In vitro assessment of the effects of cigarette smoke using an adverse outcome pathway for chronic obstructive pulmonary disease

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BAT
A BETTER TOMORROW

FOR DISCUSSION PURPOSES ONLY

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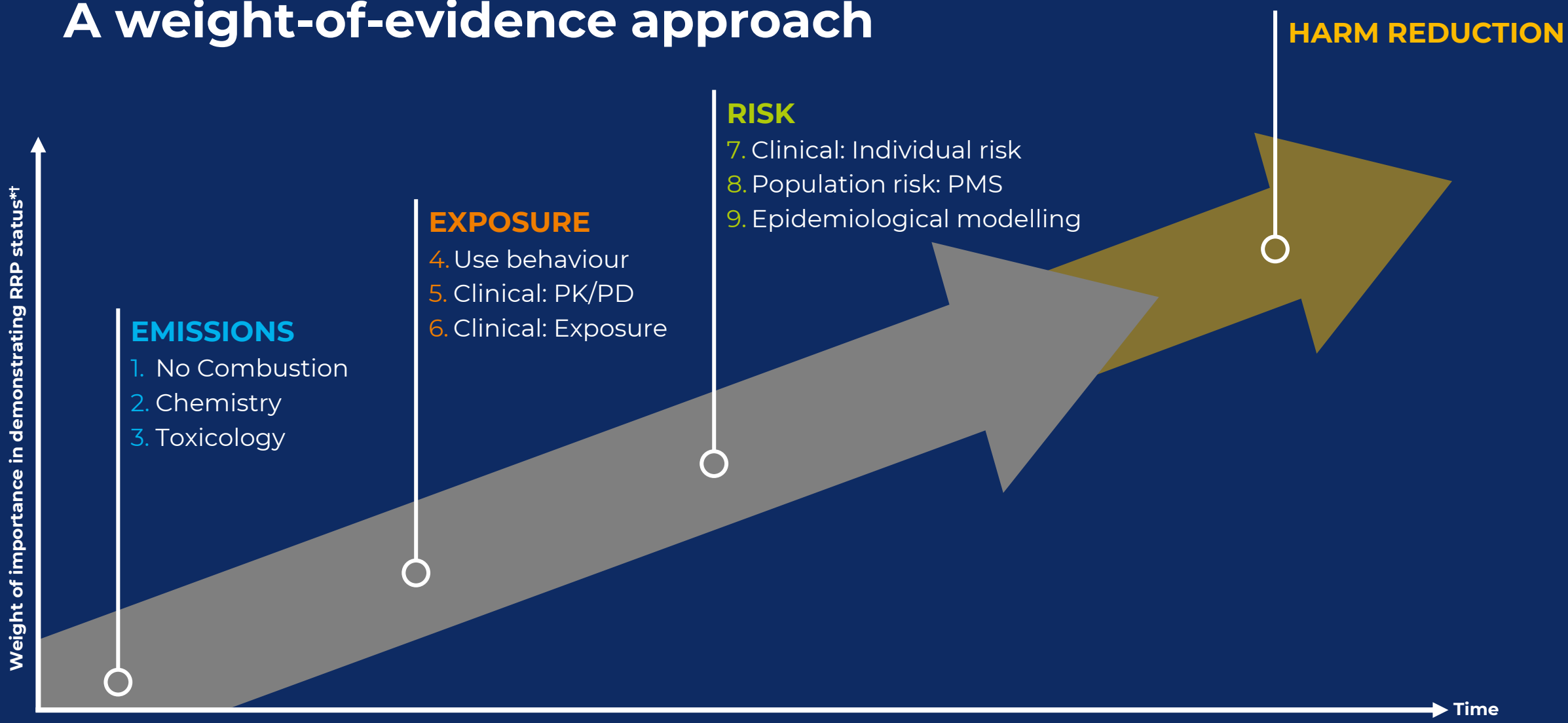
THIS PRESENTATION IS INTENDED FOR SCIENTISTS AND POLICYMAKERS. WE ENCOURAGE PERSONS INTERESTED IN TOBACCO HARM REDUCTION TO CONSIDER ALL POINTS OF VIEW AND SOURCES OF INFORMATION. BAT DOES NOT MAKE HEALTH CLAIMS REGARDING ITS BRANDS. NOTHING CONTAINED HERE SHOULD BE MISCONSTRUED TO THE CONTRARY. TO THE EXTENT THAT THIRD-PARTY SOURCES ARE REFERENCED, NEITHER THE PRESENTER NOR BAT IS RESPONSIBLE FOR THE CONTENT OF REFERENCED SOURCES AND THE VIEWS EXPRESSED MAY NOT REPRESENT THE VIEWS OF THE PRESENTER OR BAT. NO TOBACCO PRODUCT IS SAFE, ALL TOBACCO PRODUCTS CONTAINING NICOTINE ARE ADDICTIVE. YOUTH SHOULD NEVER USE TOBACCO. SMOKERS WHO ARE CONCERNED ABOUT THEIR HEALTH SHOULD QUIT.

THE TOPICS, CONCEPTS AND OTHER INFORMATION DISCUSSED HEREIN ARE NOT FINAL, ARE SUBJECT TO CHANGE AND/OR CANCELLATION AND MAY BE FOR ILLUSTRATIVE OR THEORETICAL PURPOSES ONLY. NO DEFINITIVE PLANS OR COMMITMENTS SHOULD BE INFERRED FROM THESE MATERIALS AND ANY PROPOSED PLANS OR COMMITMENTS ARE SUBJECT IN ALL RESPECTS TO APPLICABLE INTERNAL REVIEW AND GOVERNANCE REQUIREMENTS AND LAWS AND REGULATIONS IN RESPECTIVE JURISDICTIONS.

BAT's 9-Step Risk Assessment Framework: A weight-of-evidence approach



* Based on the weight of evidence and assuming a complete switch from cigarette smoking. These products are not risk free and are addictive.

