Utility of biomarkers of potential harm as end-points for the disease relevant assessment of novel tobacco and nicotine products as potentially reduced risk products

Dr Christopher Proctor

Biomarkers of potential harm: A public workshop

FDA/CTP Scientific Workshop, Washington | 4-5th April 2016

Conflict of interest statement

I declare that this work was fully funded by British American Tobacco and that myself and my co-workers were full time employees of British American Tobacco for the duration of the research.

Agenda

- Background
- Utility of biomarkers of potential harm (BoPH) with Reduced Toxicant Prototype cigarettes
- BoPH development process using the Adverse Outcome Pathway approach
- BoPH development case studies
- Summary

Background: key milestones in potentially reduced risk tobacco product*

*Approaches are consistent in their proposed use of biomarkers of exposure, effective dose and potential harm.

Biomarker definitions*

Biomarker of Exposure

- A tobacco constituent or metabolite that is measured in a biological fluid or tissue that has the potential to interact with a biological macromolecule; sometimes considered a measure of internal dose

Biologically Effective Dose (BED)

- The amount that a tobacco constituent or metabolite binds to or alters a macromolecule: estimates of the BED might be performed in surrogate tissues

Biomarker of potential harm

- A measurement of an effect due to exposure; these include early biological effects, alterations in morphology, structure, or function and clinical symptoms consistent with harm; also includes “pre-clinical changes”

**Clearing the Smoke: Assessing the Science base for Tobacco Harm Reduction* (2001)

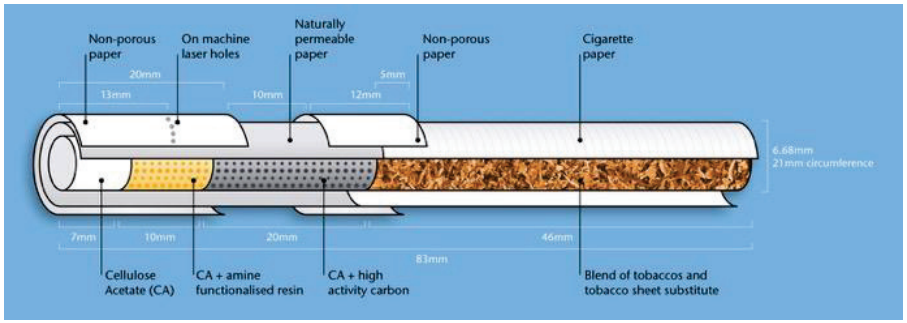
Background: rationale for BoPH selection for Reduced Toxicant Prototype cigarette studies

Biomarker	Disease end point	Observations in cessation studies	Observations in smokers versus non-smokers
high density lipoprotein cholesterol (HDL-C) in serum	Cardiovascular disease[1]	Levels can be reversed after 30 days cessation[7]	Relatively consistent differences observed between smokers and non-smokers
white blood cell total count (WBC) in blood	Inflammation[2]	Rapid and sustained decrease with cessation[8]	
soluble intercellular adhesion molecule-1 (sICAM-1) in serum	Endothelial dysfuncion[3]	Levels decline rapidly within 30 days of cessation[9]	
11-dehydrothromboxane B2 (11-DTX-B2) in urine	Platelet activtation[4]	Significant reduction after 3 days cessation[10]	
8-epi-prostaglandin F2α (8-epi-PGF2α – Type III) in urine	Oxidative Stress[5]	Significant reduction with 7 days cessation[11]	
MCP-1	Atherosclerosis[6]	Association of MCP-1 with cigarette smoking[12]	

1. Chelland Campbell *et al. Atherosclerosis* 2008 , **201:**225–35
2. Bonaterra *et al. Curr. Mol.* Med.2010, **10;**180–205
3 Gross *et al. Clin. Chem*. 2012, **58:**411–20
4. Frost-Pineda *et al. Nic. Tob. Res.* 2011, **13:**182–93
5. Milne *et al. Biomarkers*, 2005, 10 (Suppl): S10–23, Rahman, *Cell Biochem Biophys.* 2005, 43:167–88
6. Deo *et* al. *J. Am. Coll. Cardiol.* 2004, **44:1**812–88
7. Maeda *et al. Prev. Med.*2003, **37**:283-90; Moffat, Atherosclerosis 1988, **75**:85–9
8. Jensen *et al. Thorax.* 1998, **53**:784-9; Abel *et al. Mayo Clin Proc.* 2005, **80**:1022-8
9. Palmer *et al. Eur J Clin Invest.* 2002, **32**:852–7
10. Rångemark *et al. Arterioscler Thromb.* 1993, 13: 777–82; Saareks *et al. Naunyn Schmiedebergs Arch Pharmacol,* 2001, **363**:556–61
11. Pilz *et al. Thrombosis Research* 2000, **99**:209–21 Oguogho *et al. Vasa* 2000, **29**:103-5
12. Daloee *et al. Am J Mens Health.* 2015, pii: 1557988315601724. [Epub ahead of print]

