) / Sf

Where:

C = Aerosol concentration (mg/L)
T = Exposure time (min)
MV = Minute volume (L/min)*
Df = Deposition fraction*
Sf = Scaling Factor; Body weight (kg) or Lung Weight(g)**

* Estimated parameters
** Default values or study values

Alexander DJ, Collins CJ, Coombs DW, et al. **Association of Inhalation Toxicologists** (AIT) Working Party Recommendation for standard delivered dose calculation and expression in nonclinical aerosol inhalation toxicology studies with pharmaceuticals. Inhal Toxicol. 2008;20(13):1179-1189

FDA Pulmonary Division Default Standard Values

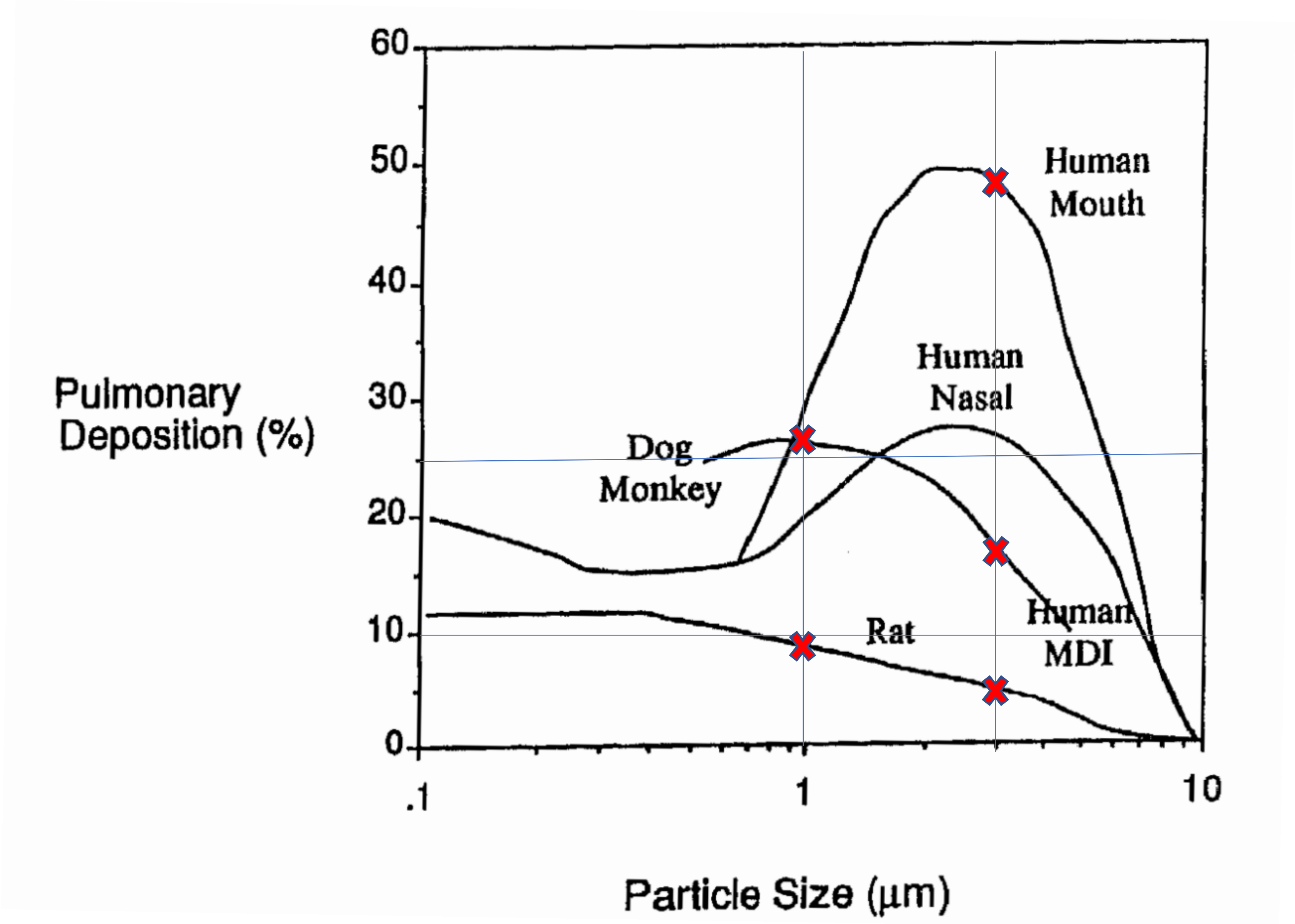
- Humans: 100% (i.e., nominal dose) is deposited in humans
- Non-Rodents: 25% of the inhaled dose is deposited
- Rodents: 10% of the inhaled dose is deposited
- Default deposition factors for rabbits, minipigs, ferrets and sheep not specified

Species	Mouse	Rat	Guinea pig	Monkey	Dog	Human*
Body Weight (kg)	0.03	0.25	0.7	2.4	10	60
MV (L/min)	0.03	0.19	0.45	1.3	4.3	20
Lung Weight (g)	0.2	1.5	4	22	110	1000
Deposition Fraction	0.1	0.1	0.2	0.25	0.25	1

* Values to be used for safety margin calculations

Tepper et. al., 2016

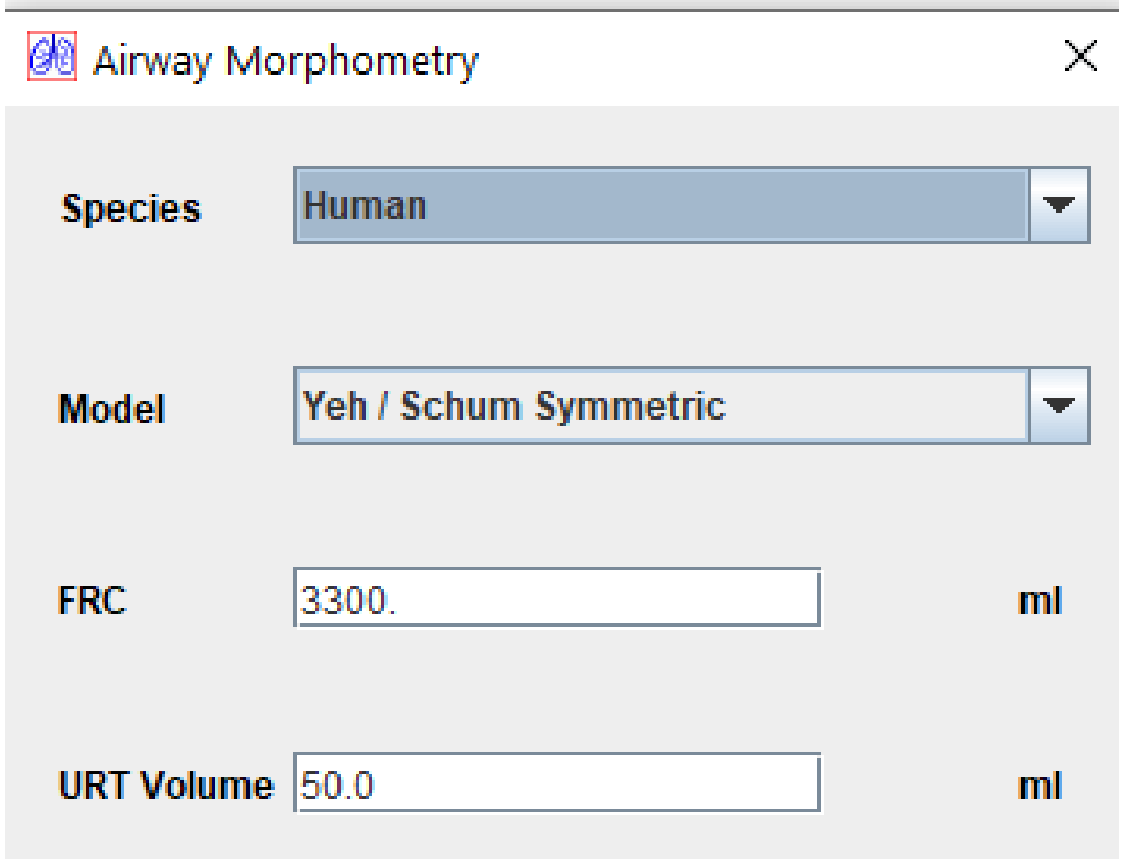
Pulmonary Deposition Varies with Particle Size



Schlesinger, R.B., Comparative deposition of inhaled aerosol in experimental animals and humans: a review.
J. Toxicol. Environ. Health, 15, 197, 1985

Wolff RK, Dorato MA. Toxicologic testing of inhaled pharmaceutical aerosols.
Crit. Rev. Toxicol. 1993; 23(4):343-69.
doi: 10.3109/10408449309104076.

In silico models, such as the MPPD model, can compute deposition fraction for humans and five laboratory species (not dogs ☹)



Applied Research Associated, Inc.
https://www.ara.com/mppd/

Scaling and Safety Margins for Inhalation FiH Dosing

- For inhaled drugs, scaling, using body weight (BWT) or lung weight (LWT), is most common

- Scaling, using BWT or LWT, shouldn't matter!
  - The equation for lung weight (g) =11.3 x BWT(kg)$^{0.99}$
  - An exponent of ~1 means that BWT scales isometrically (proportionally) with LWT

- However, it matters when calculating safety margins!
  - Default 60 kg BWT scales to a LWT of ~ 651g
  - Default LWT of 1000g scales to a BWT of ~92 kg

- This explains why normalizing to LWT provides a greater safety margin

- Required safety margin (PDD NOAEL/ Nominal Highest Clinical Dose) for the Pulmonary Division is 10-fold for rats, 6-fold for dogs and 5-fold for NHPs

Comparison of MRSD by Different Scaling Methodologies

Case example: The NOAEL is an inhaled dose of 10 mg/kg/day with the rat being the most appropriate of 2 tested species.
What are the uncertainty factors and the MRSD?

	Inhaled Dose	BSA Dose	PDD MRSD (BWT, 60kg)	PDD MRSD (LWT, 1kg)
Uncertainty Factor	10	10	10	10
Scaling Factor	1	6.2	1	0.36
Deposition Factor	1	1	10	10
Rat PDD/Nominal	1	1	2	2
MRD to MRSD factor	1	1	4	4
Total Uncertainty Factor	10	62	800	286
MRSD Dose (mg)	60	9.68	0.75	**2.10**

Summary/Recommendations

1. Standardize dose terms for inhalation to avoid confusion
2. Use the best science to determine the deposited dose in animals and humans as a method of reducing uncertainty
3. Allow NOAEL to refer only to harm to the tested animal under the conditions of the study
4. Use identifiable uncertainty factors to include potential concerns over pathogenesis, clinical relevance and monitorability
5. Harmonize by returning to the determination of the MRSD, allowing preclinical data to be used for starting dose and clinical data to determine maximum tolerated dose.

Industry Experience in Current Environment

Bill Thelin, PhD
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Per Åberg, MSc, DABT
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Jorrit Hornberg, PhD
AstraZeneca

Aidan Curran, PhD
Curran Nonclinical Consulting

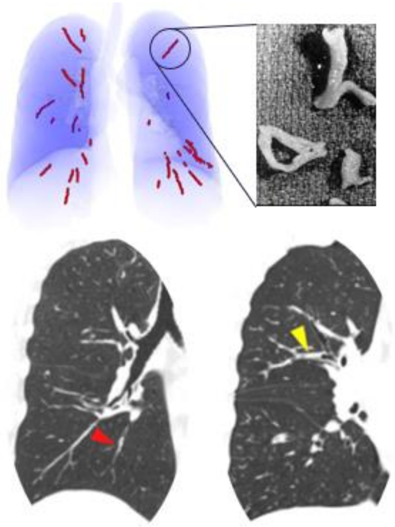
Challenges Developing Inhaled Drugs in the Current Environment – A Case Example

Bill Thelin, PhD
Aer Therapeutics

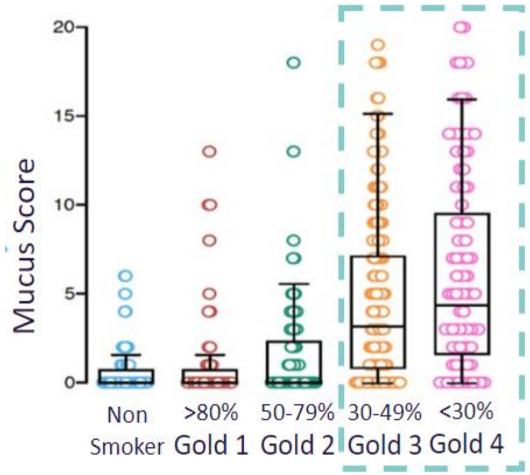
Severe COPD: A Serious Unmet Medical Need

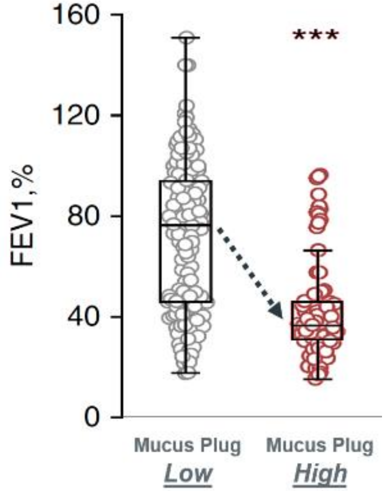
- High morbidity, mortality, and healthcare burden
- Mucus plugging is measurable and linked to worse outcomes
- Few therapies directly address structural airway obstruction
- Promising treatments for serious diseases warrant proportionate, evidence-based risk assessment

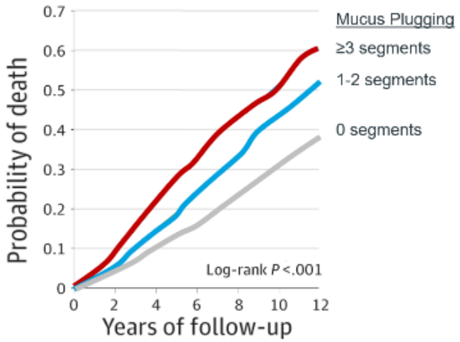
Mucus Plugs can be Visualized



Mucus Plugging Impact: Disease Severity, QoL, Lung Function, Exacerbations, and Survival







Mucus Plugs per Lung Segment	Mortality Rate (9-year, avg)
0	34.0% (±1.8%)
1-2	46.7% (±3.2%)
≥3	54.1% (±3.3%)

References:

Dunican EM et al. AJRCM. 2021; 203(8):957-968.

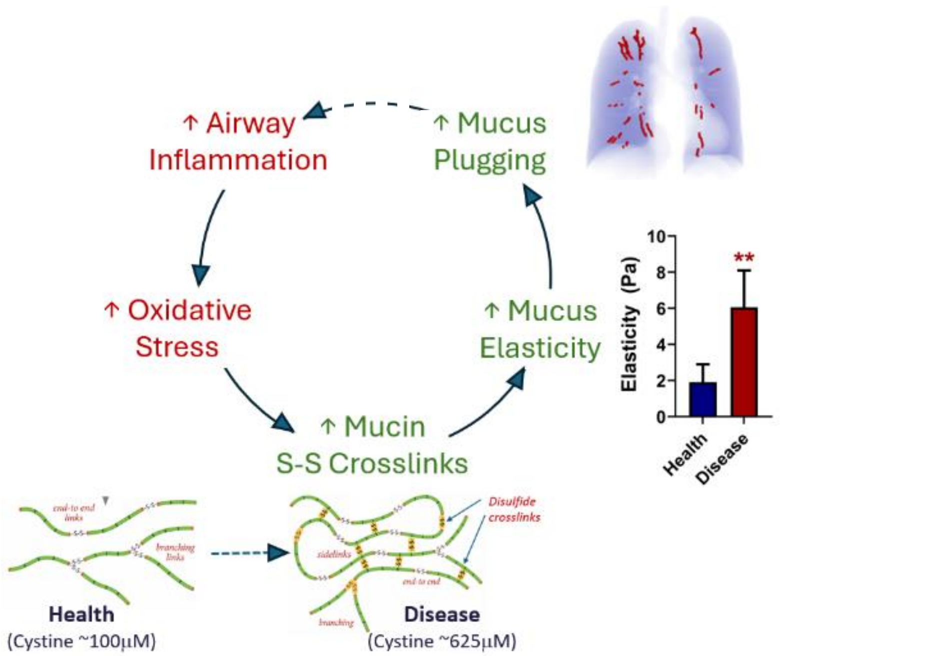
Okajima Y, et al. Chest 2020; 158:121-30.

Diaz A, et al. JAMA 2023; 329(21):1832-1839.

An Inhaled Thiol Saccharide Targeting Mucus Plugging

- AER-01 is an inhaled thiol mucolytic
- Reduces disulfide crosslinks in mucus plugs
- Thiol drugs have long-standing human use (e.g., NAC since 1960s)
- Designed to overcome the tolerability and efficacy limitations of NAC
- Innovation addresses formulation and tolerability — not a novel toxicity class

Disulfide Crosslinks are Central to Plug Formation



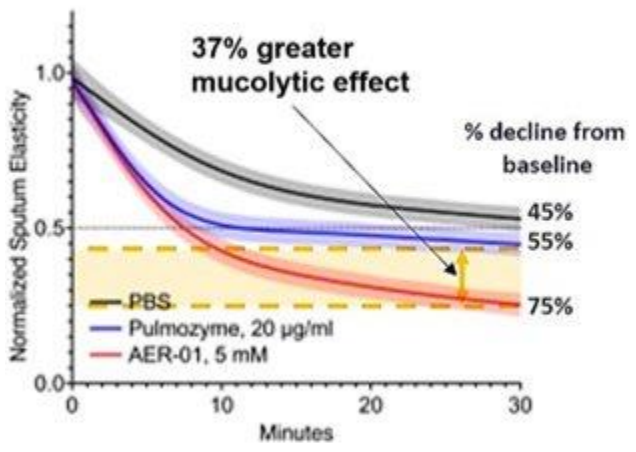
AER-01 Effect of Patient Sputum (12 min)

Translational Basis for Clinical Dose Selection

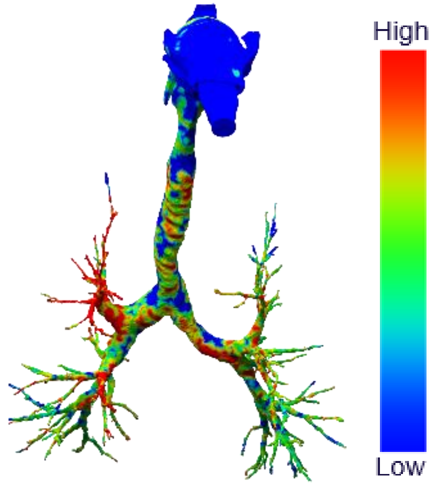
Clinical dose grounded in pharmacology and translational modeling

- Dose-dependent reduction in mucus viscoelasticity
- Defined minimum effective concentration
- Deposition modeling informed clinical dose range
- In vivo reduction in mucus burden and reduced pulmonary inflammation

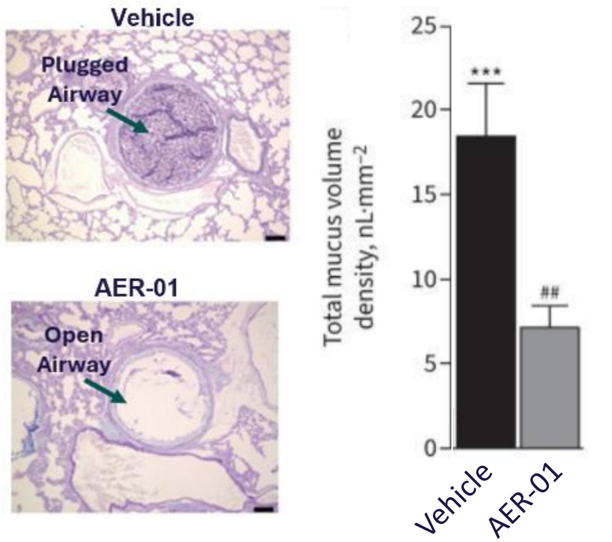
Pharmacology



Deposition and PK



In Vivo Efficacy



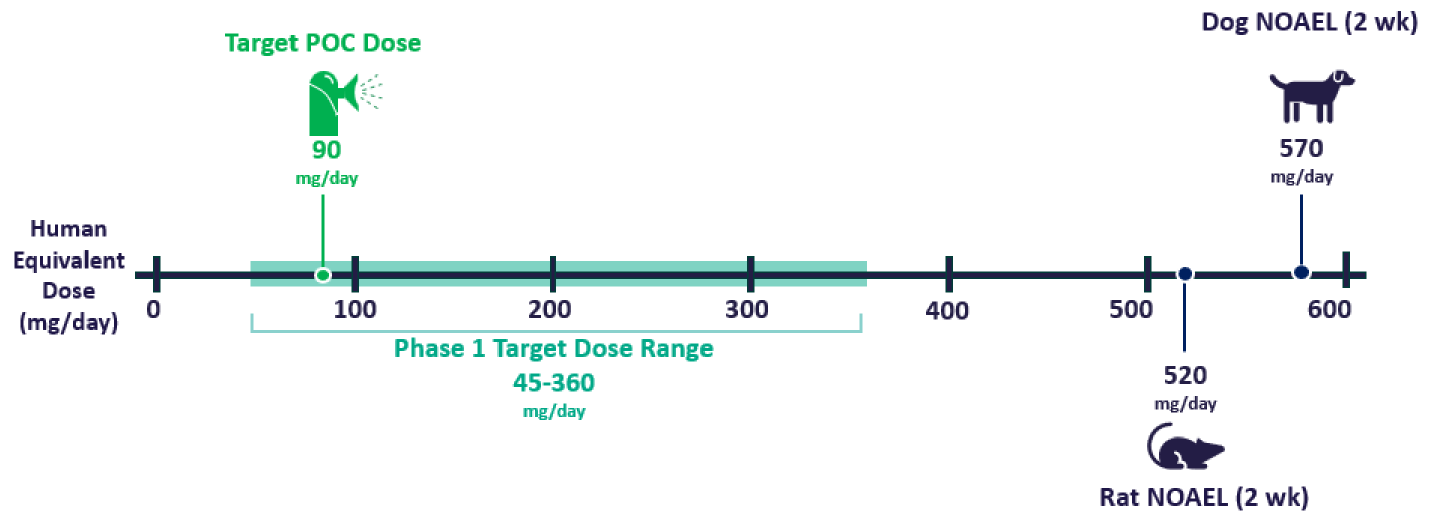
Addante et al. Eur Respir J. 2023;61(5):2202022.

Early Toxicology Supported Clinical Entry

Strong early nonclinical and clinical safety foundation

- 2-week GLP inhalation tox in rat and dog → NOAELs
- Supported Phase 1 dose range
- Phase 1 (up to 4x target dose) well tolerated

2-Week Tox Outcomes vs Clinical Dosing Targets



Phase 1 Summary (Australia)

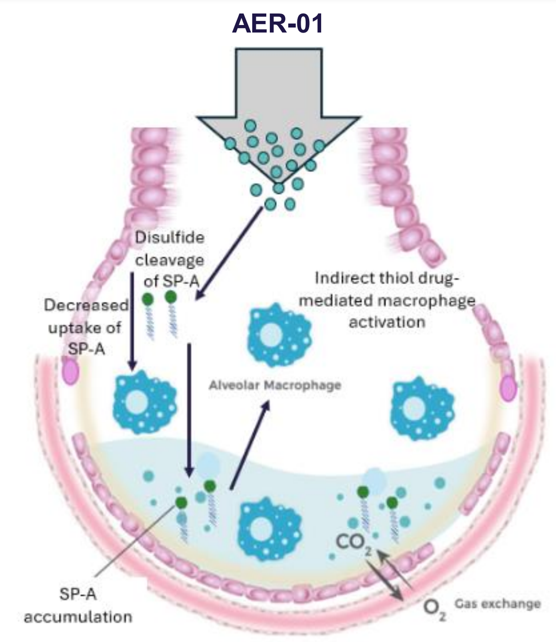
- ***Highest Dose (360 mg) well-tolerated***
  - ***4x P2 target dose***
- 96 healthy subjects evaluated
- Doses: 45 to 360 mg/day (up to 7 days)
- No pulmonary safety signals (stable spirometry)
- No systemic toxicity
- Dose-proportional PK

Chronic Inhalation Toxicology Divergence

Species divergence emerged in chronic inhalation studies

- 13-week inhalation tox studies
- Dog: NOAEL identified
- Rat: No NOAEL identified
- Rat findings: localized alveolar inflammation with protein accumulation
- Mechanistically consistent with an effect on surfactant proteins
- Non-progressive and showed reversibility

Off-Target Mechanistically Consistent with a Surfactant Effect



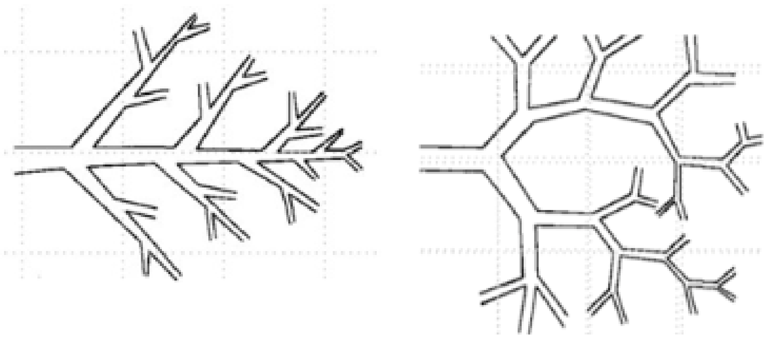
Why the Rat May Behave Differently (Than Larger Mammals)

Both Biological and regulatory factors may amplify apparent rat sensitivity

- Fewer airway generations → greater distal deposition
- Differences in mucociliary clearance
- Differences in lung mass and exposure per gram
- Rodents carry highest default regulatory safety margins (10x)

Airway Branching

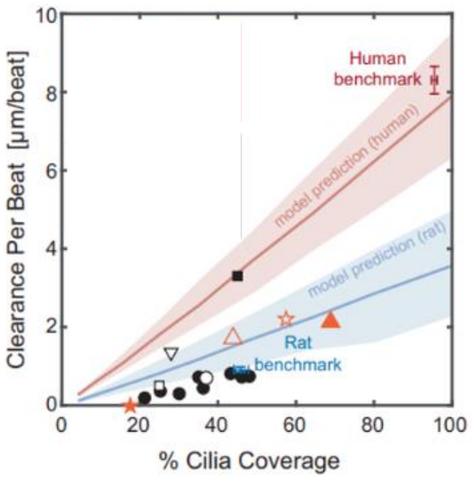
Rat (Monopodial) Human (Dichotomous)



Impacts regional aerosol deposition

Miller et al, Aerosol Sci Tech. 1993; 18(3): 257.

Mucociliary Clearance Rates



Impacts drug retention/clearance

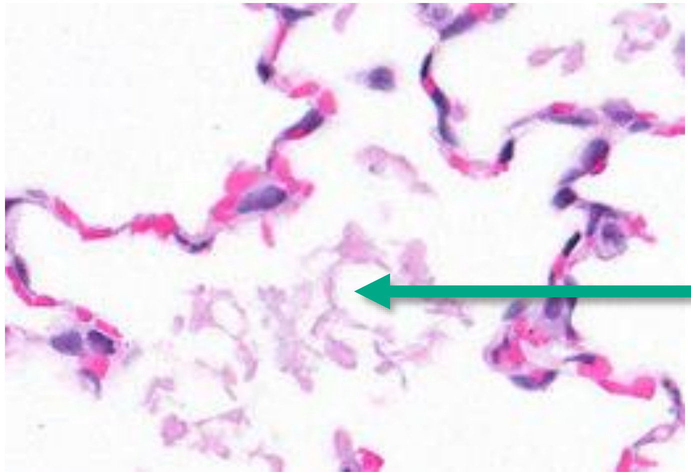
Roth et al, Nature Comm. 2025; 16(1): 2446.

Pulmonary Inflammation Is Clinically Monitorable

Rat histology signals are not inherently "non-monitorable"

- Alveolar inflammation has radiographic correlates (e.g., GGO)
- High-resolution CT detects inflammatory parenchymal changes
- Prospective monitoring and predefined stopping criteria can be implemented

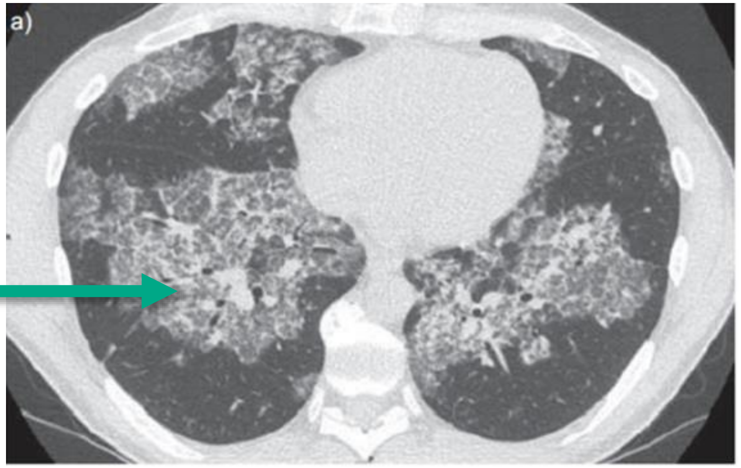
Rat Histology



Protein

Accumulation

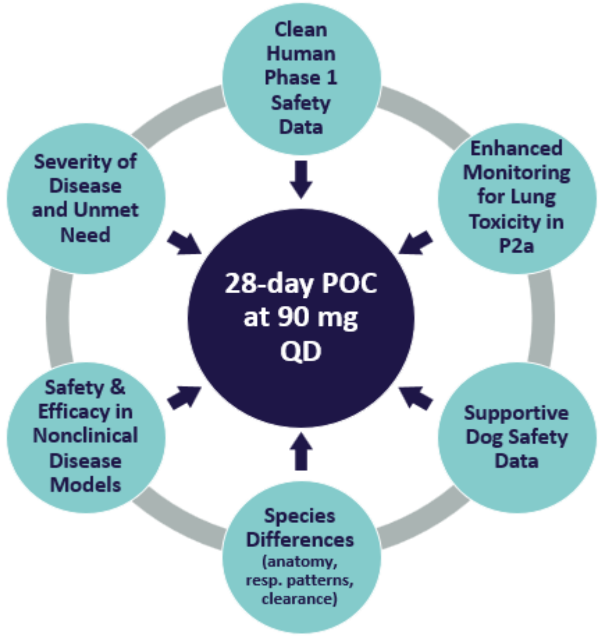
Human CT Image



When the Most-Sensitive Species Anchors Clinical Dose Limits

- Chronic rat findings frequently influence clinical dose limits, particularly for inhaled drugs
- Absence of rat NOAEL can constrain longer-duration studies
- Creates uncertainty for early-stage biotechnology companies
- Represents a recurring friction point in the current regulatory environment
- Some sponsors, including Aer Therapeutics, have conducted clinical development outside the U.S. under weight-of-evidence frameworks

Regulatory Weight of Evidence



Aer Initiated Phase 2a Development Outside the U.S.

- Status: Ongoing
- Enrollment: 100 patients with moderate–severe COPD
- Dose: 90 mg/day vs placebo for 28 days
- Efficacy: Change in FEV_1, MPS, QoL
- Safety: Spirometry, clinical labs, and HR-CT imaging
  - Prospective CT monitoring of ground-glass opacities (GGO)
  - Predefined stopping criteria triggered by inflammatory imaging signals
- Regulatory approvals: Aus, NZ, UK (MHRA), EU (EMA pending)

aer therapeutics

Implications for U.S. Innovation and Animal Use

This challenge extends beyond science — it has economic and policy implications

- Development may relocate to jurisdictions applying weight-of-evidence approaches
- ~$30M in clinical investment occurred outside the U.S. in this case
- Over reliance on a single species may drive additional animal studies
- Modern monitoring tools and NAMs/MPS may reduce need for redundant animal testing

Toward a Weight-of-Evidence Framework

- Rat toxicology signals should be respected
- But not automatically qualifying or disqualifying
- Integrate multi-species data, mechanistic understanding, and clinical monitorability
- Weight-of-evidence can *preserve* safety while *advancing* needed therapies

For serious lung diseases, integrating species biology and clinical monitorability may better balance safety, innovation, and U.S. competitiveness.

aer therapeutics

Modernizing Inhalation Toxicology

Per Åberg – Senior Director, Clinical Pharmacology and Safety Sciences, R&D, AstraZeneca Gothenburg, Sweden

Jorrit Hornberg – VP, Global Head of Safety Sciences, Clinical Pharmacology and Safety Sciences, R&D, AstraZeneca Gothenburg, Sweden

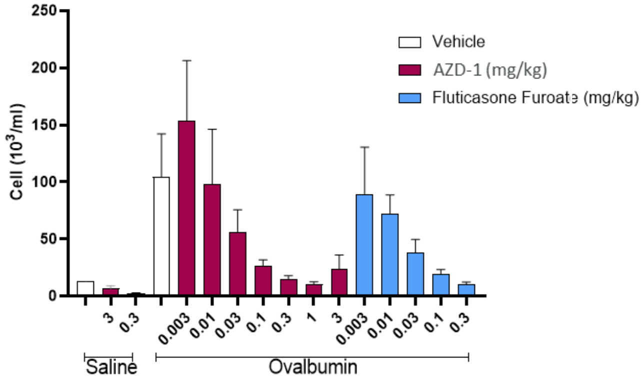
Acknowledgements to Paul Fitzpatrick and Muntasir Mamun Majumder

Contents

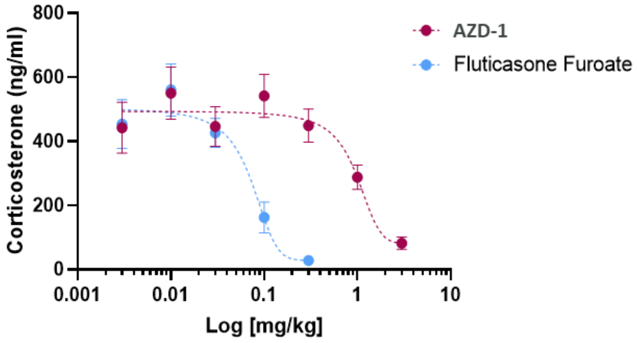
Non-steroidal glucocorticoid receptor agonist – case background

- Intended for combination treatment of respiratory disease
- Rat efficacy model: improved therapeutic index vs inhaled corticosteroids (ICS)

AZD-1 suppresses BAL eosinophilia in rats



AZD-1 has less effect on corticosterone in rats



- Therapeutic doses of 360 and 720 µg (Ph2 data)

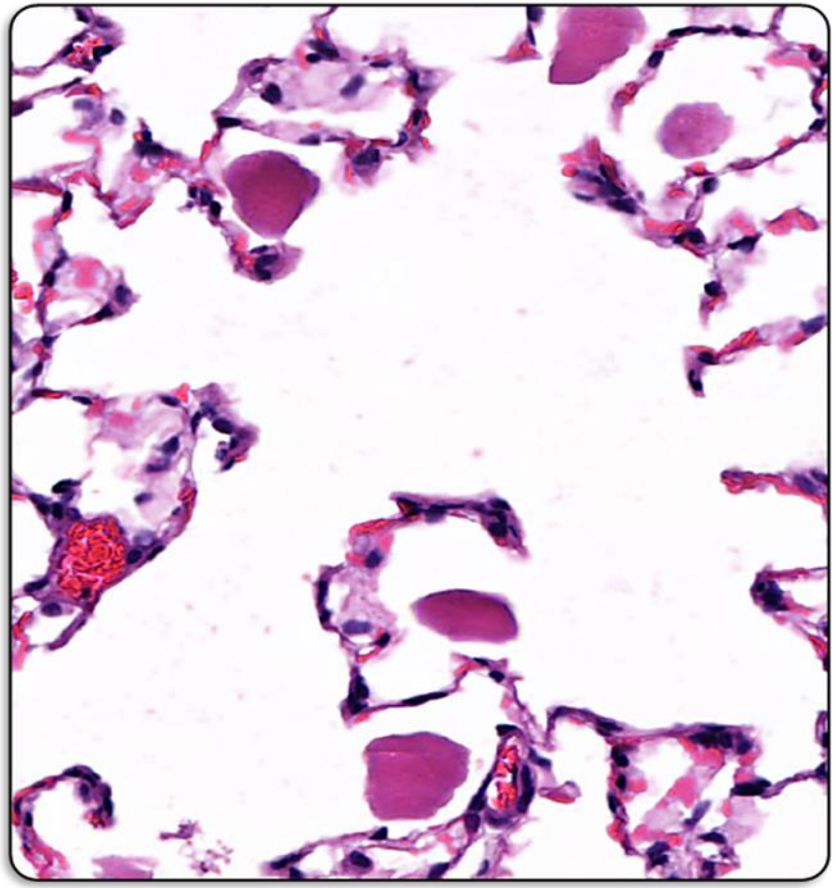
Tox program: 1- and 6-month studies in rats, 1-, 3- and 9-month in dogs

PrePh2, **FDA raised concerns around lung histopathology finding in rats and required a dose cap at 360 µg**; 10-fold margin to NOEL for finding, assuming 100% deposition in humans. Not present in dogs

AZ assessment: lesion was not representing an effect of concern for the proposed doses; no concerns raised by other Health authorities

Ph2b pursued at 360 µg in US, 720 µg in ROW; dose-related efficacy, no safety signals of concern

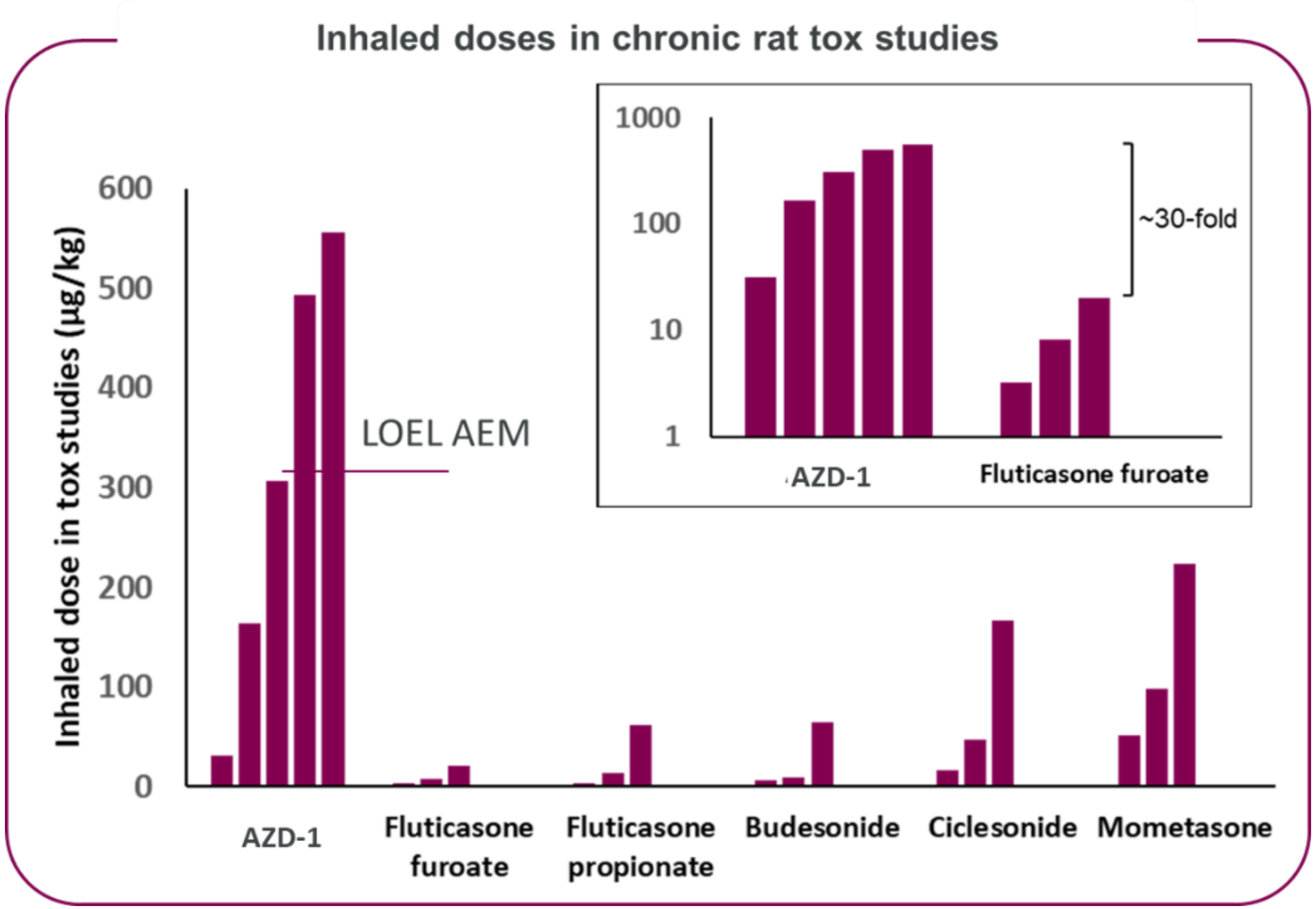
Rat lung pathology (6 month, not at 1month)



- ***Alveolar eosinophilic material (AEM)***
  - Amorphous deposits within alveolar lumina
  - PAS-positive; lipoprotein
  - TEM confirmed lamellar structure; phospholipid
  - IHC confirmed proSP-C
- Low severity, reversible
- No associated **inflammation** or **blood-air barrier damage**
- Present at an estimated dose multiple of 10-fold 720 µg (at least 19-fold accounting for human deposition)
  - Absent at 5-fold 720 µg

FDA: **AEM** not previously described with ICSs, concern it may represent alveolar proteinosis.