† Our products as sold in the U.S., including Vuse, Velo, Grizzly, Kodiak, and Camel Snus, are subject to FDA regulation and no reduced-risk claims will be made as to these products without agency clearance.

Issues with clinically establishing Reduced Risk Profile

1. Lack of Long-term epidemiology data
2. Long duration required for physiological (risk/harm) changes
3. Costs
4. Limited access to sample due to invasive nature
5. Limited validated disease predictive biomarkers

Adverse Outcome Pathway (AOP)

A structured representation of biological events leading to adverse health effects

- DNA Binding
- Protein Oxidation
- Chemical stressor

- Protein Production
- Altered signalling
- Cell-cell interactions
- Altered Tissue Function
- Altered Tissue development

Disease
Impairment

Adverse Outcome Pathway (AOP)

- Non-animal alternative
- Link clinical biomarkers potential outcomes / mechanistic narrative
- Potential biomarker evaluation
- Cost effective product screening
- Independent review and acceptance process (OECD)

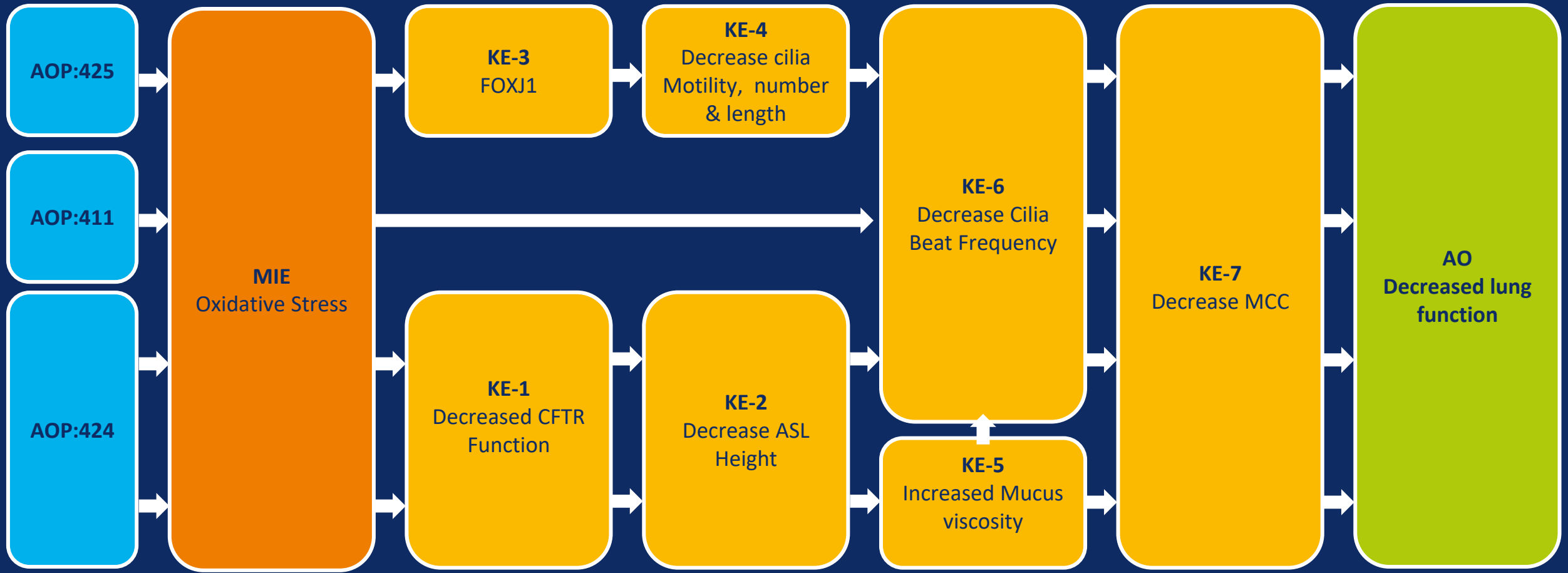
AOPs and Smoking Related Disease

Oxidative Stress - Decreased Lung Function

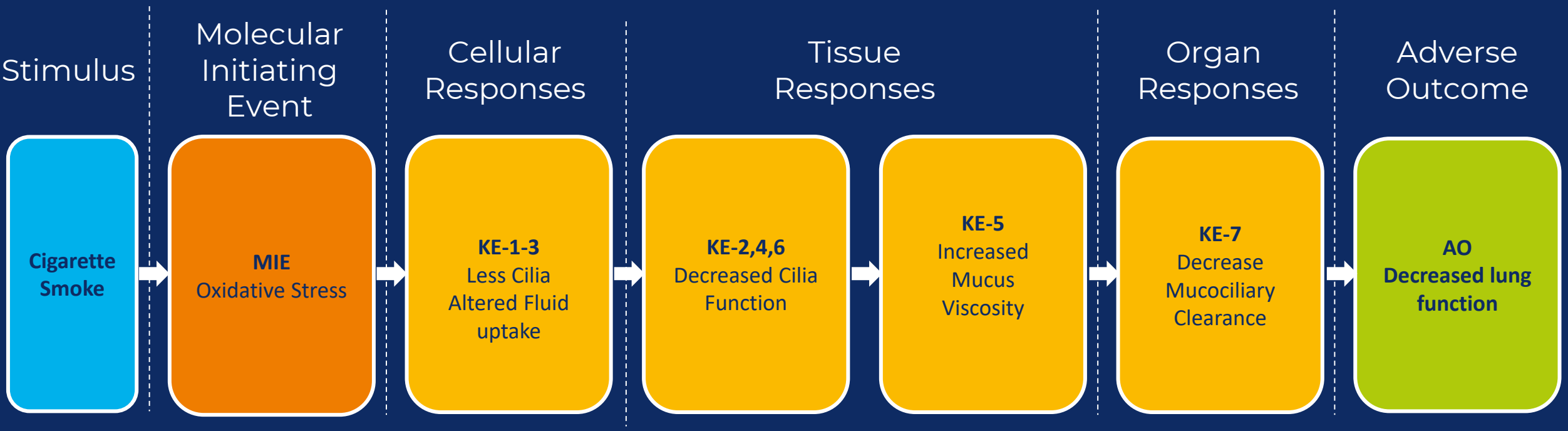
AOP 411, 424 & 425

Luettich *et al.*, Front. Toxicol. 2021

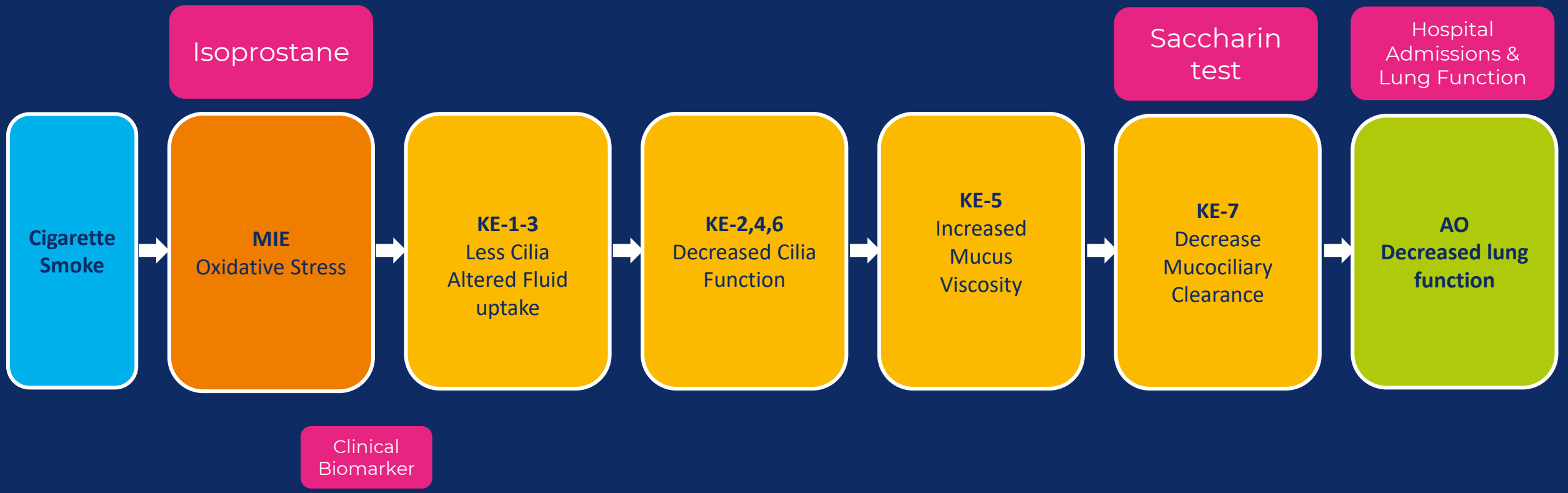