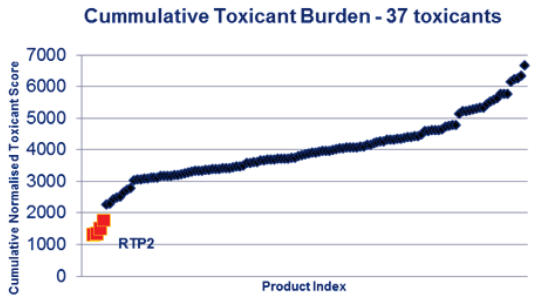
Background: Reduced Toxicant Prototype cigarette results

Product[1,2]



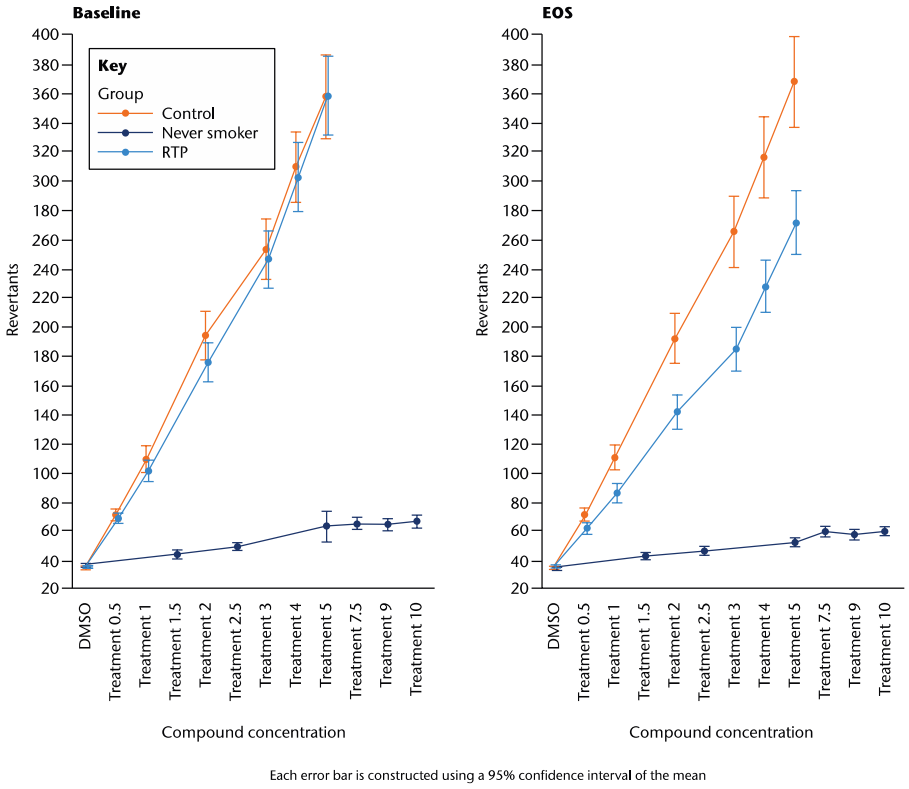
Toxicant reducing technologies in blend and filter of novel cigarette design

Chemistry[1,2]



Toxicant reducing technologies manifested reductions in toxicant yields versus conventional cigarettes

Urine Mutagenicity[1,2]