Lower systemic burden in rats than for ICS; higher dose coverage in chronic tox studies (defined by MTD)

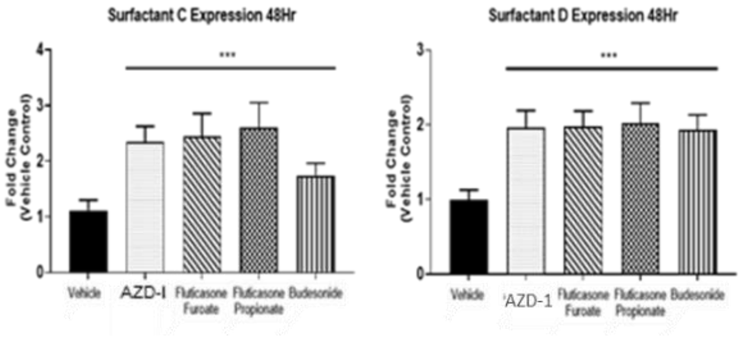


AZ hypothesis: AEM represented suprapharmacological effect on surfactant production. GR agonist stimulatory effect on surfactant production *(Lewis, 2014; Eik-Nes, 1987; Young and Silbajoris, 1986)*

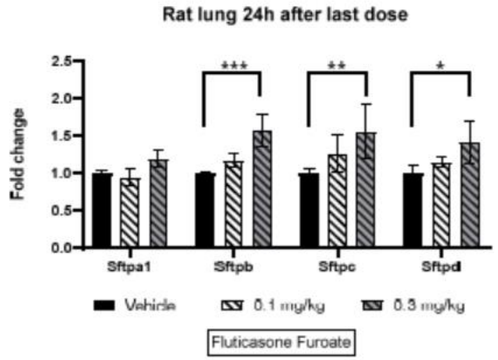
Investigative package during Ph2b to support high dose globally

GR-induced surfactant upregulation demonstrated

in **human alveolar** cells

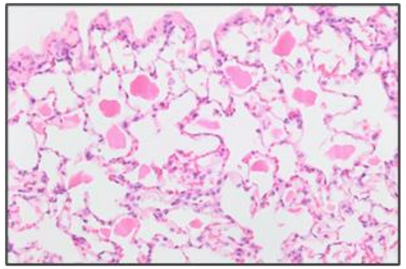


in **rats** following 7-day IT administration

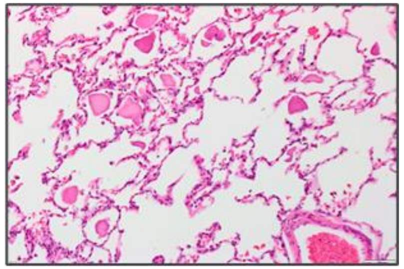


Other supportive information

Similar lesions present in air control dogs in 9-month study



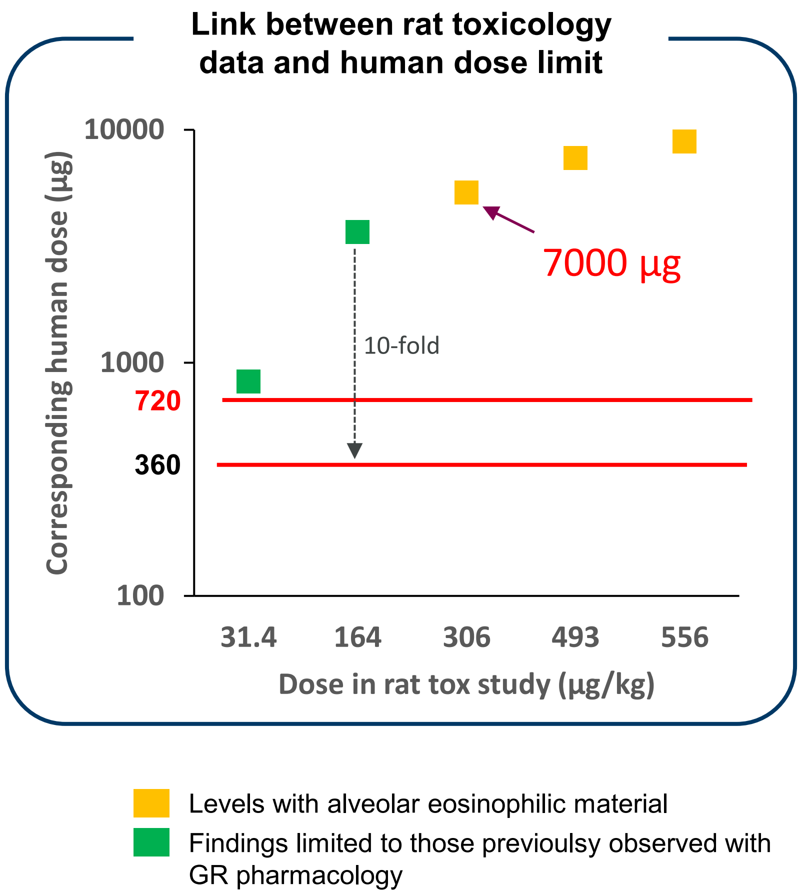
Rat lung: slight amorphous eosinophilic deposits within alveoli following dosing of AZD-1 for 6 months



Dog lung: similar amorphous eosinophilic deposits occurring spontaneously in control animal

- Max dose/NOAEL for ICS in chronic rat studies would not provide 10-fold lung dose coverage of therapeutic doses
- Phenotype not considered consistent with alveolar proteinosis

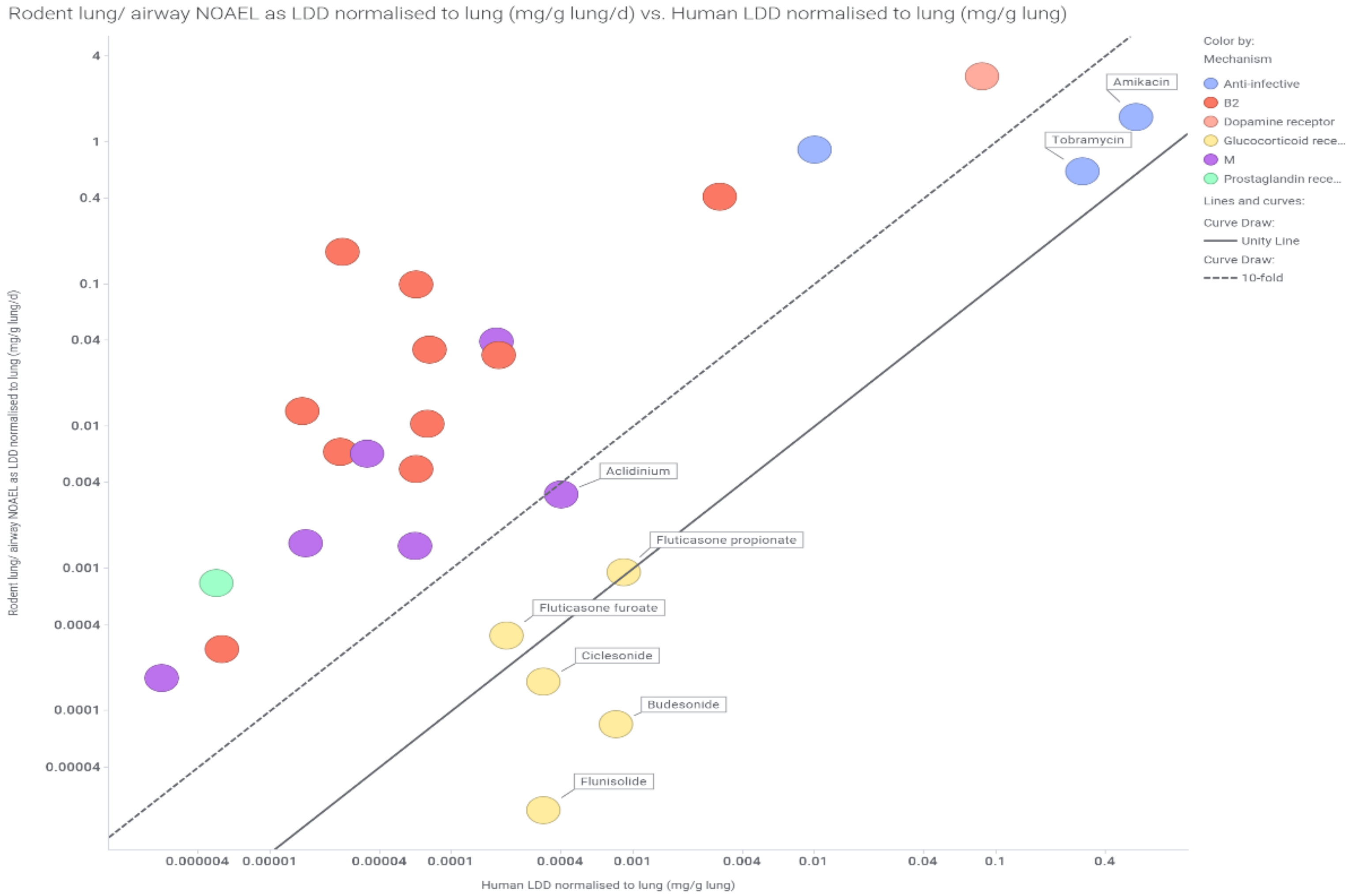
Context for risk assessment



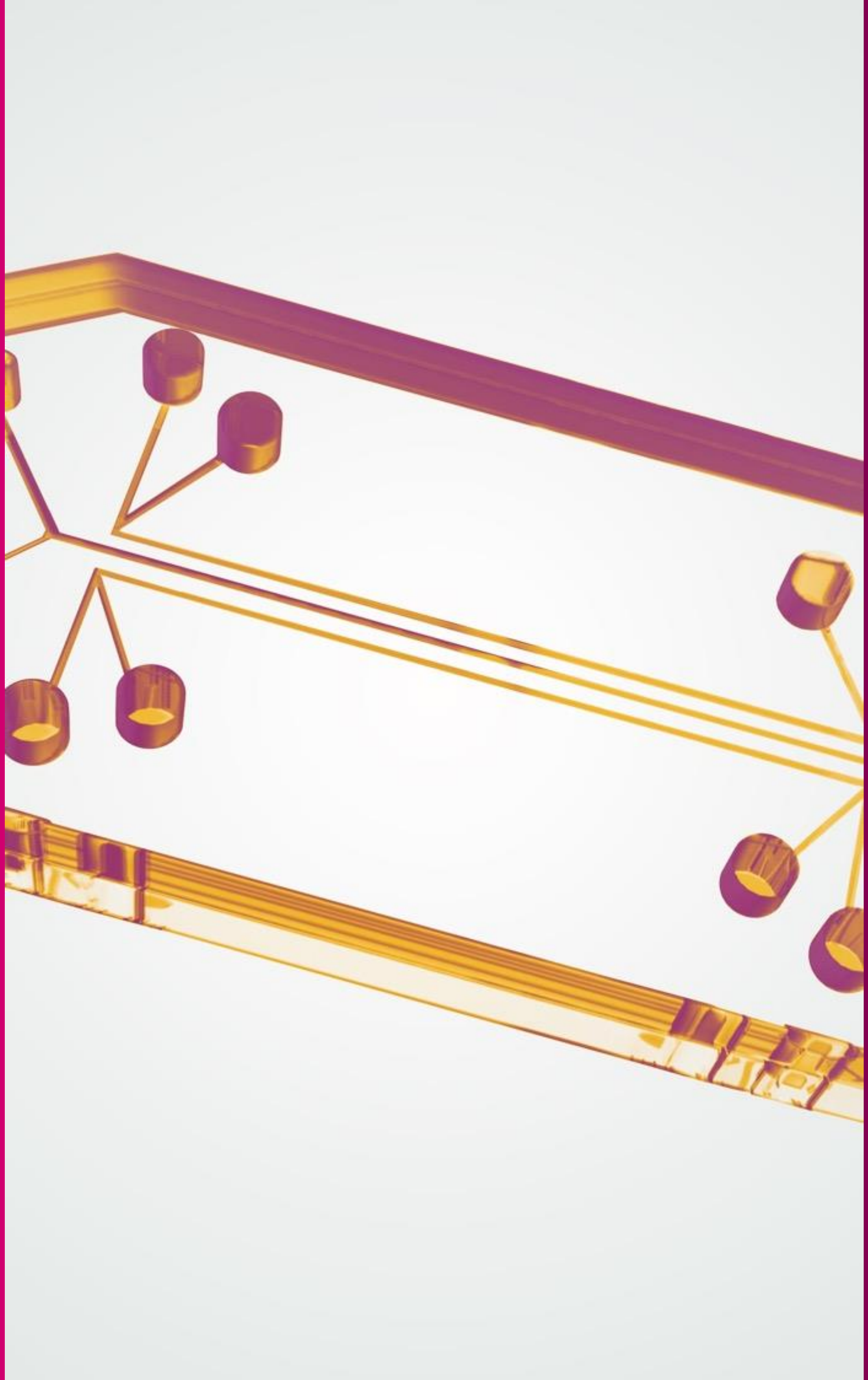
- AEM occurred in rat lung at a dose corresponding to 7000 µg in humans – vs 720 µg delivered dose, estimated ~360 µg deposited with human device
- AEM was not associated with epithelial/inflammatory lesions
- Investigative studies supported hypothesis for GR-induced surfactant expression at suprapharmacologcial levels
- Similar histopathology in non-treated dogs

10-fold margin + 100% lung deposition assumption created a very high bar for this type of lesion/molecule

Approved inhaled molecules analysis: NOAEL from chronic rat studies do not always reach 10-fold lung dose coverage



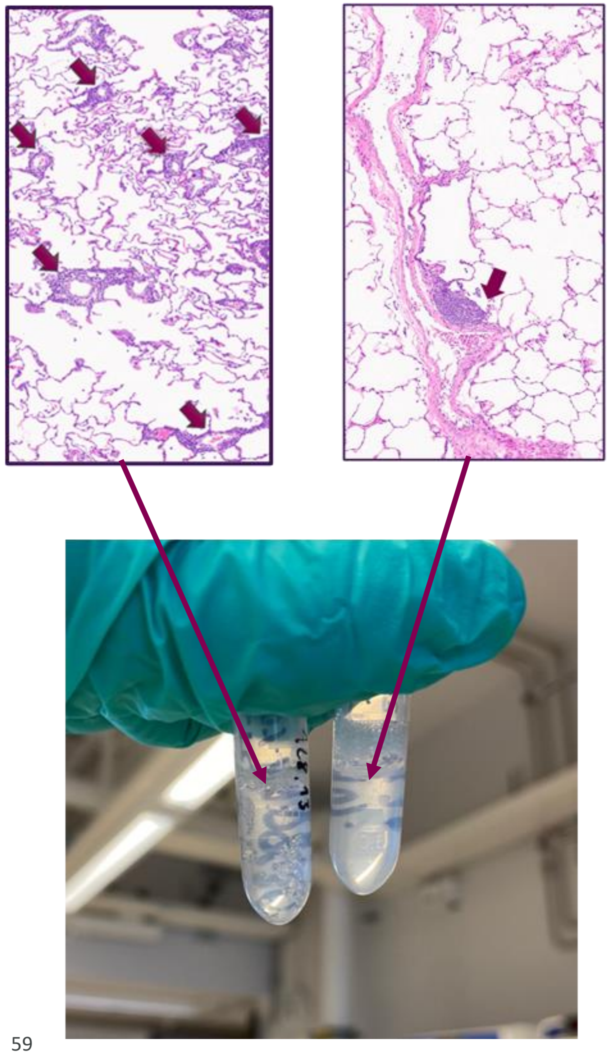
Data from FDA approval packages. Lung dose calculation according to Tepper et al, 2016. NOAEL rodent vs max approved dose



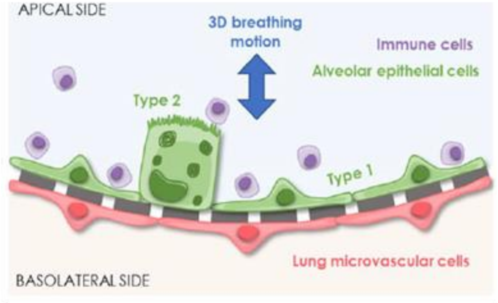
Can we predict lung histopathology with *in vitro* methods?

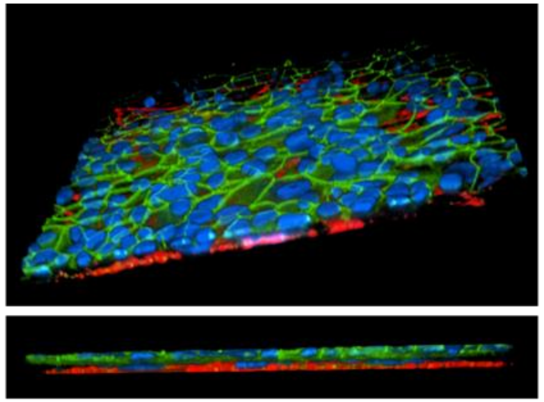
Breathing Lung-on-Chip model predicts lung irritancy and inflammatory response in vivo

Formulation 1 | Formulation 2



A lung co-culture model with a pressurized system to enable cyclic breathing movement



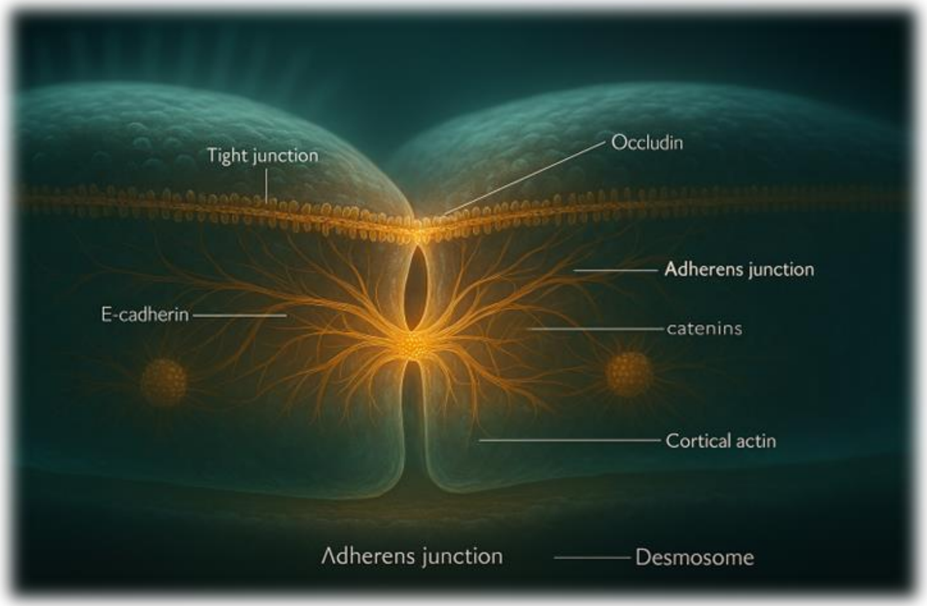


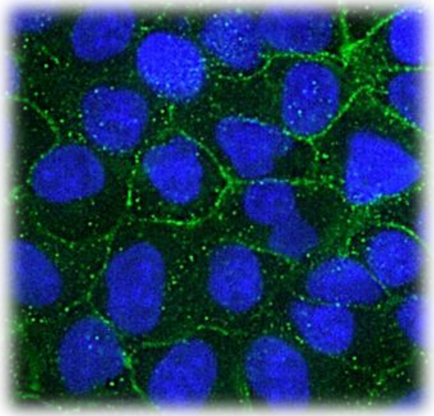
AZD1: Cytokines

	Formulation 1			Formulation 2		
	Low	Med	High	Low	Med	High
G-CSF	0.6	0.6	113.7	1.0	0.6	1.6
IL-10	1.0	1.0	24.2	1.0	1.0	1.0
IL-1b	1.0	1.0	140.7	1.0	1.0	1.0
IL-6	0.4	0.4	65.4	0.4	0.4	0.4
IL-8	0.6	0.4	3.6	1.0	0.6	0.7
IP-10	0.8	1.0	1.9	0.9	0.8	1.1
MCP-1	0.7	0.4	2.8	1.4	0.8	1.1
MIP-1a	1.6	1.0	63.7	3.4	1.6	1.0
MIP-1b	1.3	0.4	7.9	1.8	1.2	1.6
TNF-a	1.1	0.6	24.1	3.6	0.7	1.4

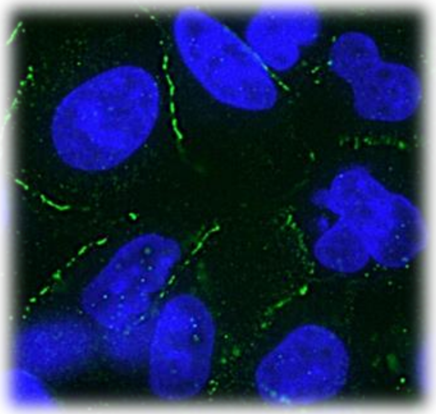
L. Johansson et al 2026

Tight-junction imaging assay predicts respiratory irritancy



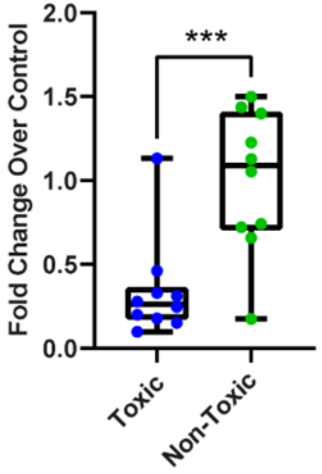


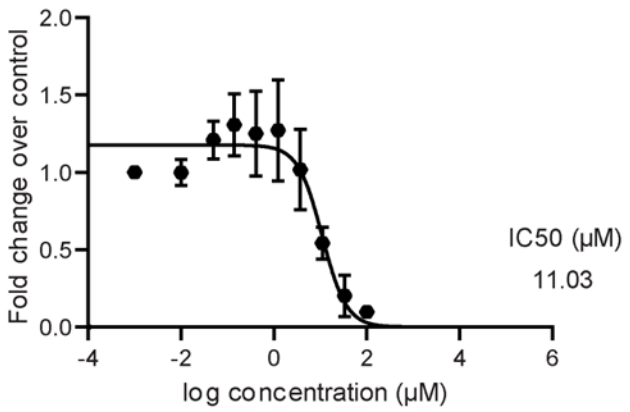
Cobblestone staining pattern indicative of TJ



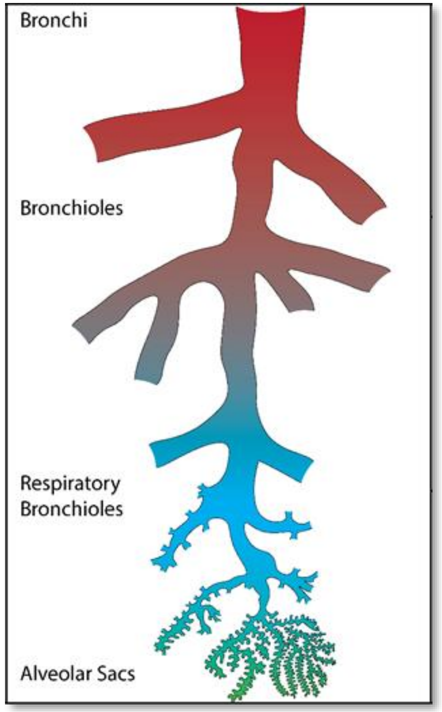
Discontinous staining pattern indicative of barrier disruption

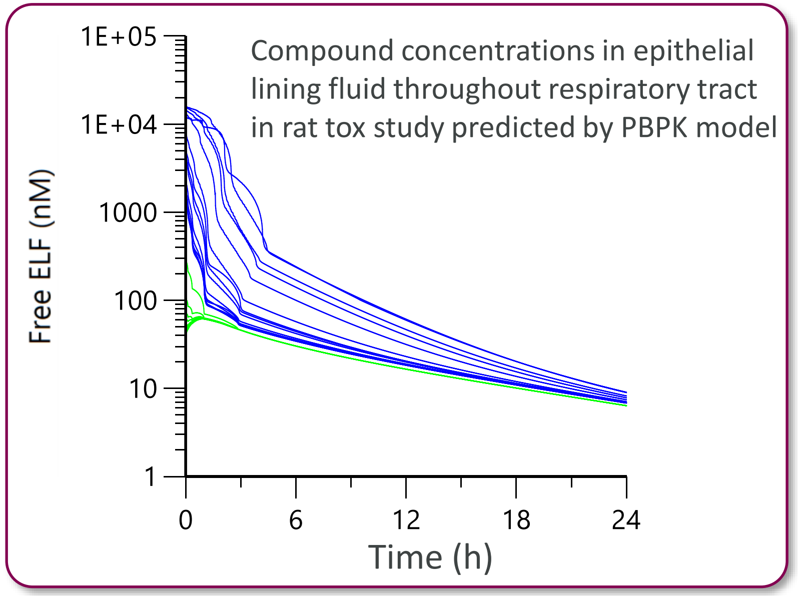
- Junctional proteins maintain strong cell-cell and cell-ECM connections
- These connections play a pivotal role in various barrier formations (e.g. blood-brain, air-blood and gut-blood barriers)

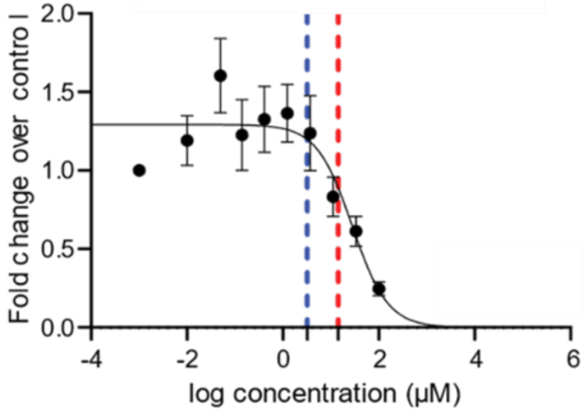


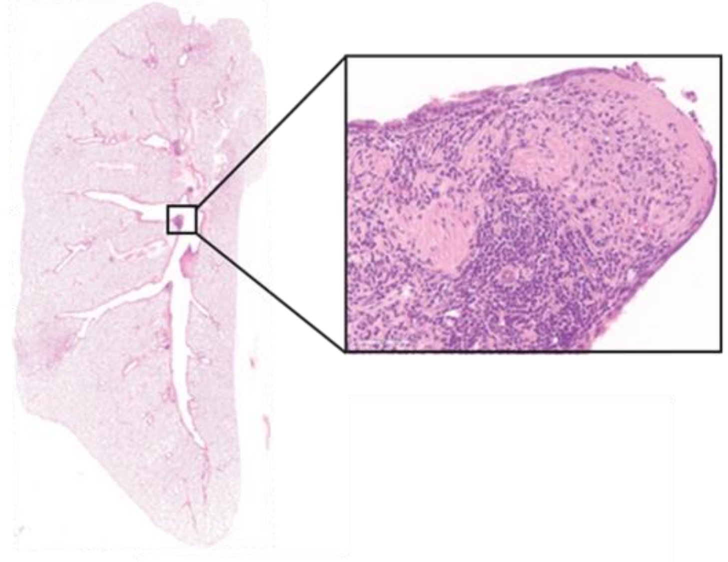


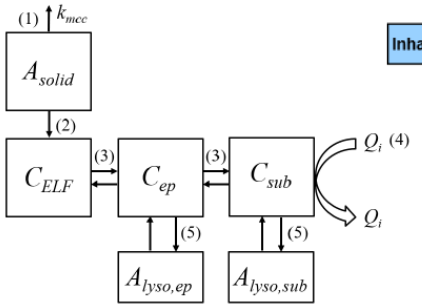
Combining in vitro occludin imaging assay data with PBPK modeling predicts localization of lung histopathology

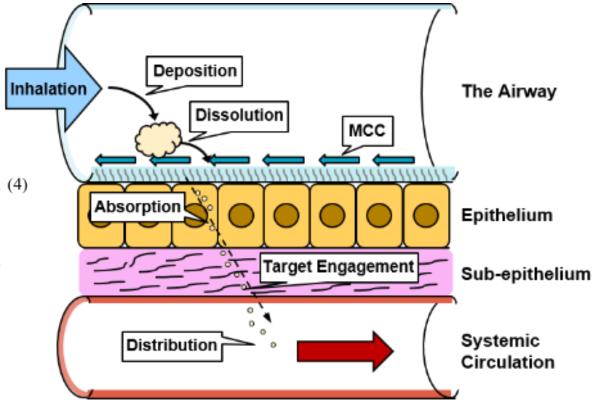


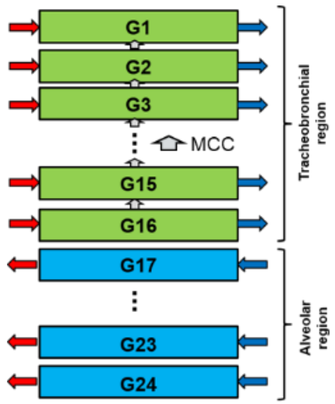




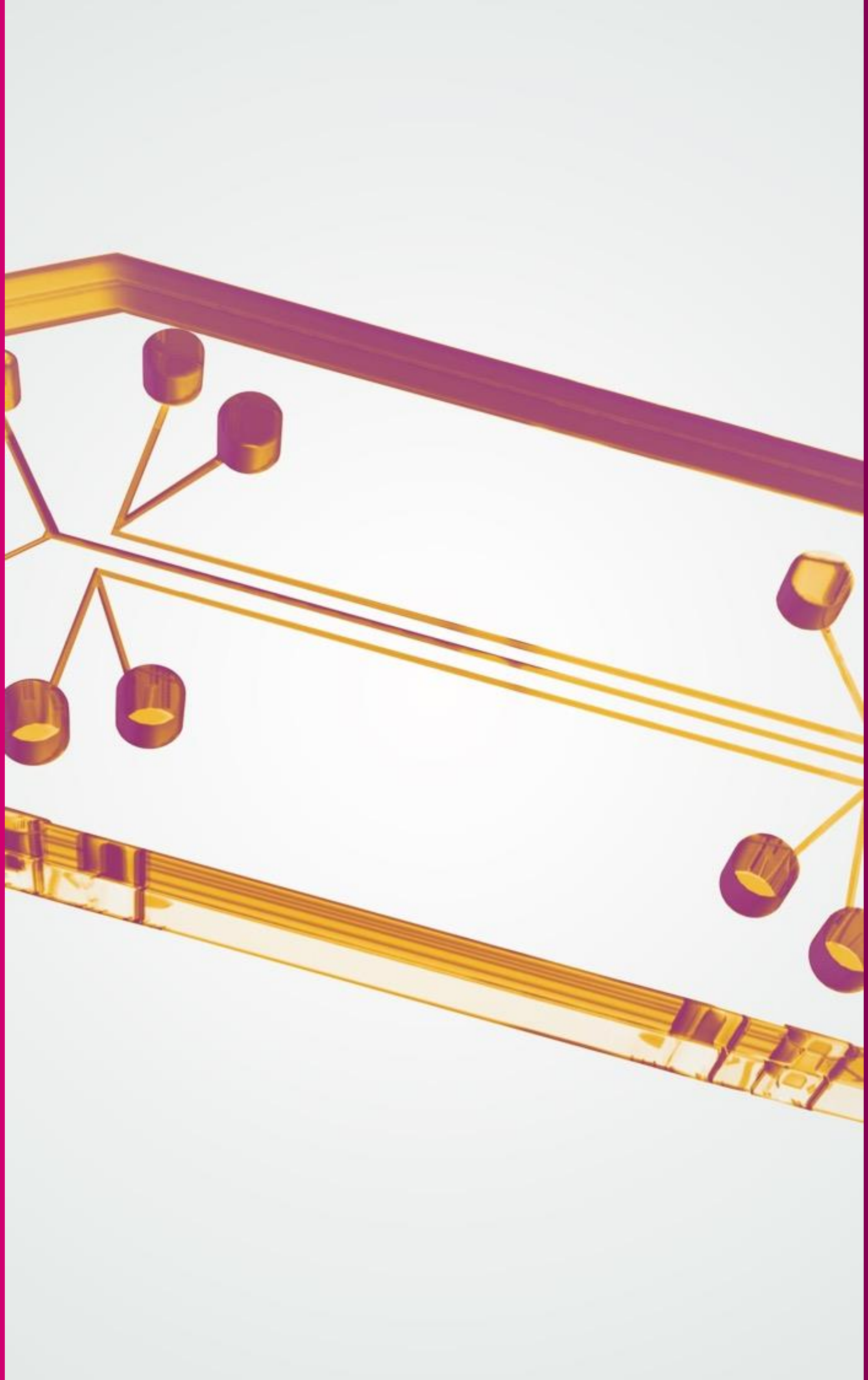








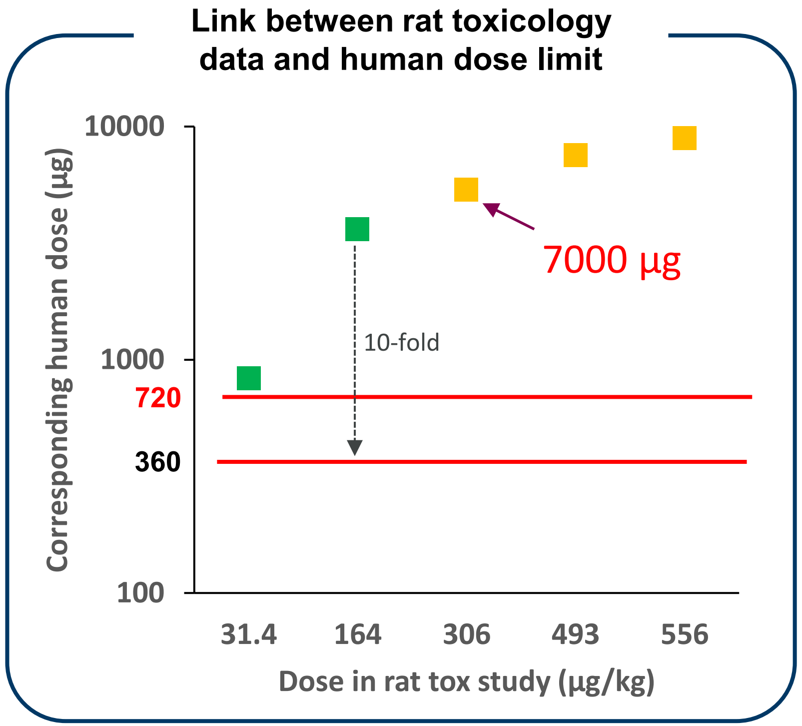
PA Fitpatrick et al 2024



Can we apply novel technology to re-define a safe dose limit?

What if we could monitor with biomarkers…

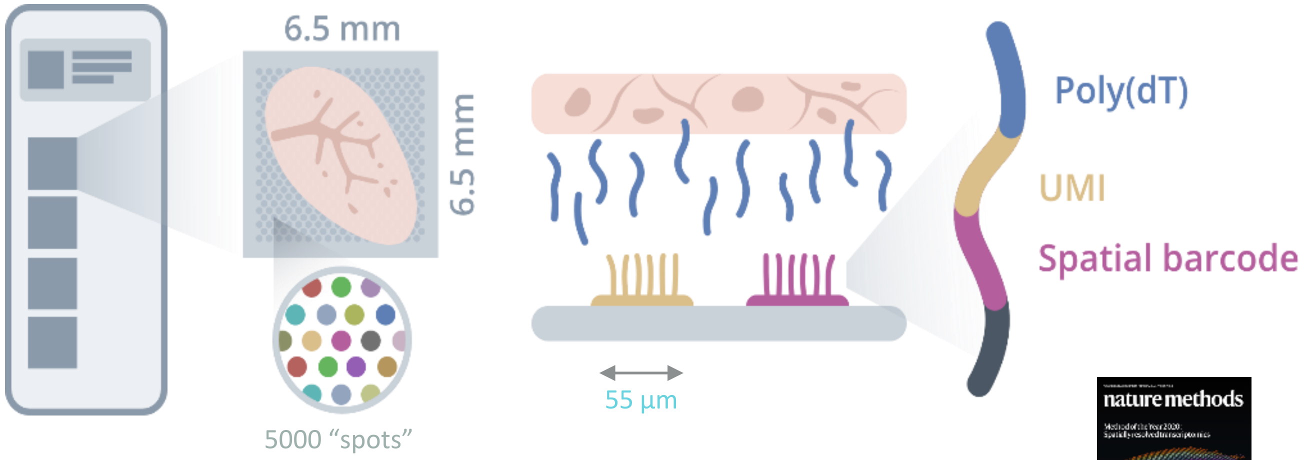
Recall Per's case study – the 10-fold…

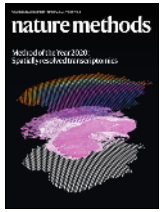


Levels with alveolar eosinophilic material

Findings limited to those previoulsy observed with GR pharmacology

Unravelling molecular mechanism of lung pathology using spatial transcriptomics

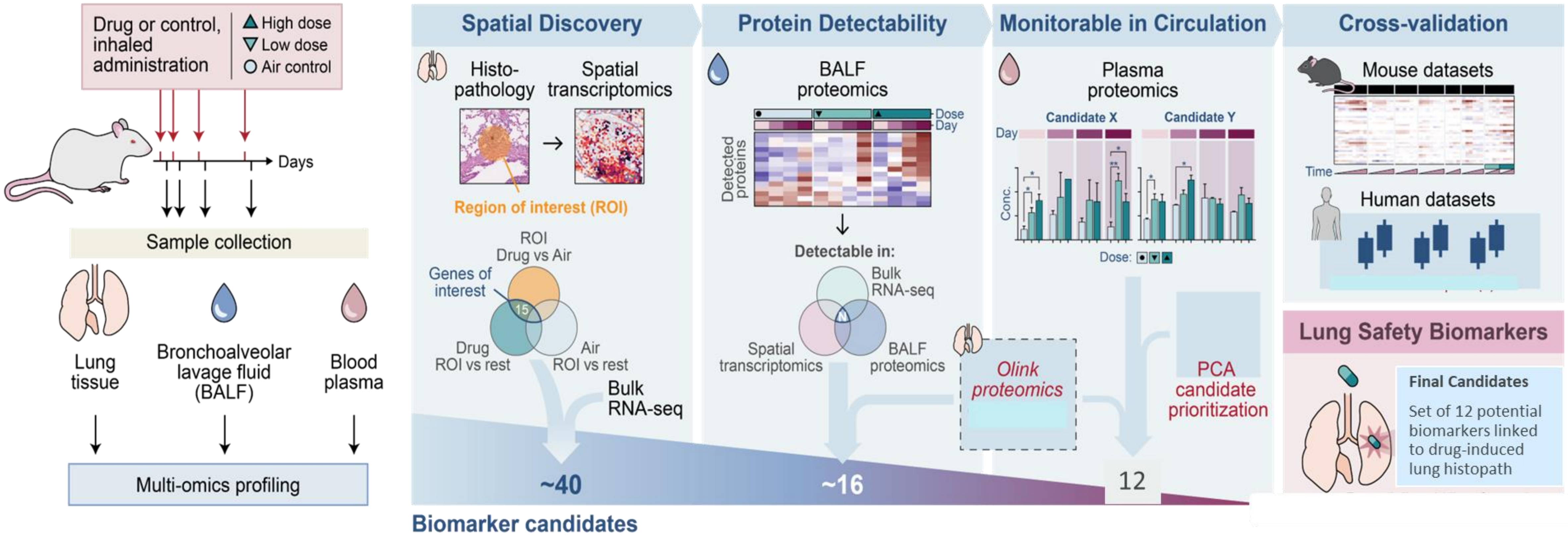




From histopath to molecular mechanism to biomarkers

Our multi-omics framework identifies circulating biomarkers for real-time toxicity monitoring

Lesion-enriched signals translate to biofluids and human serum to enable non-invasive monitoring



L Franzen et al Nat Genet (2024)

MM Majumder et al BioRxiv (2025)

AstraZeneca PLC, 1 Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge, CB2 0AA, UK
+44(0)203 749 5000
www.astrazeneca.com

Advancing Drug Development by Reducing Reliance on Animal Testing
Case Example: Preclinical Animal Models in Lung Toxicology

Industry Perspectives

Aidan K Curran PhD
Principal, Curran Nonclinical Consulting

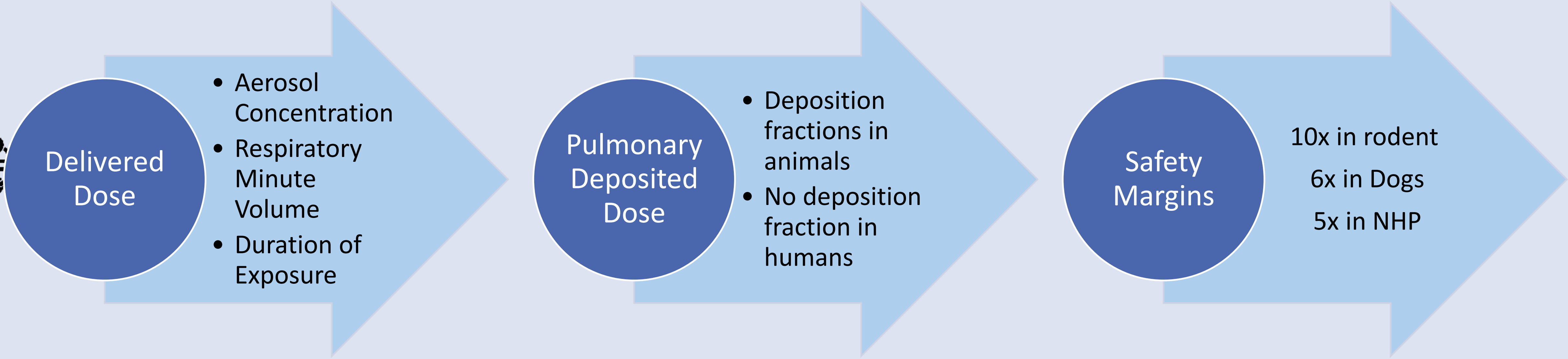
Problem Statement

- Early development of novel inhaled therapeutics through Phase I remains and will remain dependent on animal toxicology data but interpretation of findings often creates uncertainty
  - Individual findings in a single species (laryngeal findings in the rat)
  - Findings of minimal incidence or severity (NOAEL versus NOEL)
  - Stringent safety margin calculations
- Uncertainty around these issues lead to a series of preventable issues
  - Additional studies result in increased animal use
  - Program timeline delays and increased cost put small pharma at particular risk
  - Early clinical work is often moved overseas

Disclaimer: I have no direct conflict of interest - As an independent consultant I assist multiple clients but the thoughts expressed here reflect only my opinions are designed to stimulate discussion

Safety Margin/Starting Dose Calculations

- We obviously need adequate safety margins to support safe clinical dosing.
- However, margin calculations include multiple layers.