Experimental targets



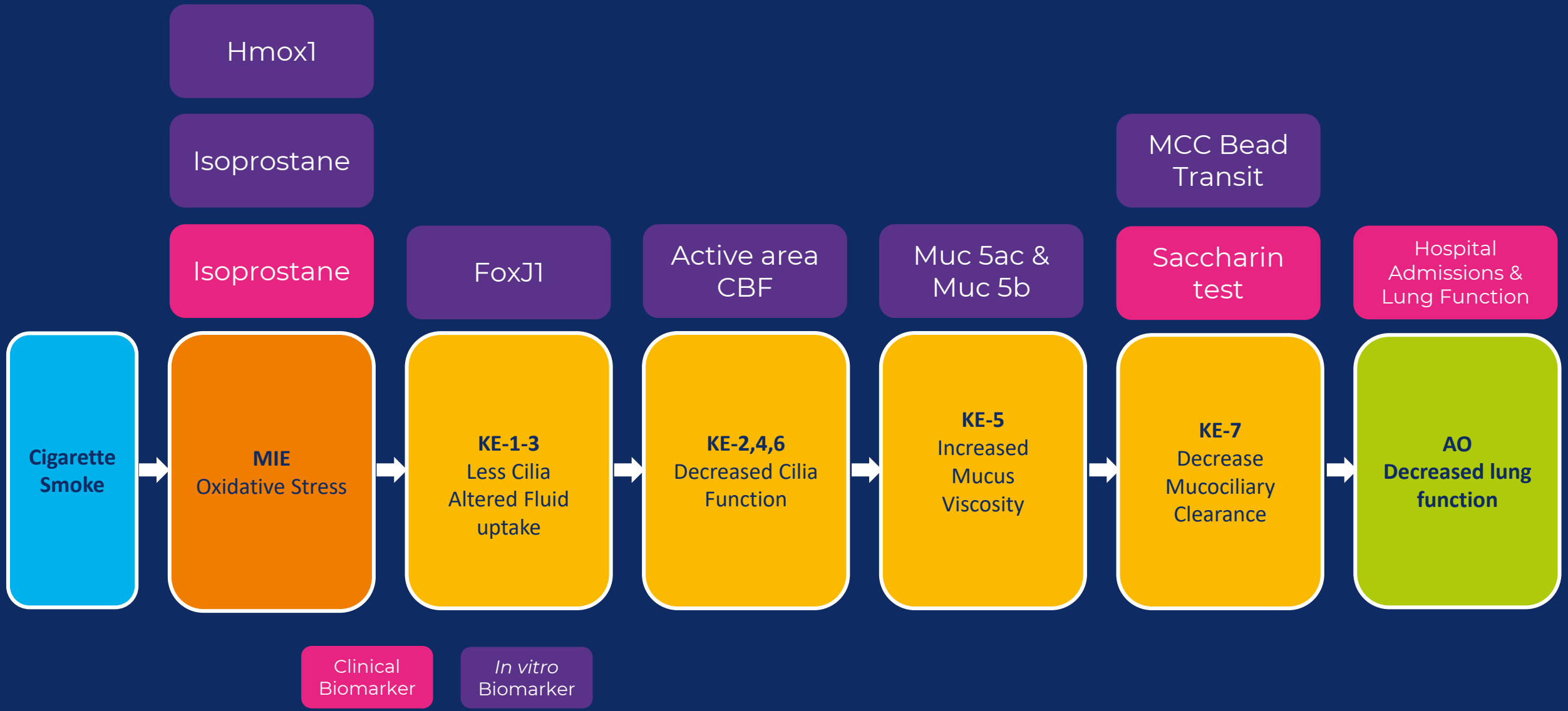
Condensed 411, 424 & 425 AOP



Biomarkers

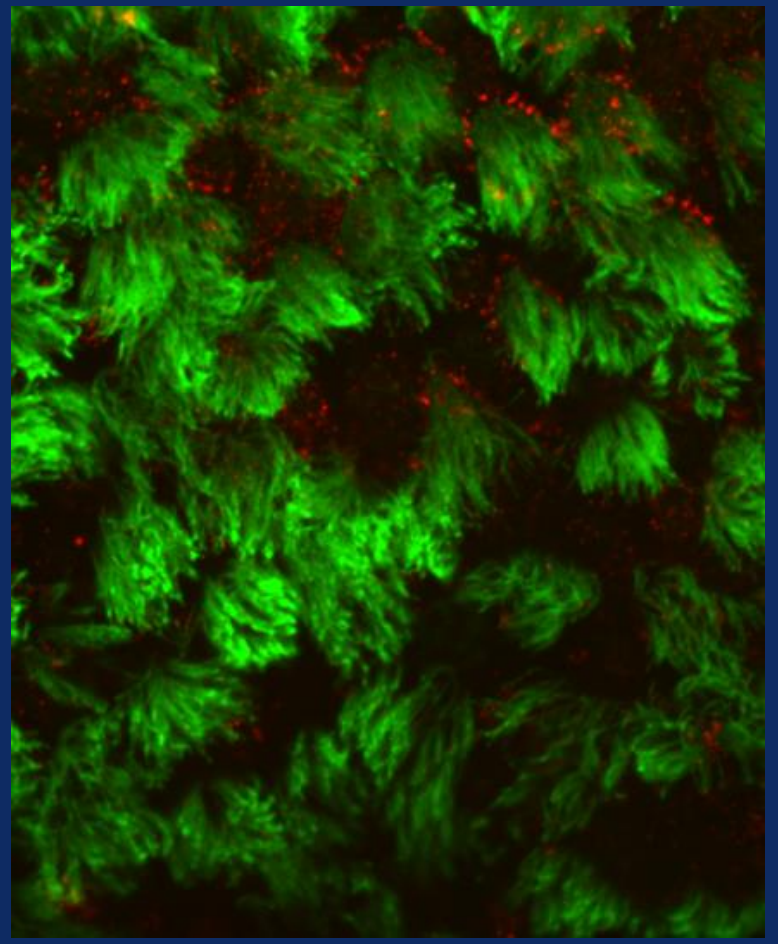


Biomarkers



Best Practice Approach to an *In vitro* Method

- Differentiated 3D lung tissues (MucilAir™)
- Sub-acute toxicity exposure (>90% viability)
- Endpoint specific exposures (Range finders)
  - Duration
  - Dilution
  - Single or Repeat dosing



Exposure Method

Vitrocell Systems GmbH, Vitrocell® VC10® Smoking engine and dilution system

1R6F Reference Cigarette puffing HCI regimen

Single Exposure

Number of puffs : 56
Length of exposure : 28 minutes

7-day Exposure (3 x per week)

