



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

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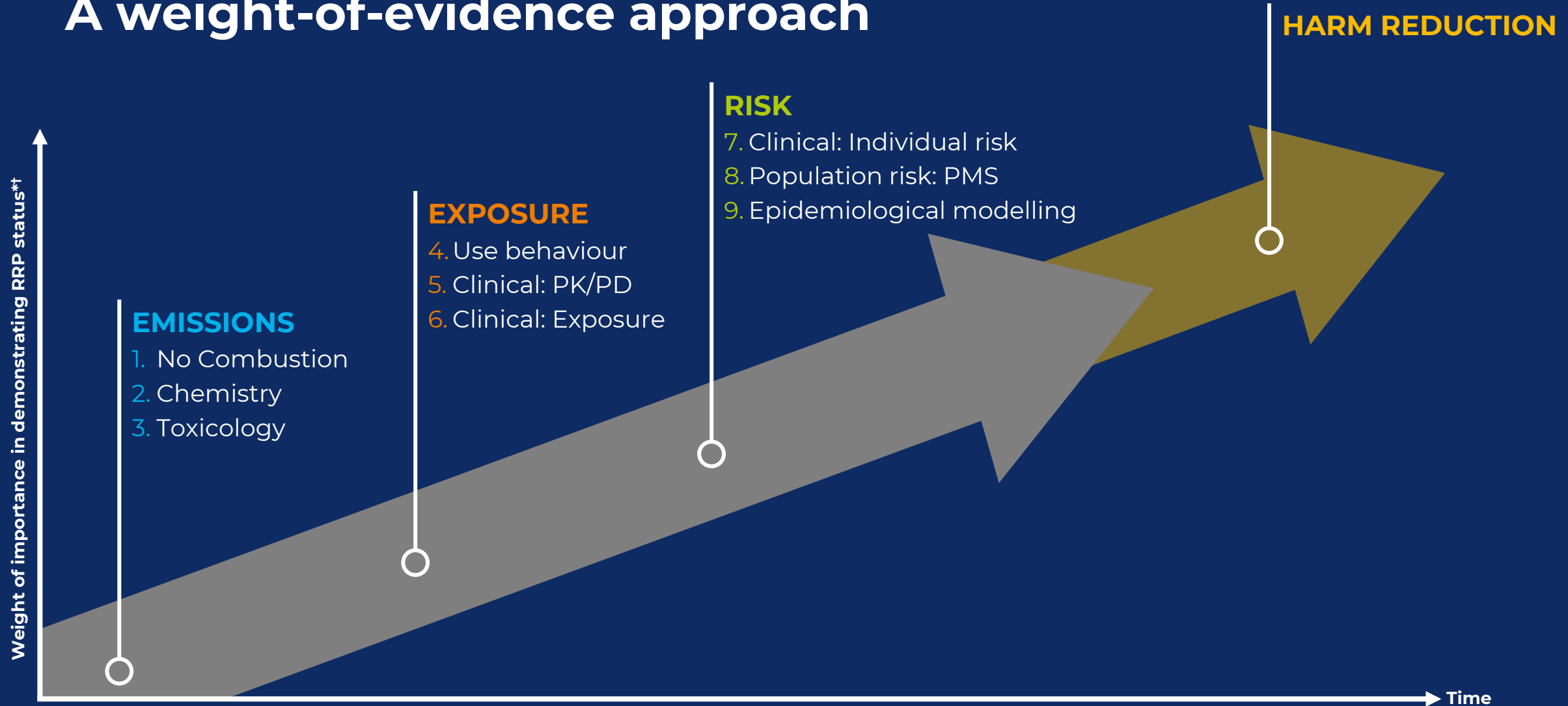
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BAT's 9-Step Risk Assessment Framework: A weight-of-evidence approach



* 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.

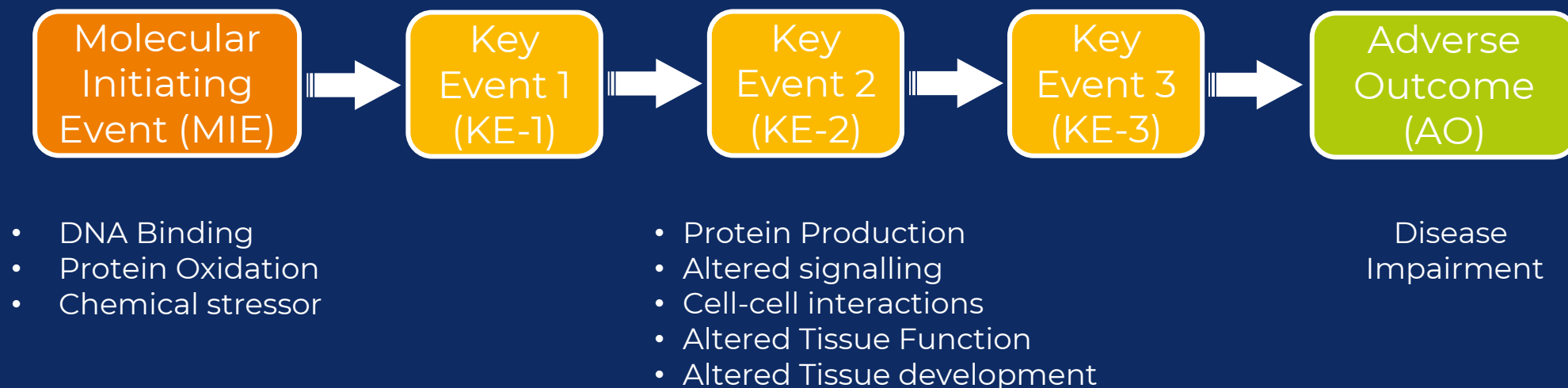
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Issues with clinically establishing Reduced Risk Profile