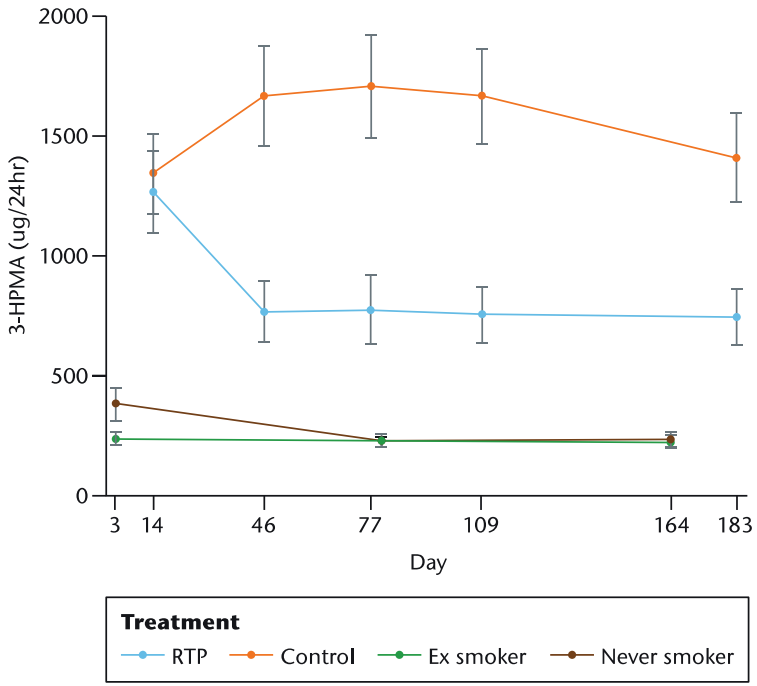


Significant reduction in urine mutagenicity at end of study between smokers of control and RTP cigarette

1. Proctor, C *et al. 68th Recent Advances in Tobacco Science* 2014: 1–34
2. Dittrich *et al.*, Approaches for the design of reduced toxicant emission cigarettes, *SpringerPlus*, **3**(2014), p. 374

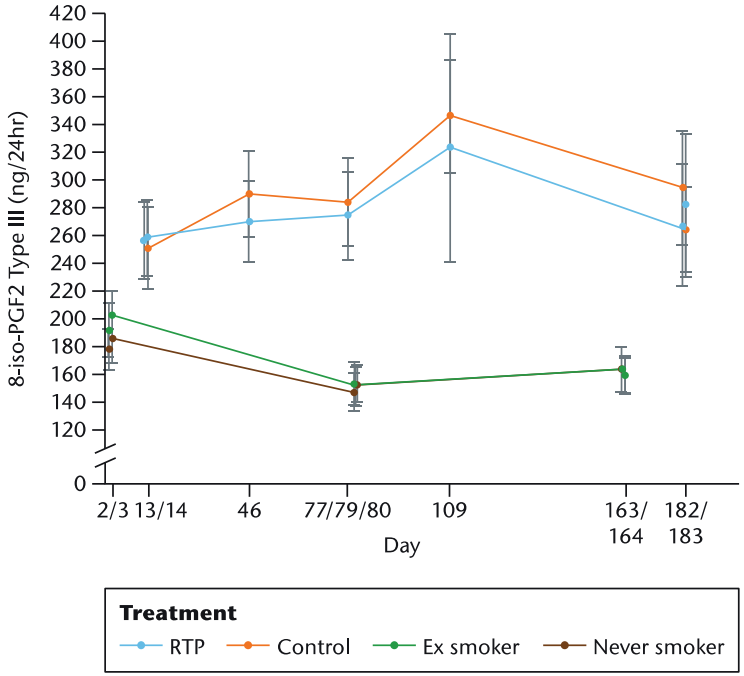
Background: Reduced Toxicant Prototype cigarette results

Biomarkers of Exposure[3]



Toxicant reductions between the RTP cigarette and control were substantial enough to reduce biomarkers of exposure

Biomarkers of Potential Harm[3]



Difference between smokers and non-smokers but no difference between RTP and control over time