Number of puffs : 48 puffs
Length of exposure : 24 minutes

Exposure level

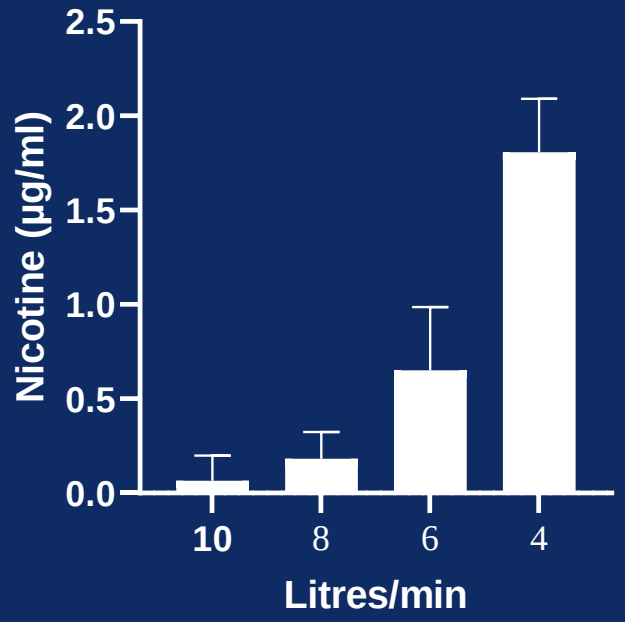
Litres/min

10 8 6 4

High Dilution Flow = Low Exposure

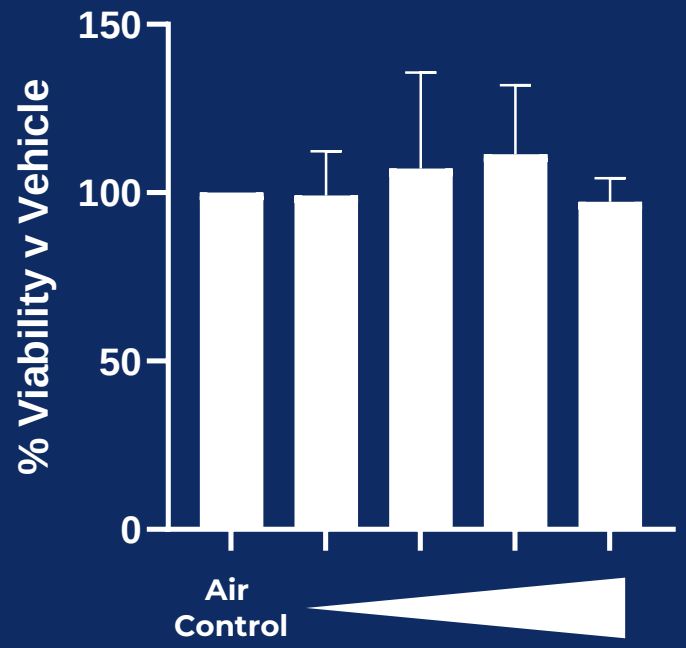
Low Dilution Flow= High Exposure

Increasing Smoke exposure

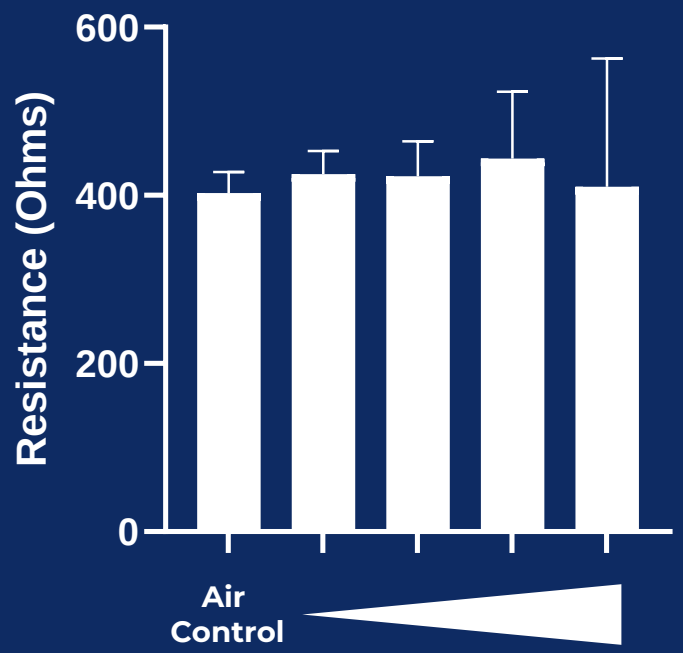


Subacute toxicity (single exposure)

Viability (WST-8)

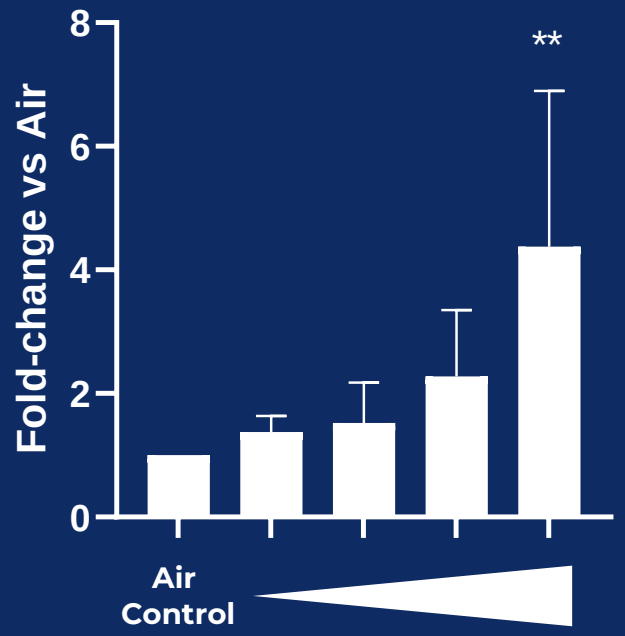


TEER

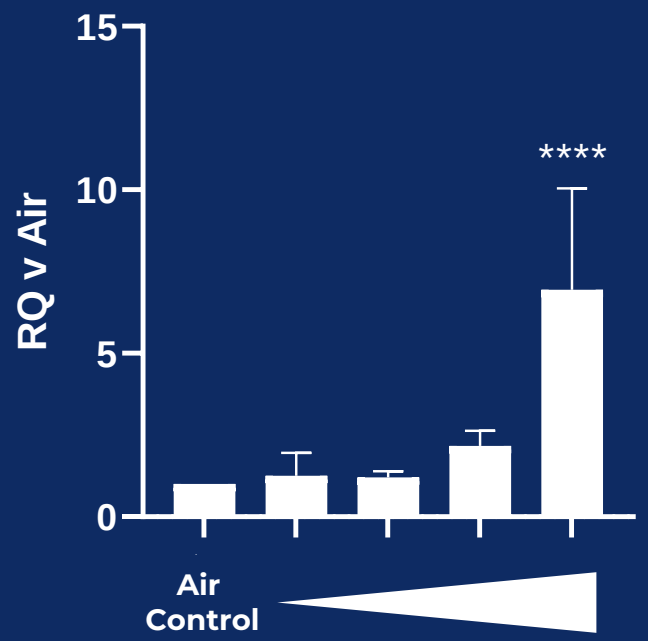


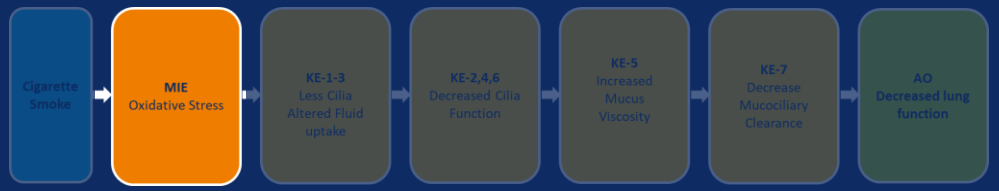
MIE: Oxidative Stress (Single Exposure)