- 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



Adverse Outcome Pathway (AOP)

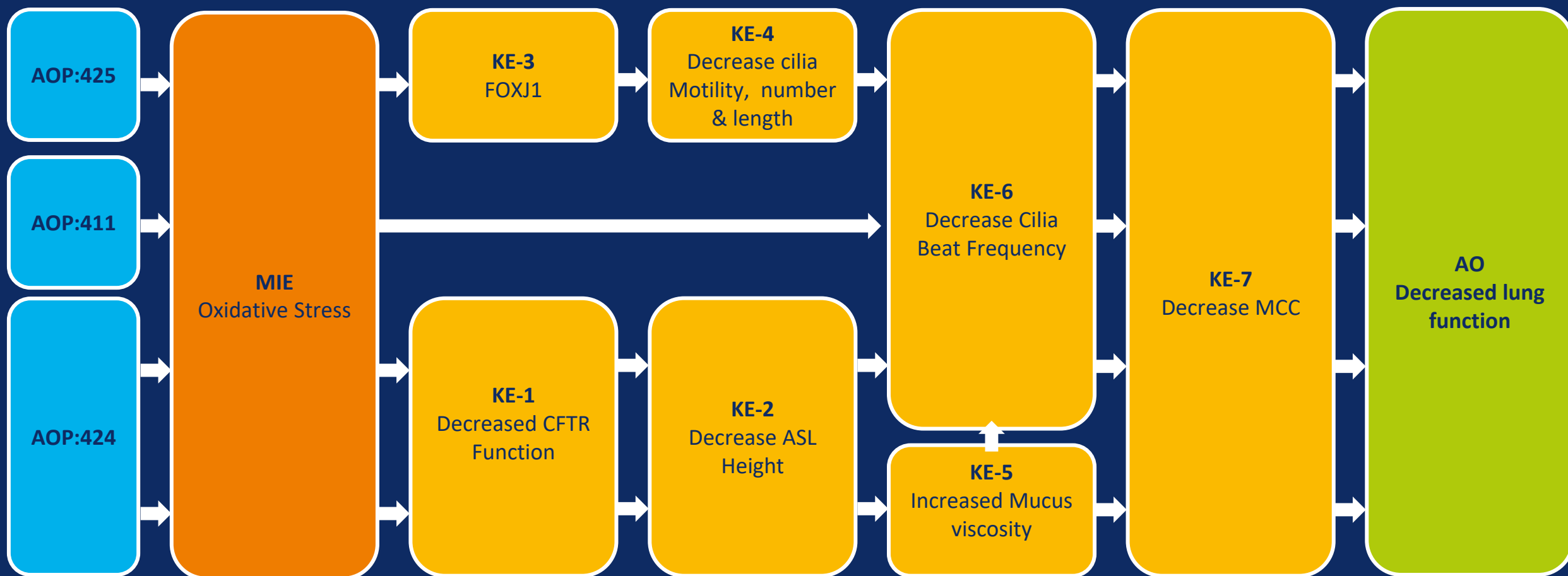
- Non-animal alternative
- Link clinical biomarkers potential outcomes / mechanistic narrative
- Potential biomarker evaluation
- Cost effective product screening
- Independent review and acceptance process (OECD)