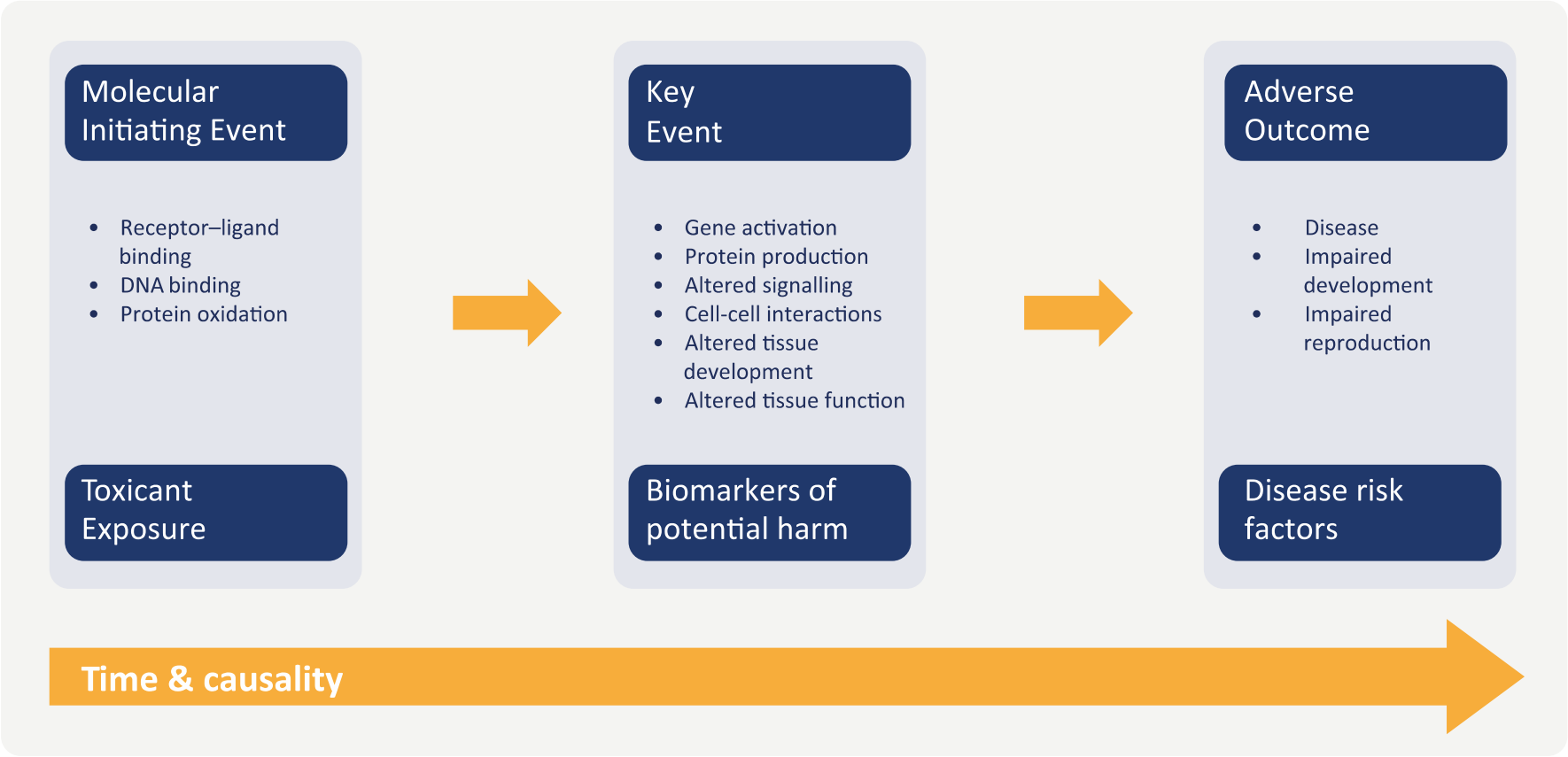
3. Shepperd, C *et al. Regulatory Toxicology and Pharmacology* 2015 **72**: 273-291

Background: BoPH results from RTP cigarette studies

Biomarker	Disease end point	Observations from RTP study
High density lipoprotein cholesterol (HDL-C) in serum	Cardiovascular Disease[1]	**No significant difference between RTP and Control group:** • Levels varied in Control Smoker and Ex-Smokers groups.
White blood cell total count (WBC) in blood	Inflammation[2]	**No significant difference between RTP and Control group:** • Levels stable throughout study for each group
Soluble intercellular adhesion molecule-1 (sICAM-1) in serum	Endothelial dysfuncion[3]	**RTP group significantly higher than control smoking group by EOS:** • Levels increased in smoking groups and RTP levels were significantly higher than control by EOS
11-dehydrothromboxane B2 (11-DTX-B2) in urine	Platelet activation[4]	**RTP smoking group significantly lower than control group by EOS** • RTP group lower than ex-smokers by EOS
8-epi-prostaglandin F2α (8-epi-PGF2α – Type III) in urine	Oxidative Stress[5]	**No significant difference between RTP and Control group:** • Increased levels at mid point in study reduced to baseline levels by EOS
MCP-1	Atherosclerosis[6]	**RTP group significantly higher than control group by EOS:** • Control group reduced to similar levels as NS by EOS while RTP group increased