- The deposition fractions in animals are based on research in the 80s and 90s, prior to development of modern aerosol and device engineering.
- Combined, these safety margin requirements can lead to a delivered dose in rodents > 100-fold that in humans.

Safety Margin/Starting Dose Calculations

- The need for very high nonclinical doses in tidally breathing animals can lead to high aerosol concentrations and/or long durations of exposure.
  - This approach risks findings unrelated to the API and more related to lung overload or mechanical effects.
- Lung deposition assumptions apply to laryngeal findings, despite the fact that deposition is likely higher in the larynx, particularly the rodent larynx.
- Updated deposition estimates in animals and the use of emitted dose and fine particle fraction to estimate human pulmonary deposited dose would would result in more accurate and reasonable risk assessment.
- Advancements in aerosol engineering, deposition modelling, imaging and interpretation are not reflected in current risk assumptions

Challenges:

Use Clinical pulmonary deposited dose for safety margins

Update our assumptions on nonclinical deposition and allometry using modern formulations, imaging and modelling methods

NOAELs and Defining Adversity

- We lean on NOAELs to define a safe starting dose but defining what we consider ‘adverse’ or more recently ‘potentially adverse’ or even ‘of concern’ is ill-defined.
- Findings of minimal severity have impacted progress
  - Can findings with no impact on organ function really be adverse?
  - Can we consider the patient population and indication?
- Some findings in the rodent larynx can often be acceptable but findings in the trachea, carina and lungs have been deemed ‘non-monitorable’ and become limiting regardless of severity or the likelihood for organ function impact.

Challenge: Using advanced imaging and biomarker specificity, do ‘non-monitorable’ findings really exist anymore?

Case example - data Interpretation

- Inhaled 505(b)(2) inhaled program for systemic exposure with known maximum canine dose based on systemic tolerability and known systemic exposure tolerability profile in humans
- Studies designed to match rodent and canine exposures to achieve similar margins at each dose
- No lung findings at any dose in either species – MTD based on dog systemic findings - >10-fold margins at high dose in both species
- Tox review found the rodent data unacceptable due to lack of findings and required an additional GLP study to determine rat MTD/MFD
  - Additional study cost time, money and animals, determined a MFD with a rat margin >100-fold but resulted in the same clinical dose
  - Clinical study moved ex-US with significant program delays

Case examples – finding interpretation

- Over the past few years, my clients have faced multiple hurdles due to a core of similar issues
  - Findings limited to the rodent laryngeal ventral pouch
  - 'Non-monitorable' airway/lung findings
  - Nebulous findings like 'epithelial alteration' flagged as a concern
  - Findings that do not progress in incidence and severity with chronic dosing
- Programs have been stalled for additional studies to investigate findings
- Ultimately many clinical programs move ex-US

Challenges:

Can we use AI tools and large data sets across multiple sources to properly assess translatability across nonclinical and clinical findings?

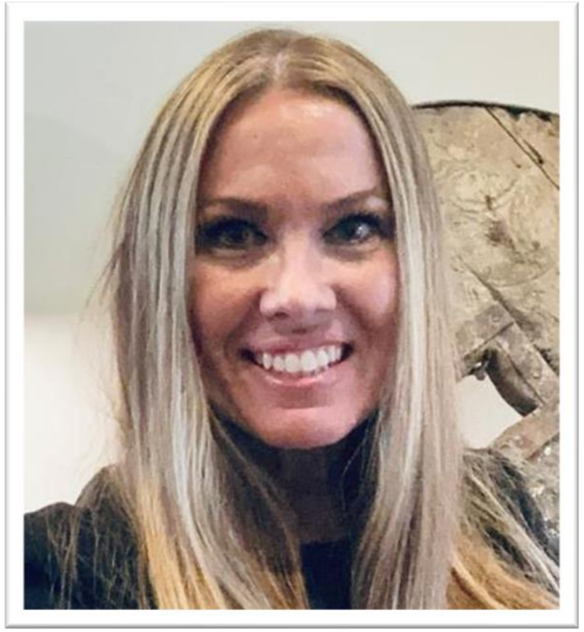
Can we assess whether the move ex-US impact patient safety?

Summary

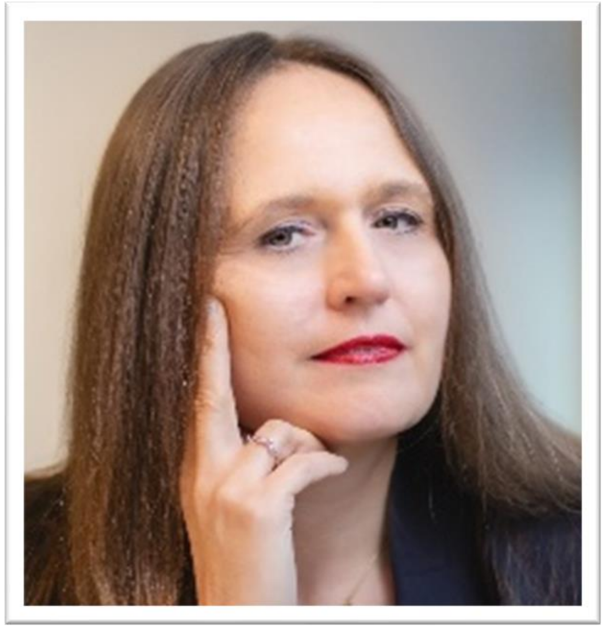
- Inhaled drug development, despite the advanced nature of engineering and delivery, retains a lot of uncertainty, unknowns and outdated assumptions.
- Conservative approaches to data interpretation and risk aversion is stalling innovation, increasing the burden on animal research and moving progress ex-US.
- There is a huge opportunity for the use of advanced tools to greatly increase our understanding of the delivery, deposition and effect of inhaled formulations.
- This will require investment and transparency from industry and a willingness on the part of regulators to support such efforts and the flexibility to utilize the findings.
- Professional organizations need to continue to promote these efforts through advocacy and active participation.

Panel Discussion

Impact of Current Environment on Product Development and Patients



Teresa Barnes
PF Warriors



Karin Hoelzer, DVM, PhD
BIO

REAGAN-UDALL FOUNDATION FOR THE FDA

Lunch

We will resume at 1:15pm ET

Innovations in Lung Toxicology Safety Studies: New Approaches in Pre-Clinical Models & Clinical Monitoring

REAGAN-UDALL FOUNDATION FOR THE FDA

Mary McElroy, PhD
Charles River Laboratories

Alexandra Maertens, PhD
Johns Hopkins University

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Embracing LUNG NAM Safety Testing: The Future Of Drug Development

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Head, Discovery Pharmacology and Safety
26 Feb 2026, Reagan-Udall Foundation

Outline

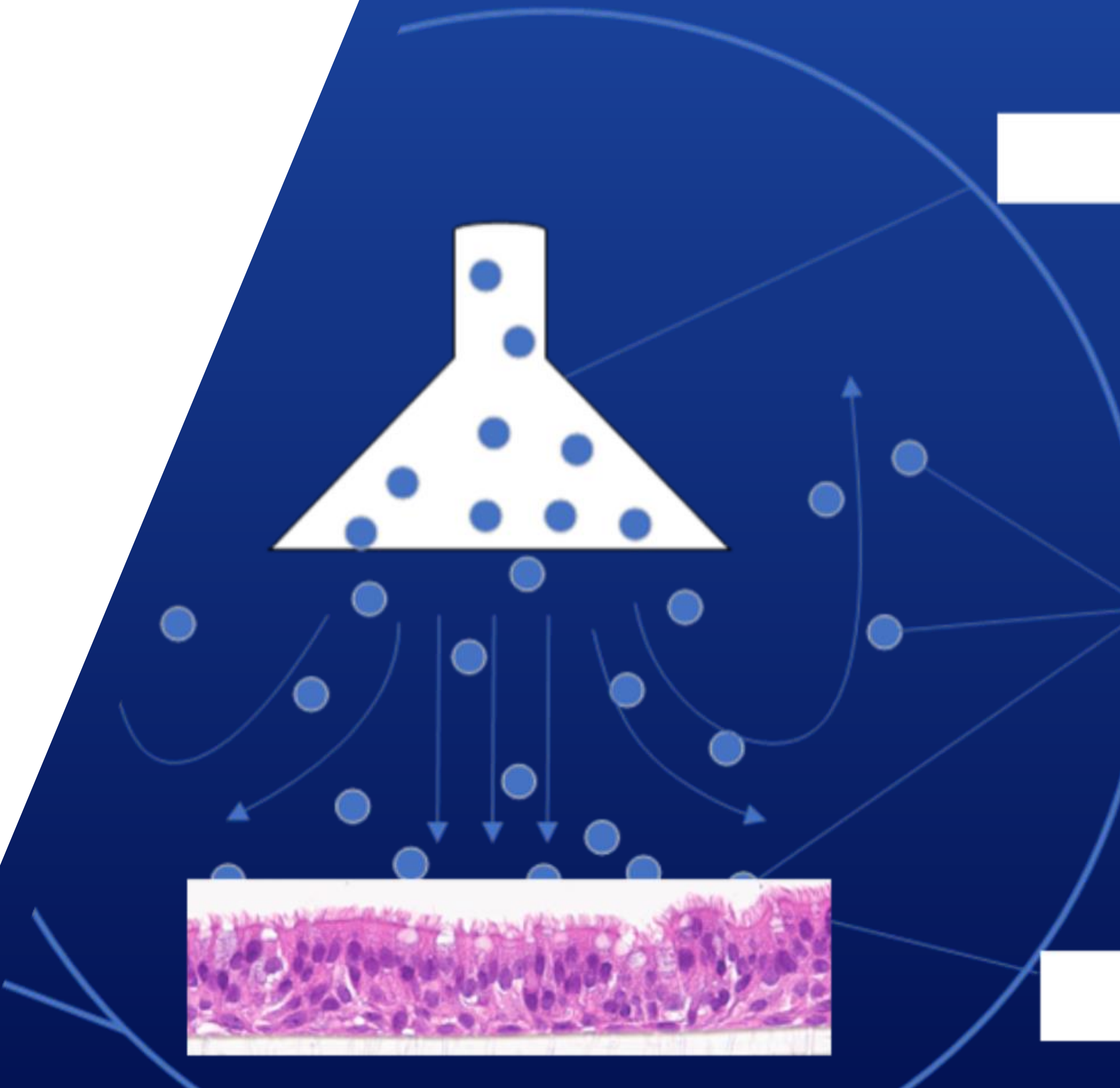
01 NAMs and AMAP

02 Lung models – Complex In vitro Models

03 Validation and Qualification of lung NAMs for inhaled risk assessment - ACC

04 Results

05 Conclusion



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NAMs and 3Rs

Definition: New Approach Methodologies

NAM refers to any technology, methodology, approach, or combination thereof that can be used to provide information on pharmaceuticals*/chemical hazard* and risk assessment and **supports replacement, reduction, or refinement of animal use (3Rs).**

*modification to include pharmaceuticals

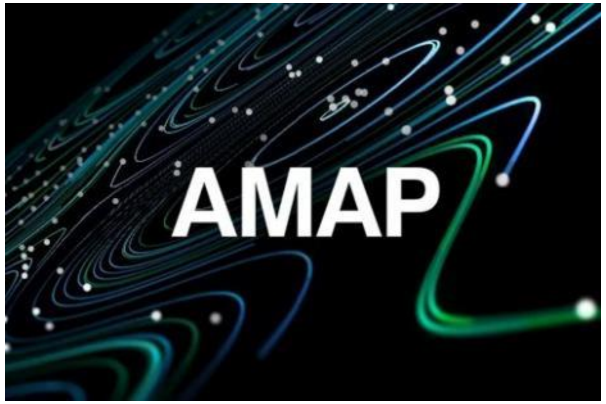
Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), 2024

Alternative Definitions
New Approach Methodologies
New Alternative Methods
Non-Animal Methods
Novel Alternative Methods

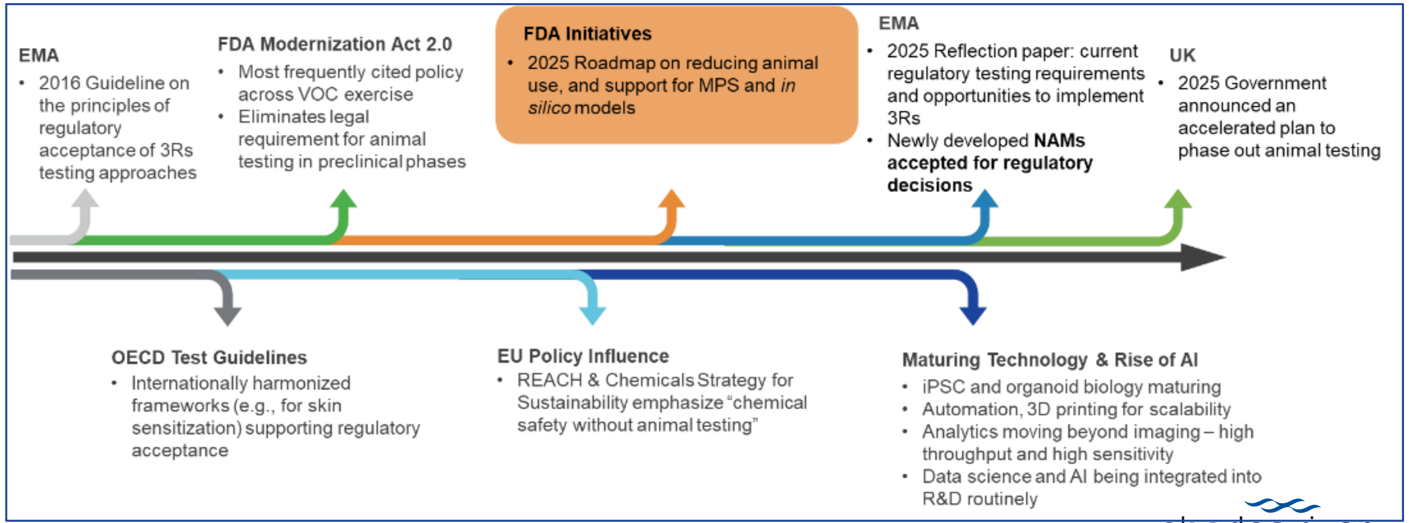
Alternative Methods Advancement Project within Charles River Laboratories

Prioritizing complex *in vitro* models for the scaled and routine adoption of NAMs

What is AMAP? AMAP is Charles River's initiative dedicated to scientific and technological innovation that helps us fulfill our commitment to the 3Rs and development of alternatives to animals in testing.



Why now? Scientific Advances – AI and Human Cell-based models – better predict toxicity without the need for animal data. **Regulatory momentum – FDA, EPA, UK Government**



NAMs adoption likely to transition from augmentation to selective replacement of animal studies over time – *Evolution rather than a Revolution*

Evolving NAMs workflow over time

Transition phase

Current state	Augmentation	Selective replacement	End state??
NAMs more commonly used in regulatory filings involving: 1. Life- threatening malignancies (e.g., ICH S9) 2. Lack of relevant animal models	NAMs are increasingly used alongside *in vivo* studies without fully replacing legacy assays. Emphasis on use in decision making in Discovery to improve candidate quality.	As validation and regulatory confidence grow, NAMs begin to selectively replace specific *in vivo* studies e.g. reduced genotox, CARC, safety pharm, single species tox?	Human risk assessment becomes integrated suite of NAMs with critical *in vivo* studies plus weight of evidence approach. Level of NAM versus *in vivo* will be case by case; NAMs become central to decision making phases.

Driven by enhanced innovation in *in vitro* and *in silico* methods, and increased regulatory acceptance

Overview of In Vitro Lung Models

- Immortalised cell-lines (A549, BEAS-2) and iPSCs
- Culture systems include 2D, air-liquid interface, spheroids/organoids
- Lung-on-a-chip (with stretch)
- Stretchable alveoli arrays
- Multiple cell types (differentiated airway/alveolar epithelial cells, macrophages, endothelial cells, fibroblasts).

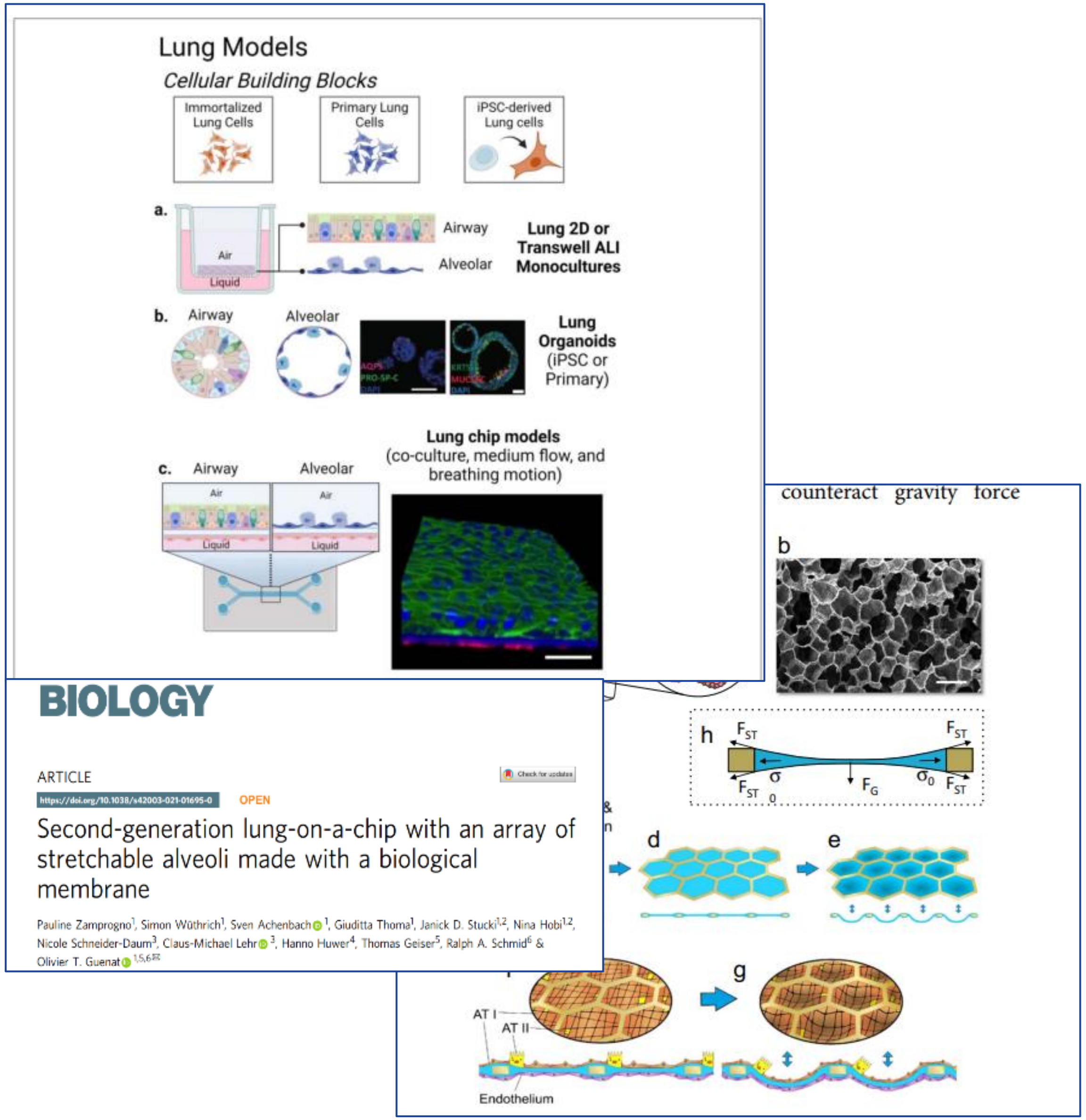
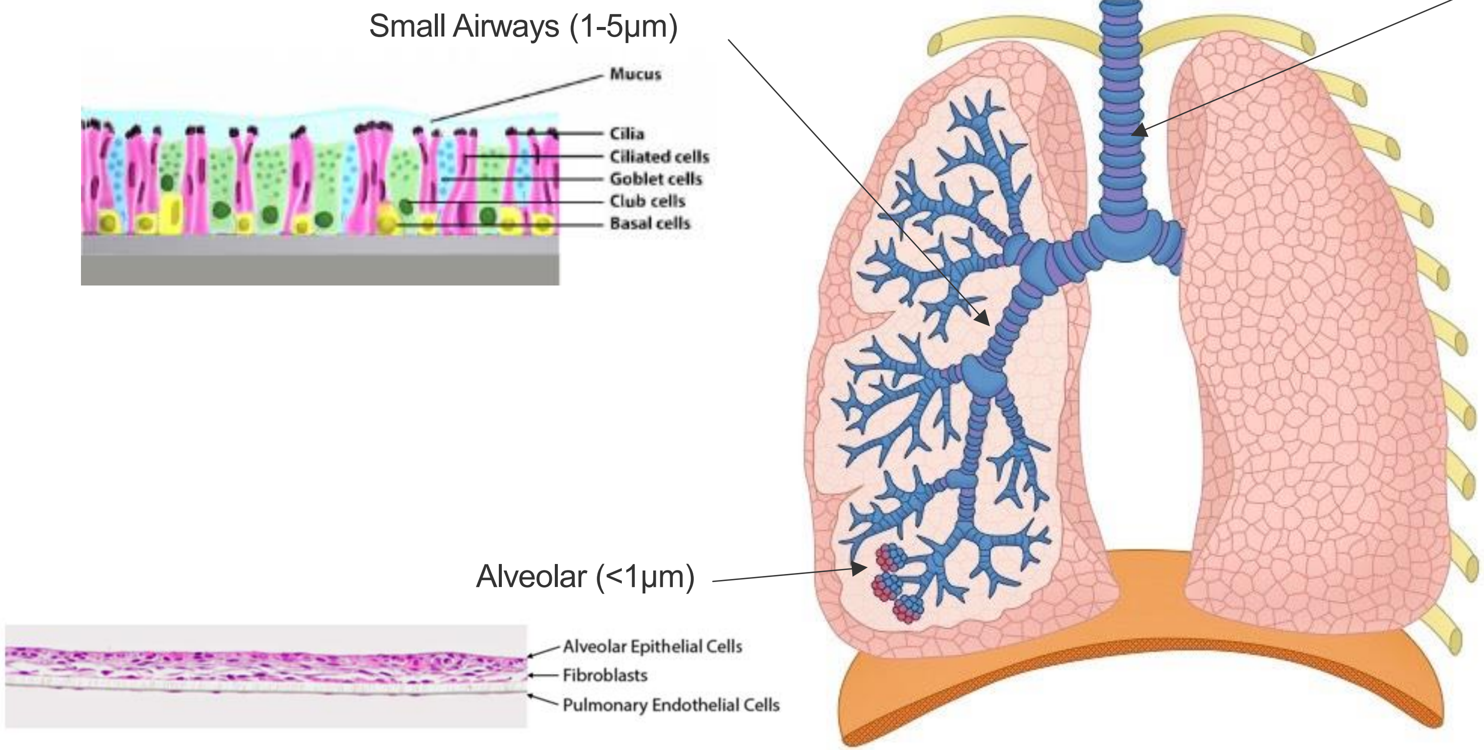


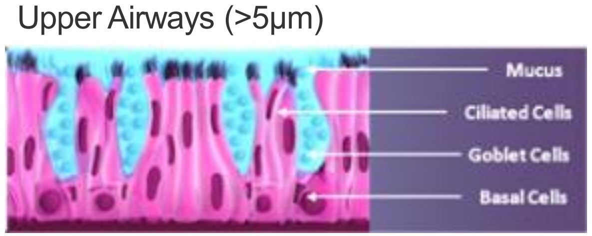
Image: Kang et al. Complex in vitro models positioned for impact to drug testing in pharma: a review. Biofabrication. 2024 Aug 27;16(4).

Different Lung Models Required Depending on Target Region in Lung

Human Airway Models

- Cell models are available at a variety of different parts of the human airway, as well as in many combinations, to represent the targeted area:





- Choosing the best target cell model (based on where you expect the greatest deposition of the test item) will give the most useful information.

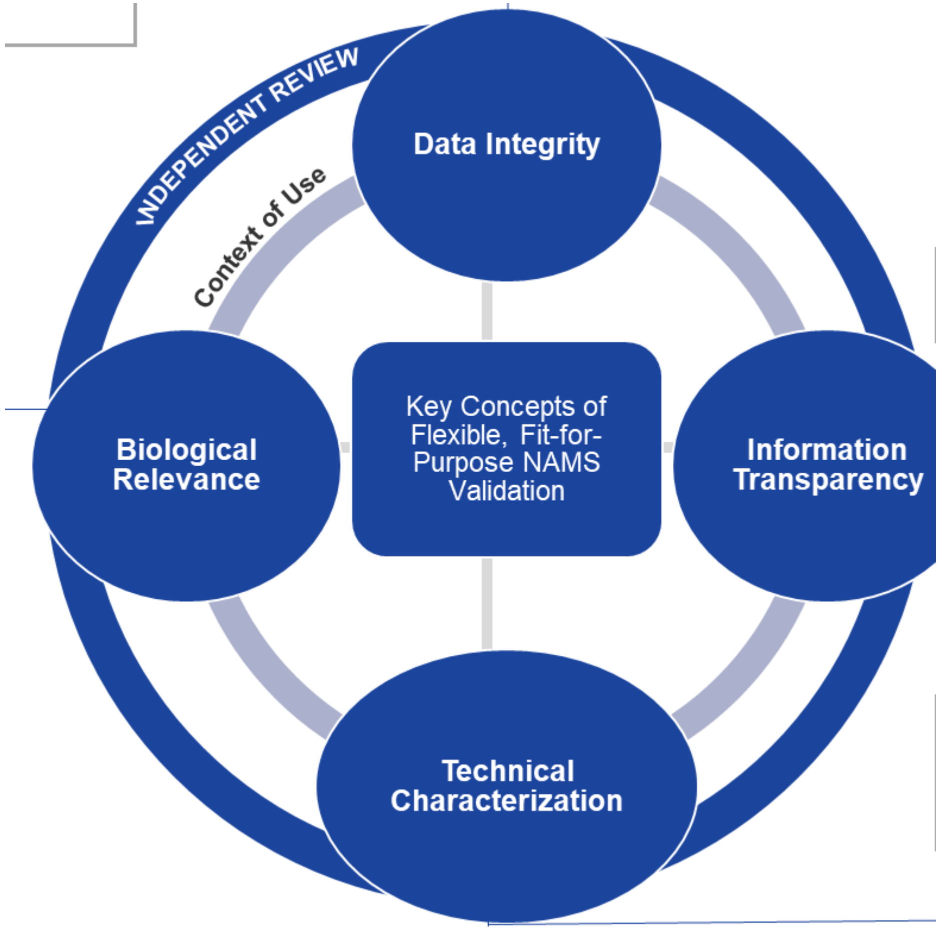
Images Reproduced from https://www.epithelix.com/ and https://www.mattek.com/

ACC Long Range Initiative Grant-
Advance the development lung NAMs for testing and assessment of chemicals for portal of entry toxicity in the respiratory tract: Collaboration with CRL Mattek/Satorius, Greek Creek Toxicokinetics and Battelle

Context of Use: Human lung upper airway model to predict toxicity of directly toxic inhaled chemicals.

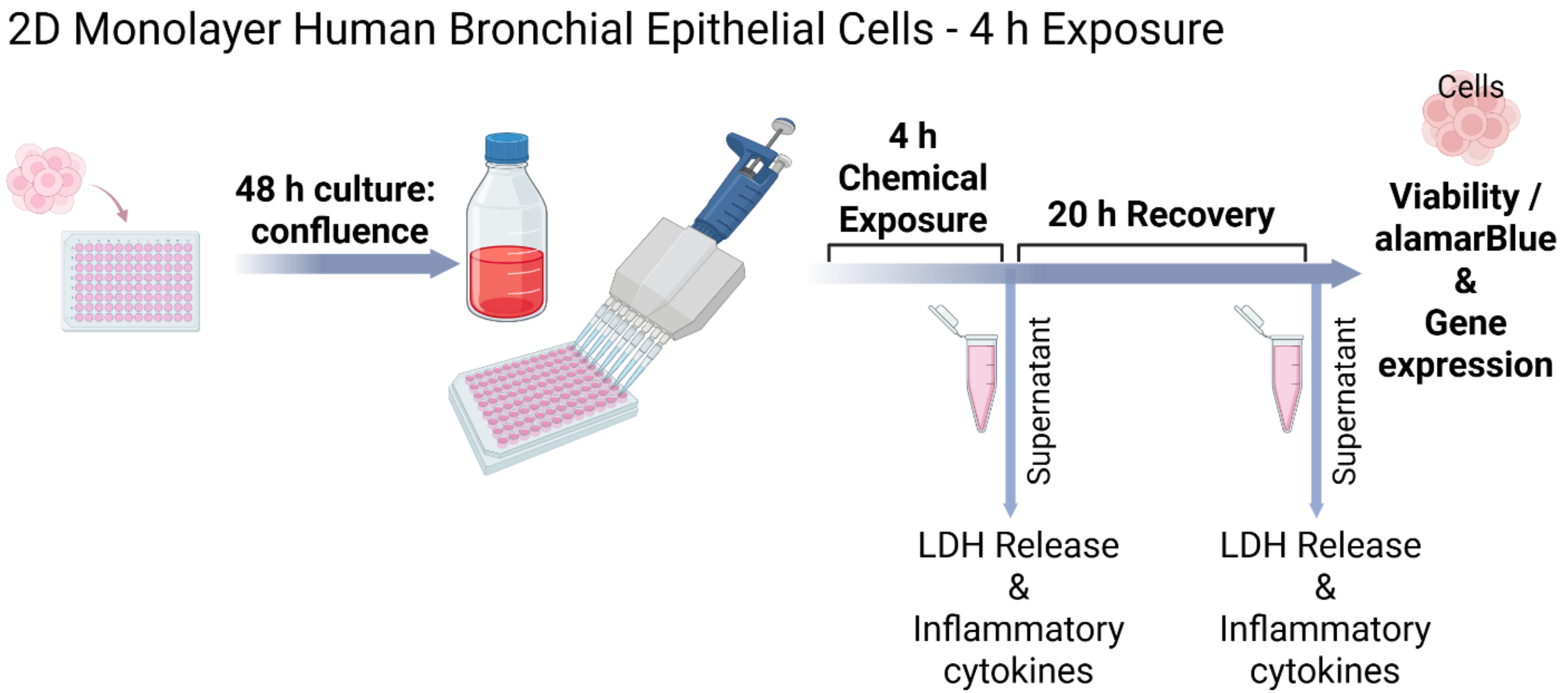
Grant objectives (June ’23 to June ’26)

1. **Evaluate the reproducibility and predictability of a single application of inhaled chemicals in 2D and 3D models (rat and human).**
2. *Repeat chemical application*
3. **In vitro aerosol delivery**
4. **Respiratory tract dosimetry model**
5. *Multiple Donors*
6. *Develop a prediction model*



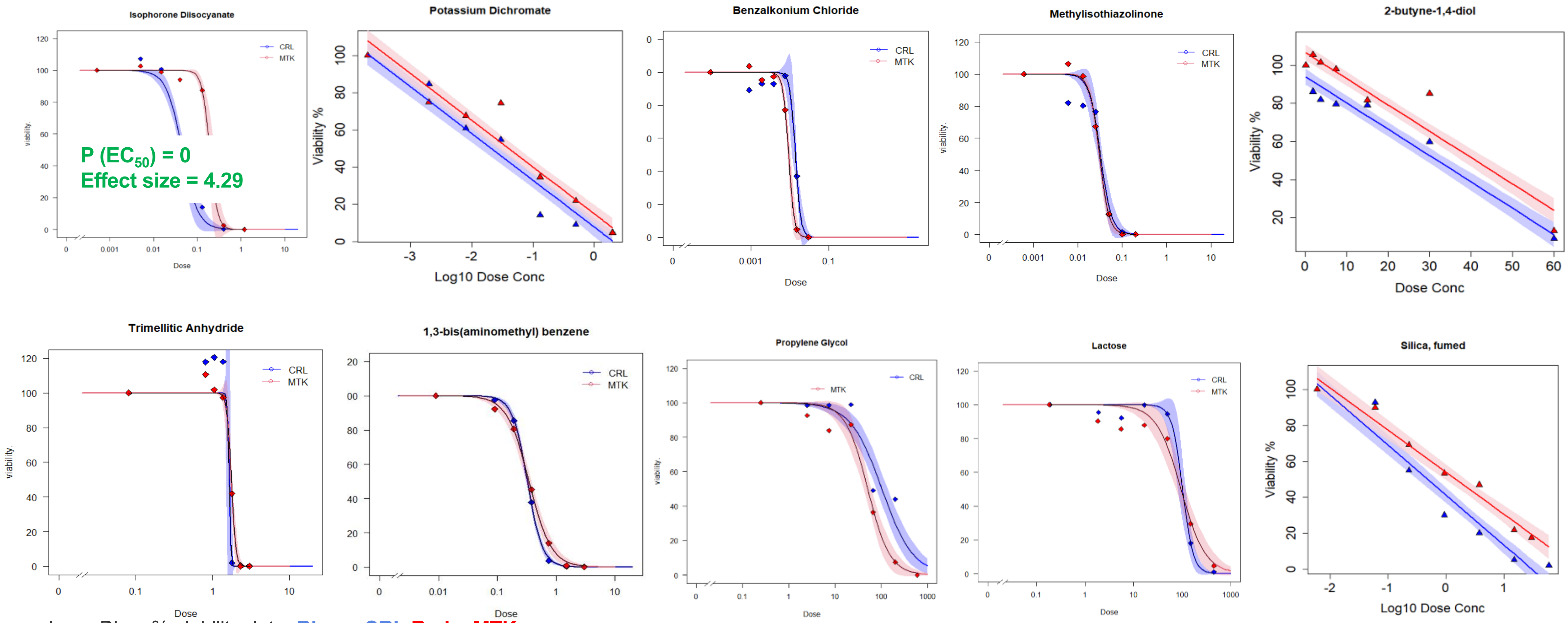
Committee on the Validation of Alternative Methods (ICCVAM) Framework

Acute (4h) Direct Liquid Application – 2D Design



Created in https://BioRender.com

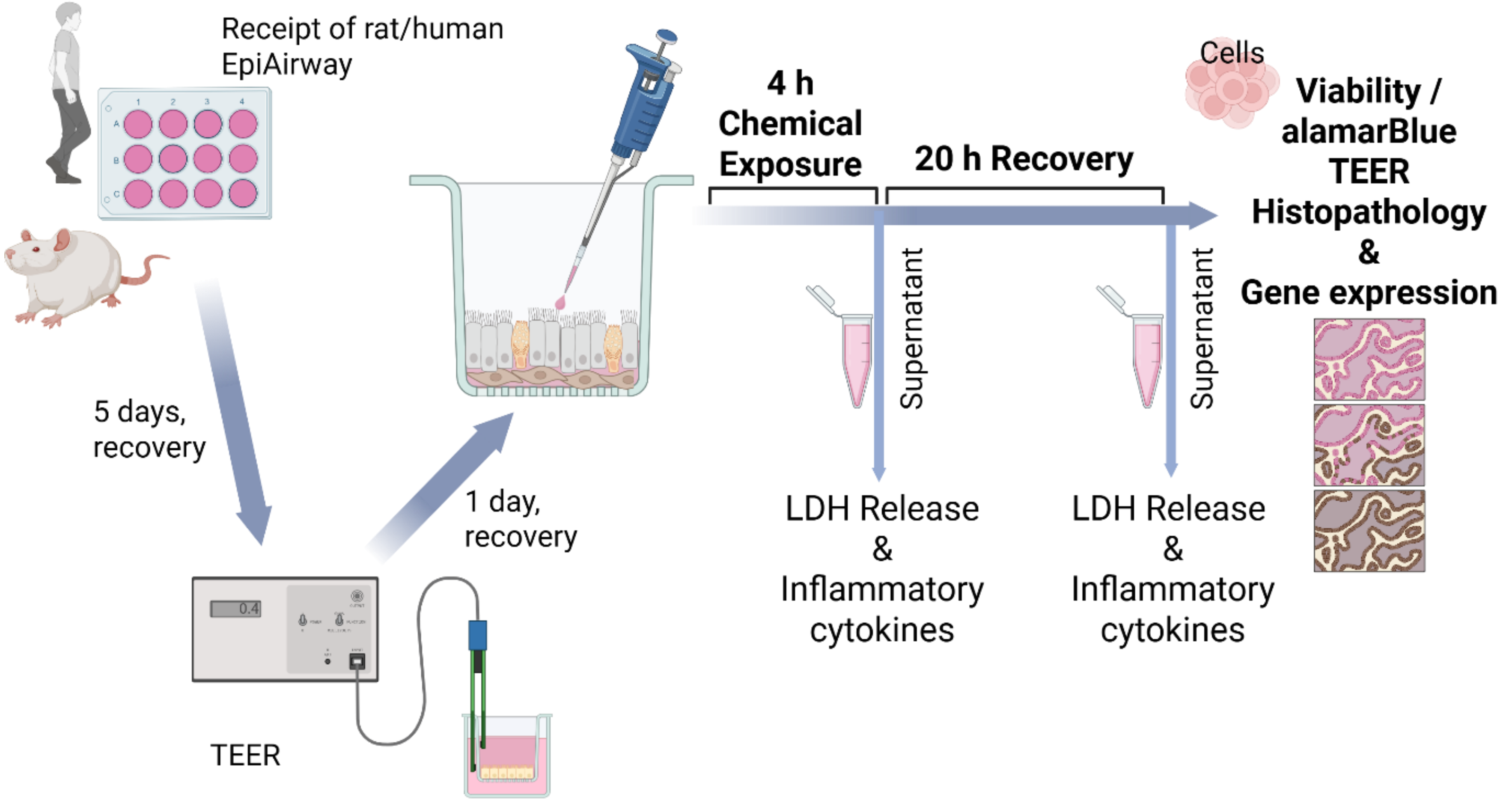
2D Acute Data: Curve Fitting



alamarBlue, % viability data. **Blue = CRL** **Red = MTK**

- The drc package was used in R to estimate and to statistically compare slope and EC50 parameters.
- Differences between CRL and MTK were significant if slope and/or EC50 parameters had a P value of <0.05 and >2.5-fold difference (i.e. biological relevance of difference) in the values themselves (**highlighted**).