Oxidative Stress - Decreased Lung Function

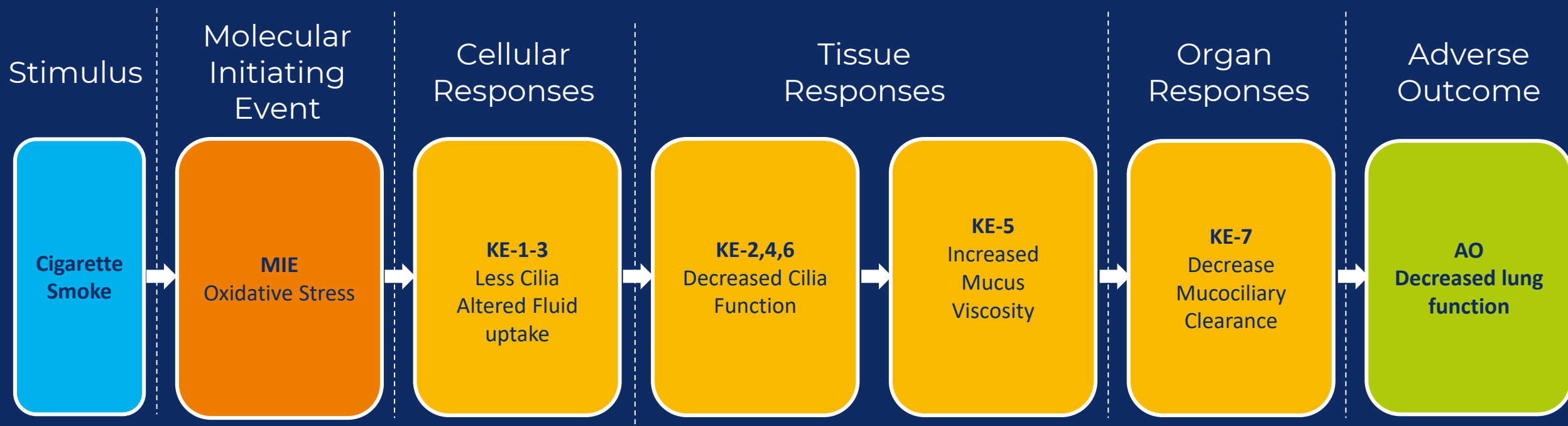
AOP 411, 424 & 425

Luettich et al., Front. Toxicol. 2021

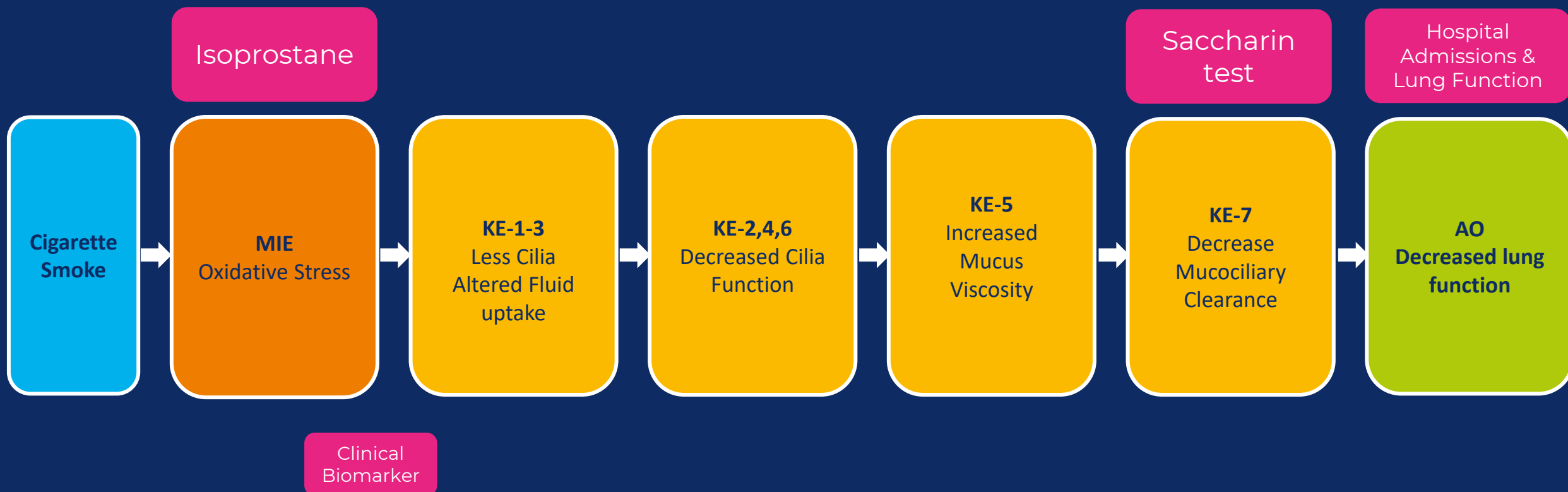
Experimental targets



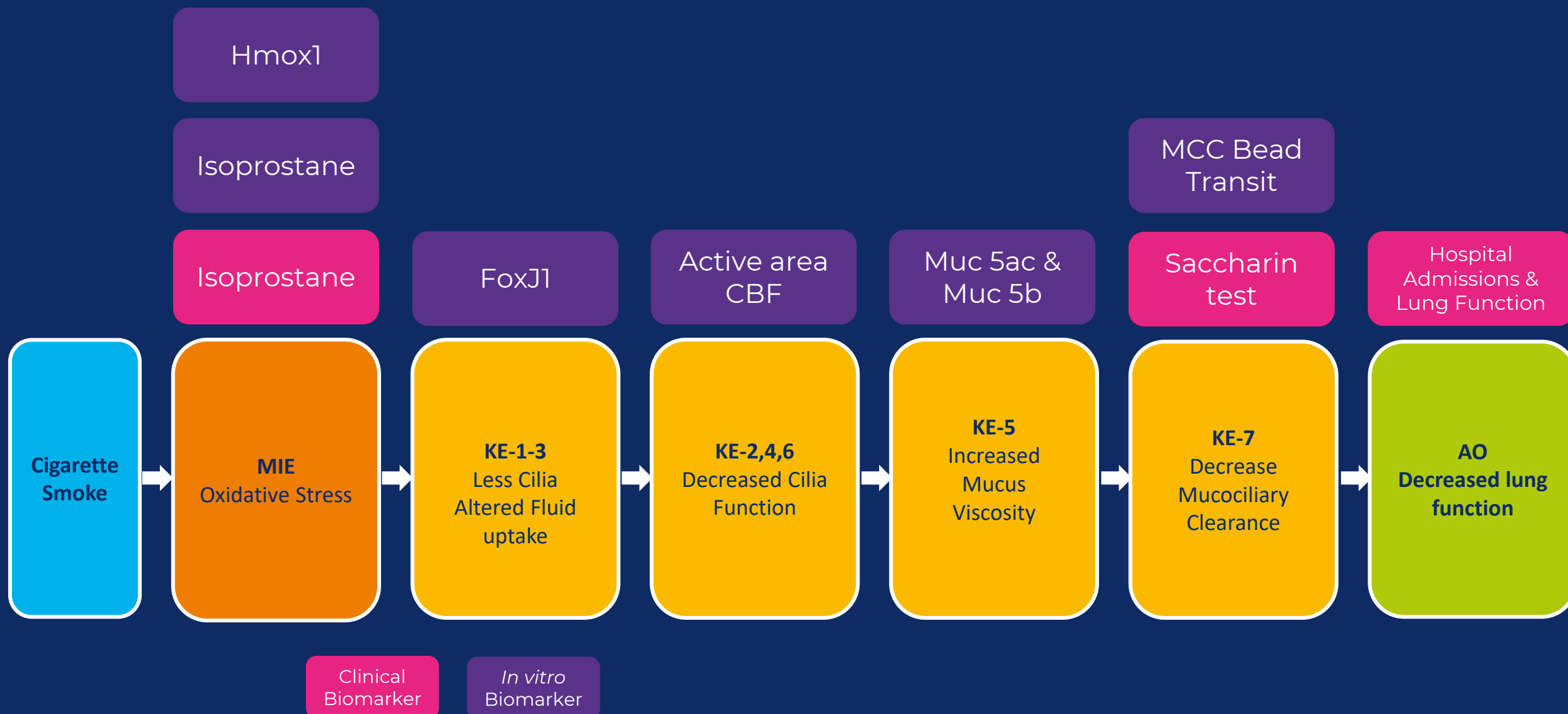
Condensed 411, 424 & 425 AOP