8-Isoprostane



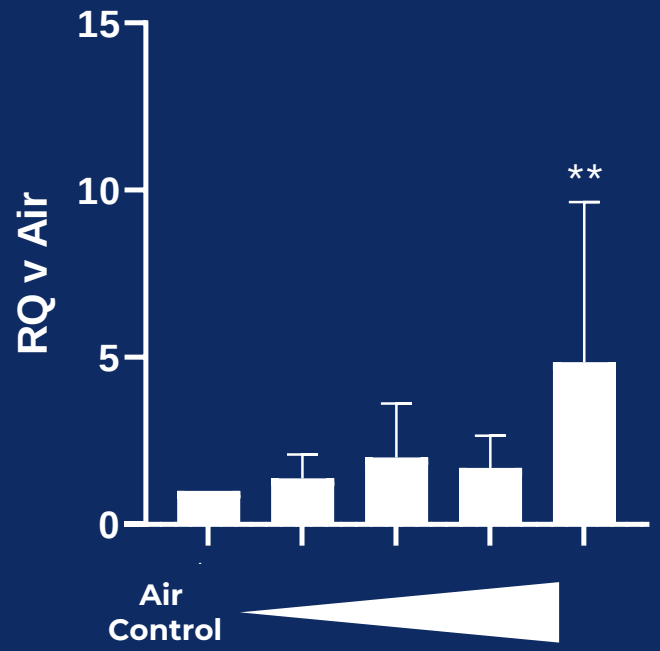
HMOX1



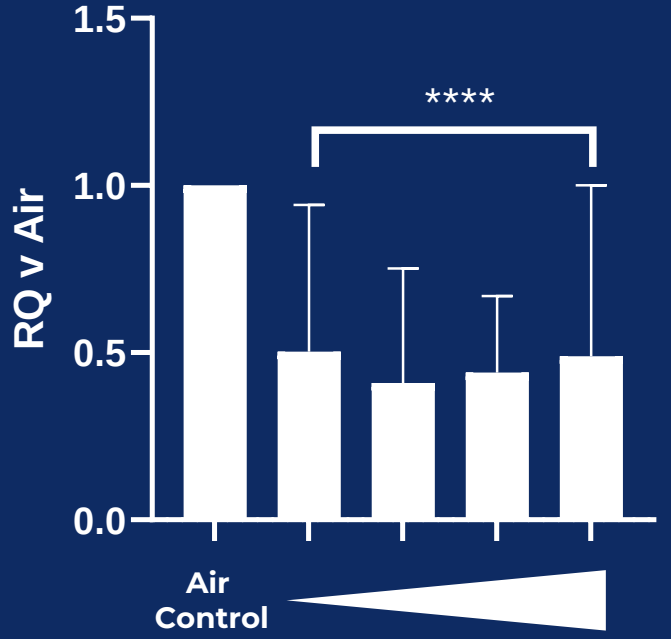


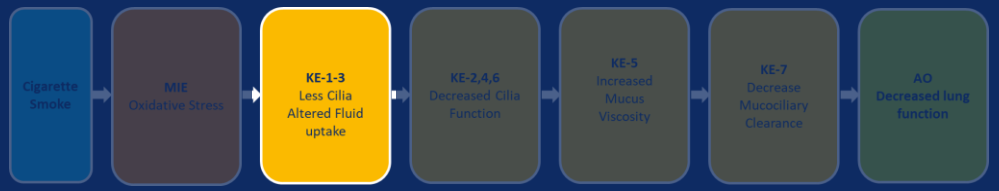
KE-1 & 3: Deceased FoxJ1

Single Exposure (FoxJ1)



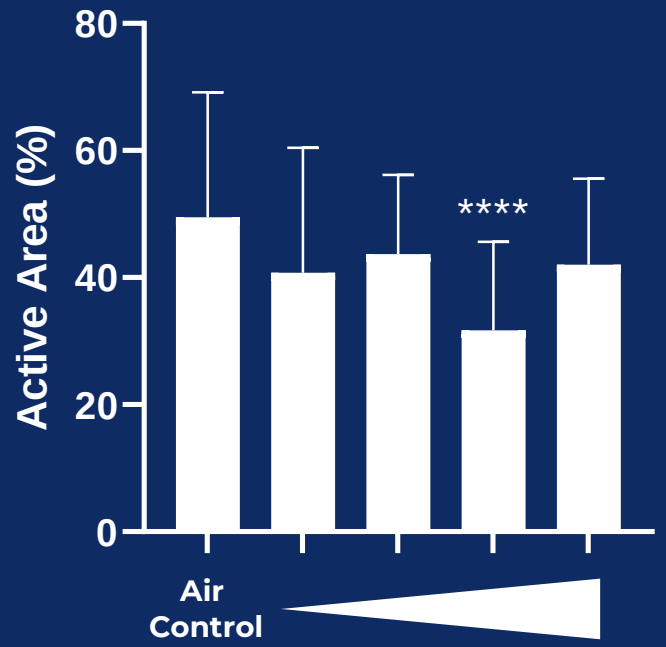
Repeat Exposure (FoxJ1)



