The developing and testing of aerosol in vitro assays for a chronic obstructive pulmonary disease adverse outcome pathway

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Introduction

Understanding the biological markers involved in the development of disease is critical to developing prediction models for product risk assessment.

Due to limited availability of validated biomarkers, investigations into the progression of chronic obstructive pulmonary disease (COPD) have proved difficult owing to accessibility, sensitivity and reliability of biomarkers. This is especially true of early disease stages.

Adverse outcome pathways (AOPs) may provide a potential solution to these shortfalls. AOPs identify the discrete molecular initiating event (MIE) and biological key events (KE), which are critical, progressive, functional and predictive in the development of disease-relevant endpoints.

Although there are other causes of COPD, smoking is the most common, with smoking-induced oxidative stress proposed as a main MIE in the development of COPD as outlined in Luettich *et al.* 2021 [1], AOP-411, 424 & 425 (see Figure 1).

It is expected that this combined AOP pathway may provide mechanistic insight into early disease, using *in vitro* models, and provide weight of evidence support to predict COPD risks for new products.

As such, we have developed and tested a series of *in vitro* assays aligning with the endpoints of the AOP.

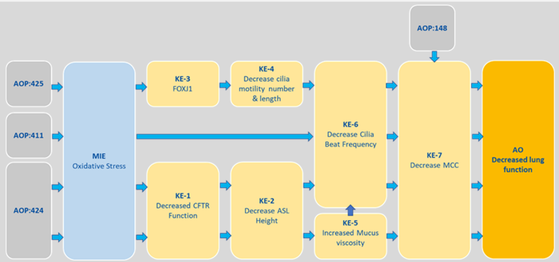

Figure 1. Alignment of AOPs associated oxidative stress impact on lung function

Methodology

The *in vitro* 3D lung model MucilAir was used in conjunction with a Vitrocell® VC10® Smoking engine (Vitrocell Systems GmbH) to test our model. It was necessary to incorporate the Vitrocell® dilution system at a range of 4 - 10 litres per minute to deliver a subacute toxicity range of cigarette (1R6F) smoke whole aerosol exposures.

The dilution, was used in combination with single and repeat exposures (3 times per week), following the puffing regimen as laid out in the table below. We identified and tested a number of endpoints throughout the AOP as identified in Figure 2, aligned with the available clinical endpoints.

	Single Exposure	Repeat Exposure
Number of puffs	56	48
Exposure duration	28 mins	24 mins
Model duration	24 hr	1 week
Dilution flow rate	10, 8, 6, 4 litres/min	10, 8, 6, 4 litres/min

Conclusion

While there are small deviations from the proposed oxidative stress-induced, reduced lung function AOP pathway, the results for cigarette smoke exposure generally aligned. Dose responsive endpoint inductions even at low levels of toxicity, were noted for both oxidative stress (MIE) and mucin (Muc5ac &b) mRNA induction, aligning with increased mucus viscosity (KE-5). Similarly, reductions were also seen with repeat exposures on cilia active area (KE-4) and in the bead tracking mucociliary clearance endpoint (KE-7).

This study demonstrates the potential of using AOPs in combination with in vitro models to inform products assessments. Aligning the endpoints with clinical biomarkers helps to add depth to a disease related mechanistic narrative.

We hope that this work will provide utility in product testing, helping to characterise product risk profiles using in vitro measures.

Results

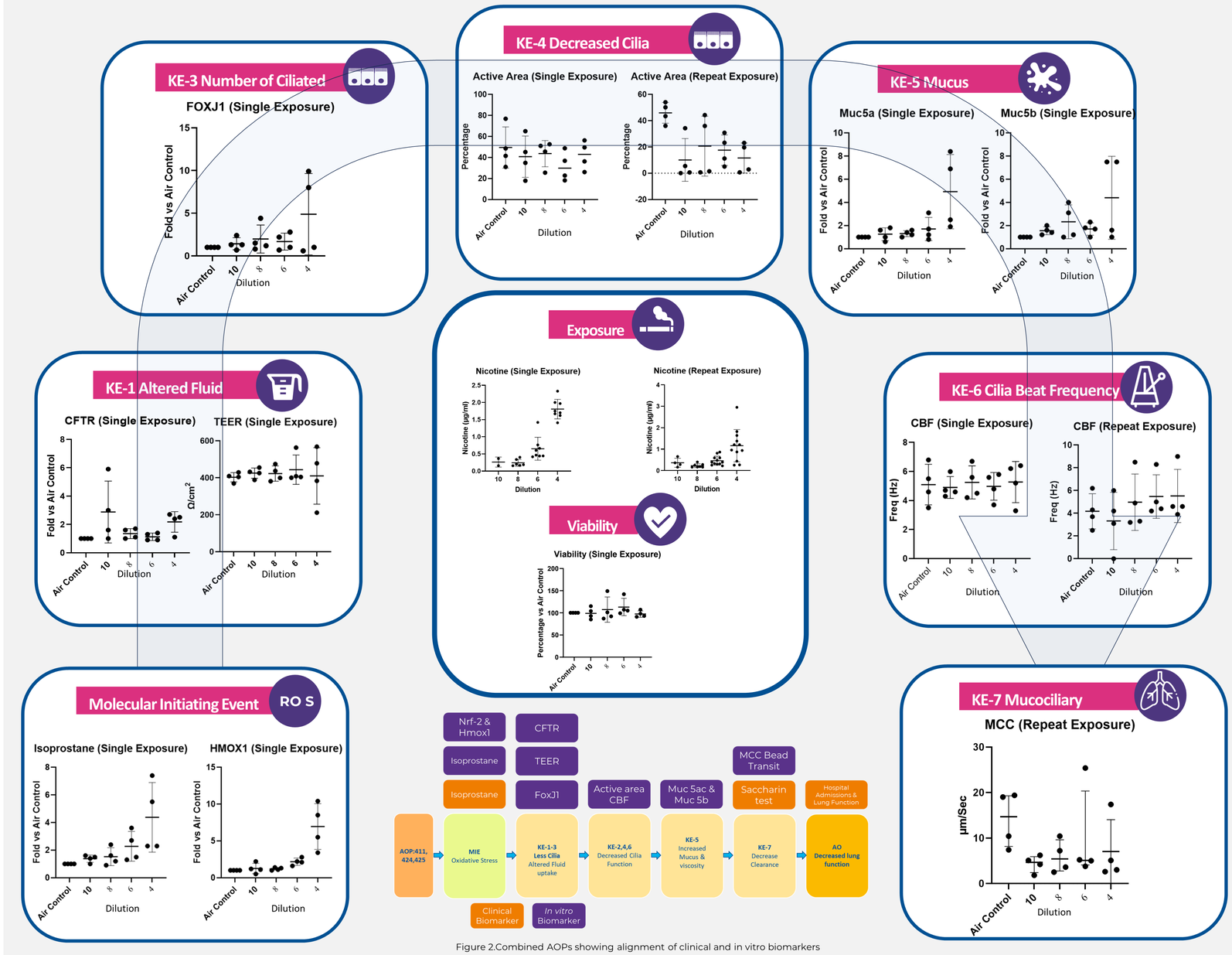

Figure 2.Combined AOPs showing alignment of clinical and in vitro biomarkers

References

1. Luettich, K et al. (2021). Outcome Pathway for Decreased Lung Function Focusing on Mechanisms of Impaired Mucociliary Clearance Following Inhalation Exposure. Front Toxicol, 3, 750254,

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