Biomarkers

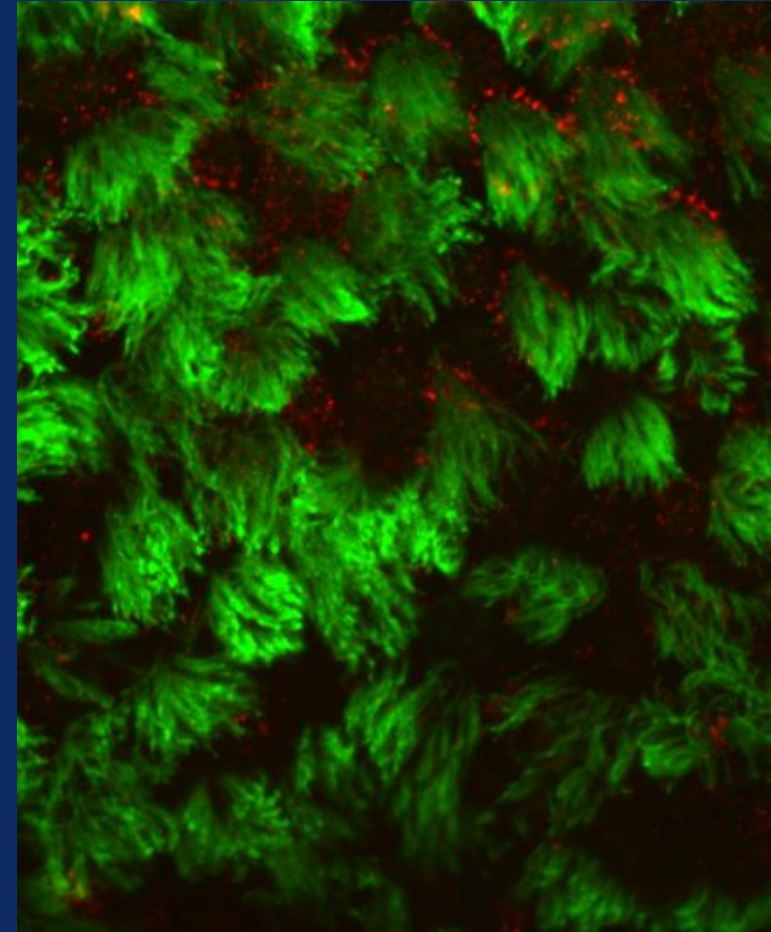


Biomarkers



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 HCl regimen

Single Exposure

Number of puffs : 56

Length of exposure : 28 minutes

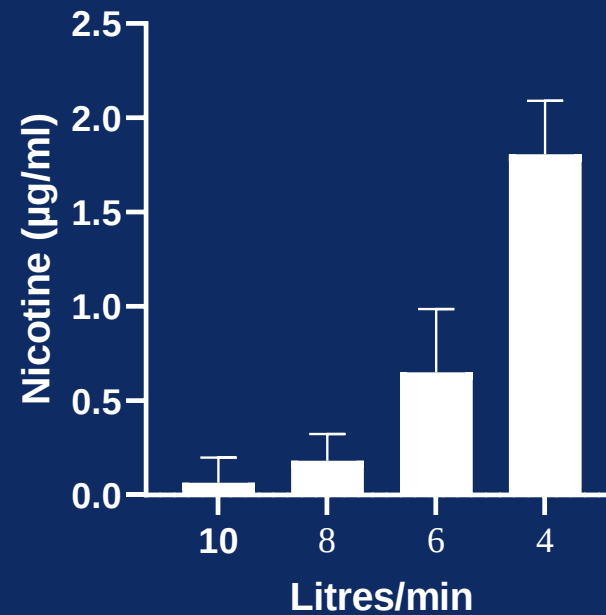
7-day Exposure (3 x per week)