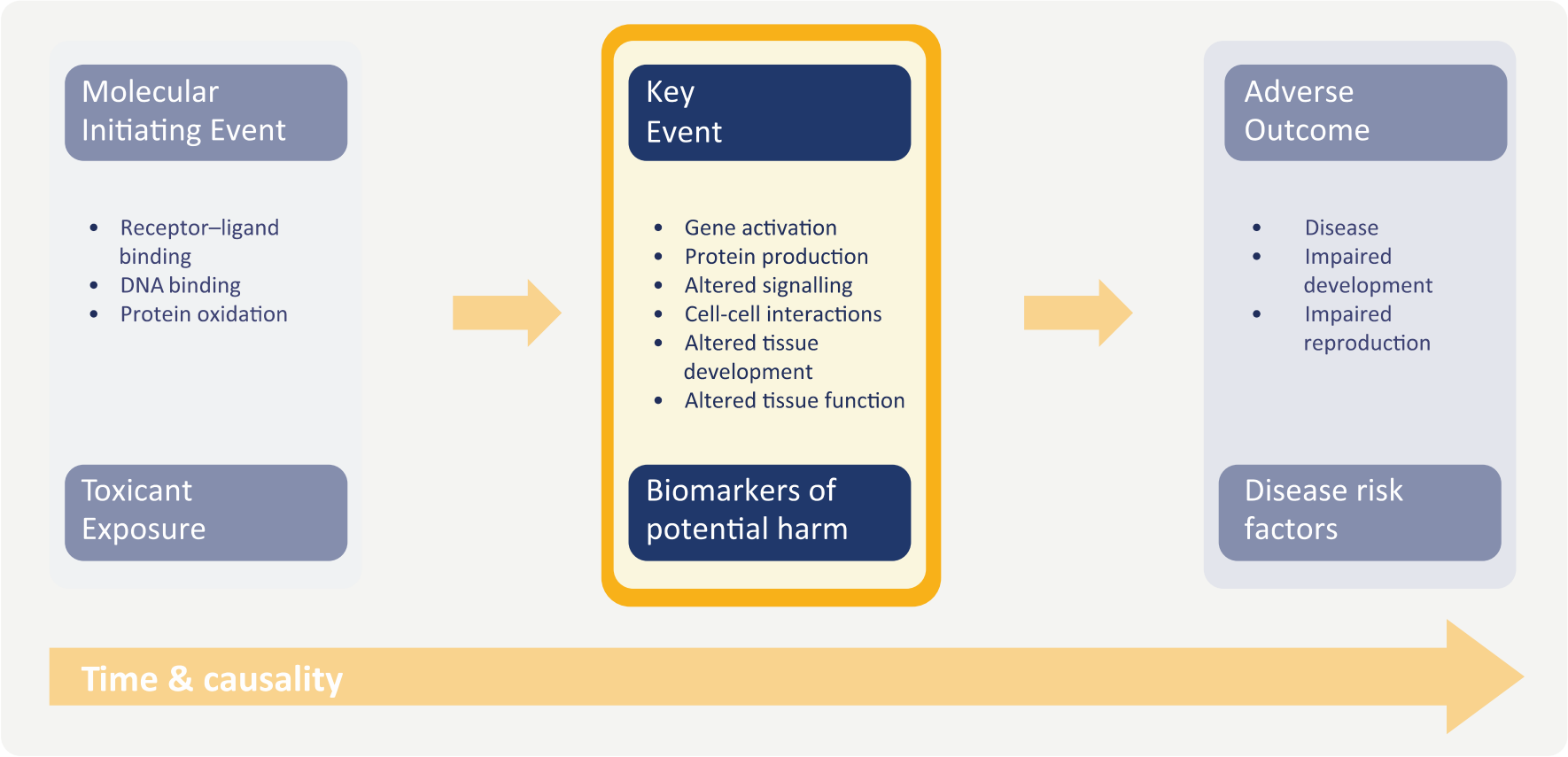
1. Chelland Campbell *et al. Atherosclerosis.* 2008 , **201**:225-35
2. Bonaterra *et al. Curr. Mol. Med.*2010, **10**;180-205
3 Gross *et al. Clin. Chem.* 2012, **58**:411-20
4. Frost-Pineda *et al. Nic. Tob. Res.* 2011, **13**:182-93
5. Milne *et al. Biomarkers*, 2005, **10** (Suppl): S10-23: Rahman, *Cell Biochem Biophys.* 2005, **43**:167-88
6. Deo *et al. J. Am. Coll. Cardiol.* 2004, **44**:1812-88