Acute (4h) Direct Application – 3D Design



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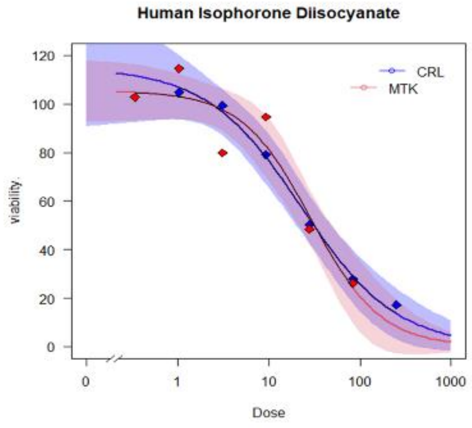
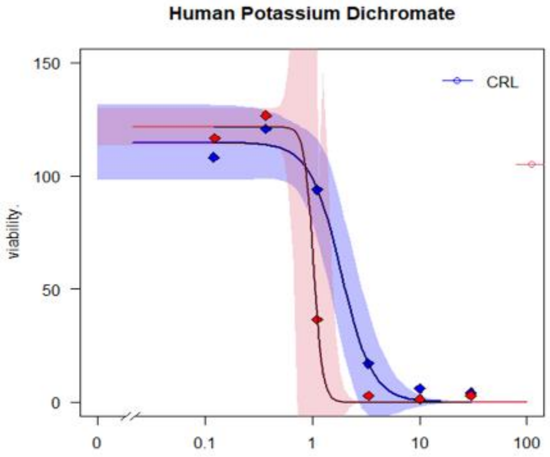
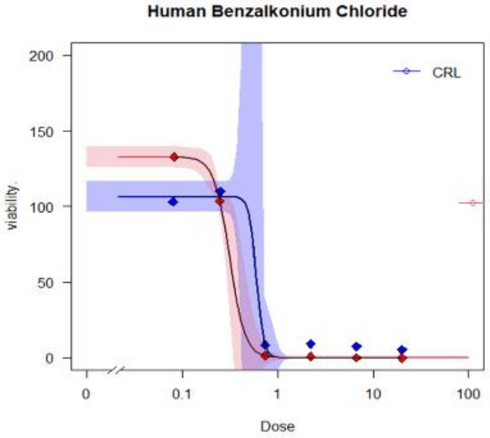
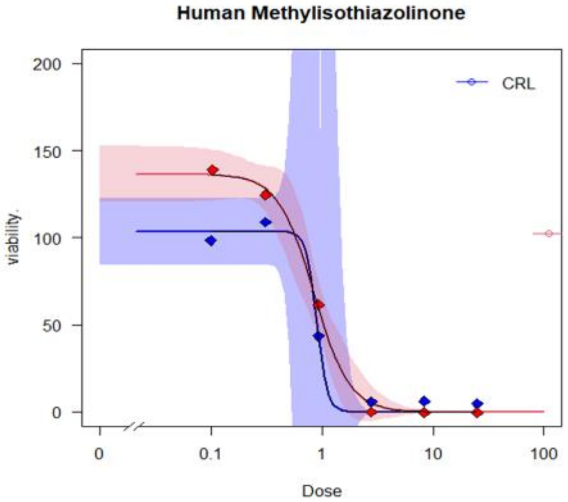
3D Human Viability Acute Data – Curve Fitting

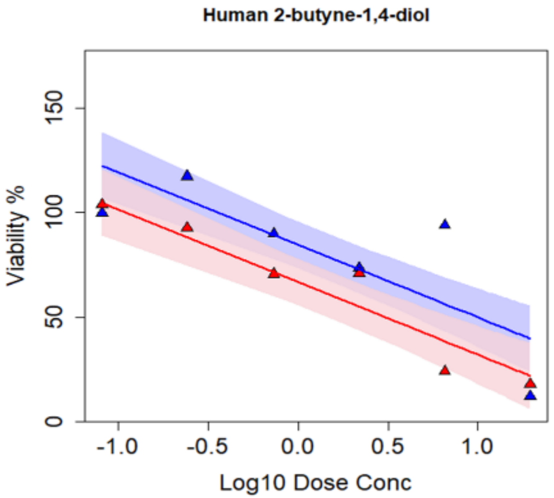
Curve fitting and comparisons between CRL and MTK – alamarBlue Data for 3D Monolayers

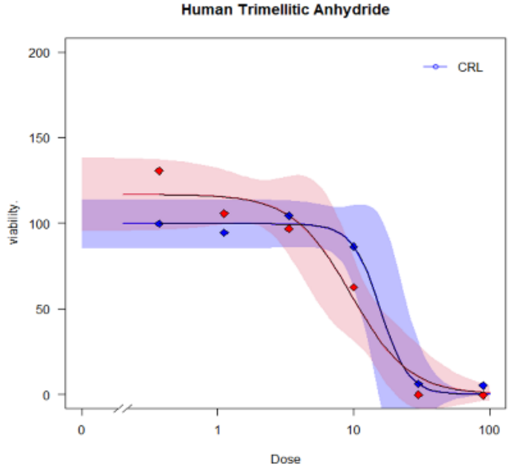


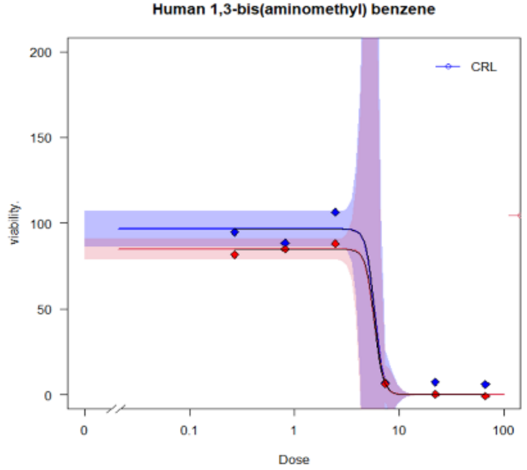












Lactose, Silica & Propylene glycol = no dose responses

alamarBlue, % viability data.
Blue = CRL **Red = MTK**

Differences between CRL and MTK were deemed significant if slope and/or inflection parameters had a *P* value of <0.05. **No significant differences between labs.**

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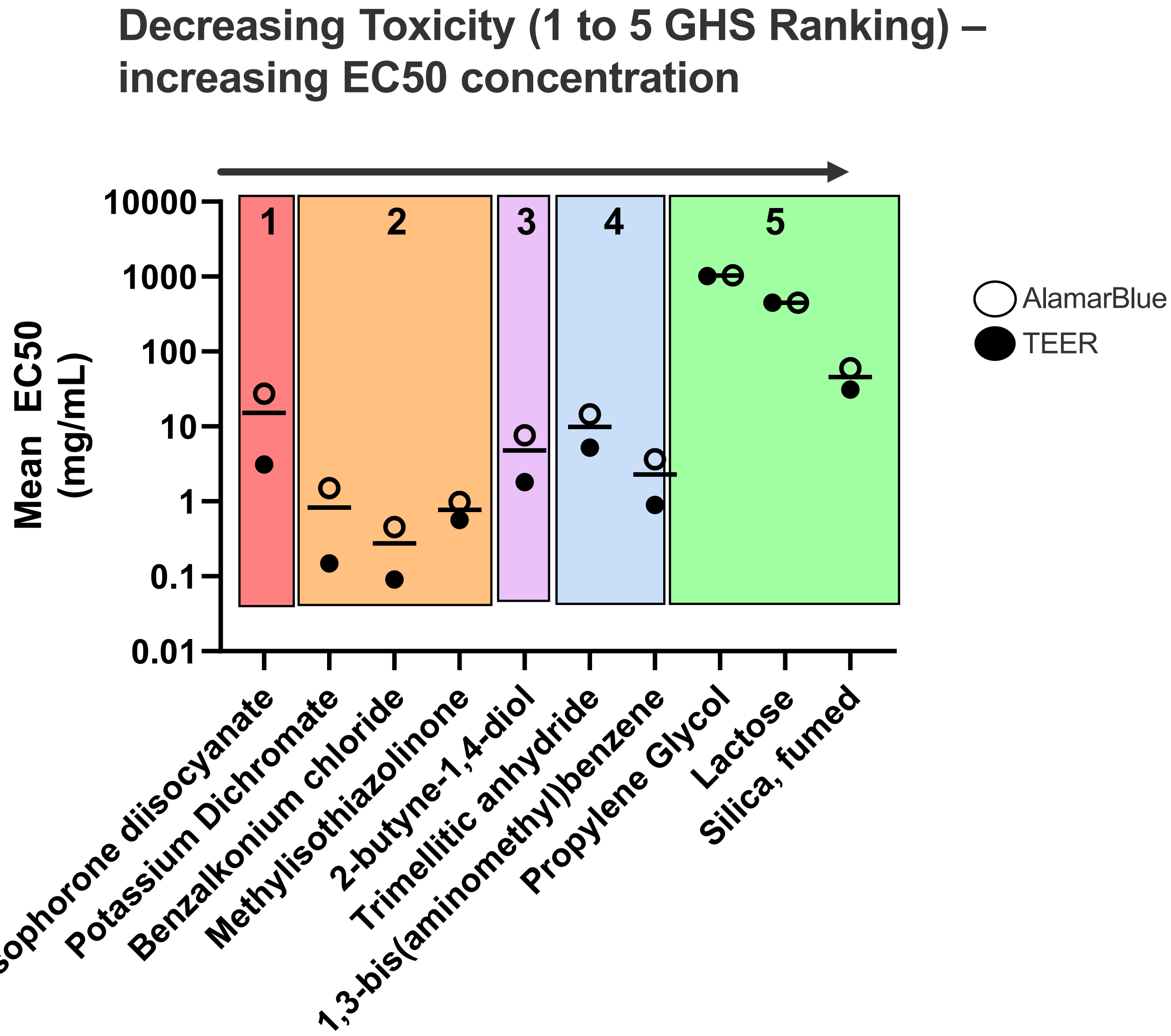
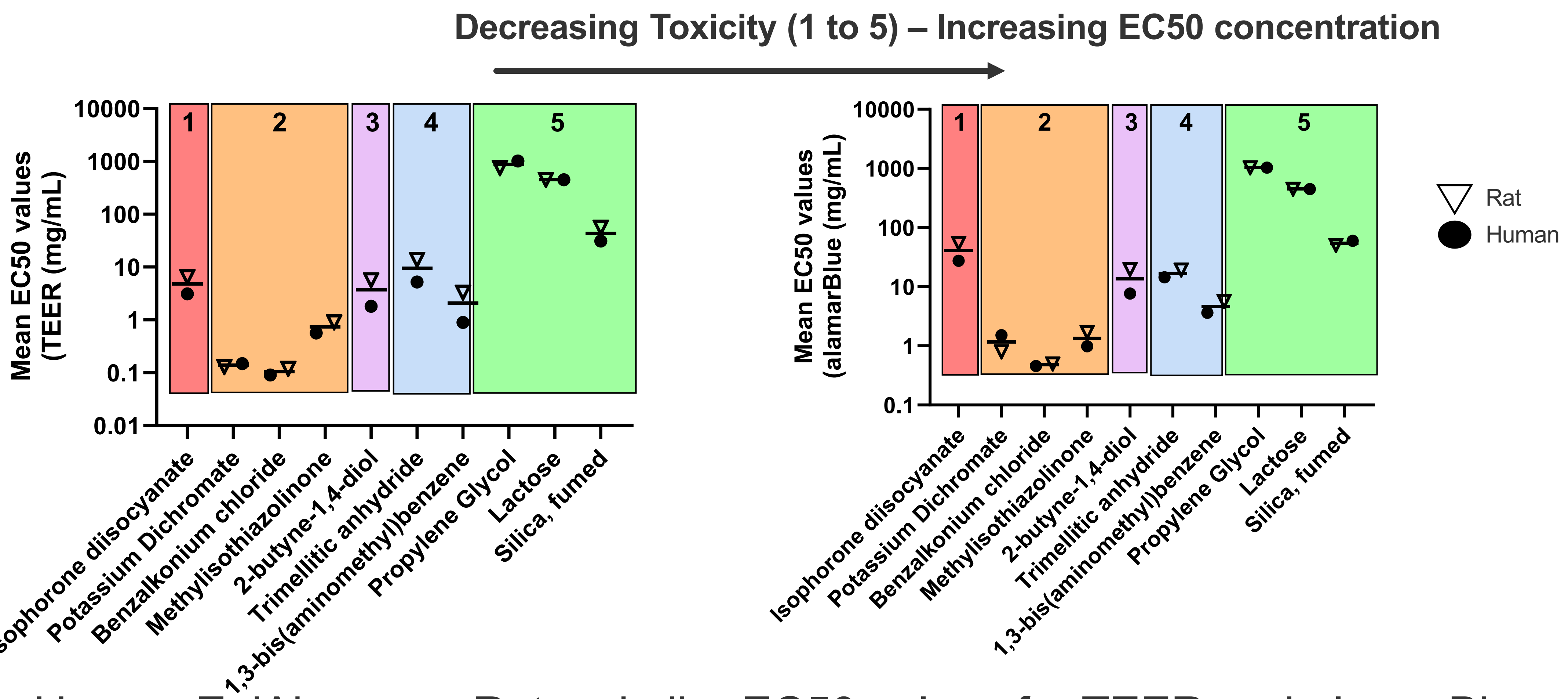
Single Acute Liquid Application: Human EpiAirway: EC50 for TEER and alamarBlue



- Generally, reduced toxicity with higher GHS rating (1 to 5) based on epithelial membrane integrity (TEER) and cell viability (alamarBlue).
- Membrane integrity (TEER) generally a more sensitive indicator of toxicity for more reactive chemicals
- **Exceptions:** Isophorone diisocyanate and 1,3, bis (aminomethyl) benzene.
- More chemicals required to develop the prediction model.

Single Acute Liquid Application: Human EpiAirway vs Rat EpiAirway: EC50 for TEER and alamarBlue



- Human EpiAirway vs Rat – similar EC50 values for TEER and alamarBlue
- EC50 concentrations increased as GHS grade increased
- Isophorone and 1,3-bis(aminomethy) benzene also exceptions in rat.

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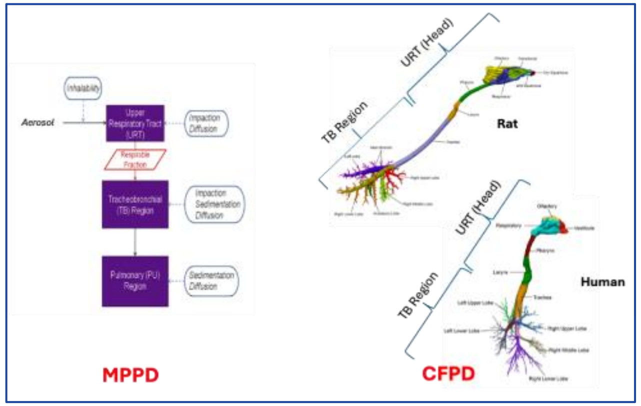
Biologically Relevance: Rat In vitro to in vivo extrapolations (Richard Corley, Kevin Yugulisk)

Current Status of MPPD
(Multiple Path Particle Dosimetry)
CFPD Modelling
(Computational fluid-particle dynamics)

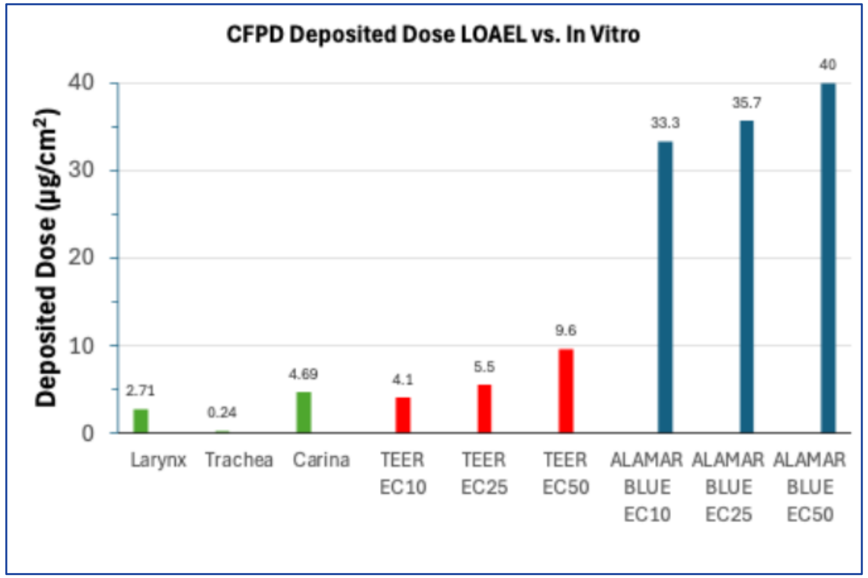


MPPD and CFPD: In Silico Tools to define local respiratory tissue dose metrics in rats and humans exposed to aerosols.

ACC Grant – Simulating respiratory dose metrics associated with aerosol exposures in rats based on inhalation tox studies in ECHACHEM database or peer-reviewed literature.

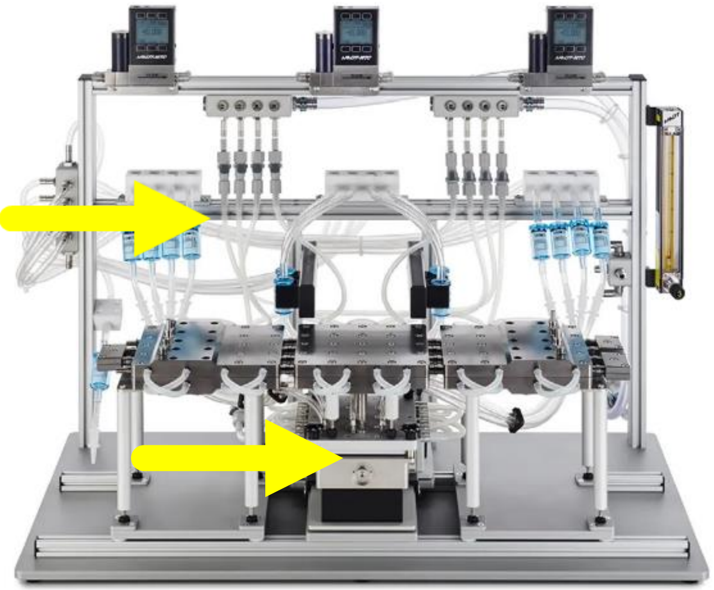
In vivo rat dose metrics were compared with dose-dependent toxicity endpoints in EpiAirway (EC values).

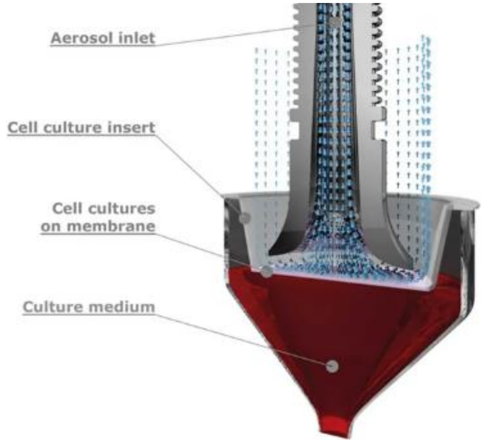
Some concordance of in vitro applied doses (Rat) with aerosol simulations of deposited dose (CFPD) e.g. Benzalkonium chloride,

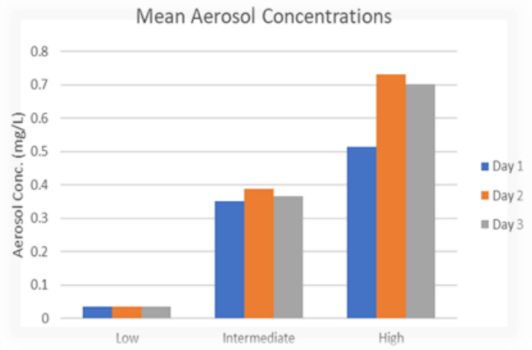


Ref: Case Study on the use of an Integrated Approach for Testing and Assessment (IATA) for New Approach Methodology (NAM) for Refining Inhalation Risk Assessment from Point of Contact Toxicity of the Pesticide, Chlorothalonil. OECD Case study, 2022

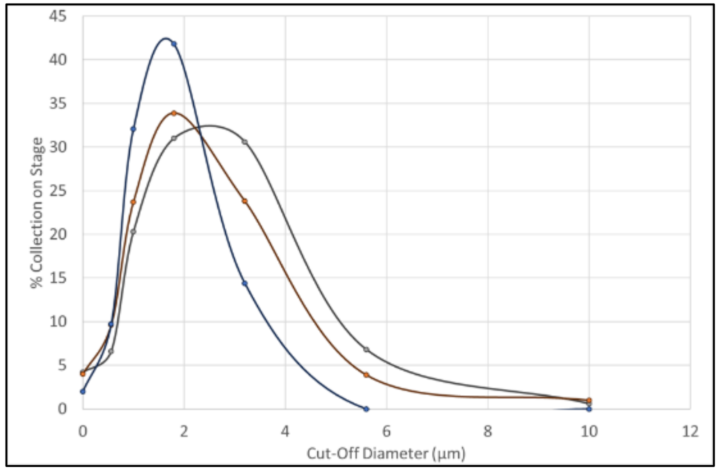
Physiological Relevance/Technical Characterisation: Liquid vs Aerosol Application

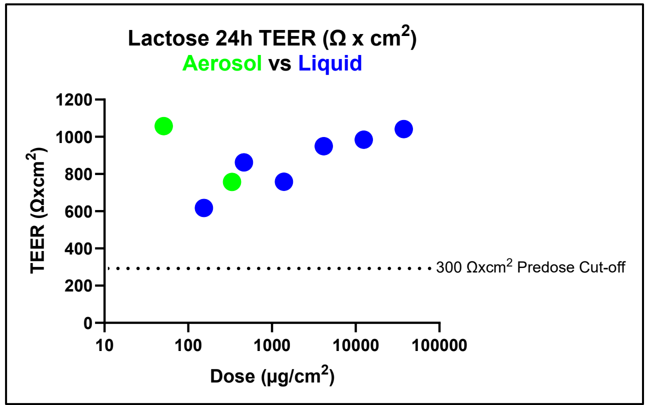


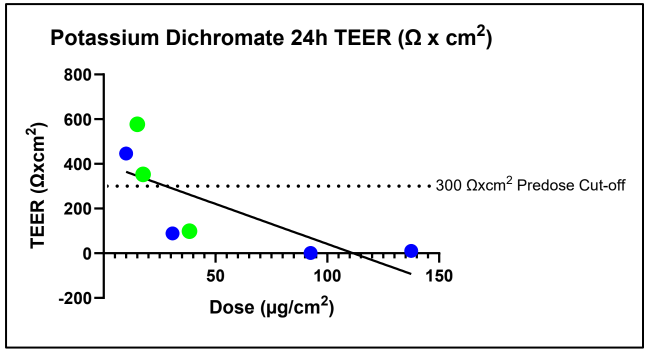


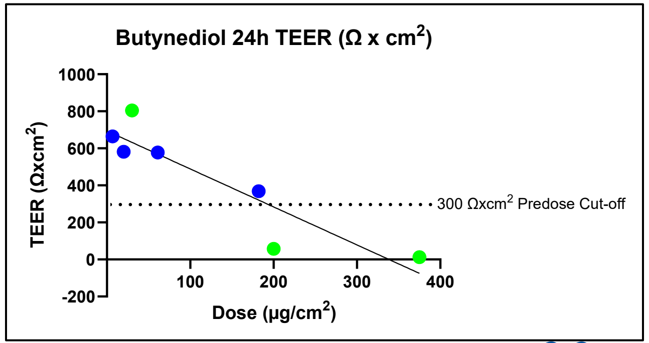


Dose	Overall Mean Aerosol Concentration (mg/L)	Variability (%RSD)
Low	0.0357	2.7
Intermediate	0.368	5.2
High	0.649	18.2









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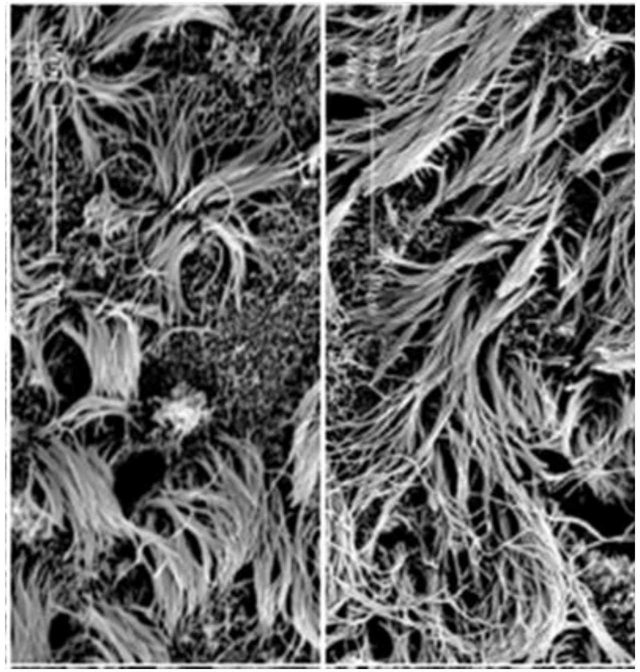
Conclusion:
Human lung upper airway model to predict toxicity of directly toxic inhaled chemicals

- Agreed protocols/SOPs resulted in similar EC50 results (2D and 3D models).
- EpiAirway (Human): EC50 from a 10-chemical test panel generally ranked chemicals in terms of GHS rating (8 out of 10).
- Rat Airway model (Mattek): EC50 values similar to human.
- Rat IVIVC extrapolations show concordance for some chemicals - but assumptions may be required to estimate in vivo aerosol exposure concentrations.
- Liquid vs aerosol application generally similar dose response but more data needed



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Kevin
Yugulisk

JOHNS HOPKINS
BLOOMBERG SCHOOL
of PUBLIC HEALTH

From environmental health to precision medicine

Alexandra Maertens, Ph.D.

Bringing Toxicology Back to the Future

- First carcinogen was discovered in 1775
- Percival Pott noticed a rare disease - scrotal cancer - was more common in chimney sweeps and connected it to soot exposure
- Traditionally, carcinogenicity was first established in humans (often through occupational exposures) and then mechanisms were verified in animals

What can environmental health teach us about clinical safety?

Atmospheric Pollution

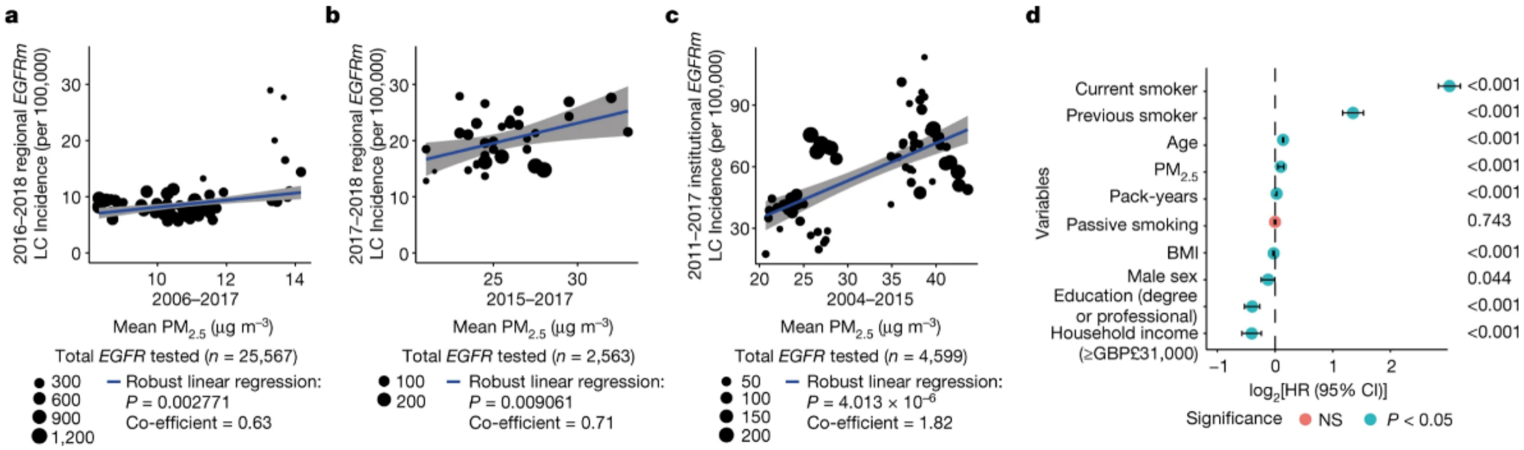
It has been argued that, since cigarette smoking is, in general, more prevalent in towns than in country districts, the comparison of different smoking groups is, in part, merely a comparison of urban and rural residents, the former being exposed to an atmospheric pollution which the latter escape. On the other hand, if the difference between the smoking habits of town and country were somewhat greater 20 to 30 years ago than it is to-day, there may be no reason at all to invoke atmospheric pollution as the explanation of the higher mortality from lung cancer in urban areas. Cigarette smoking could, in that event, be

What can environmental health teach us about clinical safety?

- Driver mutations were common in non-cancerous tissue
- Air pollution promotes lung cancer via non-mutagenic mechanisms and likely accounts for the increase in lung cancer in never-smokers

Fig. 1: Exploring the association between cancer and air pollution.

From: Lung adenocarcinoma promotion by air pollutants



Hill, W., Lim, E.L., Weeden, C.E. *et al.* Lung adenocarcinoma promotion by air pollutants. *Nature* **616**, 159–167 (2023). https://doi.org/10.1038/s41586-023-05874-3

Respiratory sensitization

- We have no validated animal model for respiratory sensitization
- In silico models of skin sensitization have approximately an 80 percent accuracy - can we reproduce this success with respiratory sensitization?
- Since the molecular initiating event was similar, how accurate was Toxtree for predicting respiratory sensitization?

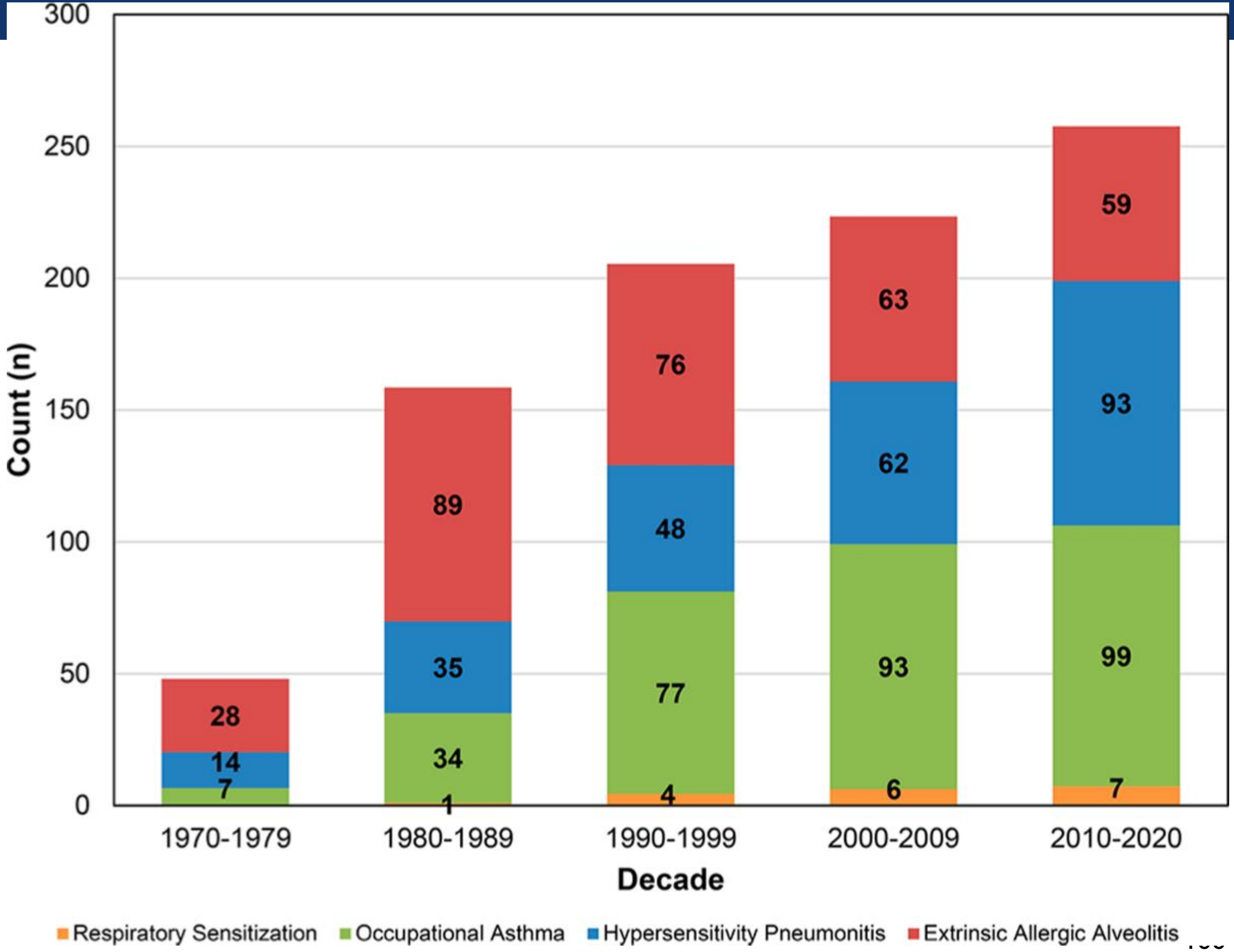
ARTICLE | December 15, 2020

Mapping Chemical Respiratory Sensitization: How Useful Are Our Current Computational Tools?

Emily Golden, Mikhail Maertens, Thomas Hartung, and Alexandra Maertens*

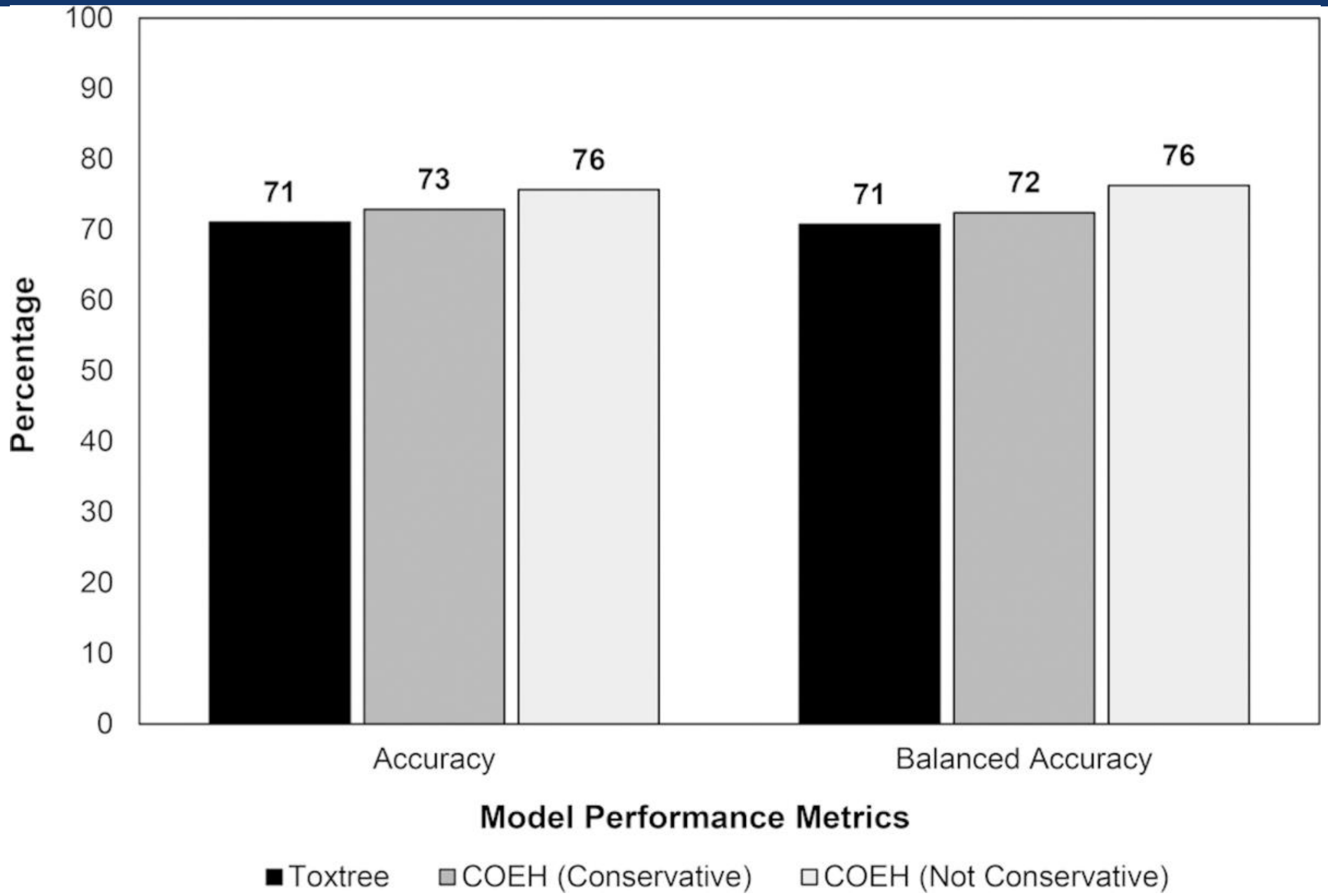
Conflicting Terminology

- Terms used in the literature varied significantly over time and were very inconsistent
- Not always clear when determining irritation version respiratory sensitization
- Respiratory sensitization - especially in the context of an occupational exposure - can easily be overlooked by a doctor



Accuracy

- Reasonable accuracy with combined models
- Structural alerts over predicted
- Some mispredictions were likely errors in the data set



Challenges - Balancing A Data Set

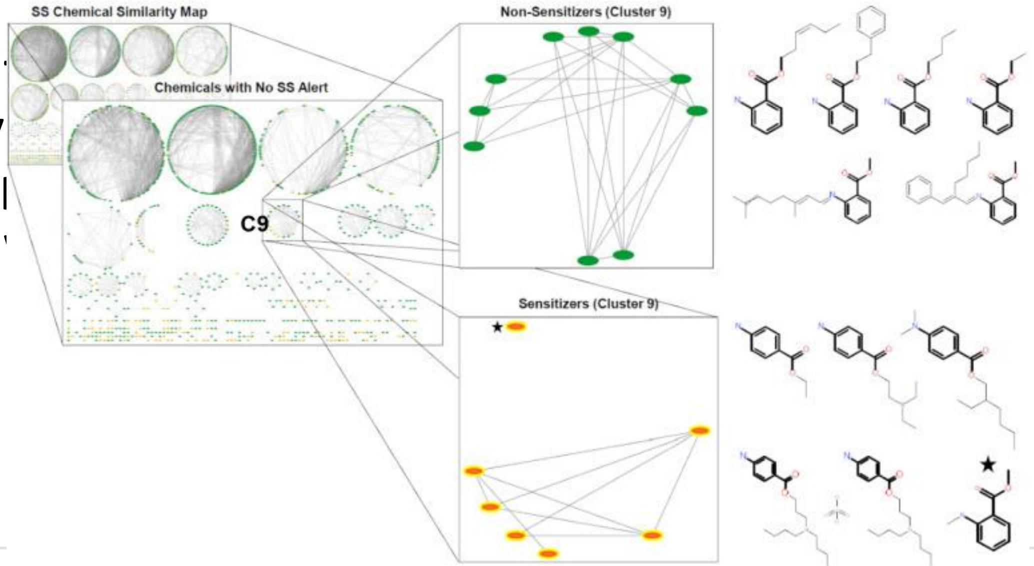
Unbalanced datasets can inflate accuracy of structural alerts in particular, but are a problem for any algorithm!

Toxtree alerts have low accuracy in a more balanced data set the best performing alert had only 18% accuracy, and and the lowest 2.4%; overall accuracy is around 65 percent in a more balanced dataset based on in vitro and human patch test studies

Activity cliffs make read-across complicated –
similar chemicals with divergent outcomes, ev
ecular i
ts that

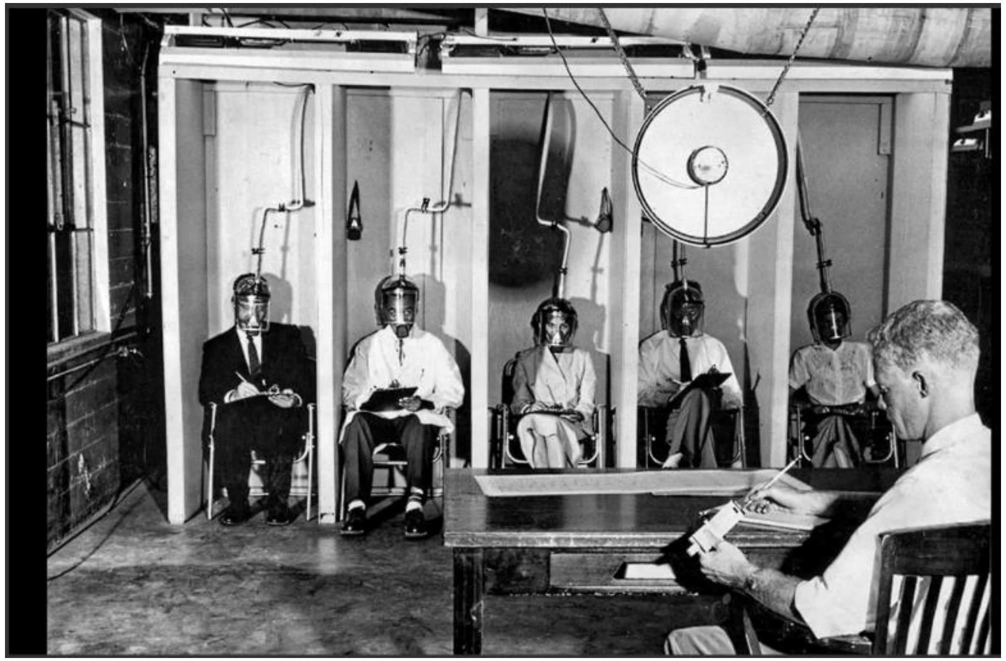
The Good, The Bad, and The Perplexing: Structural Alerts and Read-Across for Predicting Skin Sensitization Using Human Data Click to copy article link

Emily Golden, Daniel C. Ukaegbu, Peter Ranslow, Robert H. Brown, Thomas Hartung, and Alexandra Maertens*



Balancing a Data Set

- Data imputation -
  - We used HPV chemicals and looked for any reports of repiratory sensitization
  - If none, provisional negative
  - Many exposures are already taking place, but we aren't capturing the data
  - Many real-world adverse drug events are caused by co-exposures, many of which won't be documented in EHR



June 28, 1956: Chemical engineer Edward Liston, right, of Stanford Research Institute checks on five subjects in a smog chamber breathing reconstit smog and noting reactions on pads. The testing was being conducted at SRI labs in South Pasadena.