Number of puffs : 48 puffs

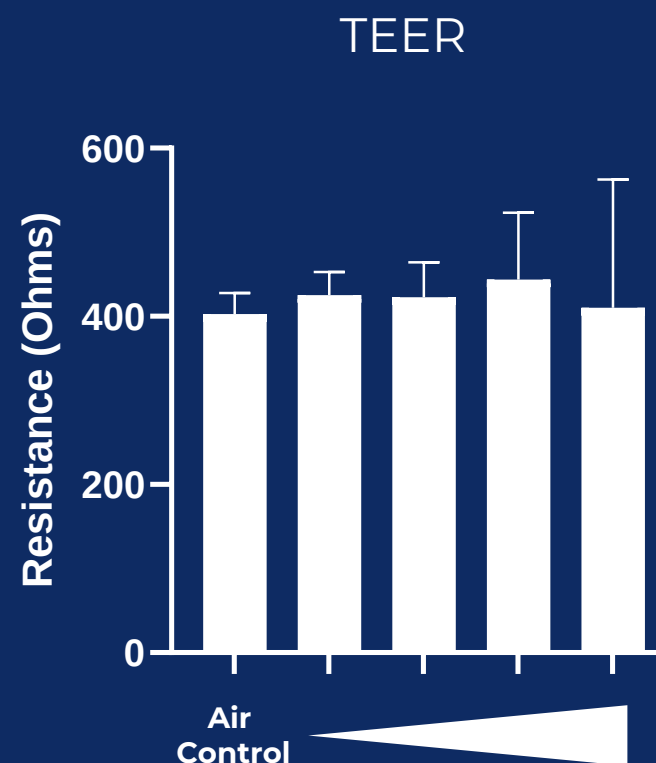
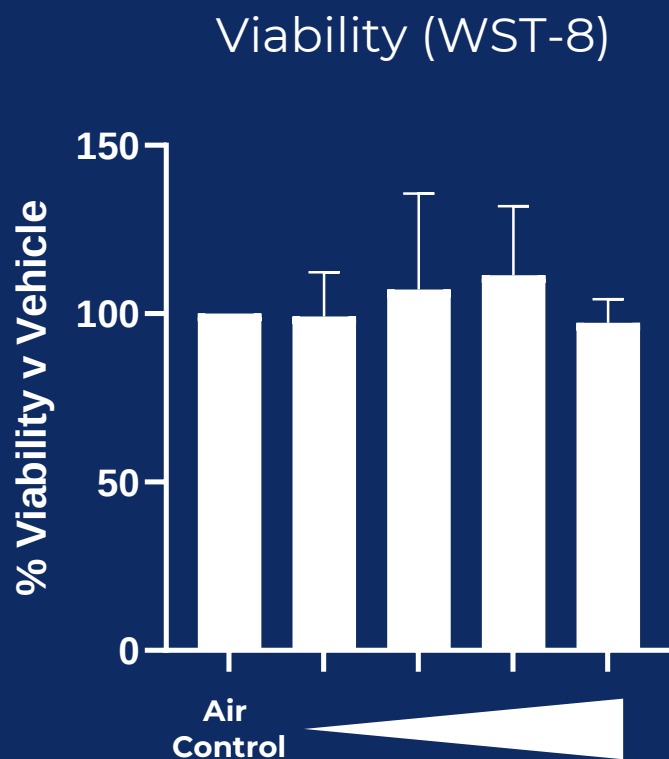
Length of exposure : 24 minutes



Exposure level

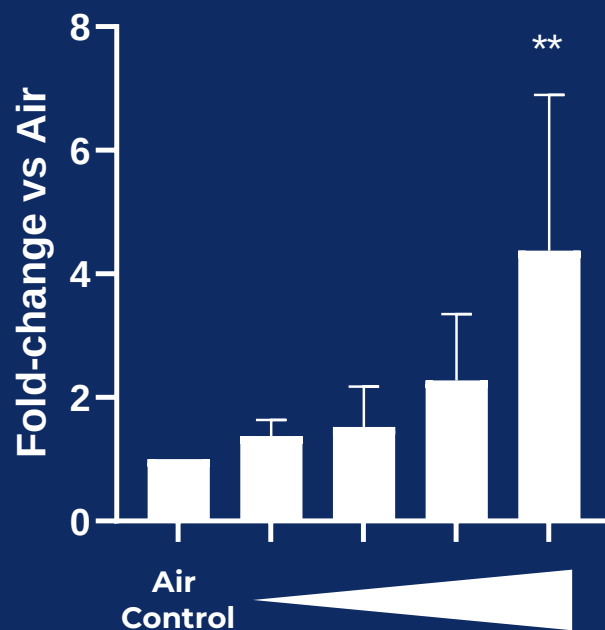


Subacute toxicity (single exposure)

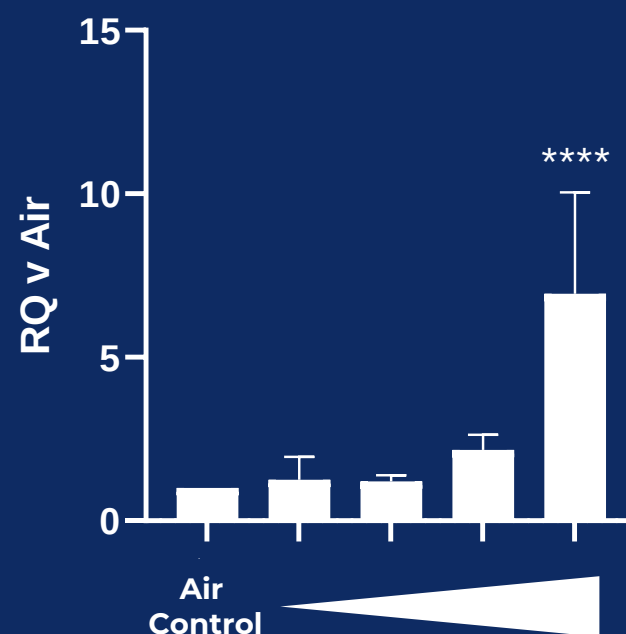


MIE: Oxidative Stress (Single Exposure)