BoPH development process using the Adverse Outcome pathway approach*



*Adapted from Ankley *et al*. *Environ Toxicol Chem*, 2010 ;**29(3):** 730-741 and Edwards *et al. J Pharmacol Exp Ther*. 2016; **356(1)**:170–81

BoPH development process using the Adverse Outcome pathway approach*



*Adapted from Ankley *et al*. *Environ Toxicol Chem*, 2010 ;**29(3):** 730-741 and Edwards *et al. J Pharmacol Exp Ther*. 2016; **356(1)**:170–81

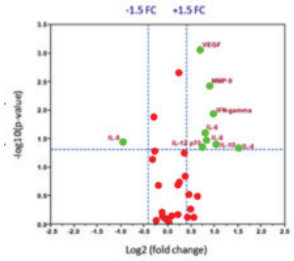
Case study OVERVIEW

In Vitro	Clinical	AOP
Transcriptomics & metabolomics	Transcriptomics & metabolomics	
Proteomics	Proteomics	
Candidate targets differentiated by exposure and non-exposure to cigarette smoke	Refine candidate targets by confirming differentiation in samples from smokers and non-smokers	• Establish Adverse Outcome (AO) • Filter candidates targets by association with AO • Filtered targets become potential key events • Identify and qualify upstream/downstream key events • Qualify key event relationships

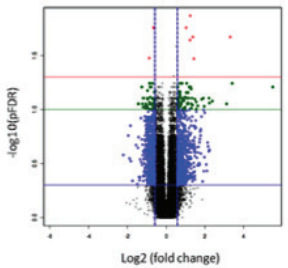
Case study 1a | RNA-seq transcriptomics & metabolomics: *in vitro*

Molecular Initiating Event → Key Event → Adverse Outcome

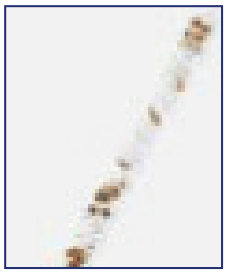
Step A: Omics profiling and phenotypic endpoints



Differential cytokine release air *vs.* smoke
(33 cytokines quantified)



Differential gene expression air *vs.* smoke[1]
(44,184 RNAs expressed)



Quantitative immunostaining (Muc-5Ac)

Step B: Enrichment analysis and ontology mapping

Knowledge-base enrichment and causal analyses to predict altered functions and link these to diseases and phenotypes[2]

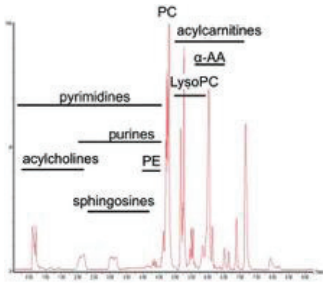
i) Identify differences between exposed and non-exposed cells to smoke
ii) Phenotype associated with disease – measure BoPH or surrogate in human

1. Banerjee *et al.* Differential Gene Expression Using RNA-seq Profiling in a Reconstituted Airway Epithelium, Mucilair™, Exposed to Conventional or Electronic Cigarettes Aerosols. SOT 2016, Abstract 3037, P179
2. Krämer *et al. Bioinformatics*. 2014, **30**: 523–530

Case study 1b | RNA-seq transcriptomics & metabolomics: clinical

Molecular Initiating Event → **Key Event** → Adverse Outcome

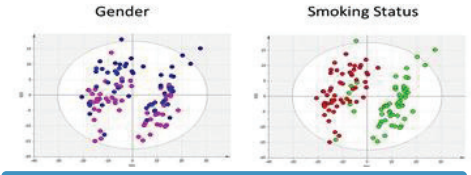
Step 1
High resolution untargeted profiling of biofluids



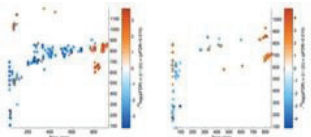
RP-UPLC-MS
Hillic-UPLC-MS
^{1}H-NMR

Separation of metabolites based on physico-chemical properties

Step 2
Identification of discriminating features smokers vs. non-smokers



PCA – group separation

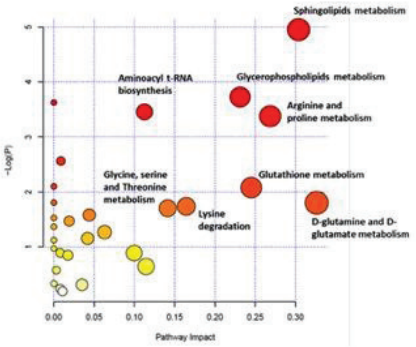


74 significant metabolites/ lipids (pFDR<0.05)