Chemical Safety for Health for the 21st Century

- Biomarker driven for both exposures and disease mechanism

- Capturing casual and non-unionized industries (agricultural workers, cleaning staff, nail salon)

- Difficult balance between privacy and highly granular genomic and exposomic data

- Require electronic health record mining, analyzing complex chemical mixtures in non-invasive biomatrices - this will necessarily be AI driven

Biased Data

- Data doesn't grow on trees - someone has to decide **who counts**, what we **quantify**, and how **we encode that**

- Because of that - **all data is biased**. We can be more thoughtful about this fact but we cannot get rid of it

- AI often makes this **less obvious**

- For respiratory sensitization: Is asthma encoded with equal precision for **men vs women?** For patients of different backgrounds? How useful is an encoding of asthmatic/non-asthmatic? Sensitizer/non-sensitizer?

Challenges: AI

A Can BPA be linked to respiratory sensitization?

Yes, there is scientific evidence suggesting BPA (bisphenol A) can be linked to respiratory sensitization and related allergic responses.

A Can lead be linked to respiratory sensitization?

Yes, lead exposure has been linked to respiratory sensitization and various respiratory health effects, though the mechanisms differ somewhat from other environmental toxins.

A Can mercury be linked to respiratory sensitization?

Mercury exposure has been associated with respiratory sensitization and allergic r
though the evidence is more complex and varied compared to some other environ
toxins.

A Can PCBs be linked to respiratory sensitization?

Yes, PCBs (polychlorinated biphenyls) have been linked to respiratory sensitization and various respiratory health effects through multiple studies.

Challenges: AI

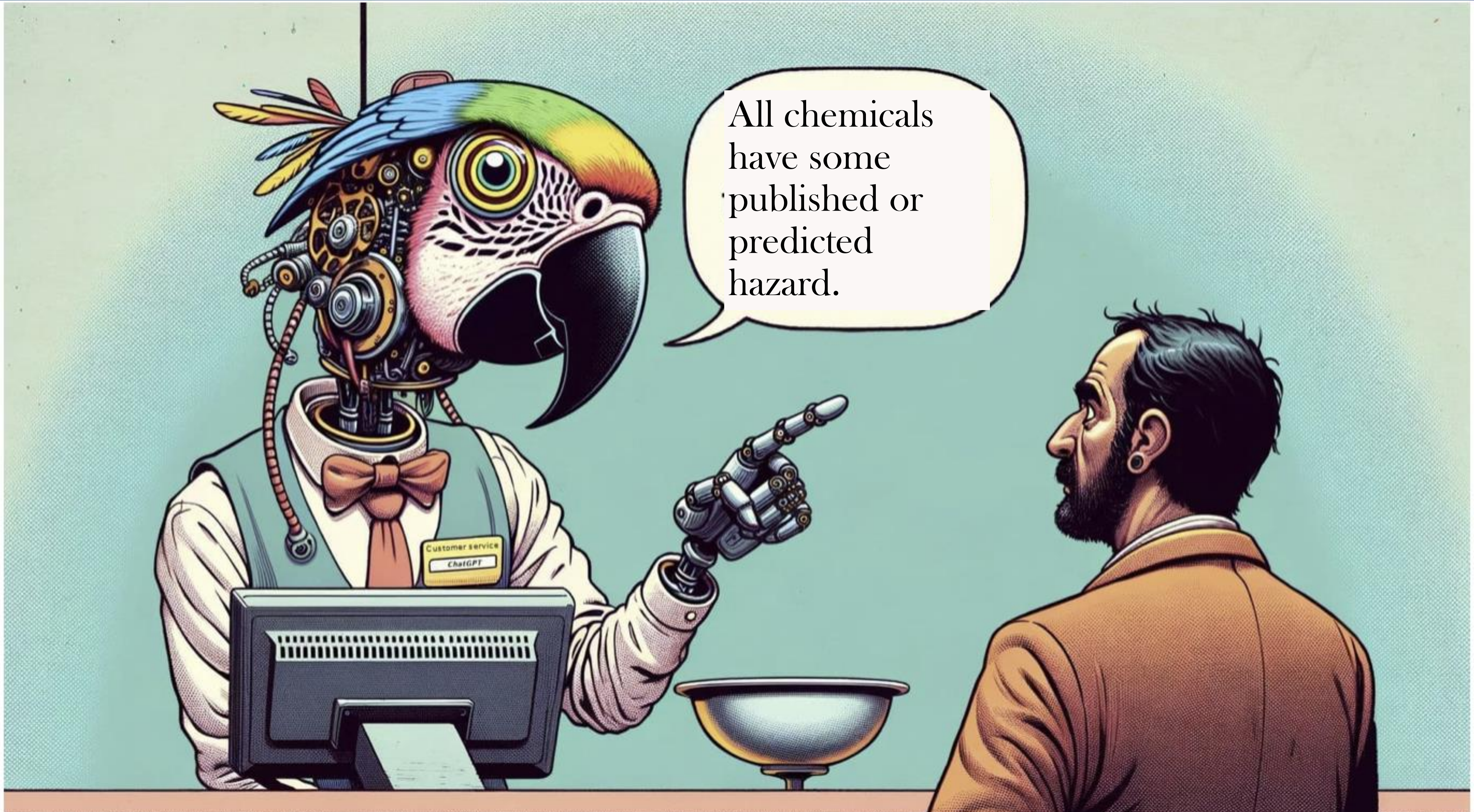


Image:Lesswrong.com

AI: Challenges - Mining the Literature

ctd *Illuminating how chemicals affect human health.*

Comparative Toxicogenomics Database

Home | Search | Analyze | Download | Commercial Users | Help

Respiratory Hypersensitivity

Basics | Chemicals | Genes | Phenotypes | Comps | Pathways | Exposure Studies | Exposure Details | References

These chemicals are associated with *Respiratory Hypersensitivity* or its descendants. A chemical has either a curated association to the disease (M *marker/mechanism* and/
tion.

Filt by Association e curated Filter

1–50 471 results.

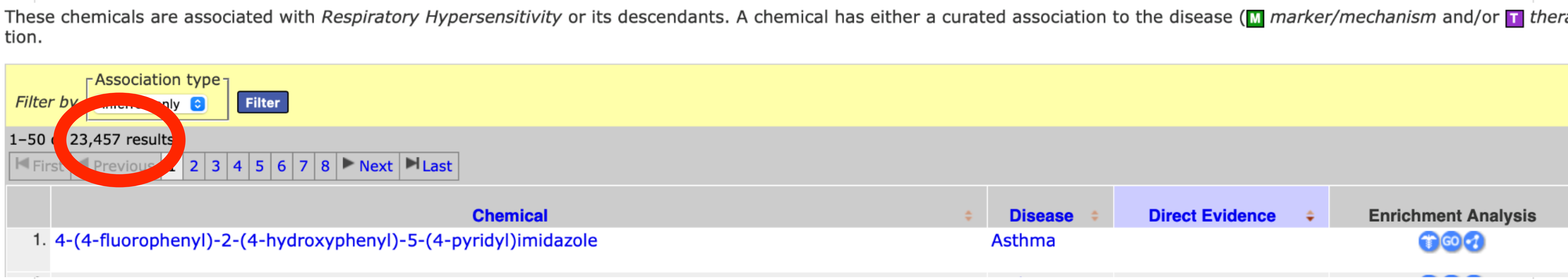
First 3 4 5 6 7 8 Next Last

	Chemical	Disease	Direct Evidence	Enrichment Analysis
1.	Dust	Asthma	M	GO
2.	Ovalbumin	Asthma	M	GO
3.	Toluene 2,4-Diisocyanate	Asthma	M	GO
4.	Nitrogen Dioxide	Asthma	M	GO
5.	Antigens, Dermatophagoides	Asthma	M	GO
6.	Carbon Monoxide	Asthma	M	GO

Dietary Fats	Asthma	M	GO

AI Challenges - Mining the Literature

- Inferred connections between asthma and 23,457 chemicals



Green Toxicology for a Sustainable Future
May 14, 2025
10am-12:30pm
Hybrid Webinar
Alex Maertens
Julie Zimmerman
Marie Studer
Julie Krzykwa
Jakub Kostal
Fenna Sillé
Join us for a hybrid webinar, featuring speakers from various fields of science, connecting Green Toxicology to sustainability and policy.
Scan the QR code below to learn more and register for free!
CAAT
Johns Hopkins Center for Alternatives to Animal Testing

Methods from the 3Rs Collaborative to Reduce Reliance on Animal Testing in Drug Development: A Focus on Inhalation Tox

Megan LaFollette | Executive Director | The 3Rs Collaborative

The 3Rs Collaborative is a US-based non-profit whose mission is to advance better science – for both people & animals

The 3RsC's efforts span across critical topics:

Culture of Care

Environmental Health Monitoring

Translational Digital Biomarkers

Artificial Intelligence

3Rs Certificate Course

Refinement (Mouse Handling & NHP)

Microphysiological Systems

Translational Digital Biomarkers

What? Non-invasive 24/7 continuous monitoring technologies

Digital biomarkers can expand the translational relevance of animal research & reduce animal numbers

The 3RsC review papers on Translational Digital Biomarkers outlines value propositions, strategies for implementation, and a validation framework that could be used for regulatory use.

frontiers
in Behavioral Neuroscience

REVIEW
published: 14 February 2022
doi: 10.3389/fnbeh.2021.758274

Emerging Role of Translational Digital Biomarkers Within Home Cage Monitoring Technologies in Preclinical Drug Discovery and Development

Szczepan W. Baran[1], Natalie Bratcher[2], John Dennis[3], Stefano Gaburro[4], Eleanor M. Karlsson[5], Sean Maguire[6], Paul Makidon[7], Lucas P. J. J. Noldus[8,9], Yohann Potier[10], Giorgio Rosati[4], Matt Ruiter[11], Laura Schaevitz[12], Patrick Sweeney[13,14] and Megan R. LaFollette[15]*

frontiers | Frontiers in Toxicology

TYPE Review
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Validation framework for *in vivo* digital measures

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Artificial Intelligence

Broad scope, great potential

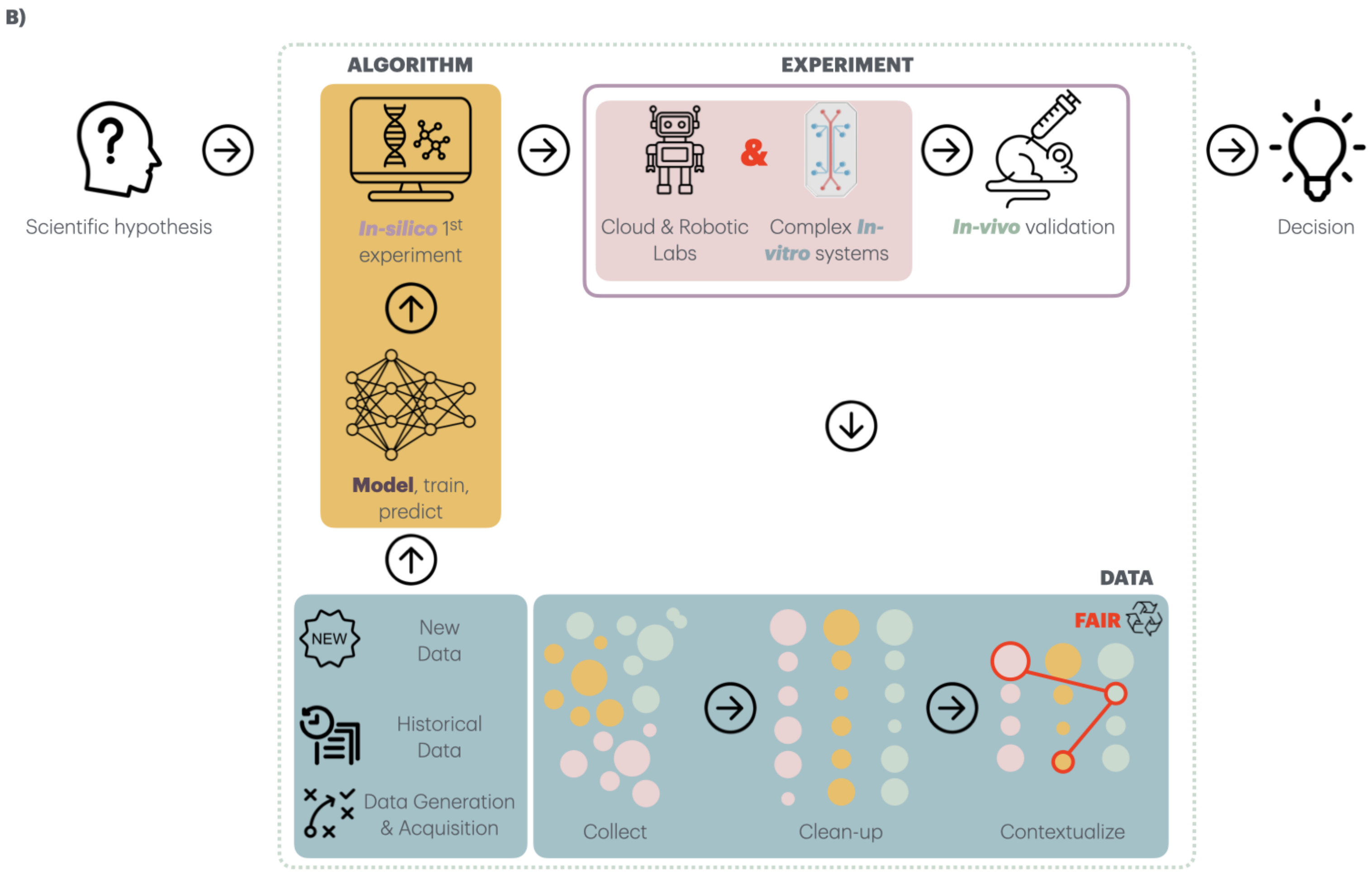
The 3RsC Artificial Intelligence initiative advances global awareness & harmonization.

Scope: Using predictive & generative AI for the **risk assessment & safety of drugs**, environmental, and industrial chemicals.

Focus: Facilitate **productive interactions between stakeholders (end-users, developers, regulators**) while encouraging appropriate use of AI as an independent NAM.

Co-Leads: Szczepan Baran & Weida Tong

AI can be used to integrate data across experiment types



Microphysiological Systems

What?

Complex in vitro models, organ-on-a-chip, spheroids, organoids

Microphysiological systems are an impactful emerging technology that are already being used to assess inhalation toxicity & efficacy.

The 3RsC MPS Initiative includes >49 member institutions (>100 members), mostly commercial developers

aimbiotech
Alcyomics
ALTIS BIOSYSTEMS
AlveoliX In-vitro models inspired by nature
久浪智醫 Anivance ai
Aracari Biosciences
ATCC

Axiom
AXOL
AZAR innovations
BIOIVT ELEVATING SCIENCE
BiomimX THE BEATING ORGANS-ON-CHIP
Bioprinting Laboratories
BrainXell

CanChip
CN-BIO
CRITICAL PATH INSTITUTE
curi bio
cyprotex AN EVOTEC COMPANY
DefiniGEN DISEASE MODEL INNOVATION
DYNAMIC42

emulate
esqLABS WE EMPOWER HEALTH CARE
EXIR BIO
GSK
HESPEROS THE HUMAN-ON-A-CHIP COMPANY
ibidi cells in focus
ImmuONE Lung & Immune *In Vitro* Assay Solutions

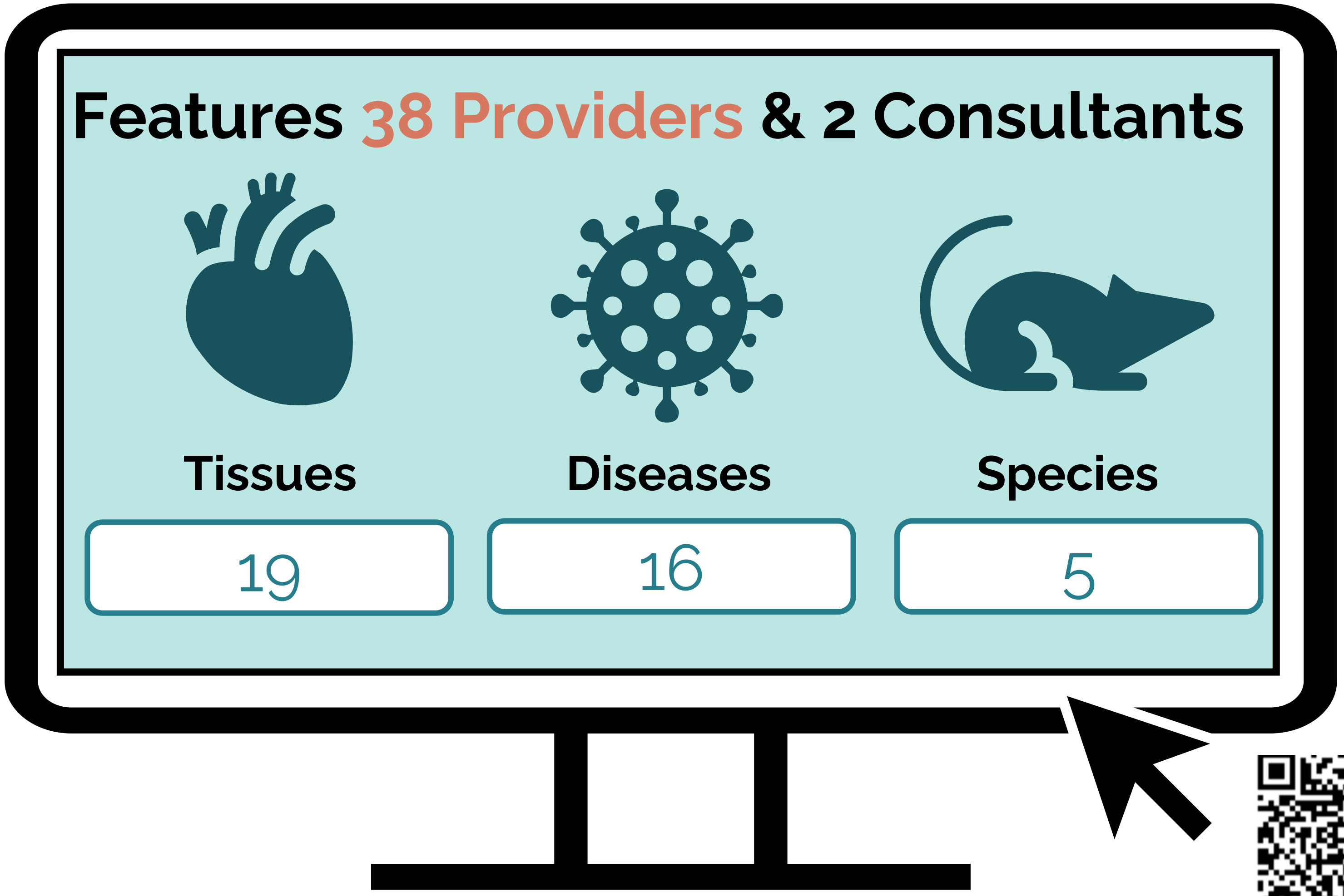
innoVitro
insphero
KIYATEC
LENABIOSCIENCES
MEPSGEN Micro Engineered Physiological Systems Guiding and Enabling the Next
microtissues

MIMETAS the organ-on-a-chip company
mo:re
nagi bioscience
Ncardia
NETRI
NILOGEN ONCOSYSTEMS
Newcells BIOTECH

Obatala
OPTICS11 life
Organo Consulting
PredictCan Biotechnologies
Quris ai
Roper Toxicology Consulting Limited

SYNVIVO
TheWell BIOSCIENCE
TISSUSE Emulating Human Biology
VoxCell
Xellar biosystems

Access our MPS tech hub to find relevant commercial tech providers, consultants, & enabling companies.



NEW:

Publication Hub: explore publications from commercially available companies using the same filters!

15 Lung MPS Providers + 2 consultants +2 enabling companies

Roper Toxicology Consulting Limited

BIOVETALITY

ALTIS BIOSYSTEMS

AlveolIX

Anivance ai

BiomimX THE BEATING ORGANS-ON-CHIP

CN-BIO INNOVATIONS

DYNAMIC42

emulate

ImmuONE Lung & Immune *In Vitro* Assay Solutions

MIMETAS

Ncardia Stem cell experts

NEWCELLS BEST BIOLOGY DRIVING *IN VITRO* INNOVATION

NILOGEN ONCOSYSTEMS

SYNVIVO

TISSUSE Emulating Human Biology

VoxCell

Our 2023 October Lung Workshop features presentations below:

- Respiratory Toxicology Services – Clive Roper, **Roper Toxicology Consulting**
- Unveiling animal-free tools for inhalation risk assessment: make every breath safer – Louis Scott, **ImmuOne**
- ^{AX}Lung-on-Chip and its applications – Giulia Raggi, **Alveolix**
- PhysioMimix OOC Lung MPS – Emily Richardson, **CN-Bio**
- Small Airway Epithelial Cells: A in-vitro model for the study of airway physiology & disease – Megan Websiter, **Newcells BioTech**
- Lung-on-Chip Models for Drug Safety & Efficacy – Deborah Ramsey, **SynVivo**
- Uncovering SARS-CoV-2 Pathogenic Insights & Screening Therapeutics in a High-Fidelity and High-Throughput BSL3 Human Lung MPS – Ashley Gard, **Draper**
- The OrganoPlate as a platform for lung-on-a-chip research – Iris Schilt, **Mimetas**

3rc.org/mps/presentations

Case Studies & Highlights

Note: this is a very high-level, simplified overview of a few models. Please check out prior webinars and talk to company representatives for more info.

Overview of lung MPS providers

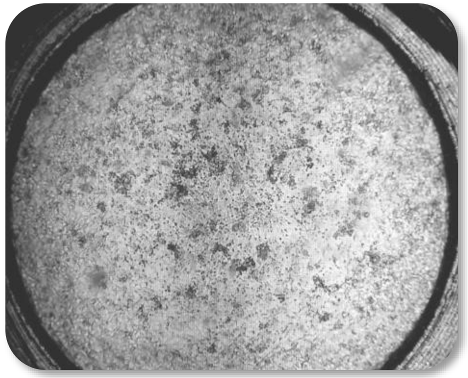
All except ImmunONE & Aracari have mechanical stretch / dynamic airflow

Company	Cell Types	Notes
Alveolix	Epithelial cells (alveolar or bronchial), endothelial cells, Lung Fibroblasts, Macrophages and other cells	Immunocompetent models. Compatible with exposure vial aerosol, dry powder continuous flow.
Anivanceai	Epithelial cells (alveolar or bronchial)	FNIH NAMs VQN RFI
Aracari	Epithelia cells (alveolar or bronchial), Endothelial cells, Pneumocytes, Lung Fibroblasts, Macrophages	Vascularized MPS, Often with tumors
Dynamic42	Endothelial cells, Epithelial cells (alveolar or bronchial),	Optionally immunocompetent with monocytes. Lung-Liver model.
Emulate	Endothelial cells, Epithelial cells, Macrophages,	Vascularized & immunocompetent model
ImmuONE	Epithelia cells, Alveolar Macrophages	Immunocompetent. Advanced dosing capabilities (e.g., liquid vs aerosolized)
TissUse	Endothelial cells, Epithelial cells, Macrophages	Immunocompetent model Multi-organ Lung-Liver & lung-lymph node-liver models

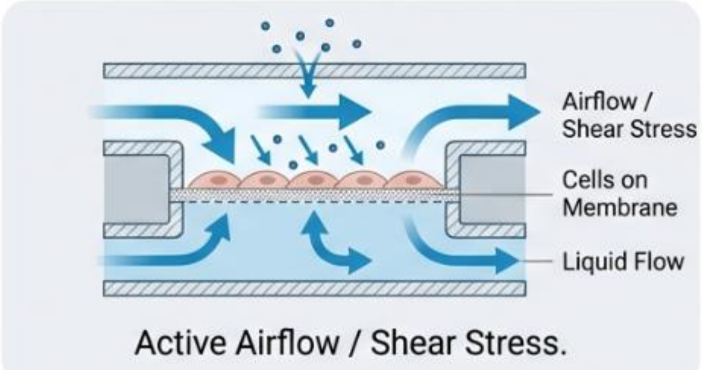
Regulatory Applications of Lung MPS

- Safety studies: discover mechanisms & explain in vivo results
- Clinical studies to investigate variability in patient-patient response
- Immuno-oncology
- Barrier functionality
- Model healthy or diseased lungs
- Drug repurposing
- Personalized medicine
- Characterizing early lung inflammation to determine whether a response is adaptive or adverse.

Images & diagrams of MPS

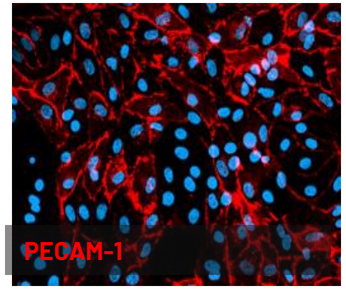


AlveoliX

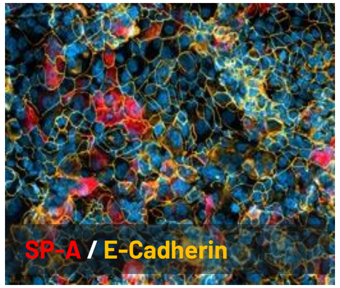


Anivance ai

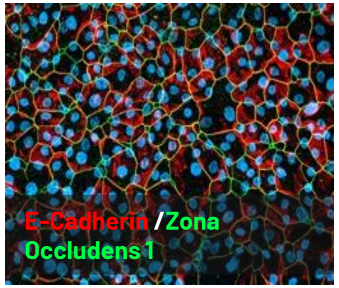
Vascular



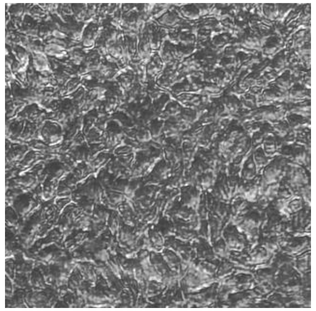
Alveolar



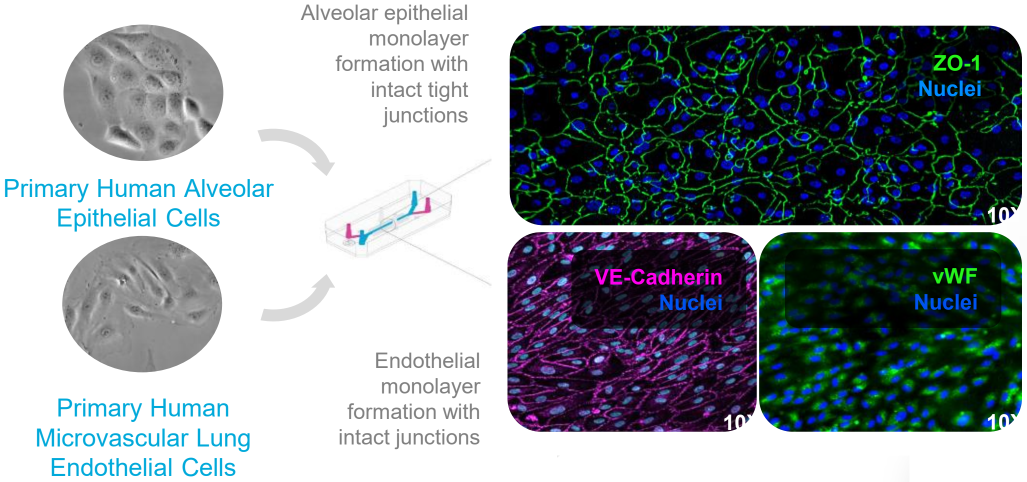
Bronchial

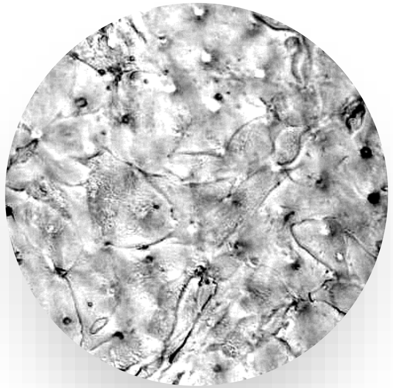


Bronchial Cilia Beating

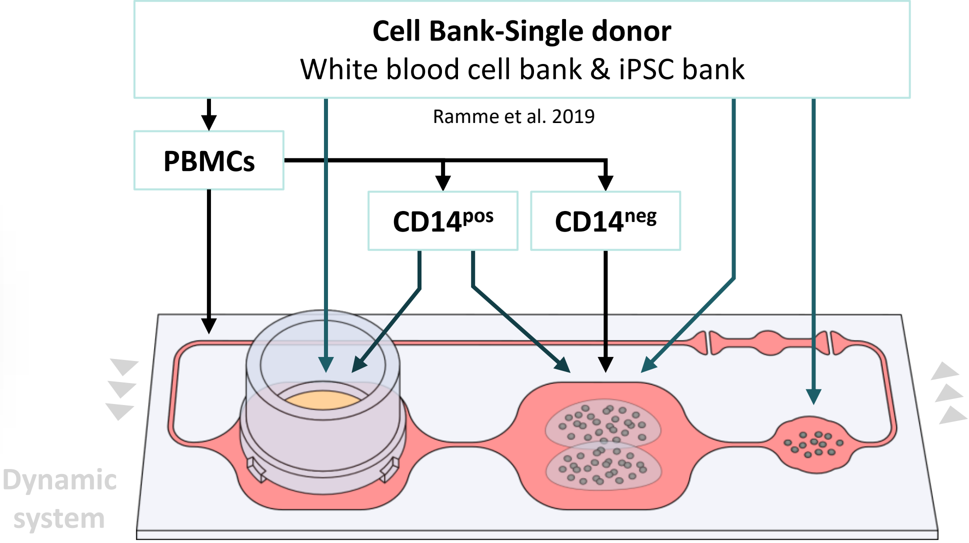


DYNAMIC42



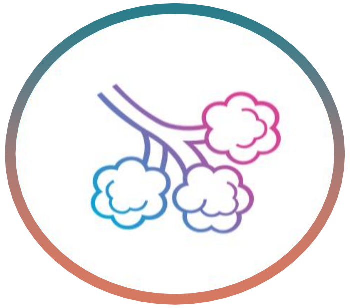


emulate



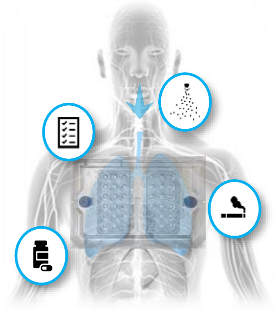
TISSUSE
Emulating Human Biology

Emulate Case Study: Drug Repurposing



1. Identified target gene following viral infection: Receptor for Advanced Glycation End Products (RAGE)
2. Identified previously safe small molecule RAGE inhibitor: *Azeliragon (Aze) (previously found to be safe, but not effective) in Alzheimer's trials)*
3. Tested drug efficacy to rescue infected lung MPS
4. **Result: 12 months from study initiation to successful licensing. Currently in clinical trials for covid-19.**

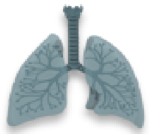
Alveolix Case Study:
IND Submissions & More



Data used in several IND submissions

- Safety and Efficacy testing (e.g.: Immunotherapy, ADC, ASO, ARDS, and more)
- Inhalation studies (Nanoparticles & toxic chemical (such as PHMG, MIT, pesticides, ZnO, TiO2 etc.), HTP & smoke pollution, dry powder, Aerosol drug)
- Accurate disease Modeling (e.g.: IPF, COPD, VLS, PAH, Emphysema)
- Infection Modeling (Virus, Bacteria (Tuberculosis, LPS, Streptococcus pneumoniae), Fungus)
- Personalized medicine

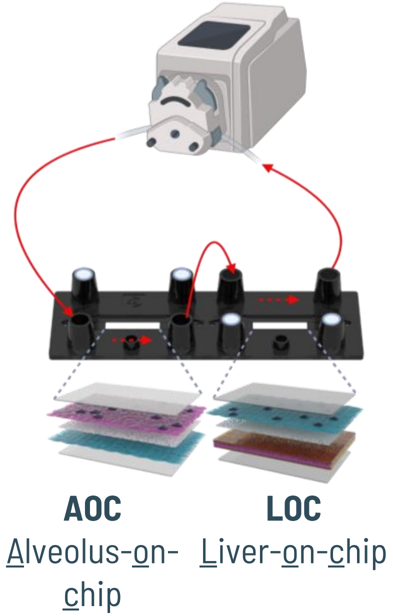
Dynamic 42 Case Study: Paraquat



DYNAMIC42

BASF We create chemistry

- Two organ model lung – liver to investigate tox
- Project partner: BASF
- Paraquat (PQ) used as reference compound
- Very low binding (adsorption) in platform
- Demonstrated lung-specific cytotoxicity in two organ setup



ImmuONE Case Study: De-Risking Antibody-Drug Conjucates (Ads)

ImmuONE
Lung & Immune *In Vitro* Assay Systems

- Problem: Drug-induced interstitial lung disease (DIILD) is a **leading cause of clinical holds** for ADCs (used for oncology), but **conventional models fail to predict** late stage failures due to **lack of predicted Fcy receptor-mediated** uptake in human alveolar macrophags
- Solution: **ImmuPHAGE alveolar macrophages with confirmed Fcy receptor** expression that can more accurately predict DIILD

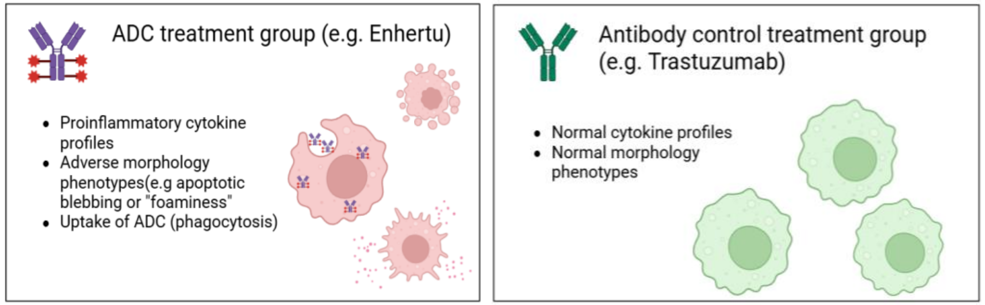


Fig 1: ADCs induce proinflammatory profiles and apoptotic phenotypes in alveolar macrophages compared to antibody controls.

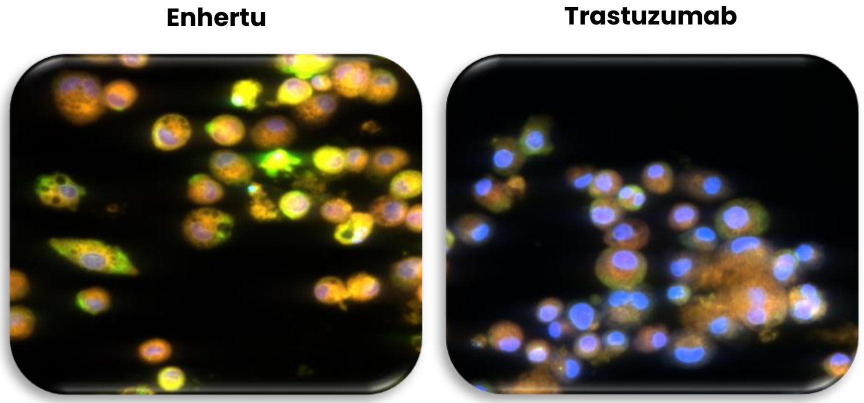


Fig 2: Representative image of macrophages treated with 100 µg/mL ADC and displaying foamy phenotype.

Overall strengths of lung MPS

- MPS can recreate tissue-tissue interfaces, provide relevant drug dosing & vascular infusion, include immune cells
- MPS have physiologically relevant barriers in the presence of vascular flow, air-liquid interface, and often mechanical stretch that emulates breathing
- Human cells to predict human-specific outcomes. Can use primary tissue from healthy or diseased patients with various features depending on scientific question
- Animal cell models can remove the major anatomical differences between rodent & human respiratory systems *(e.g., rodents being obligate nose breathers with nasal passages that can filter out inhaled chemical)*
- Mechanistic insights
- Reduction of animal use
- Can require less test substance for dosing compared to animal models

Lung MPS still have limitations

- Limited cell types do not fully mimic the full respiratory tract
  - However, focus on particular regions can still be beneficial
- Limited capacity for long-term exposure
- General lack of consensus/confidence/guidance on best practices
- Although in vitro models CAN model diverse populations, they may currently only include limited donors
  - Note this could also be a concern with animal models

Regulatory Readiness of Lung MPS

- Depends greatly on the context of use & validation data provided. Needs to be focused on a regulatory COU.
  - Lung MPS results have already been included in IND and clinical trial applications
  - Unlikely to fully replace animal studies unless MPS can show all relevant readouts
- Lung MPS could be submitted to FDA ISTAND program is good for broad qualification
  - 3RsC has an accepted LOI for liver MPS that includes 8 platforms
- Note: platforms do NOT need to be qualified to be used in regulatory submissions.

Call to Action: Join the 3RsC in advancing better science - for both people & animals.

Sign up for our newsletter to stay up to date & join us

Reach out to me meglafollette@3rc.org to join a specific initiative.

CN-BIO

REAGAN-UDALL FOUNDATION for the Food and Drug Administration

Advancing Drug Development by Reducing Reliance on Animal Testing:

Case Example: Pre-Clinical Animal Models in Lung Toxicology

Dr Emily Richardson

Biology Group Leader - Toxicology

Building predictive *in vitro* assays

New approaches to add to the preclinical toolbox are required.

Factors to consider when building new pre-clinical assays

- Reliable and robust
- Cost
- Throughput
- Representative of human biology
- Clinically relevant (translational) endpoints
- Mimic *in vivo* pharmacokinetics
- Acute vs chronic dosing
- Effects of various modalities (small molecules, biologics, gene therapy agents)
- Immunological effects
- Population variability

What is OOC / MPS?

Organ-on-a-chip / microphysiological systems

MPS come in many shapes and sizes but have some common features:

Human cells and tissue

Typically grown in three dimensions and with geometrical confinement or patterning

Fluidic flow

Mimic flow of blood, provide O_2 and nutrient supply and provide biomechanical stimuli

Environment control

Mechanical cues and sheer stress, electrical stimulation, control of gas exchange and growth factors

MPS enhances non-clinical safety toxicology studies

By addressing two key areas

1. Refine your pre-clinical experimental design

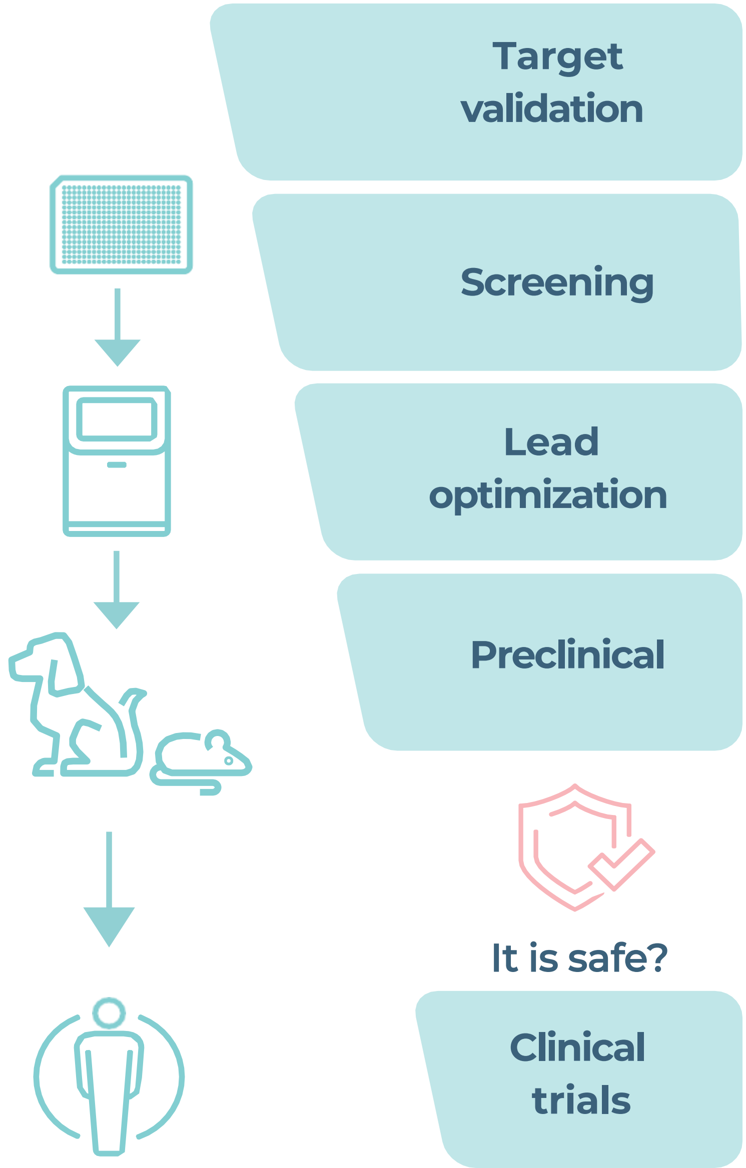
Human relevant, mechanistic data enables you to:

- make informed stop/go decisions while there's an opportunity to modify drug design.
- de-risk drug development earlier.
- narrow down molecule selection before *in vivo* testing.

2. Be better prepared for the clinic

Predict human outcomes for new drug modalities that lack suitable *in vivo* models.

Generate translatable data to predict clinical observations & confidently progress to human trials.