8-Isoprostane

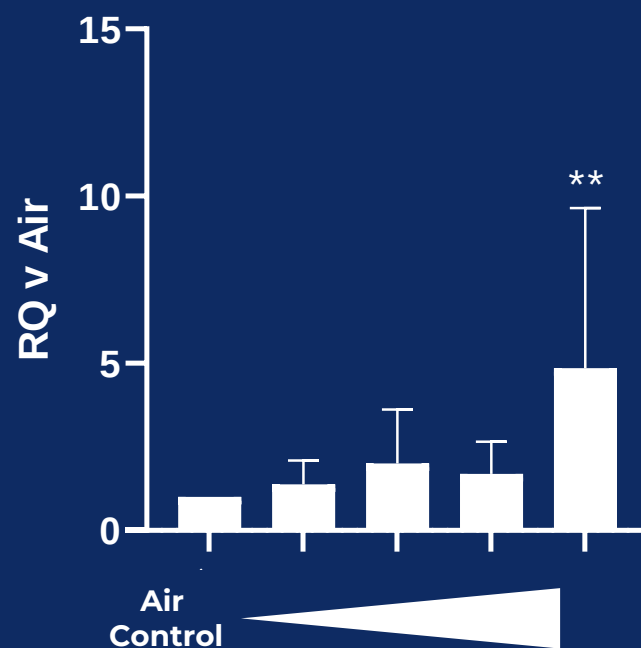


HMOX1

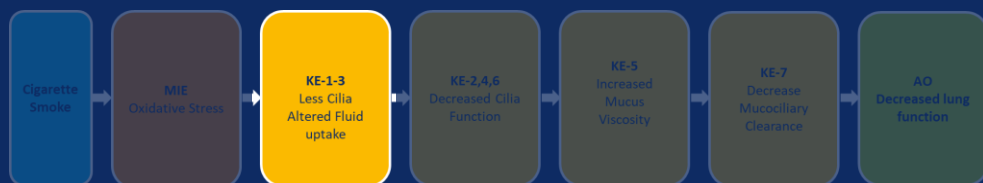
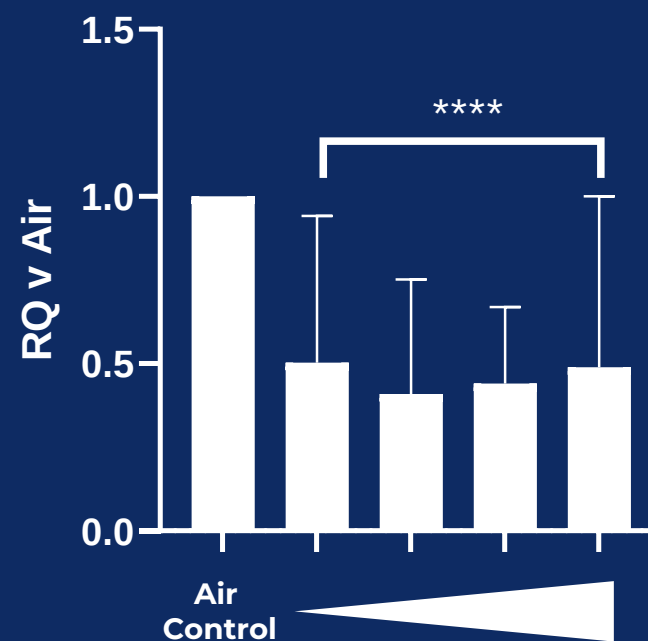


KE-1 & 3: Deceased FoxJ1

Single Exposure (FoxJ1)

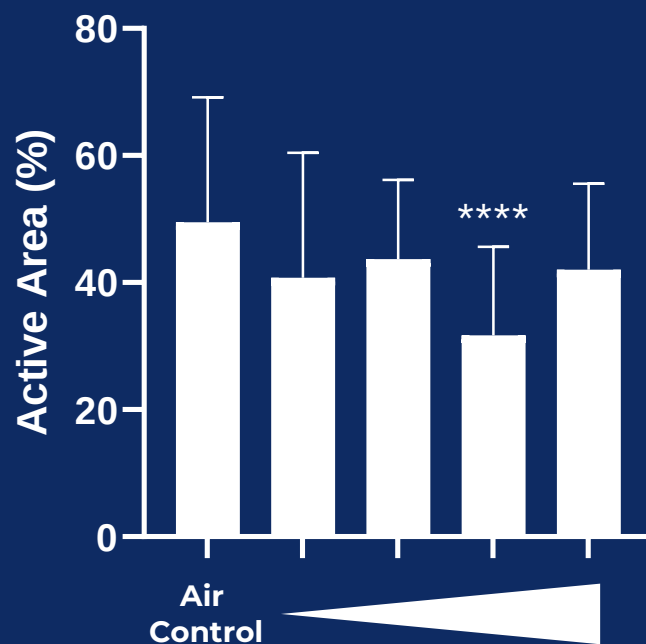


Repeat Exposure (FoxJ1)