Statistically significant metabolites ID

Ranking of metabolites based on robustness of identification and statistical significance

Step 3
Enrichment analysis and ontology mapping



Ranking of altered functions based on metabolite role and pathway match

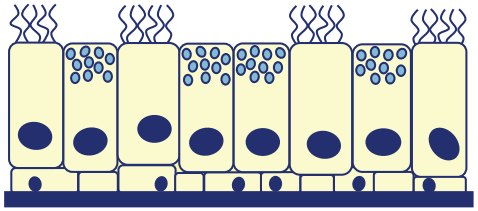
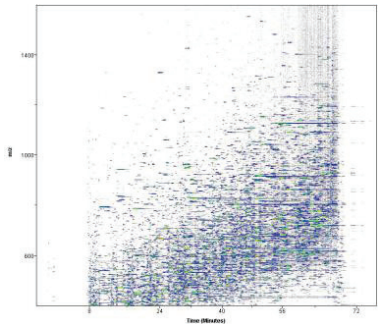
Combining metabolomics profiling with knowledge base mapping gives mechanistic insights into adverse biological effects and diseases

- Garcia-Perez *et al*. *Bioanalysis* 2014, 6: 2733-2749
- Kaluarachchi *et al*. A Multiplatform Metabolic Phenotyping Approach Integrated with Pathway Mapping to Identify Biochemical Differences Between Healthy Smokers and Non-smokers. SOT 2016, Abstract 1107 – P136,
- Shepperd *et al*. *BMC Public Health* 2013, 13:690

Case study 2a | *In vitro* proteomics

Molecular Initiating Event → Key Event → Adverse Outcome

Phase I: Characterisation of proteins in *in vitro* airway surface liquid

	LC-MS/MS  + Database search	Extracellular region **14%** Inflammation immune response **13%** Signaling **13%** Cytoskeleton **11%** Cell adhesion – wound healing **8%** Energy Metabolism **8%** Redox homeostasis stress response **7%** Calcium ion binding **7%** Protein degradation **5%** Nuclear organisation transcription **4%** Lipid metabolism **3%** Translation **2%** Misc. **5%**
In vitro lung samples	2,414 unique peptide corresponding to 487 proteins	Biological processes associated with ASL proteins

***in vitro* airway surface liquid provides a rich source of information to study tissue homeostasis**

1. Haswell *et al*. The effect of cigarette smoke exposure on the proteomic composition of human bronchial epithelial cell air surface liquid.
• Society of Toxicology meeting 2014 (Abstract # 1530)

Case study 2a | *In vitro* proteomics smoke exposure

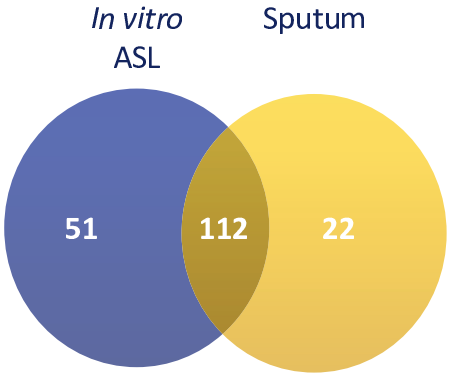
Molecular Initiating Event → Key Event → Adverse Outcome

Comparison of *in vitro* ASL samples with healthy sputum

Phase II: Targeted comparison MRM

171 proteins in targeted LC-MS/MS

163 proteins detected



A strong overlap of 112 common proteins

Good similarity between protein profiles from clinical samples and 3-D *in vitro* lung models

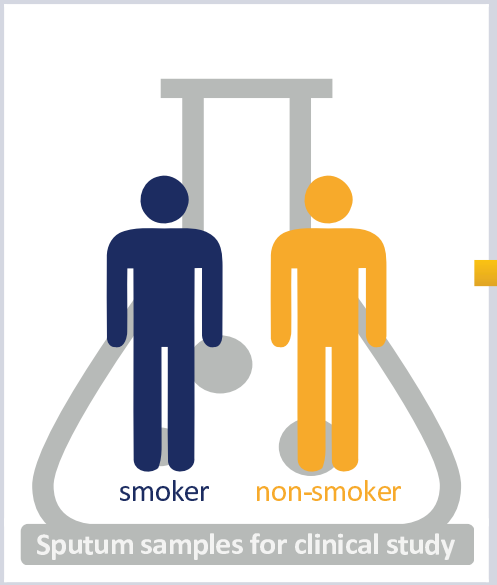
•Haswell *et al*. The effect of cigarette smoke exposure on the proteomic composition of human bronchial epithelial cell air surface liquid. Society of Toxicology meeting 2014 (Abstract # 1530)