PhysioMimix® Core

CN-BIO

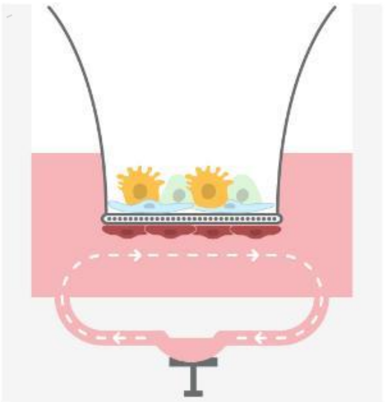
12 individual Chips
per Barrier plate

open well
600 µL media
100,000+ cells

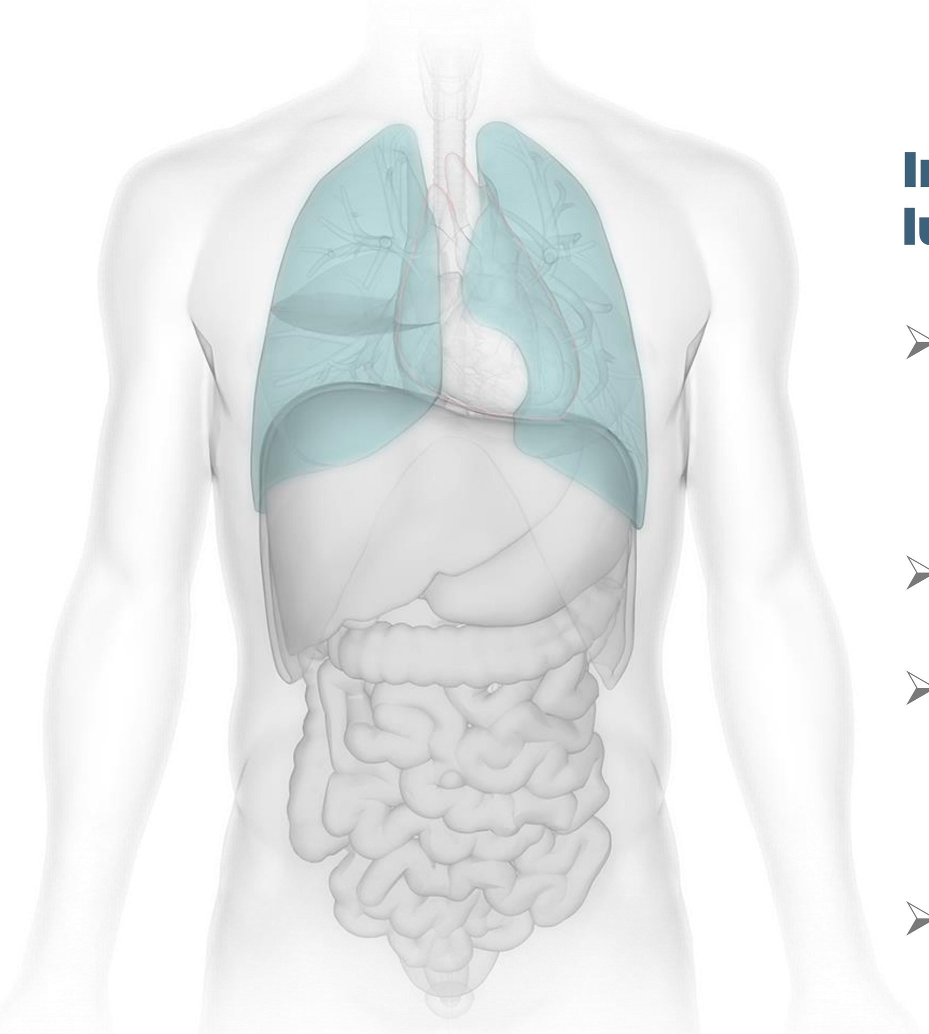
→ Total of 72 Chips
per Controller

6 Multi-chip plates
per Controller

Fluidic flow +
inserts for ALI
(e.g., Transwells)



PhysioMimix® Lung MPS



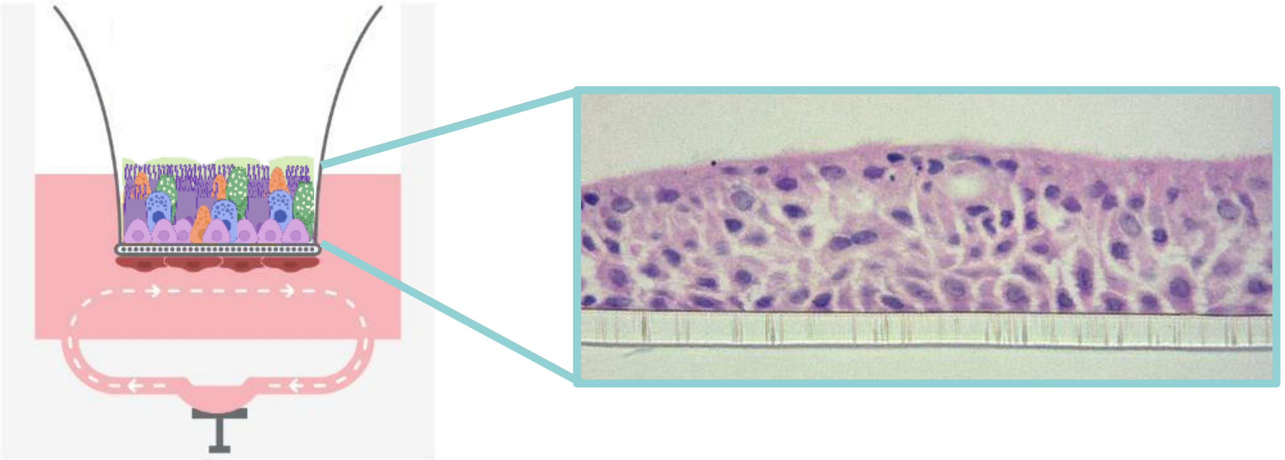
Important aspects of the lung to model *in vitro*:

- Phenotypes of disparate areas of lung (nasal vs airways vs alveoli)
- Air-cell-liquid interface
- Maintained functional viability – overall barrier and cell type
- Immune components

PhysioMimix® Lung MPS

Bronchial MPS

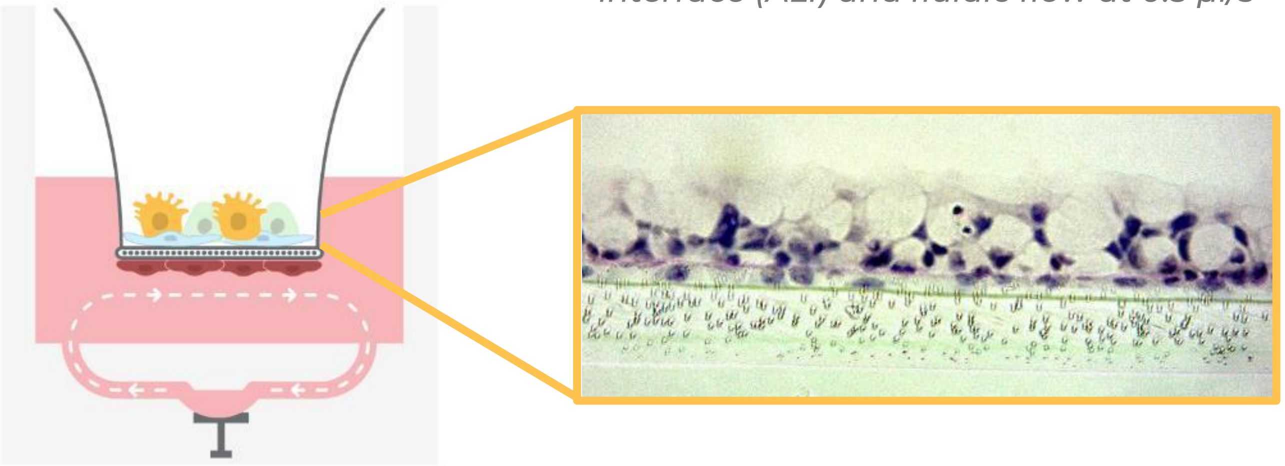
Primary bronchial epithelial cells
NHBE
(Ciliated, Goblet, Club, Basal)
+
Primary lung microvascular cells



14 days differentiation at air-liquid interface (ALI) and fluidic flow at 0.5 µl/s

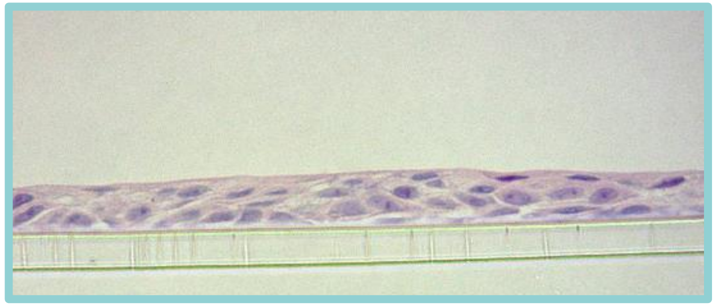
Alveolar MPS

Primary small airway cells
SAEC
(AT1, AT2)
+
Primary lung microvascular cells

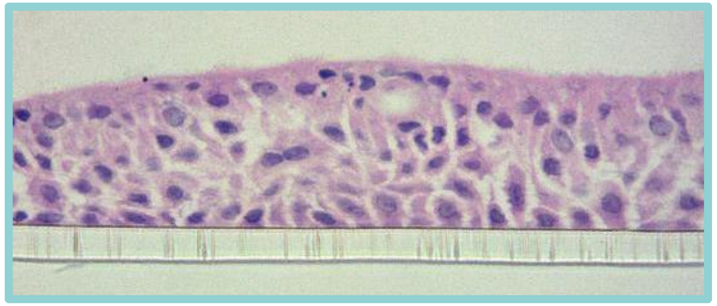


PhysioMimix® Lung MPS

Static Bronchial

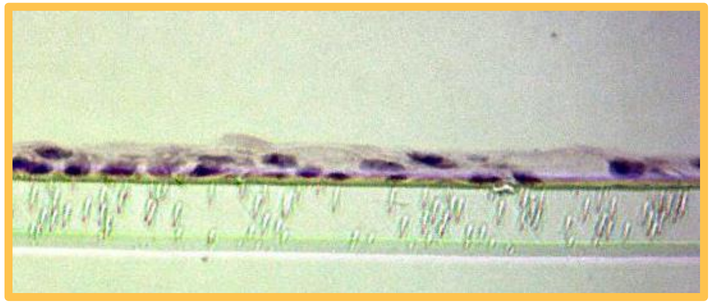


Bronchial MPS

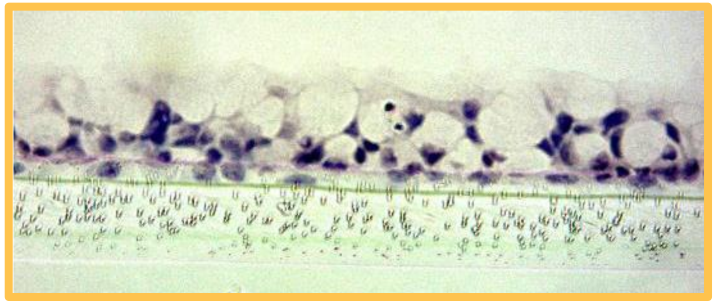


14 days differentiation at air-liquid interface (ALI) and fluidic flow at 0.5 µl/s

Static Alveolar



Alveolar MPS



Fluidic flow

↓

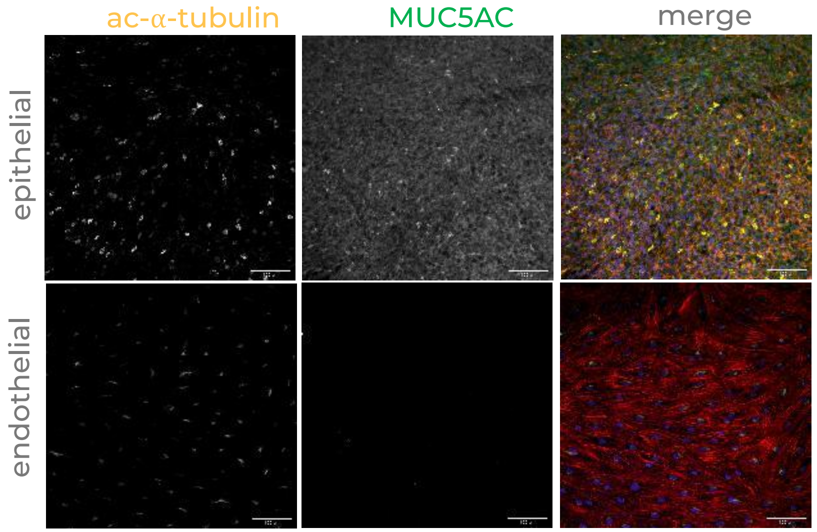
Increased nutrient / oxygen availability

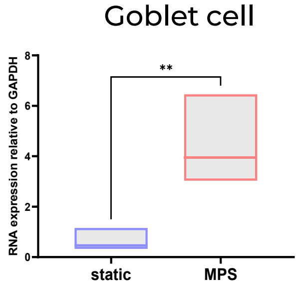
↓

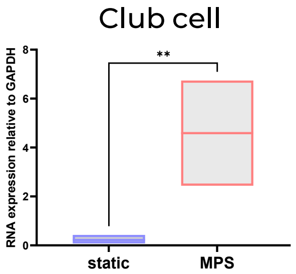
Faster differentiation

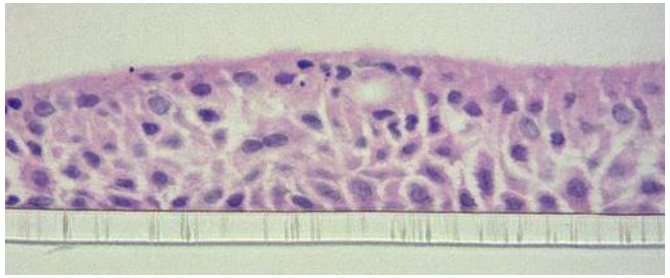
Lung MPS recapitulate human biology

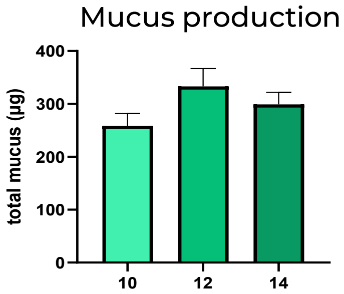
Bronchial MPS







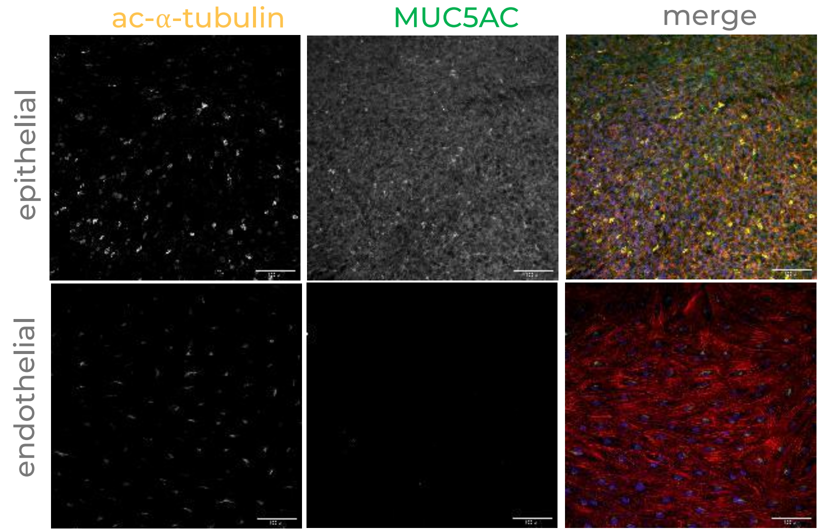


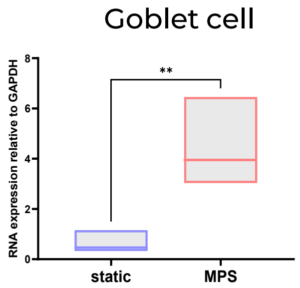


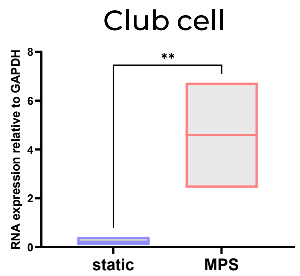
Alveolar MPS

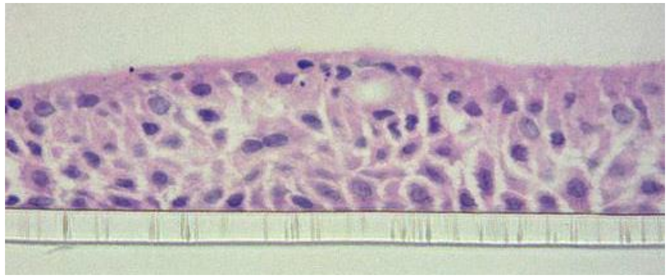
Lung MPS recapitulate human biology

Bronchial MPS

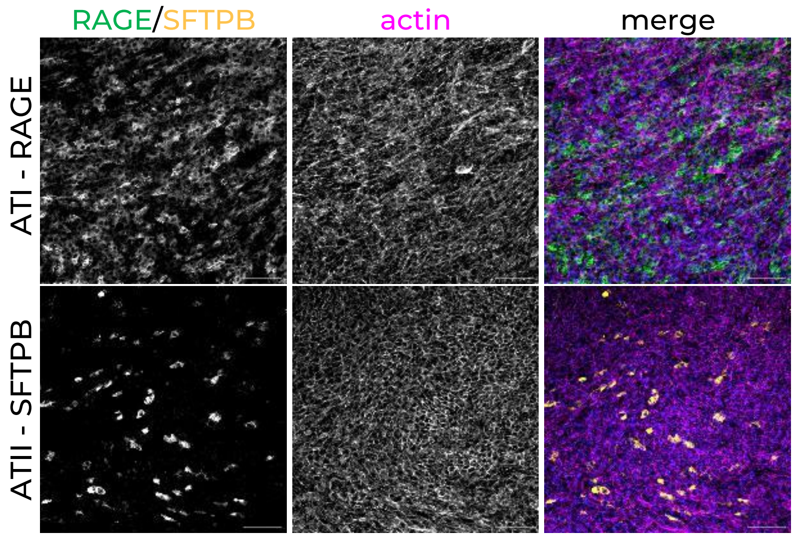


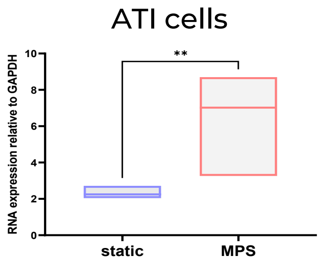


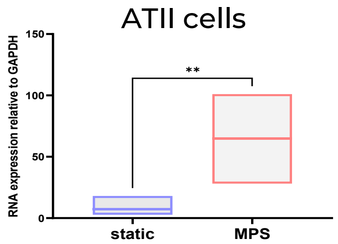


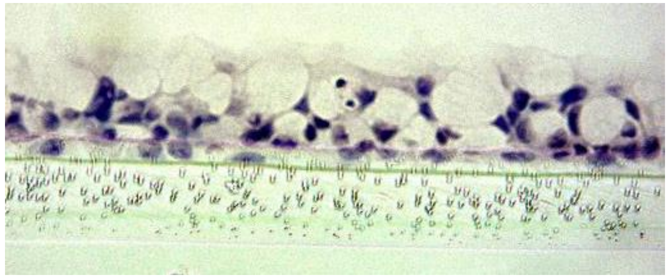


Alveolar MPS

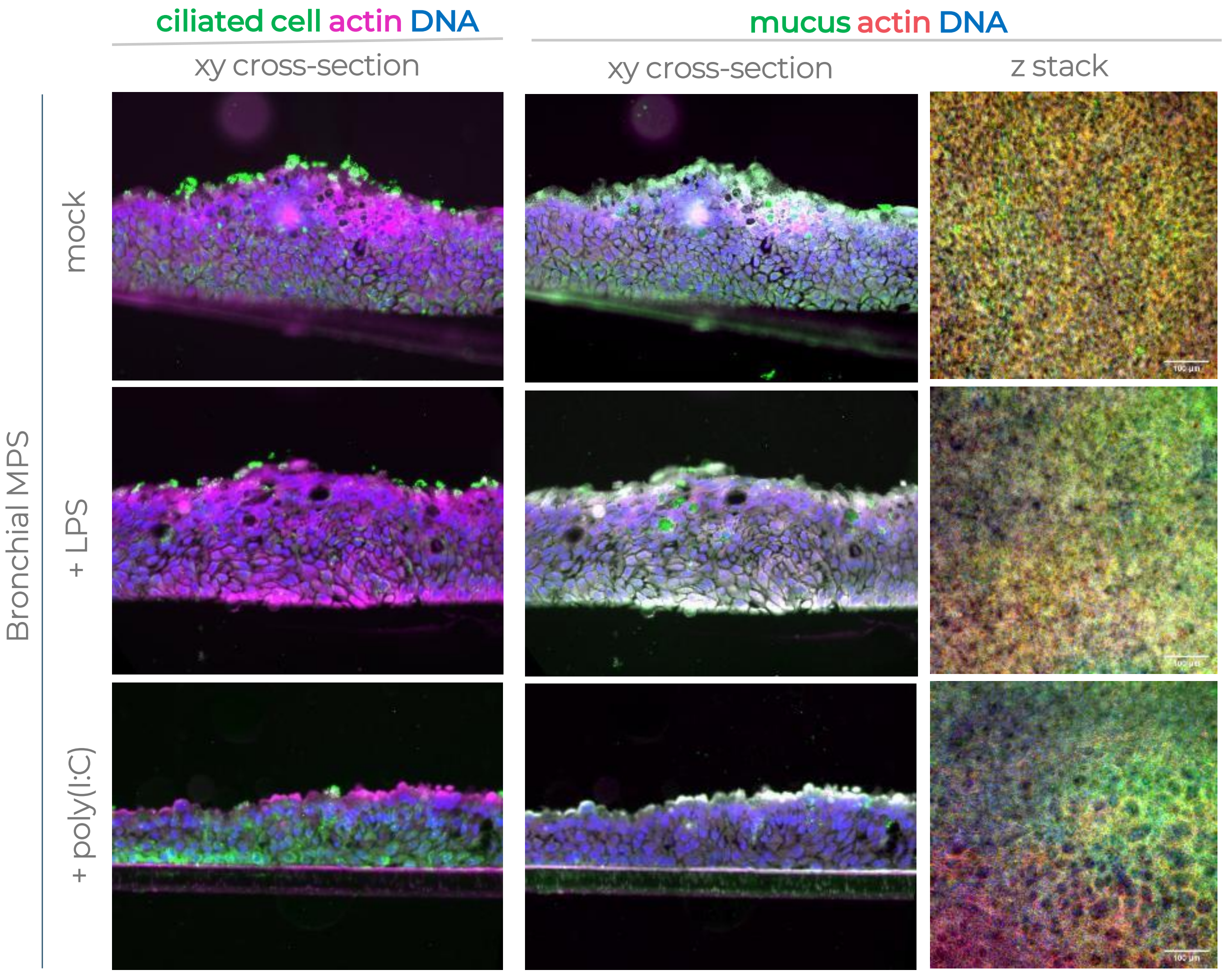


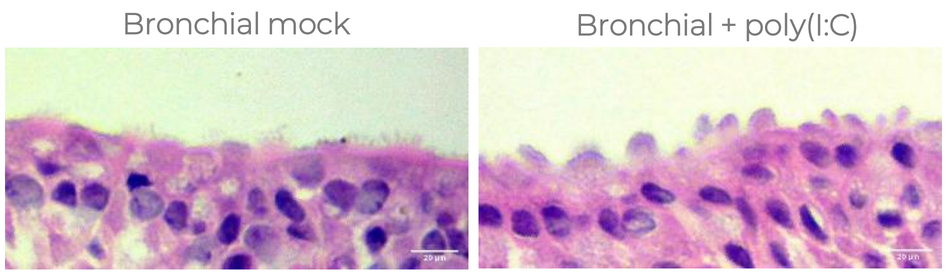


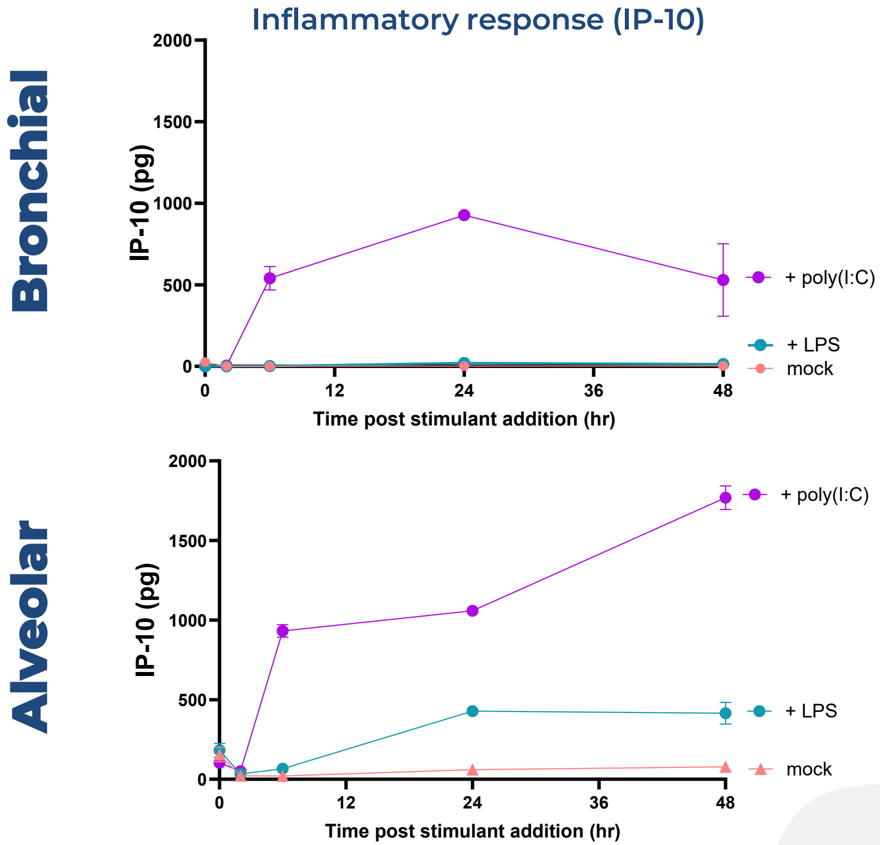




Lung MPS models predict inflammatory responses

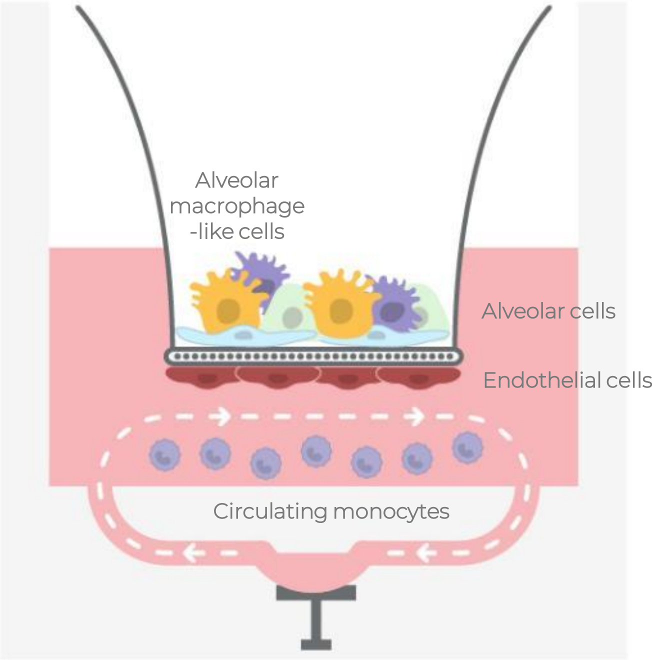




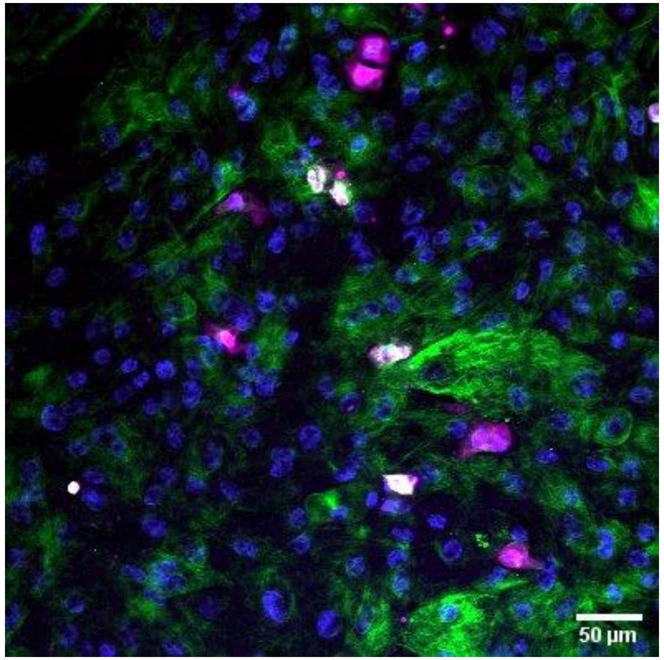


CN-BIO

Lung-Immune MPS models predict inflammatory responses

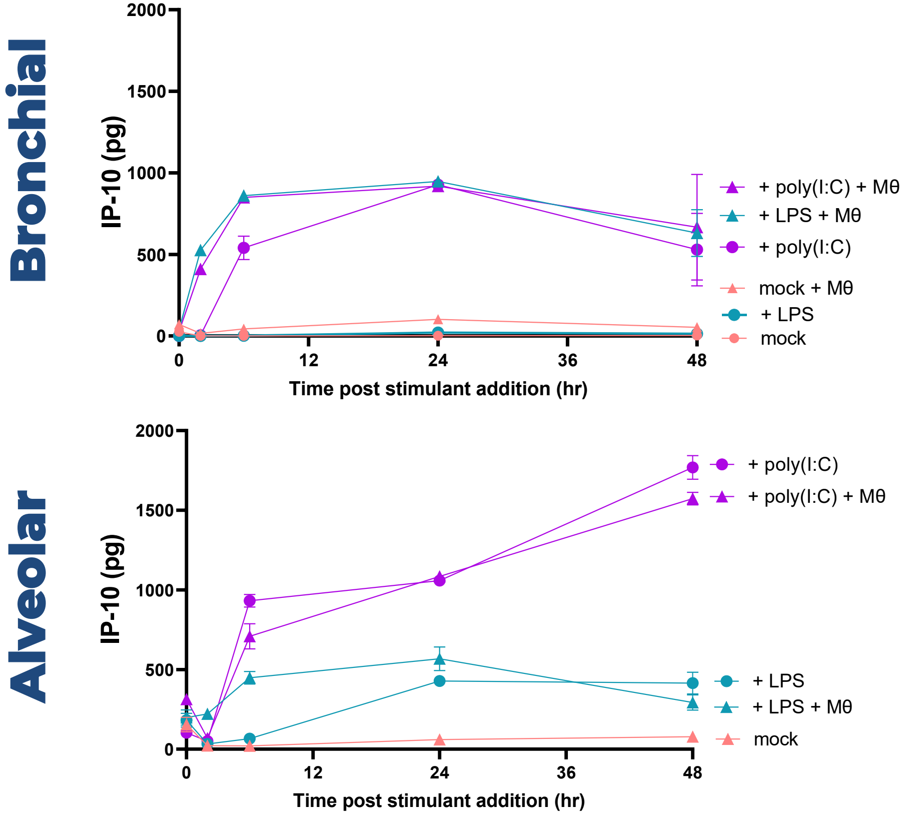


Alveolar model + monocytes



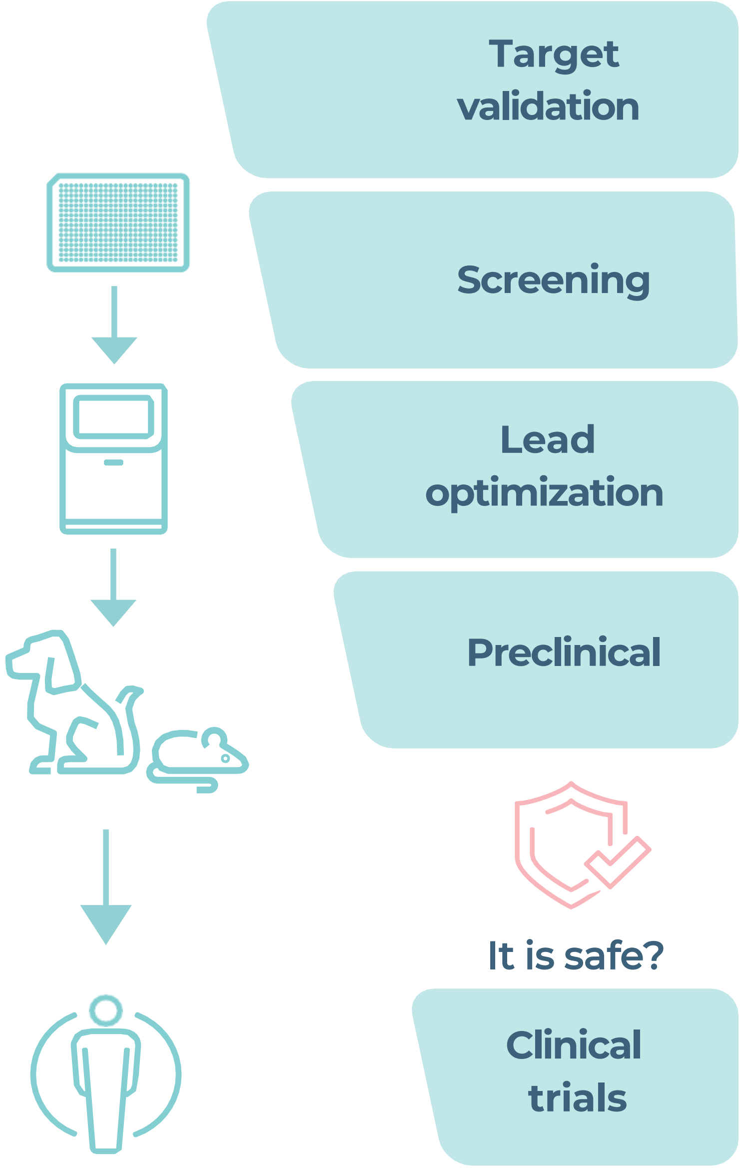
monocytes actin DNA

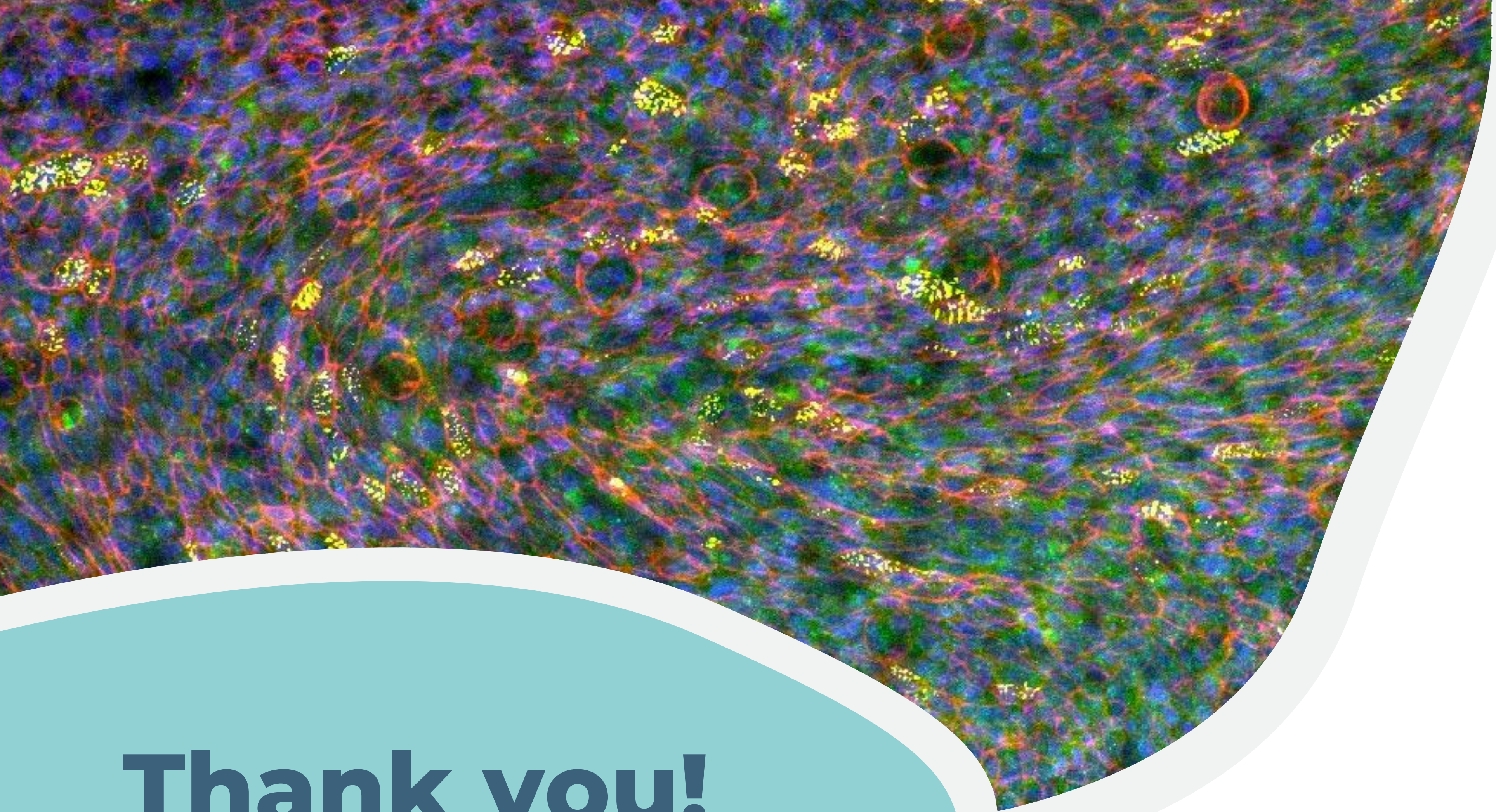
Inflammatory response (IP-10)



Lung MPS for non-clinical lung toxicology safety studies

- Lung MPS recapitulate human biology and response
- Circulating fluidic flow enables faster differentiation and incorporation of immune cells
- Insert-based cultures allow liquid or aerosolised drug administration.
- Open-well format for ease of drug administration and sampling over time.
- Adaptable for cell types and donors
- Human relevant, mechanistic data used to make informed stop/go decisions
    - De-risk earlier
    - Reduce candidates/concentrations for *in vivo* testing
    - Translatable data to confidently progress to clinical trials





Thank you!

visit **cn-bio.com**

For further info please contact

Dr Emily Richardson

Biology Group Leader

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Quantitative CT Imaging to Evaluate Lung Structure and Function

Rachel L Eddy, BEng, PhD

Assistant Professor, Departments of Radiology and Pediatrics, University of British Columbia, Vancouver, Canada

Imaging Scientist, VIDA Diagnostics Inc.