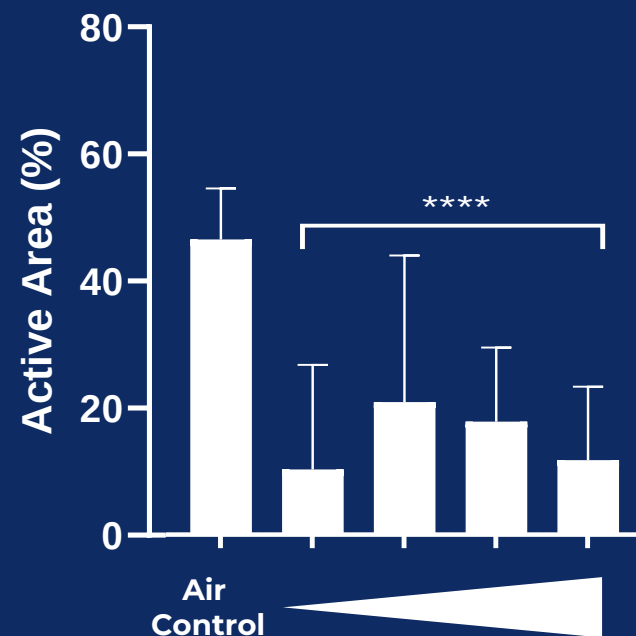


KE-5: Decreased Cilia Function

Single Exposure Active Area

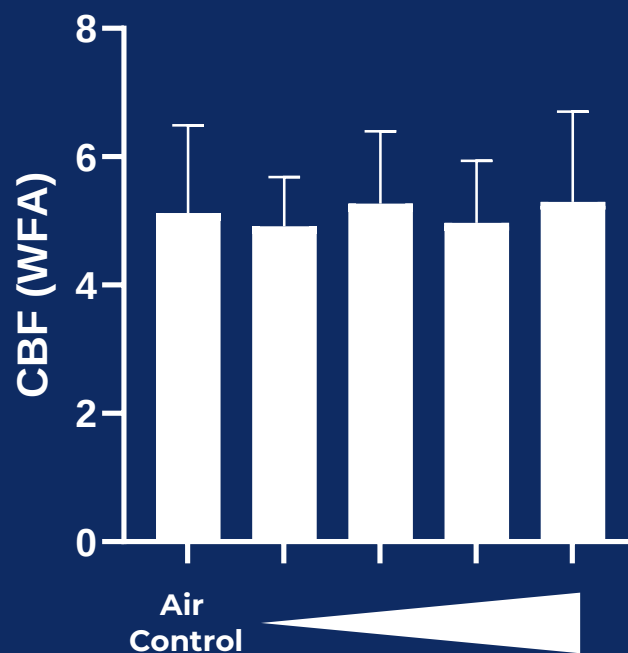


Repeat Exposure Active Area

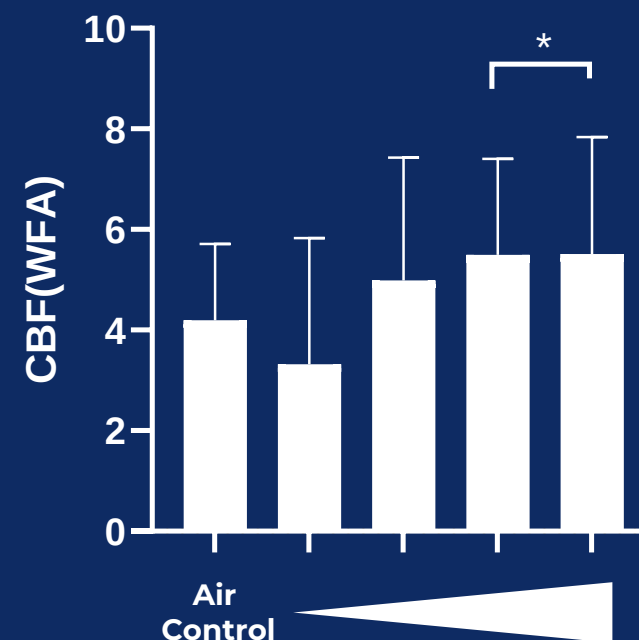


KE-6: Decreased Cilia Beat Frequency

Single Exposure

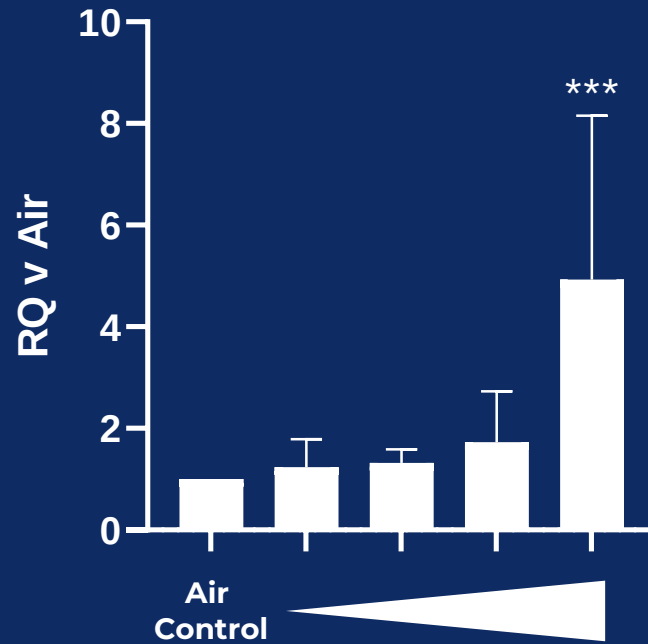


Repeat Exposure