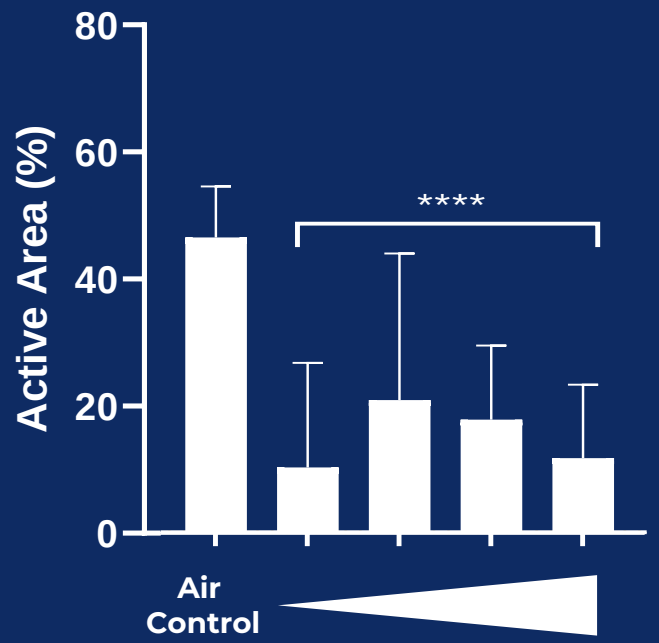


KE-5: Decreased Cilia Function

Single Exposure Active Area

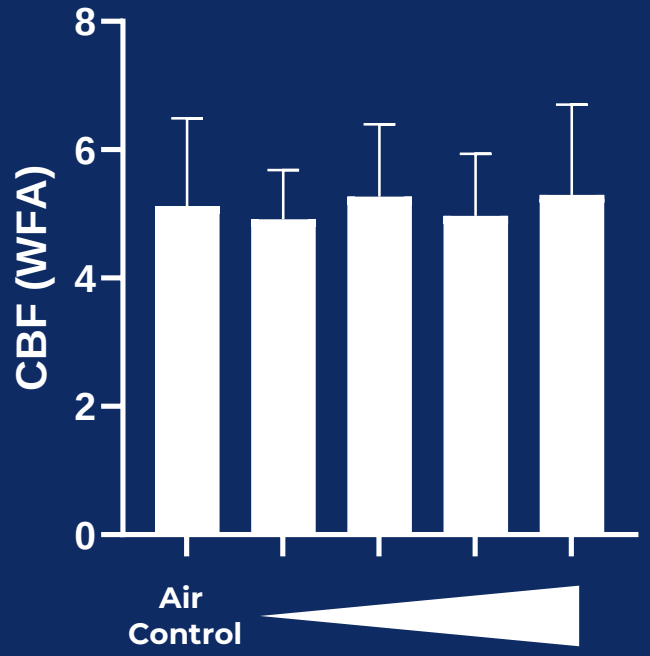


Repeat Exposure Active Area

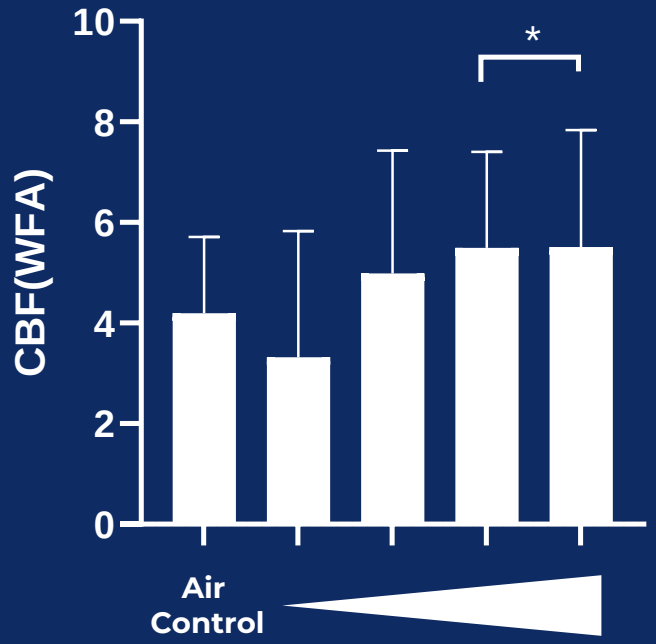


KE-6: Decreased Cilia Beat Frequency

Single Exposure

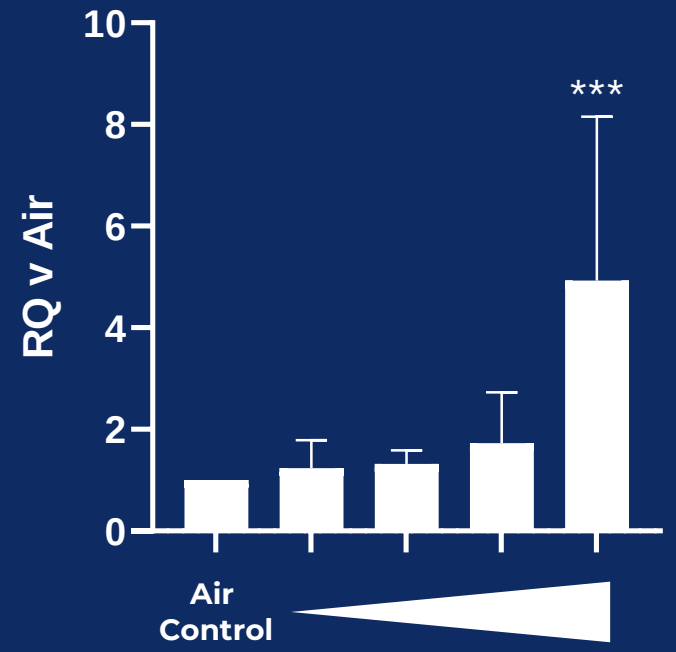


Repeat Exposure

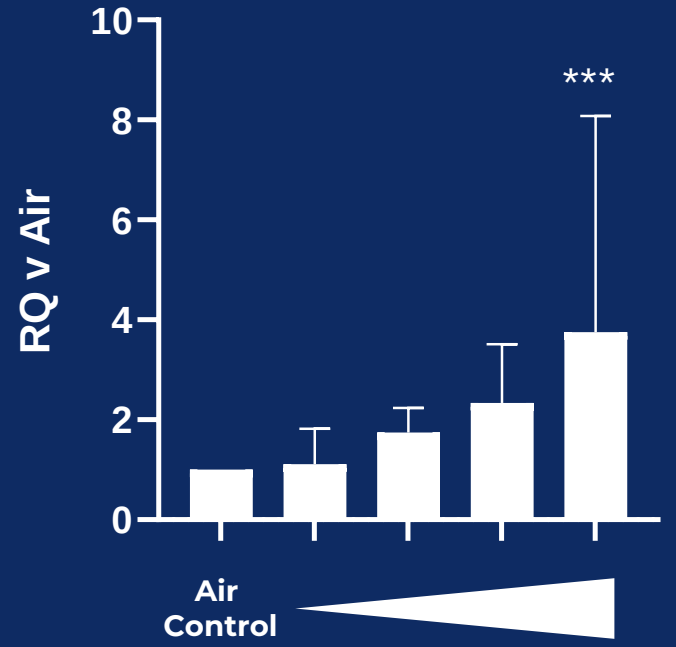


KE-5: Increased mucus viscosity

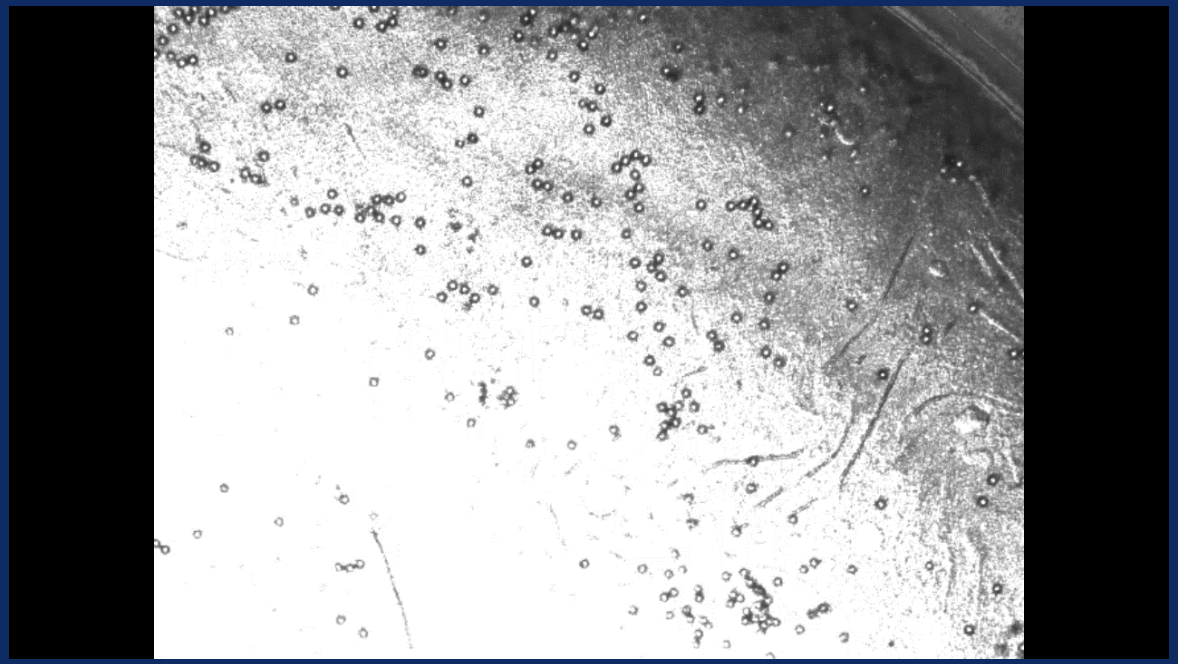
Single Exposure (Muc5ac)

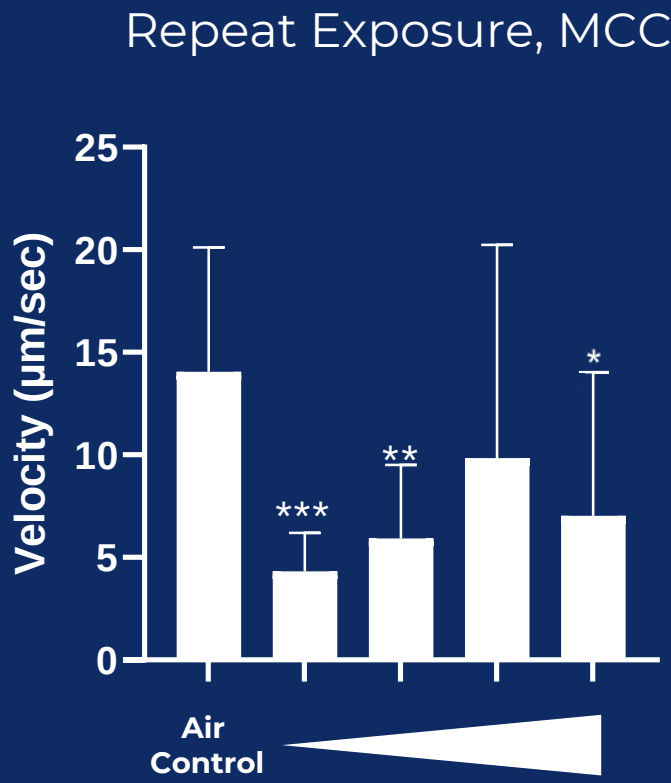


Repeat Exposure (Muc5ac)



KE-7: Mucociliary Clearance





Summary

- We have demonstrated positive readouts across the AOP
- AOPs show promise to link exposure with disease outcome
- AOPs can support the mechanistic narrative
- There is utility to assess other products using this AOP

Future Work

- Further validation, testing oxidative stressors in the absence of cigarette smoke
- Testing other nicotine delivery product categories

Acknowledgments

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Brian Keyser & Robert Leverette

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