February 26 2026 – Reagan-Udall Foundation

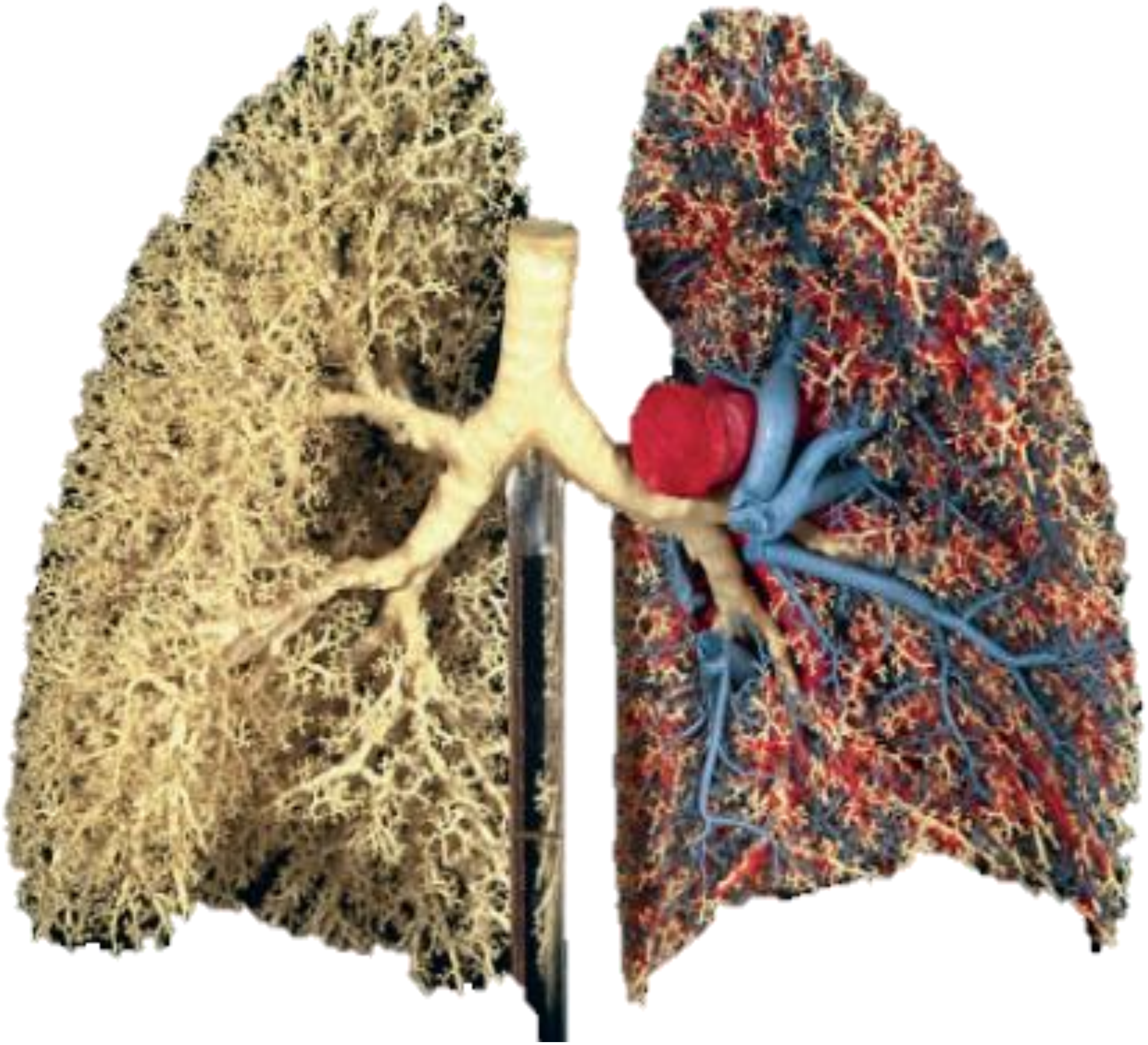
Monitoring for Lung Toxicity

- Symptoms
  - Validated questionnaires

- Lung function tests
  - Spirometry
  - Lung volumes
  - Diffusing capacity

1500 miles of airways

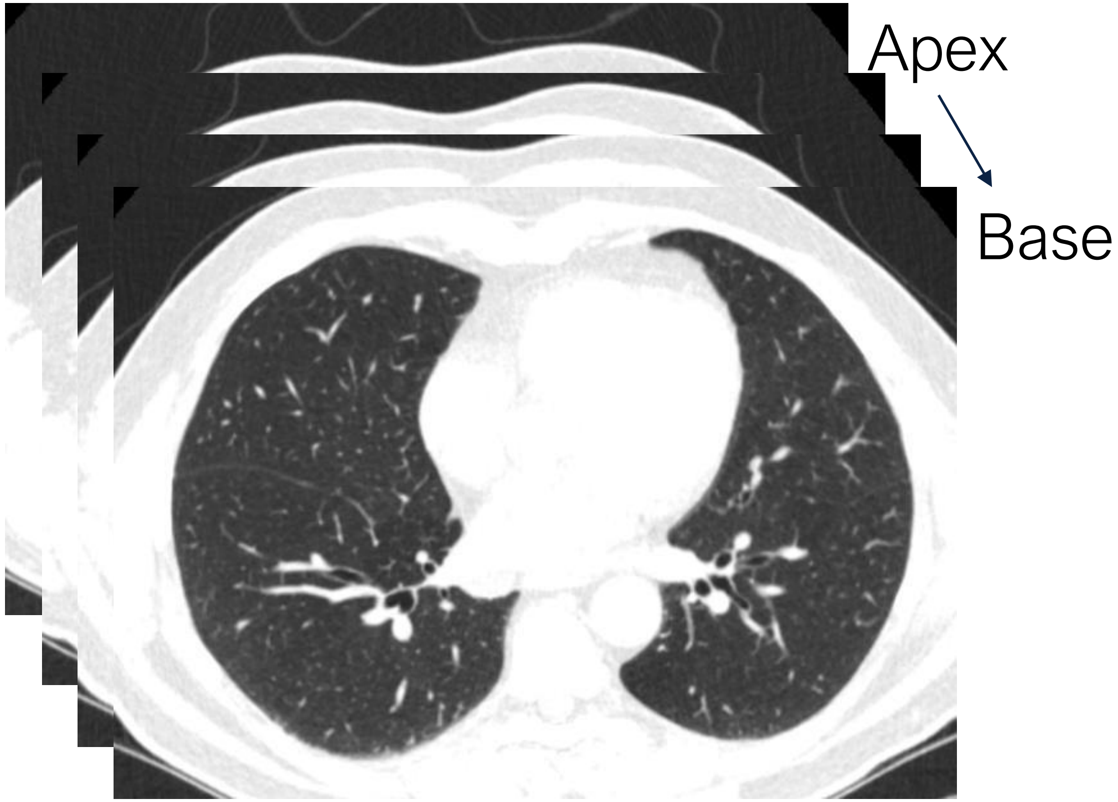
500 million alveoli



Weibel. *Swiss Med Wkly* 2009

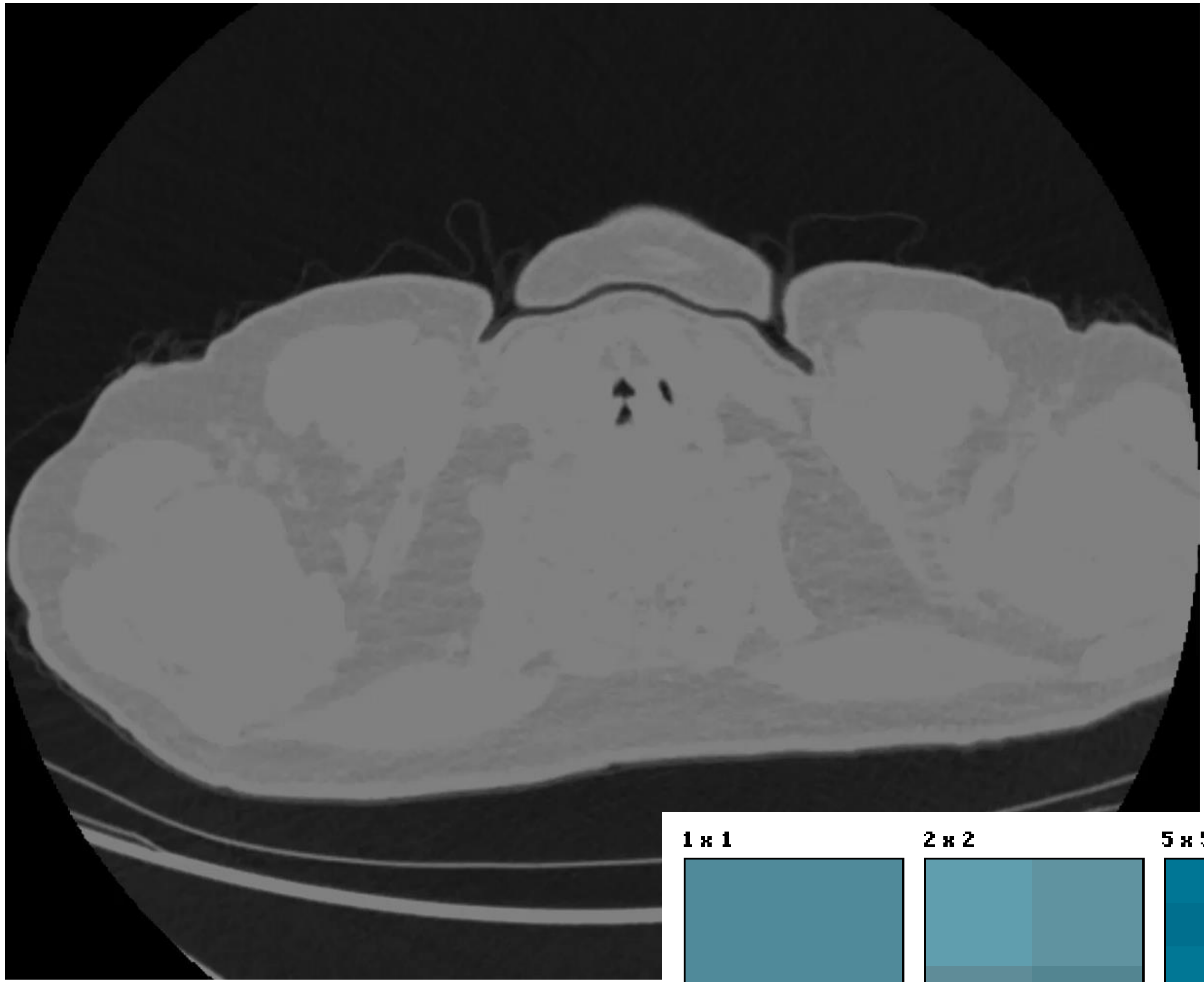
CT Imaging of the Lungs

Computed tomography (CT)



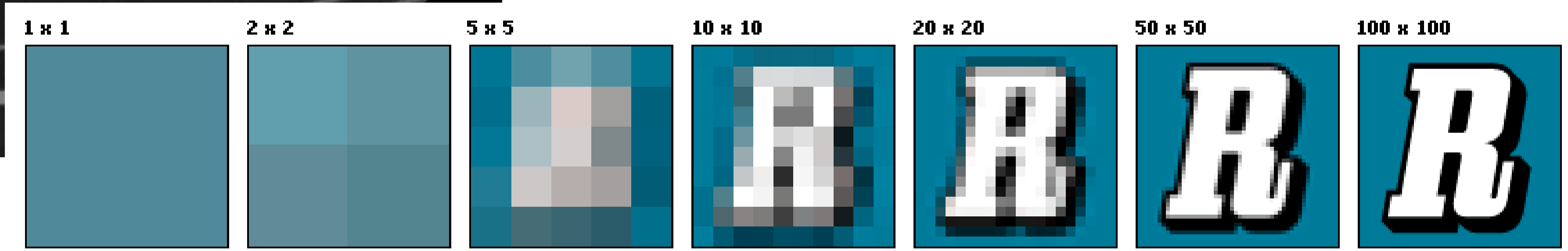
CT Imaging of the Lungs

Computed tomography (CT)



Terminology:

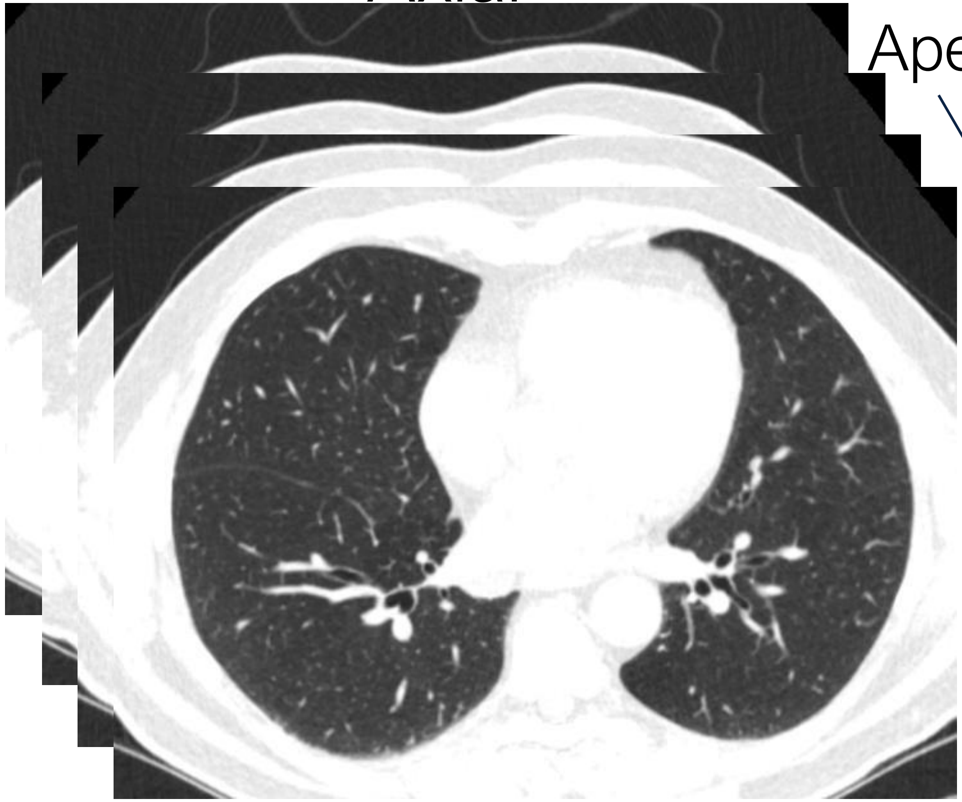
- CAT Scan
  - Computerized axial tomography
- MDCT
  - Multi-detector CT
- HRCT
  - High-resolution CT



CT Imaging of the Lungs

Computed tomography (CT)

Axial

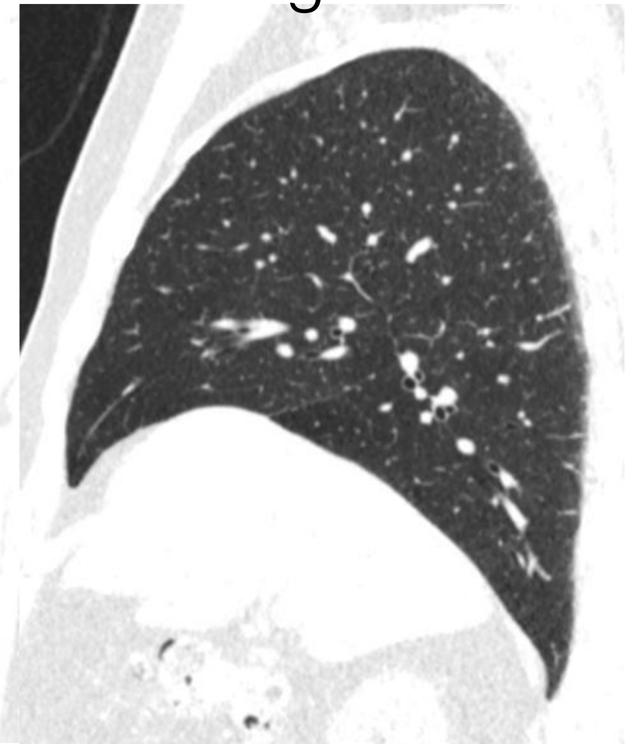


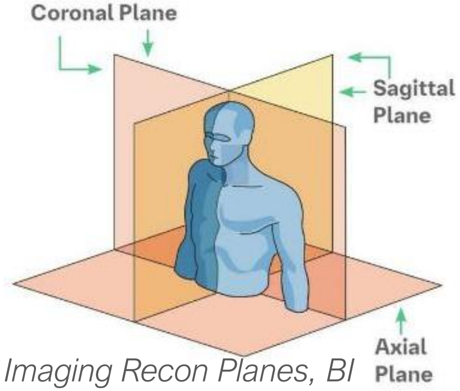
Apex

Base

Coronal

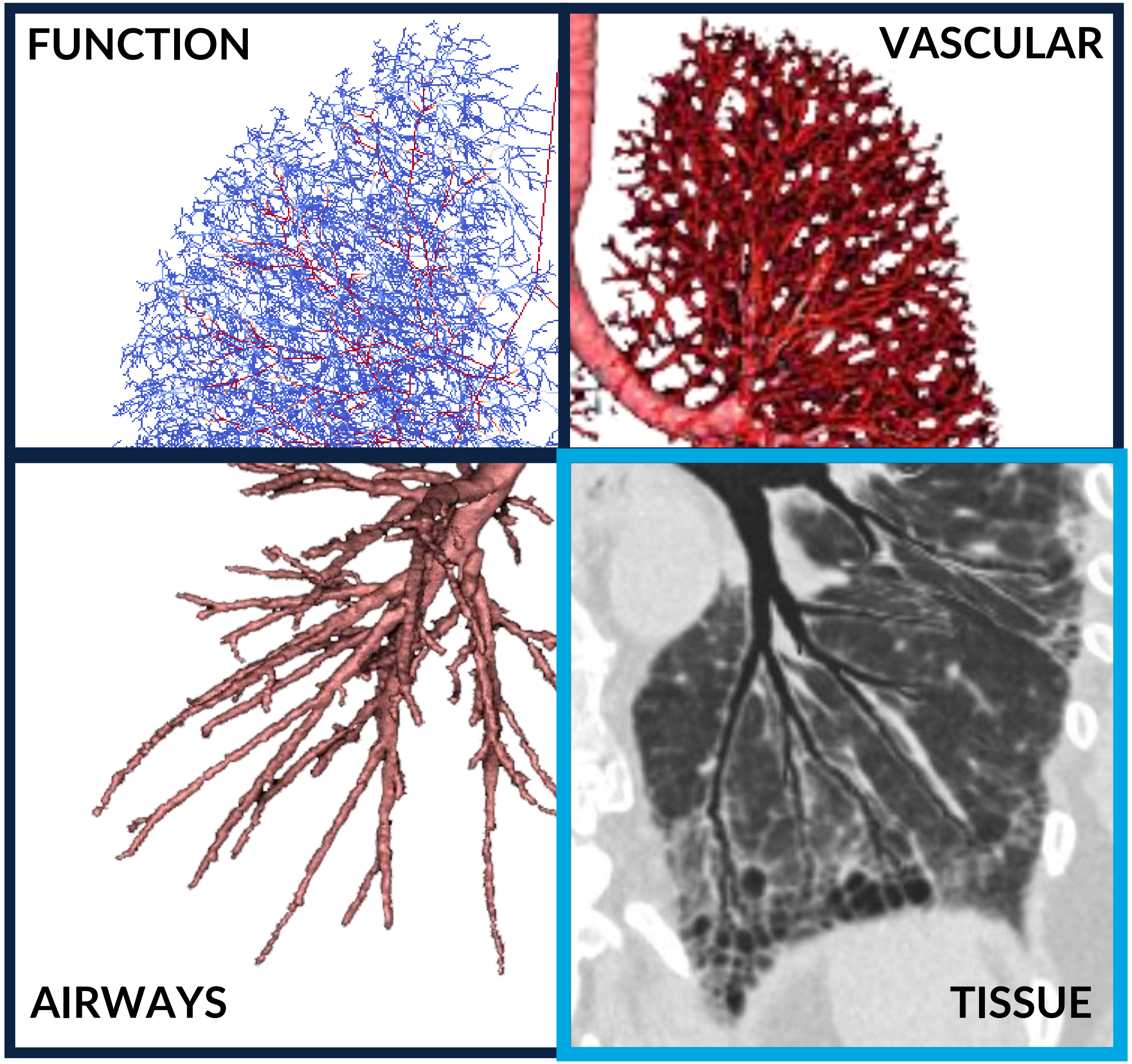
Sagittal





Imaging Recon Planes, BI

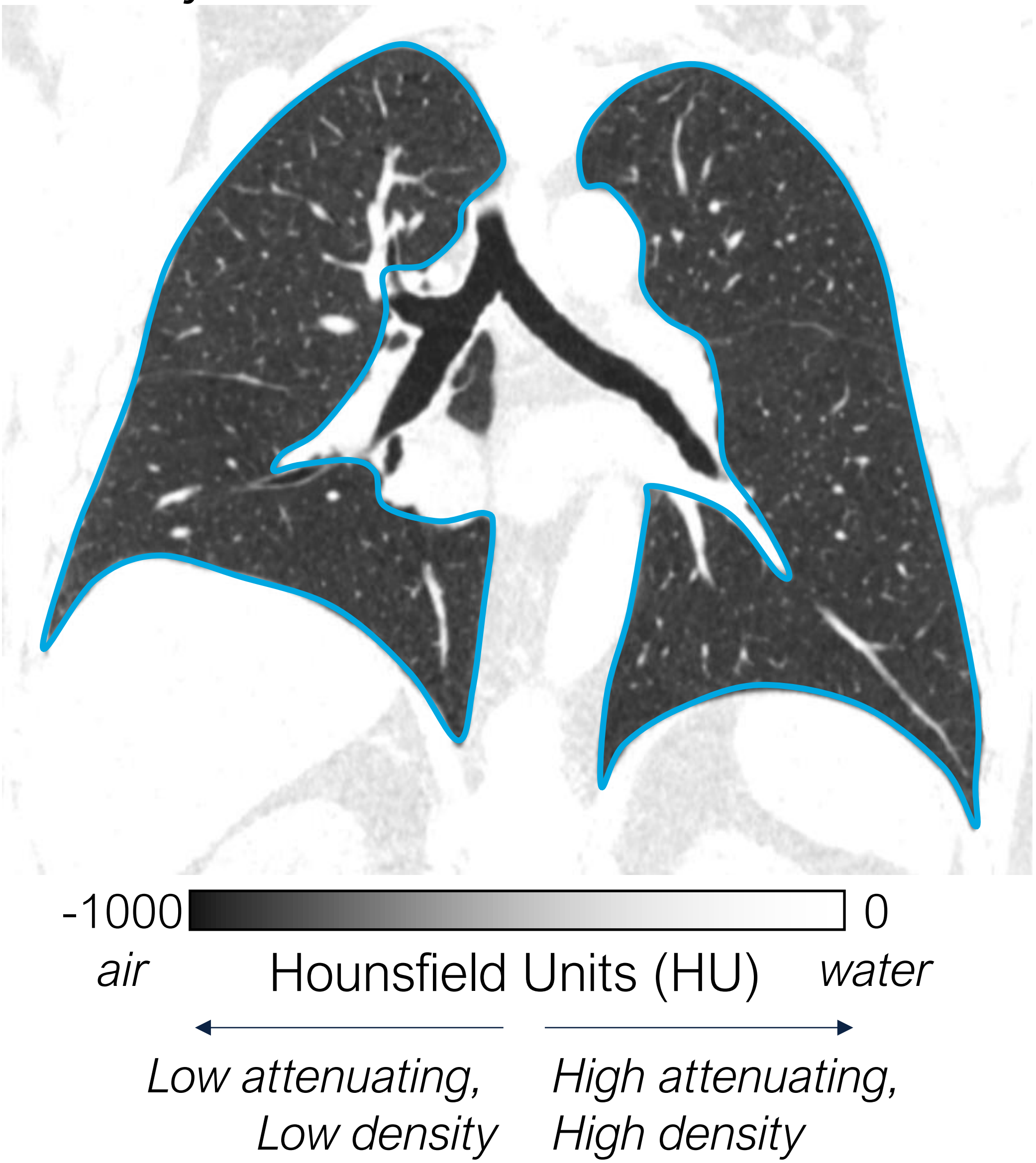
Quantitative CT for Lung Structure-Function

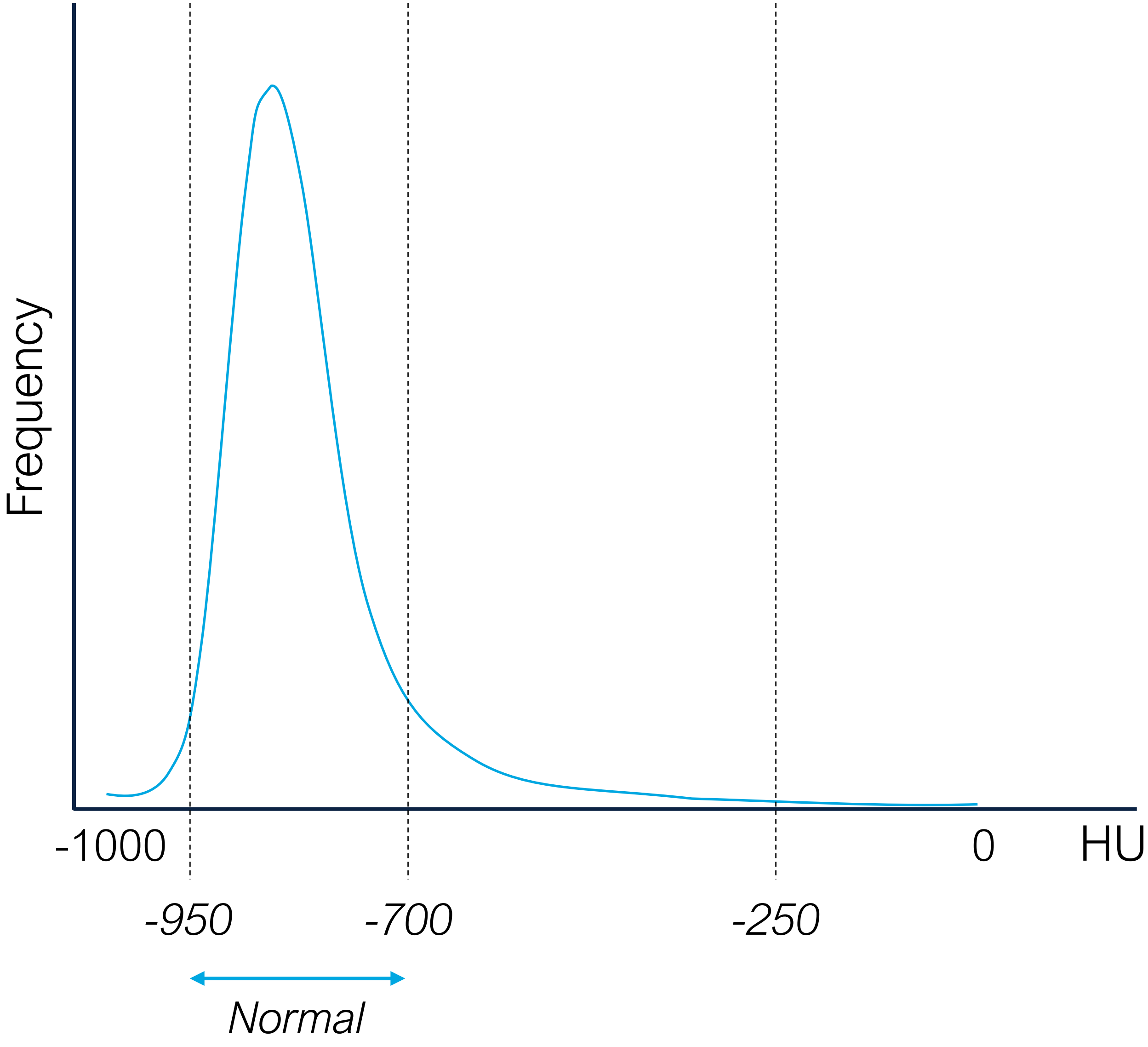


Tissue measures for lung toxicity

Quantitative CT: Tissue Measurements

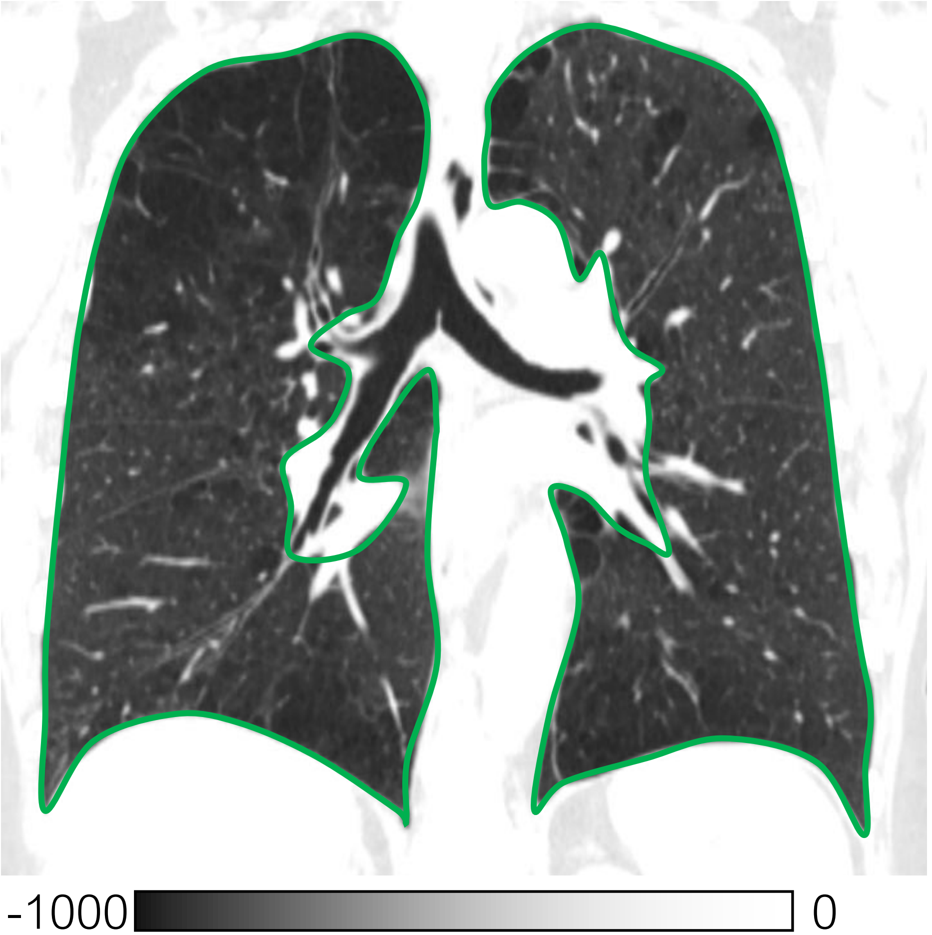
Healthy

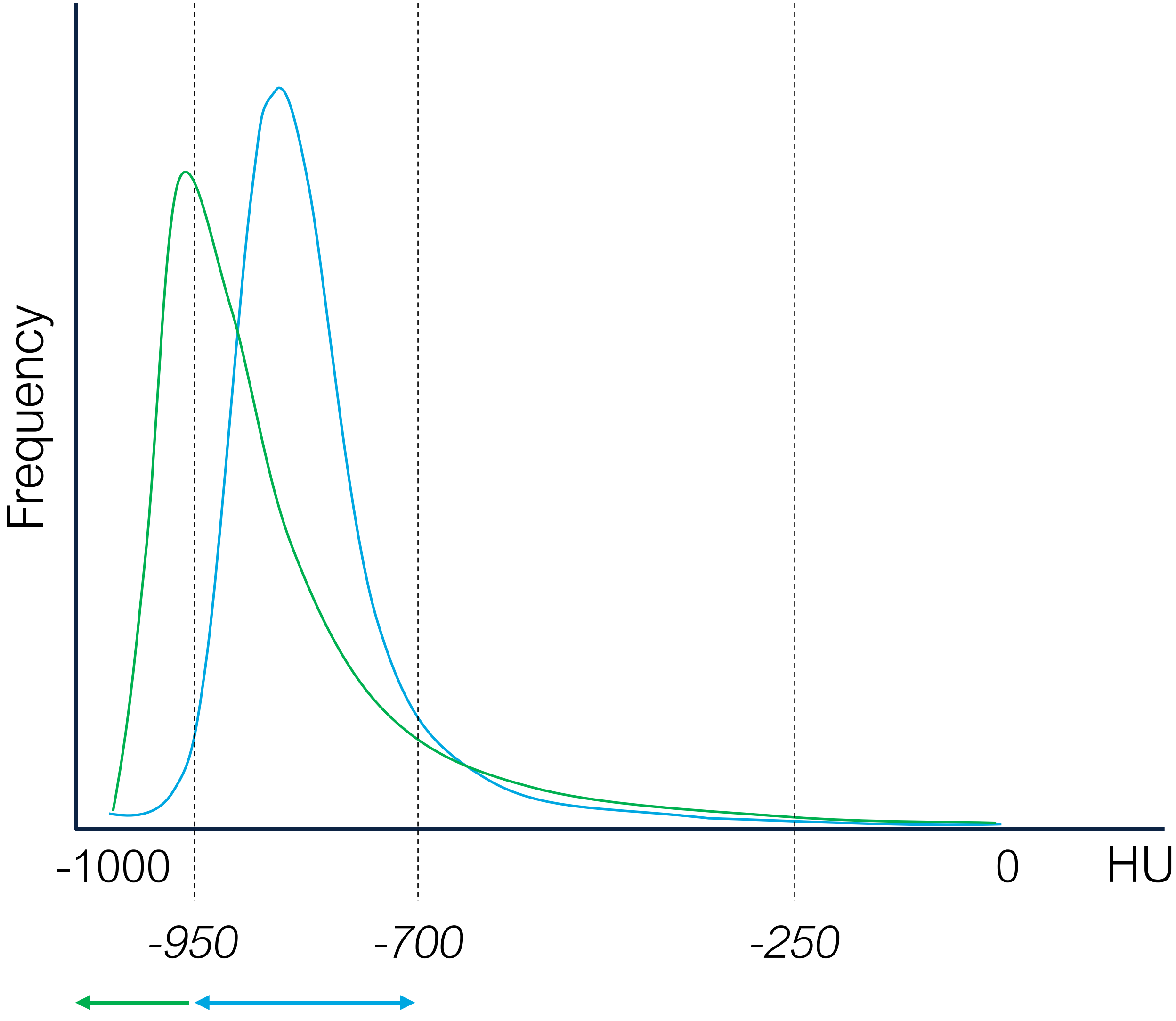




Quantitative CT: Tissue Measurements

Emphysema

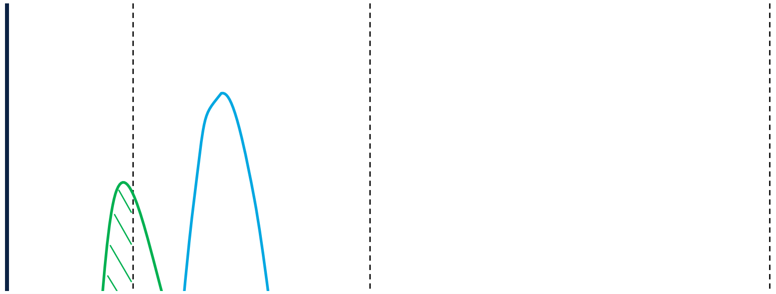




Quantitative CT: Tissue Measurements

Emphysema

Quantitative biomarker:
LAA950: low attenuating areas <-950 HU



STATE OF THE ART

Am J Respir Crit Care Med. 2025;211(5):709-28.

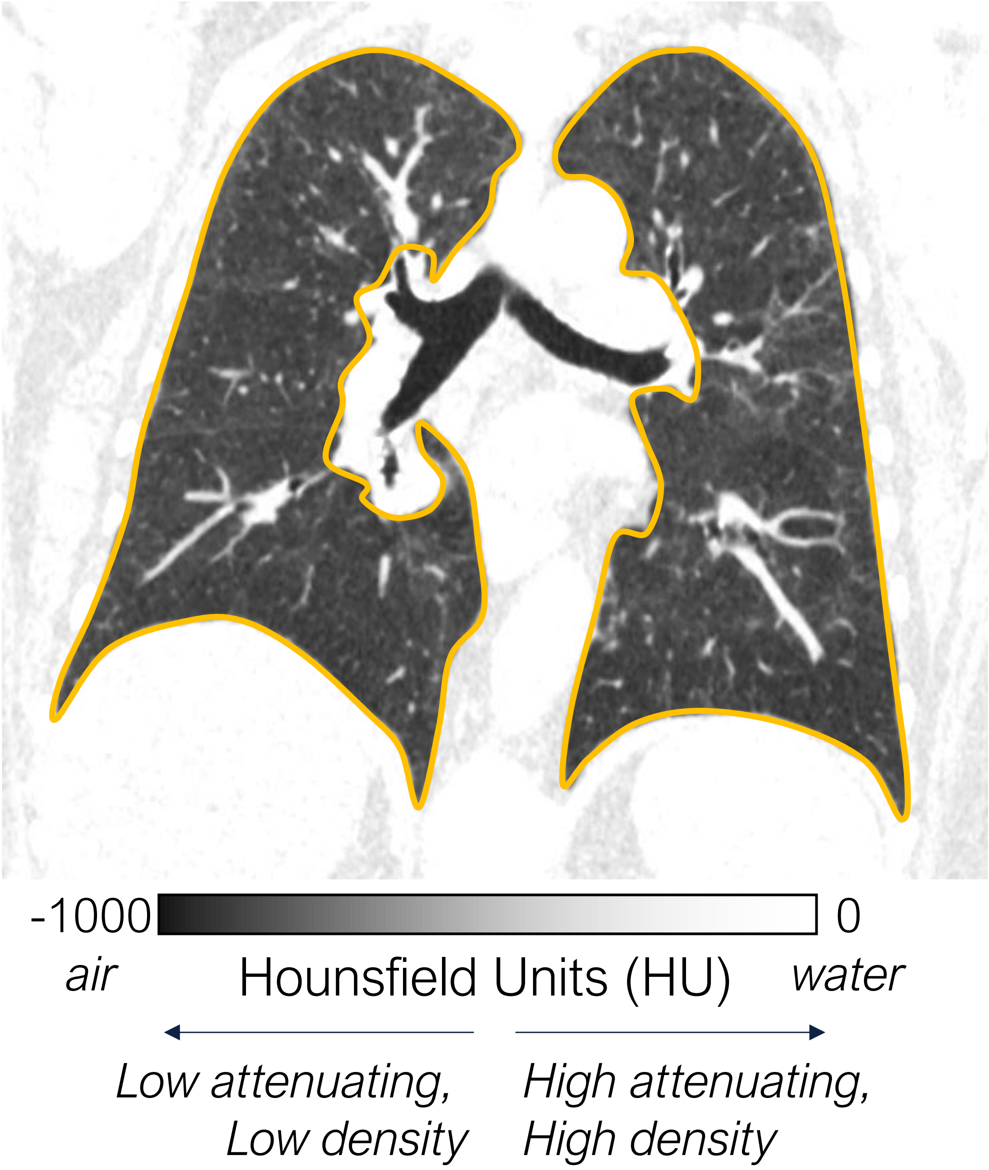
The Use of Computed Tomography Densitometry for the Assessment of Emphysema in Clinical Trials

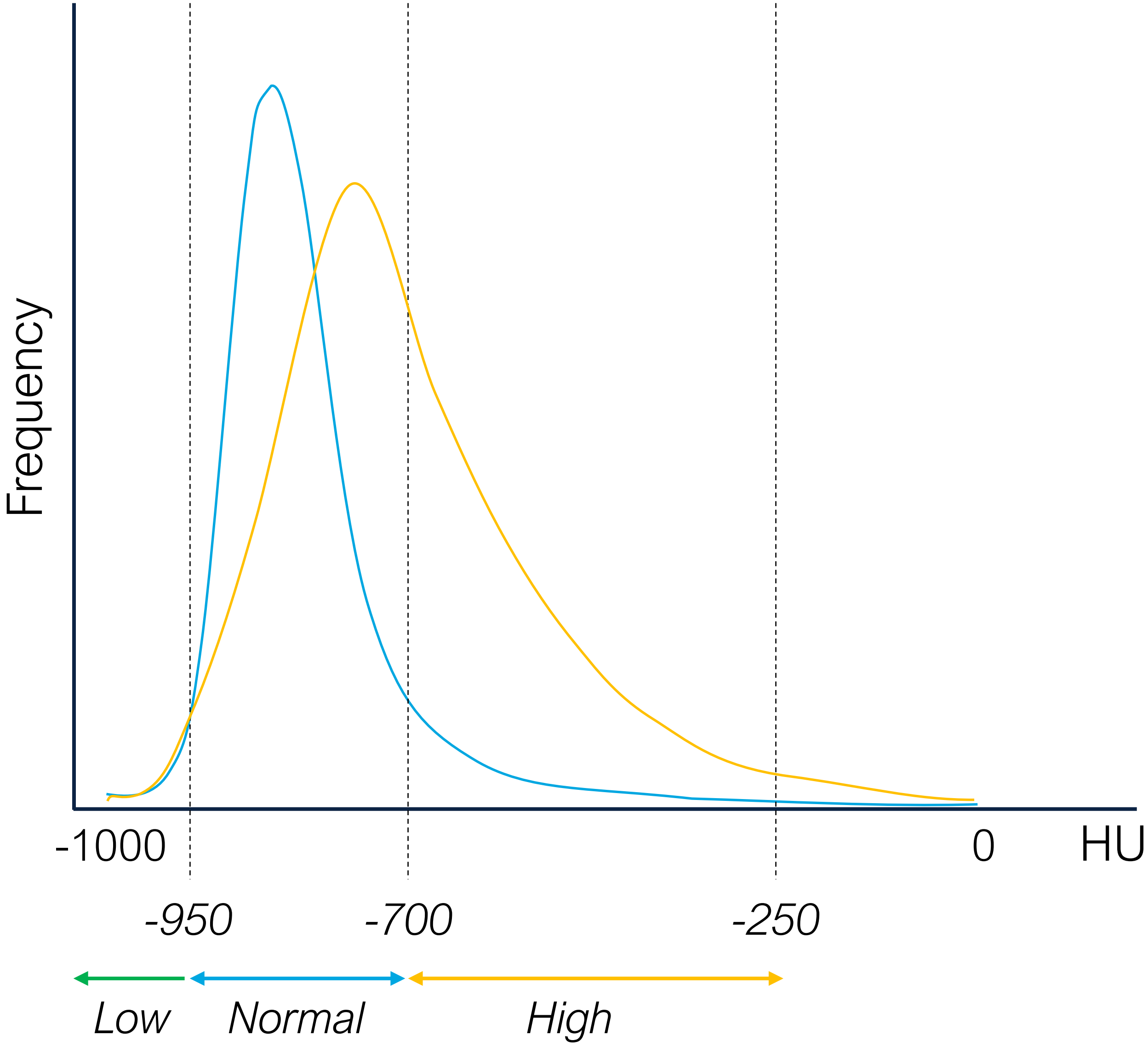
A Position Paper from the Fleischner Society

Raúl San José Estépar[1], R. Graham Barr[3], Sean B. Fain[4], Philippe A. Grenier[5], Eric A. Hoffman[4], Stephen M. Humphries[15], Miranda Kirby[6], Nancy Obuchowski[7], Christopher J. Ryerson[8], Joon Beom Seo[9], Ruth Tal-Singer[10], Samuel Y. Ash[11], Alexander A. Bankier[12], James Crapo[13], MeiLan K. Han[16], Liz Kellermeyer[14], Jonathan Goldin[17], Cynthia H. McCollough[18], John D. Newell, Jr.[4], Bruce E. Miller[19], Lars H. Nordenmark[20], Martine Remy-Jardin[21], Mathias Prokop[22], Yoshiharu Ohno[23], Edwin K. Silverman[2], Charlie Strange[24], George R. Washko[2], and David A. Lynch[15]