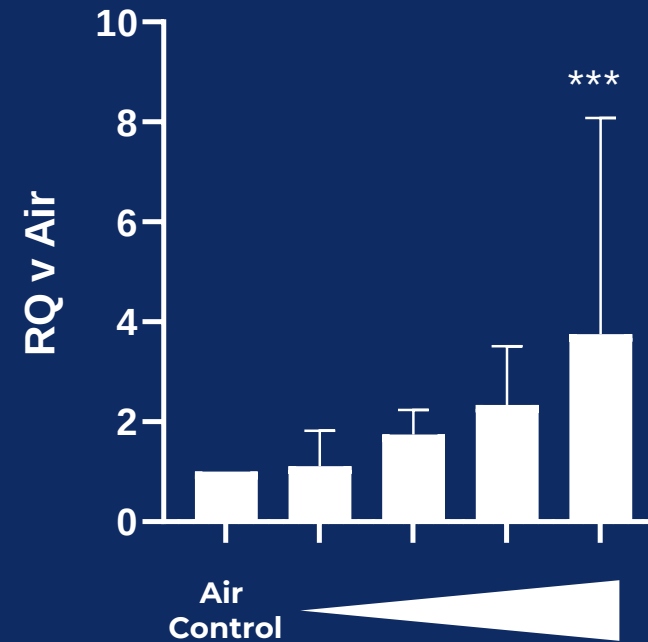


KE-5: Increased mucus viscosity

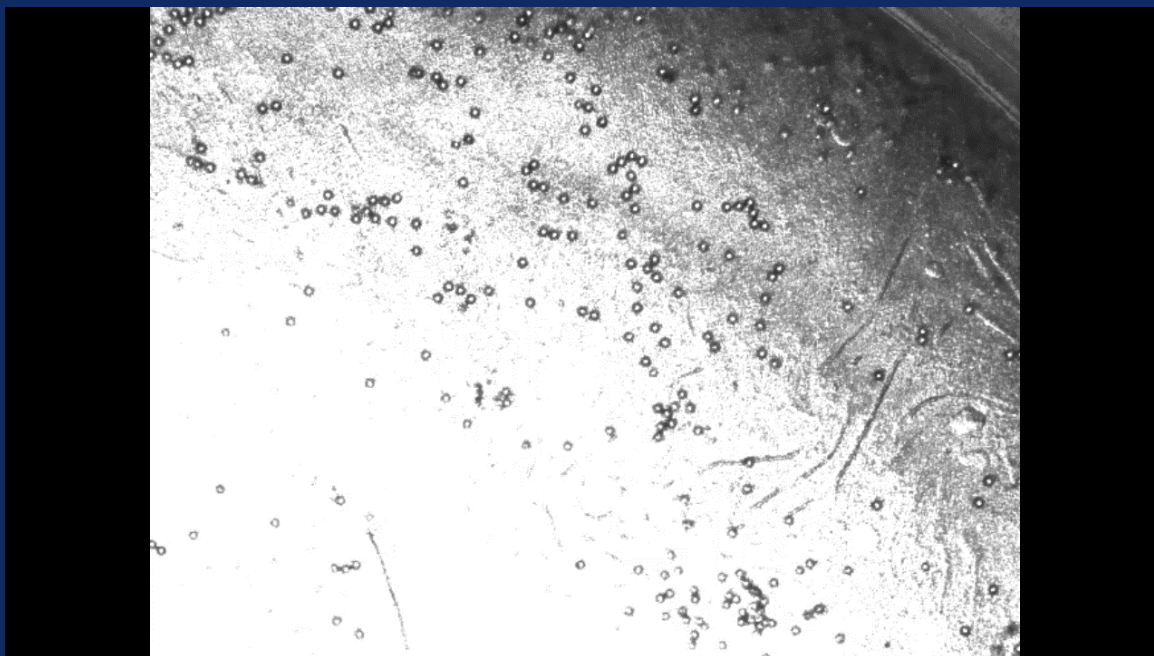
Single Exposure
(Muc5ac)



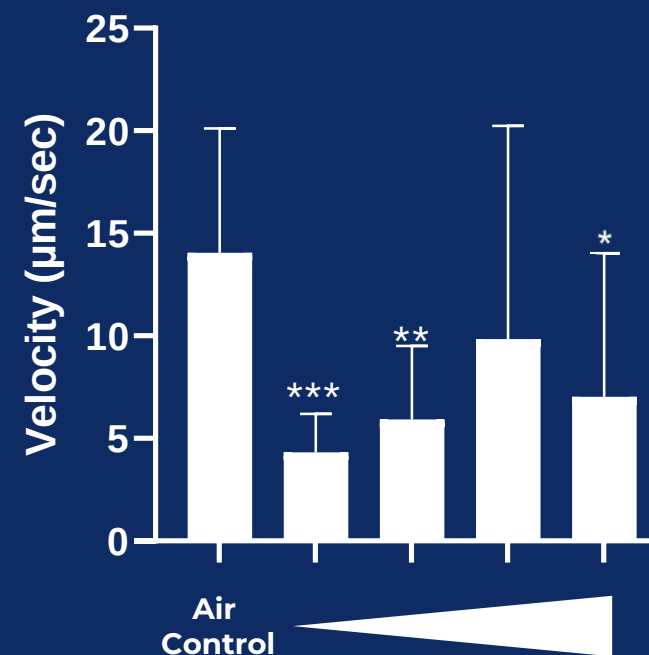
Repeat Exposure
(Muc5ac)



KE-7: Mucociliary Clearance



Repeat Exposure, MCC



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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