Quantitative CT: Tissue Measurements

Infiltrates/inflammation





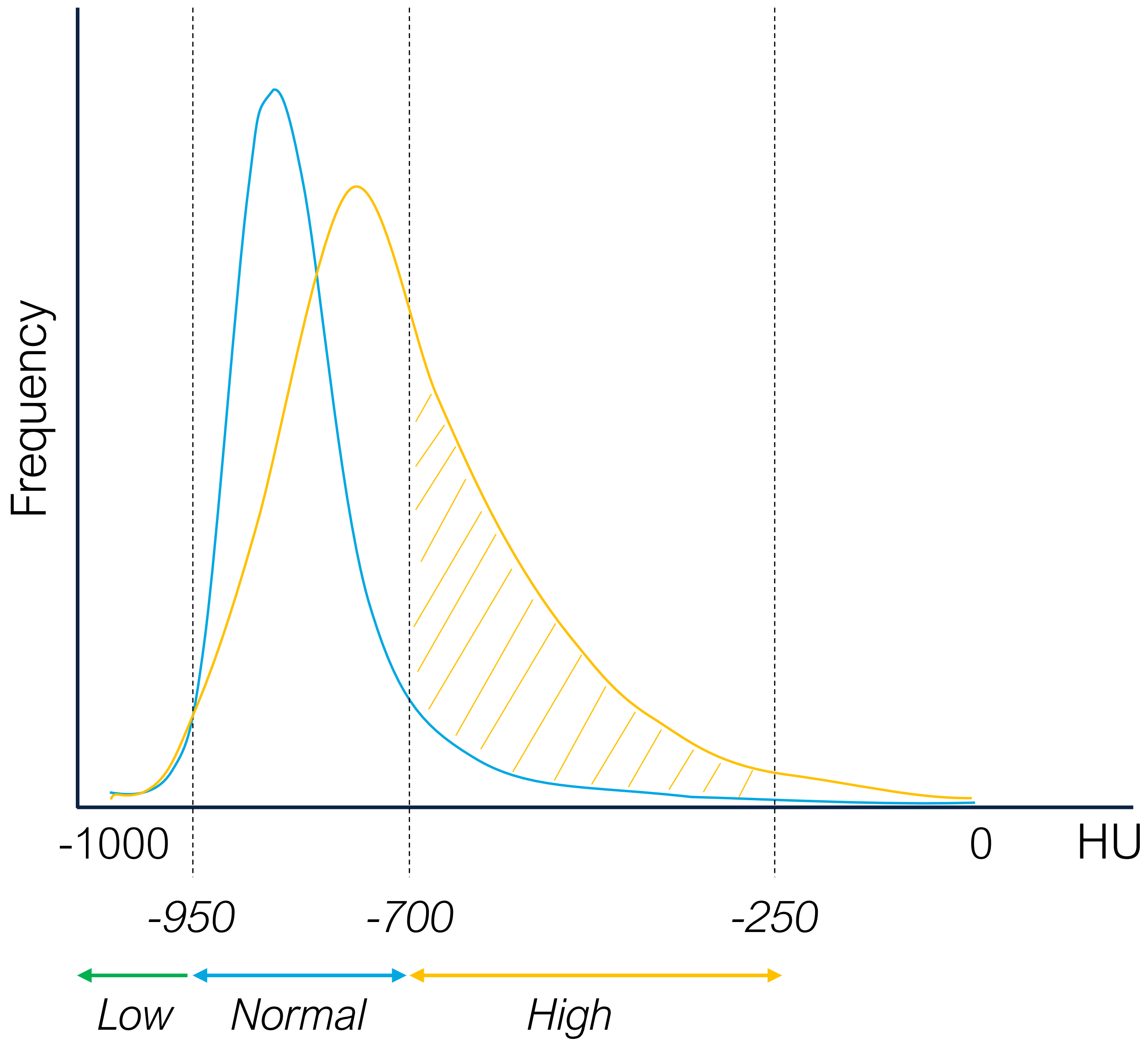
Quantitative CT: Tissue Measurements

Infiltrates/inflammation

Quantitative biomarker:
HAA: high attenuating areas

***HAA of ≥10% is sensitive and specific for clinically relevant abnormalities*[1]**

***Lung density measures are highly repeatable*[2]**



1. Lederer *et al. AJRCCM* 2009 2. Motahari *et al. AJRCCM* 2023.

Quantitative CT: Tissue Measurements

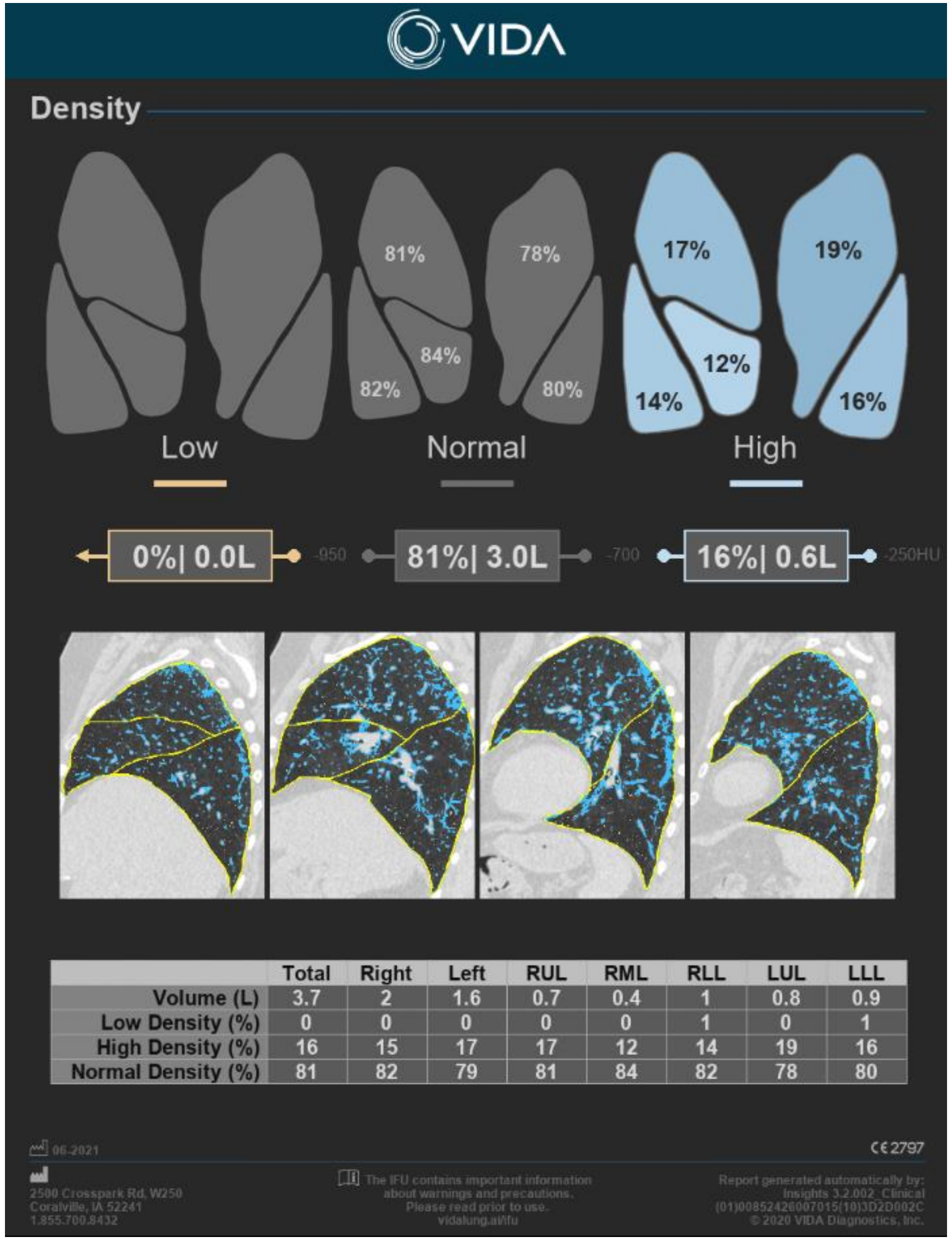
Infiltrates/inflammation

Quantitative biomarker:
HAA: high attenuating areas

HAA of ≥10% is sensitive and specific for clinically relevant abnormalities[1]

Lung density measures are highly repeatable[2]

Fit-for-purpose, industry standard reports



	Total	Right	Left	RUL	RML	RLL	LUL	LLL
Volume (L)	3.7	2	1.6	0.7	0.4	1	0.8	0.9
Low Density (%)	0	0	0	0	0	1	0	1
High Density (%)	16	15	17	17	12	14	19	16
Normal Density (%)	81	82	79	81	84	82	78	80

1. Lederer *et al. AJRCCM* 2009. 2. Motahari *et al. AJRCCM* 2023.

On Radiation Dose

	Estimated Equivalent Dose
Annual background in USA[1]	3.1 mSv*
Typical cross-USA flight[2]	0.035 mSv
Clinical Chest X-Ray[3]	0.1 mSv
Clinical Chest CT[3]	7 mSv
Research Chest CT[4,5]	<5 mSv

Measured in sieverts (Sv) or gray (Gy)

CT technology is continuously improving to enable quantitative measures at minimized radiation doses[4,5]

1. US EPA. 2. US CDC. 3. Mahesh *et al*, *Radiology* 2023. 4. Sieren *et al*, *AJRCCM* 2016. 5. Atha, Eddy *et al*, *Med Phys* 2025.

Summary

CT has evolved to a standardized, quantifiable and repeatable technique to measure lung structure and function

- Lung density measures are rapid, repeatable
- High attenuating areas ≥10% define abnormal alveolar infiltrates
- Quantitative CT can be implemented in a standardized way
- Radiation doses continue to decrease, reducing risk to study participants

Monitoring drug-induced lung toxicity in patients

February 26, 2026

John V. Fahy, M.D., M.Sc.
Professor of Medicine, Division of Pulmonary and Critical Care Medicine
University of California San Francisco
Founder, Aer Therapeutics

UCSF

UCSF Airway Clinical Research Center

Outline

1. Review reasons for relatively high safety margins for inhaled drugs.

2. Review monitoring methods for drug-induced lung toxicity in clinical trials of investigational drugs

3. Review lessons from use of CT lung scans in clinical medicine and in monitoring of lung toxicity from approved drugs.

4. CT lung scanning to monitor lung toxicity from investigational drugs.

Safety Margins to Guide Dosing of Inhaled Drugs

Division of Pulmonary, Allergy, and Rheumatology Products (DPARP) criteria are that safety margins (SM) based on mg/kg body weight or mg/g lung weight should be at least 10 (mice and rats), 6 (dogs), and 5 (monkeys).

Reasons for high safety margins:

1. **High risk**: The FDA classifies oral inhalation, along with injections, as having the highest risk among drug administration methods because it delivers substances directly to mucous membranes.

2. **Unique Toxicity Concerns** (non-monitorable effects): It is considered that inhaled drugs can cause inflammatory responses/tissue damage in the lung that are *not easily monitored* in human clinical trials.

Monitoring for lung toxicity

1. **Symptoms and quality of life**: Validated questionnaire instruments

   ➔ St George's Respiratory Questionnaire (SGRQ)

   ➔ Specific asthma and COPD questionnaires (Asthma Control Test, COPD assessment test).

   ➔ Specific interstitial lung disease questionnaires (King's Brief Interstitial Lung Disease)

2. **Lung function tests**: Spirometry, lung volumes, diffusing capacity, oxygen saturation.

3. **Lung imaging:** Chest X ray; *Computed Tomography lung scans*; Magnetic resonance imaging (MRI) scans

Monitoring for lung toxicity

1. **Symptoms and quality of life**: Validated questionnaire instruments

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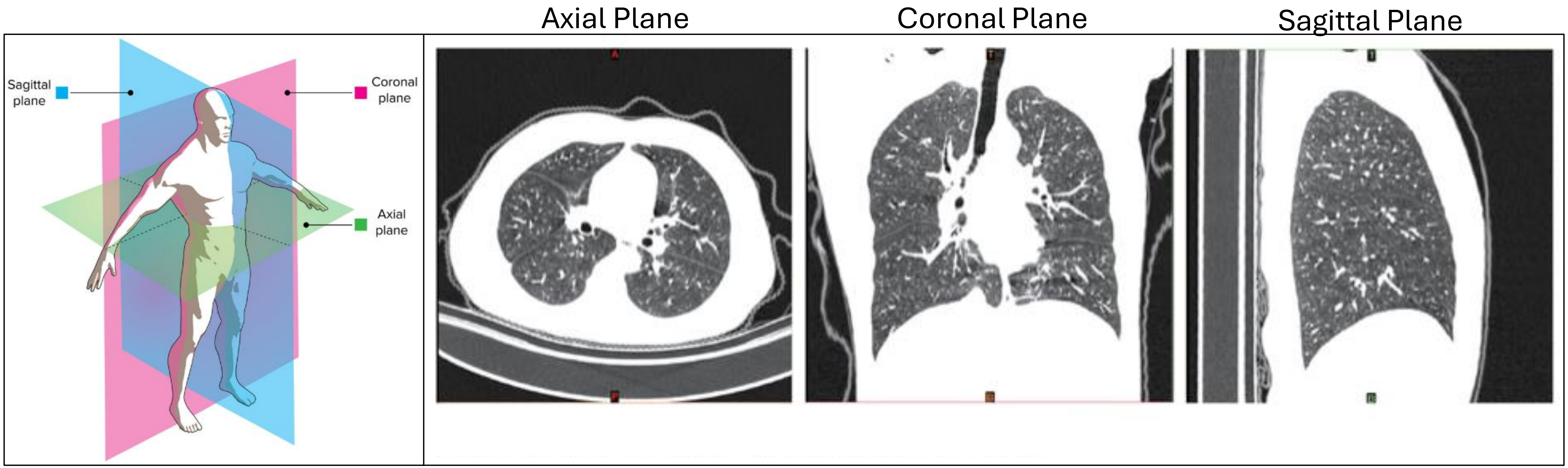
3. **Lung imaging:** Chest x ray; *Computed Tomography lung scans*; Magnetic resonance imaging (MRI) scans

Computed tomography (CT) lung scans

-> a CT lung scan is superior to a chest X-ray because it provides high-resolution, 3D cross-sectional images, allowing for the detection of small lesions and subtle abnormalities.

-> high sensitivity & detailed pattern-based assessments in multiple planes.



CT lung scans have largely replaced lung biopsies in the diagnosis and monitoring of interstitial lung diseases

Interstitial lung disease (ILD): a heterogeneous group of lung disorders characterized by inflammation and/or fibrosis affecting the lung interstitium and parenchyma.

CrossMark

Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper

David A Lynch, Nicola Sverzellati, William D Travis, Kevin K Brown, Thomas V Colby, Jeffrey R Galvin, Jonathan G Goldin, David M Hansell, Yoshikazu Inoue, Takeshi Johkoh, Andrew G Nicholson, Shandra L Knight, Suhail Raoof, Luca Richeldi, Christopher J Ryerson, Jay H Ryu, Athol U Wells

Lancet Respir Med 2018; 6: 138–53

Published Online November 15, 2017 http://dx.doi.org/10.1016/S2213-2600(17)30433-2

See Comment page 82

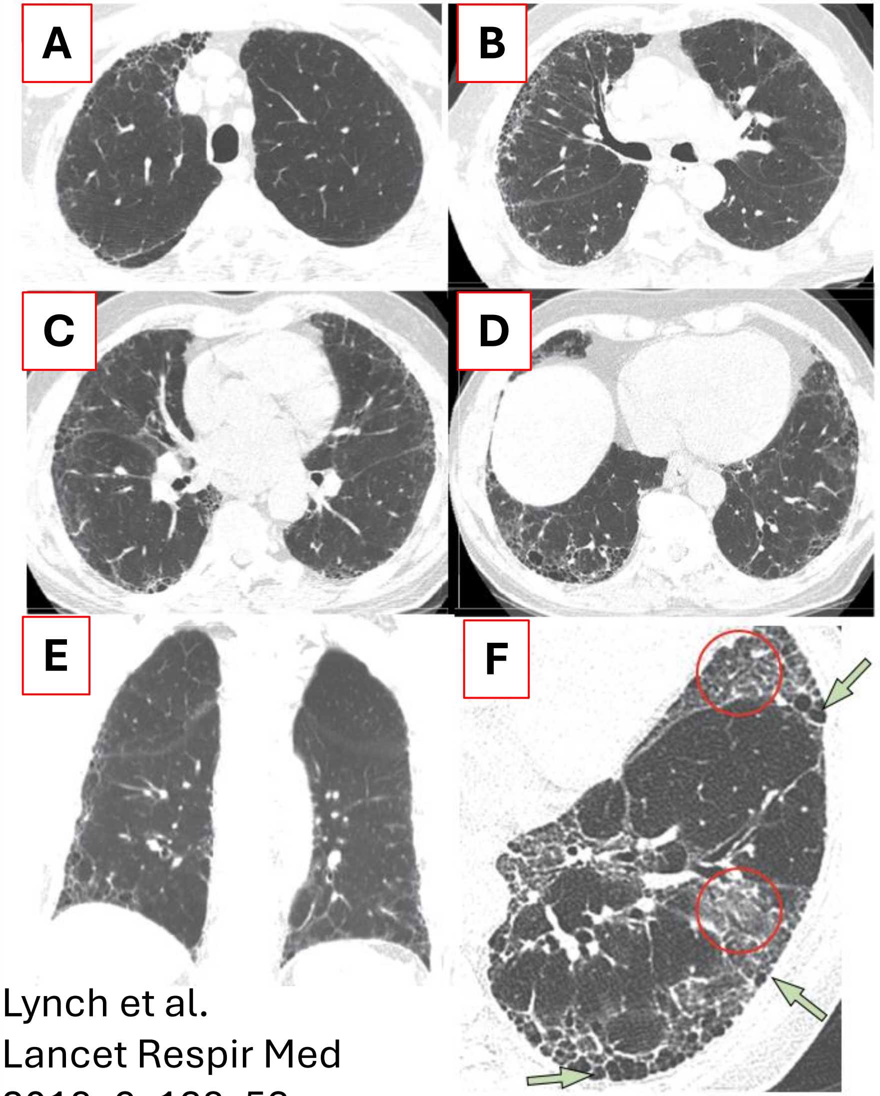
Department of Radiology (Prof D A Lynch MB), **Department of Medicine** (Prof K K Brown MD), **and Library and Knowledge Services** (S L Knight MS),

This Review provides an updated approach to the diagnosis of idiopathic pulmonary fibrosis (IPF), based on a systematic search of the medical literature and the expert opinion of members of the Fleischner Society. A checklist is provided for the clinical evaluation of patients with suspected usual interstitial pneumonia (UIP). The role of CT is expanded to permit diagnosis of IPF without surgical lung biopsy in select cases when CT shows a probable UIP pattern. Additional investigations, including surgical lung biopsy, should be considered in patients with either clinical or CT findings that are indeterminate for IPF. A multidisciplinary approach is particularly important when deciding to perform additional diagnostic assessments, integrating biopsy results with clinical and CT features, and establishing a working diagnosis of IPF if lung tissue is not available. A working diagnosis of IPF should be reviewed at regular intervals since the diagnosis might change. Criteria are presented to establish confident and working diagnoses of IPF.

The Fleischner Society

- an international, multidisciplinary medical organization focused on thoracic radiology, dedicated to improving the diagnosis and treatment of chest diseases.

Example: CT lung scans can diagnose idiopathic pulmonary fibrosis



Lynch et al.
Lancet Respir Med
2018; 6: 138–53

Typical CT pattern of Idiopathic Pulmonary Fibrosis (IPF)

A–E. Axial and coronal CT images show subpleural predominant reticular abnormality with traction bronchiectasis & honeycombing.

F. Magnified view shows areas of honeycombing occurring in single and multiple layers (arrows). Two areas of apparent ground glass abnormality (circles) contain dilated bronchi (traction bronchiectasis) representing fibrosis.

-> These CT lung image patterns are considered diagnostic of IPF

Many drug toxicities manifest as interstitial lung disease

= drug-induced interstitial lung disease (DI-ILD)

- Multiple molecularly targeted and immuno-oncology agents for cancer are associated with DI-ILD

- Drugs with the highest incidence and prevalence of DI-ILD include:

➔ immune checkpoint inhibitors;
➔ targeted cancer therapies (EGFR-directed therapies, mTOR inhibitors, tyrosine kinase inhibitors, anti-ErbB2 antibody-drug conjugates, and CDK4/6 inhibitors.

- Frequently seen radiological patterns in DI-ILD include:

-> organizing pneumonia;
-> nonspecific interstitial pneumonia;
-> hypersensitivity pneumonitis
-> diffuse alveolar damage;
-> pulmonary eosinophilia.

Conte et al. ESMO Open 2022; 2: 1-15.
Spagnolo et al. European Respiratory Journal 2022 60(4): 2102776.

Radiologic patterns of drug-induced interstitial lung disease (DI-ILD) on Chest CT Scan

Pattern	Imaging Manifestations at Chest CT Scan
Non-specific interstitial pneumonia	Starting from patchy areas of GGO, progression to irregular reticular opacities, architectural distortion, and traction bronchiectasis, with or without associated areas of consolidation; bilateral and symmetric, with predominant lower-lung involvement
Organizing pneumonia	Multifocal patchy alveolar opacities typically with peribronchovascular and/or peripheral distribution; may demonstrate reversed halo sign
Hypersensitivity Pneumonitis	Poorly defined small centrilobular nodules, bilateral GGO, large or lobular areas of decreased attenuation and vascularity (mosaic attenuation)
Diffuse alveolar damage	Extensive bilateral areas of GGO and dependent airspace consolidation in exudative phase; traction bronchiectasis and decreased lung volumes in organizing and fibrotic phases
Simple pulmonary eosinophilia	Nonsegmental consolidation or GGO, unilateral or bilateral; transient and migratory; spontaneous resolution within 4 wk is common

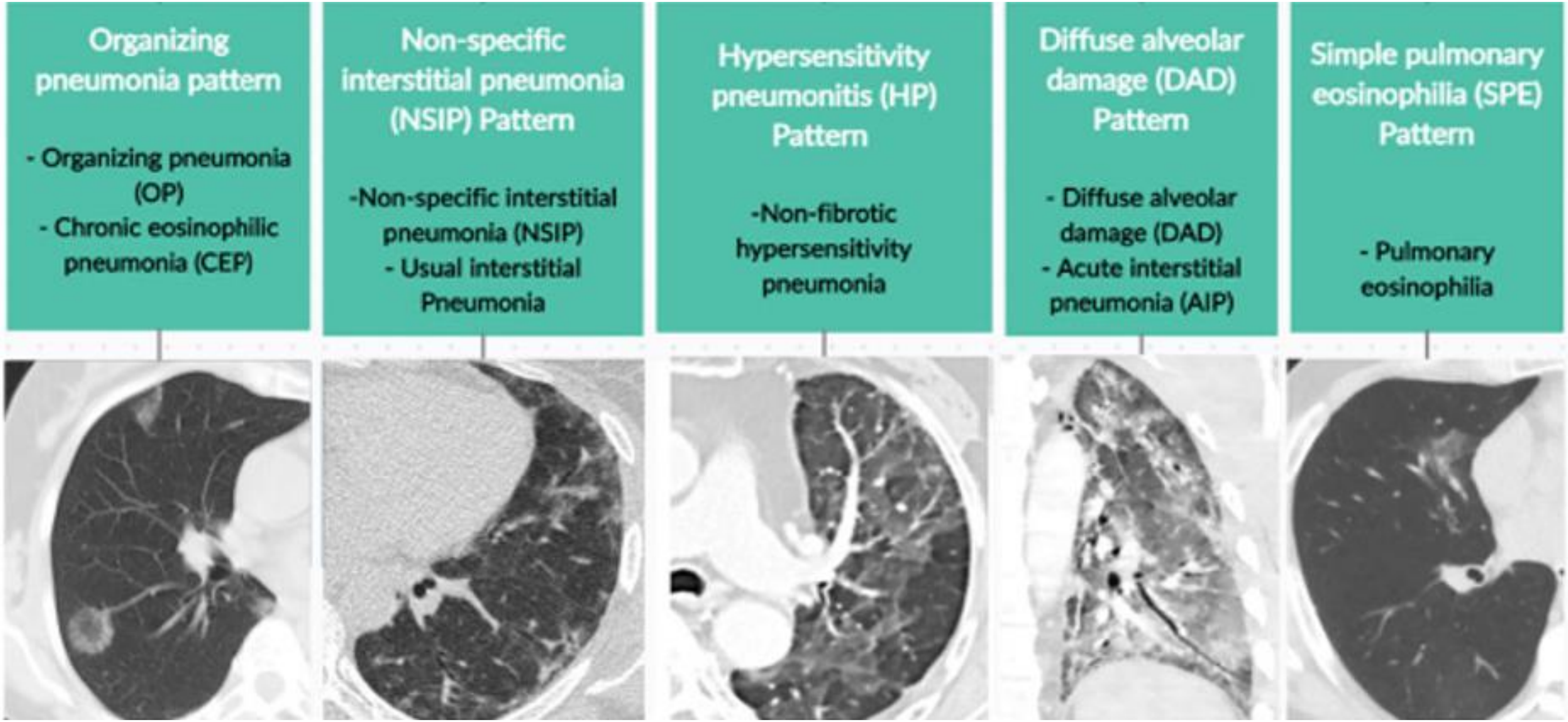
AIP = acute interstitial pneumonia; DAD = diffuse alveolar damage; GGO = ground-glass opacity; HP = hypersensitivity pneumonitis; NSIP = nonspecific interstitial pneumonia; OP = organizing pneumonia; PEo = pulmonary eosinophilia.

-> Note emphasis on ground glass opacities (GGO)

-> GGO% can be measured automatically in CT lung scans.

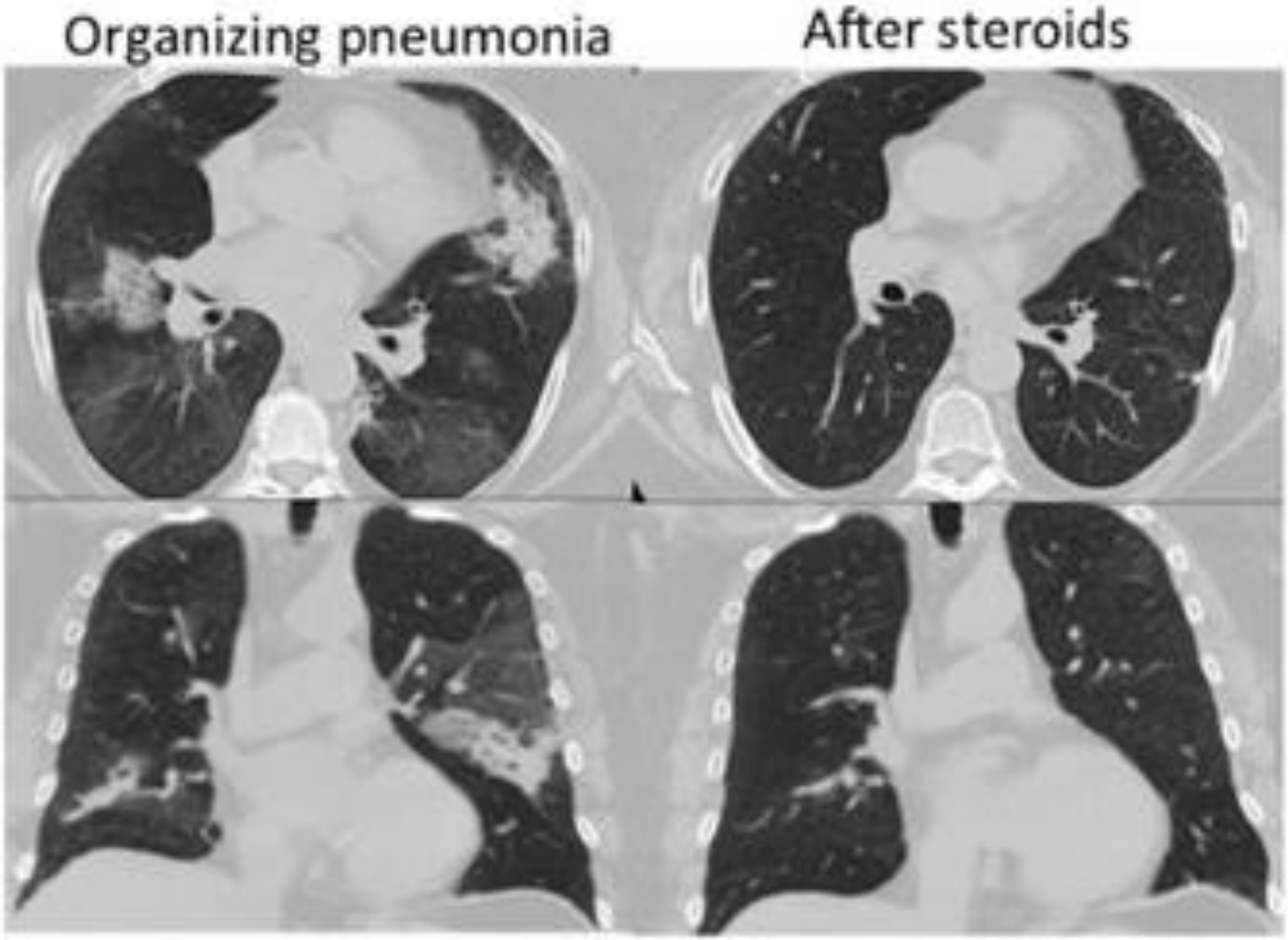
Johkoh et al.
CHEST 2021; 159(3):1107-1125

Radiologic patterns of drug-induced interstitial lung disease (DI-ILD) on Chest CT Scan



Chest CT Scan can determine if (DI-ILD) is reversible

-> Organizing pneumonia is a toxicity associated with checkpoint inhibitors and mTOR inhibitors
-> DI-ILD is usually reversible with drug discontinuation or with corticosteroid treatment.

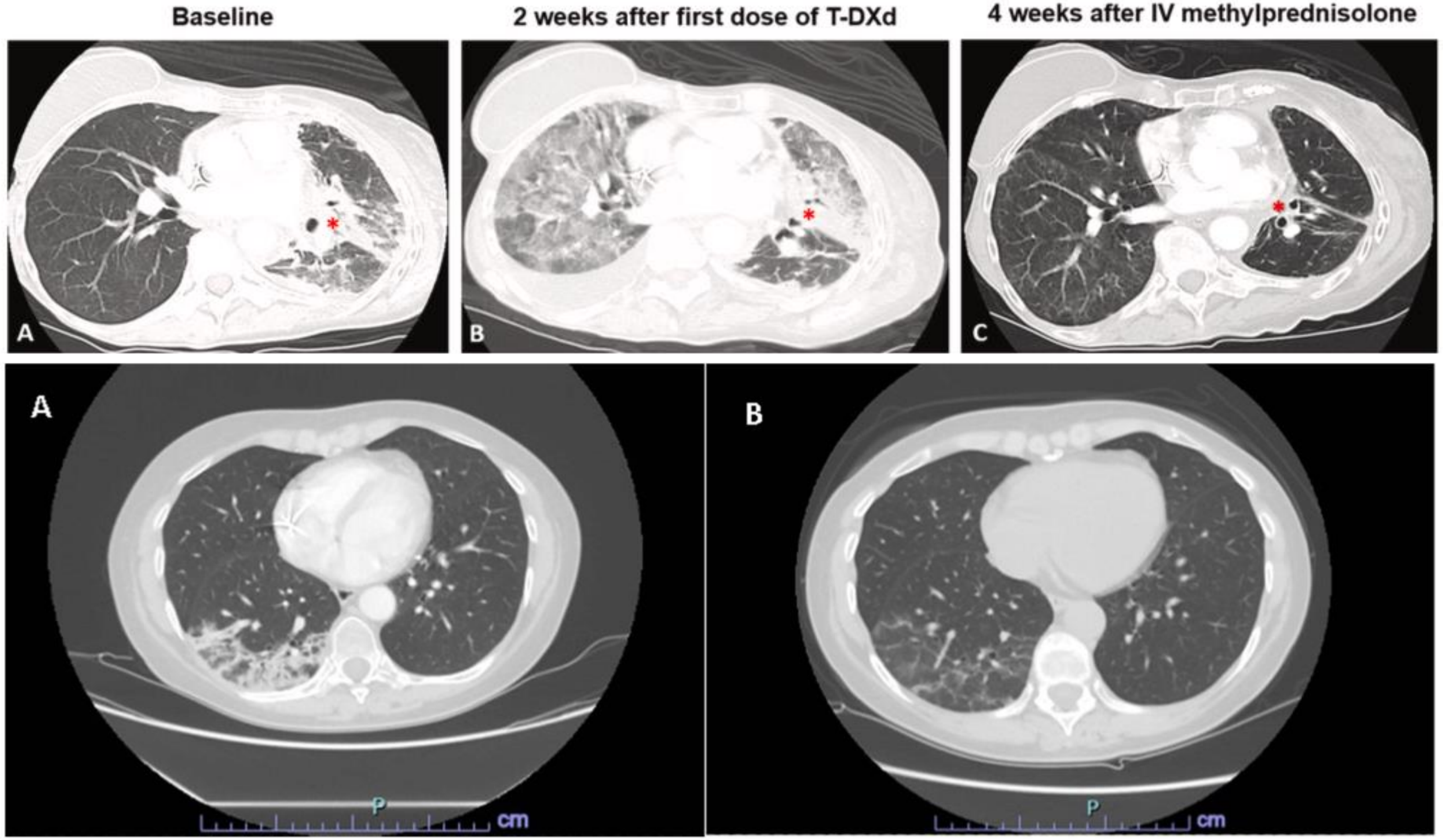


Chest CT Scan can determine if (DI-ILD) is reversible

- Trastuzumab deruxtecan (T-DXd, “Enhertu”), antibody-drug conjugate targeting HER2.
- Interstitial lung disease occurs commonly with T-DXd treatment.

Case #1

Case #2

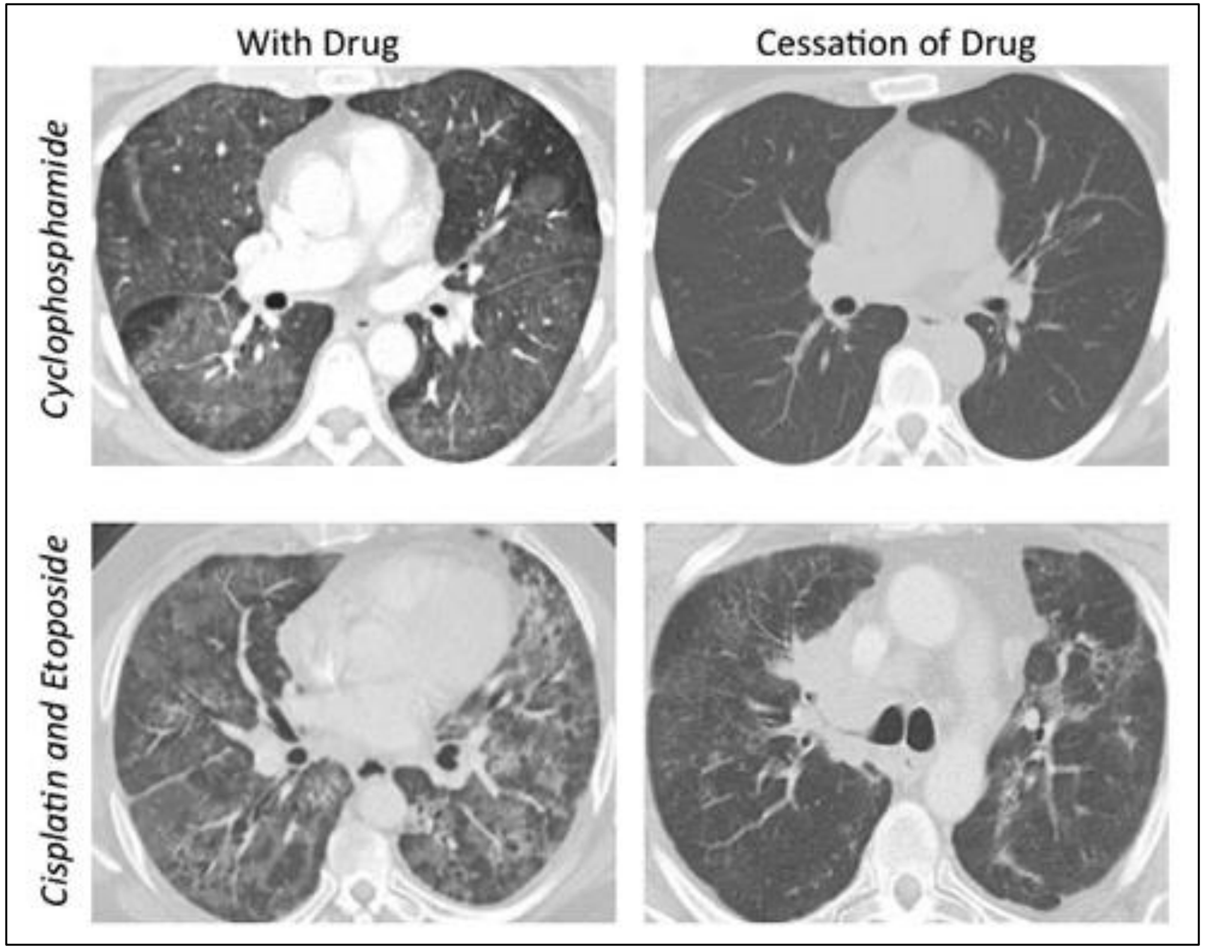


Swain et al. Cancer Treatment Reviews 2022: 106: 102378

Chest CT Scan can determine if (DI-ILD) is reversible

- Cyclophosphamide, Etoposide, Sorafenib

Traditional chemotherapeutic agents



Sorafenib (Nexavar), an oral multikinase inhibitor

Presymptomatic diagnosis of DI-ILD using CT Lung imaging

Some forms of DI-ILD require early, presymptomatic diagnosis to avoid severe toxicity.

There is consensus that patients on Trastuzumab deruxtecan (T-DXd) treatment should have a *proactive monitoring strategy* aimed at early detection.

Proactive patient monitoring

- ➔ Symptoms (cough and dyspnea) monitoring
- ➔ CT lung assessments every 6 - 12 weeks
- ➔ Lung function measures, as necessary

-> A similar monitoring strategy could be employed for investigational drug development

Swain et al. Cancer Treatment Reviews 2022; 106
Henning et al. Current Oncology 2023;30:8019 -8038
Sousa et al. Clinical Drug Investigation 2024;44:801-810

Summary - 1

The concern that inhaled drugs can cause lung inflammation responses or tissue damage that are *not easily monitored* in human clinical trials can be addressed by using CT lung imaging to tests for toxicity.

<u>CT lung imaging has multiple advantages</u>:

- Widely available and sensitive test of drug-induced interstitial lung disease.

- CT lung patterns of drug toxicity can be rigorously quantified and normal ranges are established.

- Multiple CROs have enabled incorporation of CT into clinical trials as a measure of drug efficacy. These systems and measures can be applied to enable safety monitoring.

- CT lung images can be done repeatedly

- Radiation exposure in research protocols is less than with clinical CT protocols, and advances in imaging technology are leading to ever decreasing radiation exposure levels.

Summary - 2

1. Advances in CT lung imaging have reduced reliance on tissue histology tests in the diagnosis and treatment of lung disease.

2. Incorporation of CT lung imaging into drug development and drug safety monitoring provides a method to improve monitoring for lung toxicity and reduce reliance on animal data.

   -> the availability of a sensitive and reliable imaging test of lung health should allay concern that lung toxicity from investigational drugs is not easily monitored.

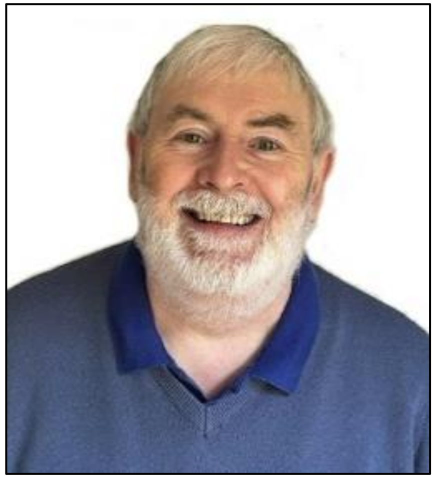
   -> lung imaging could be incorporated into animal studies of lung toxicity to allow cross species comparisons of drug related lung findings between rats and dogs and animals and humans.

What the Future Might Look Like

Opportunities for Regulatory Harmonization and Global Alignment

Lorna Ewart, PhD, DSc
Emulate

Andrew Goodwin, PhD
FDA



David Jones, FRSB, FBTS
ApconiX

Timothy McGovern, PhD
White Oak Regulatory Tox, LLC

Steven Rowe, MD
Cystic Fibrosis Foundation

Closing Remarks

Susan C. Winckler, RPh, Esq.
Chief Executive Officer
Reagan-Udall Foundation for the FDA

The recording & transcript will be posted on our website www.ReaganUdall.org

Thank you for joining us!

Advancing Drug Development by Reducing Reliance on Animal Testing

Case Example: Pre-Clinical Animal Models in Lung Toxicology

Hybrid Public Meeting
February 26, 2026 | 10am-4pm (eastern)

Aer Therapeutics, Avalyn Pharma, Inc., Biotechnology Innovation Organization, Charles River Laboratories, Endeavor BioMedicines, Ionis Pharmaceuticals, and VIDA provided funding for this meeting.