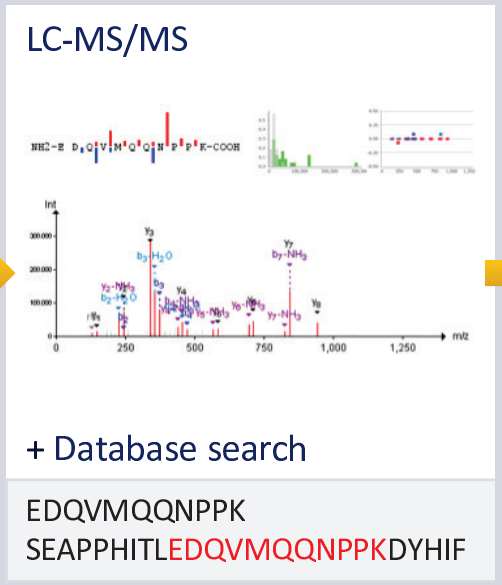
•Haswell *et al*. A targeted proteomic comparison of human induced sputum from smokers and non-smokers. Society of Toxicology meeting 2016 (Abstract # 3041)

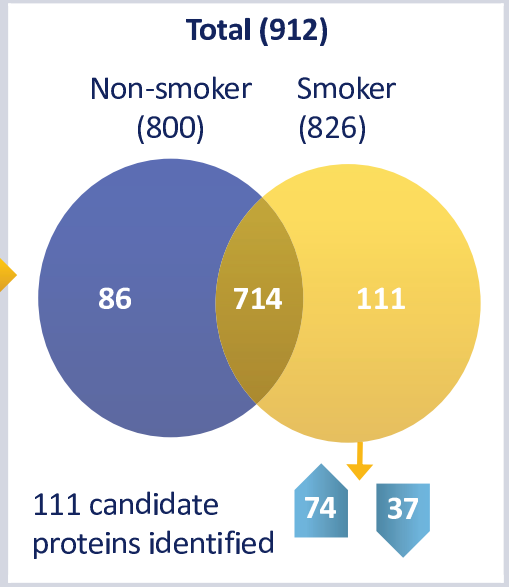
Case study 2b | Proteomics: clinical protein identification

Molecular Initiating Event → Key Event → Adverse Outcome

Phase I: Characterisation of proteins in sputum







Difference in proteins from smokers and non-smokers in clinical samples – next step, monitor samples in longitudinal study

- Haswell *et al*. A targeted proteomic comparison of human induced sputum from smokers and non-smokers. Society of Toxicology meeting 2016 (Abstract # 3041)
- Camacho *et al*. A targeted proteomic comparison of human-induced sputum from smokers and non-smokers. Submitted for publication.

Case study 2b | Proteomics: quantification

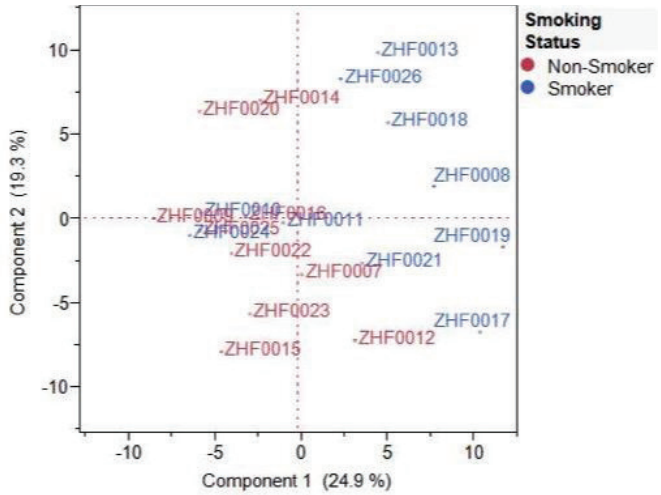
Molecular Initiating Event → **Key Event** → Adverse Outcome

Phase II: Targeted comparison MRM

143 proteins in targeted LC-MS/MS

↓

139 proteins detected



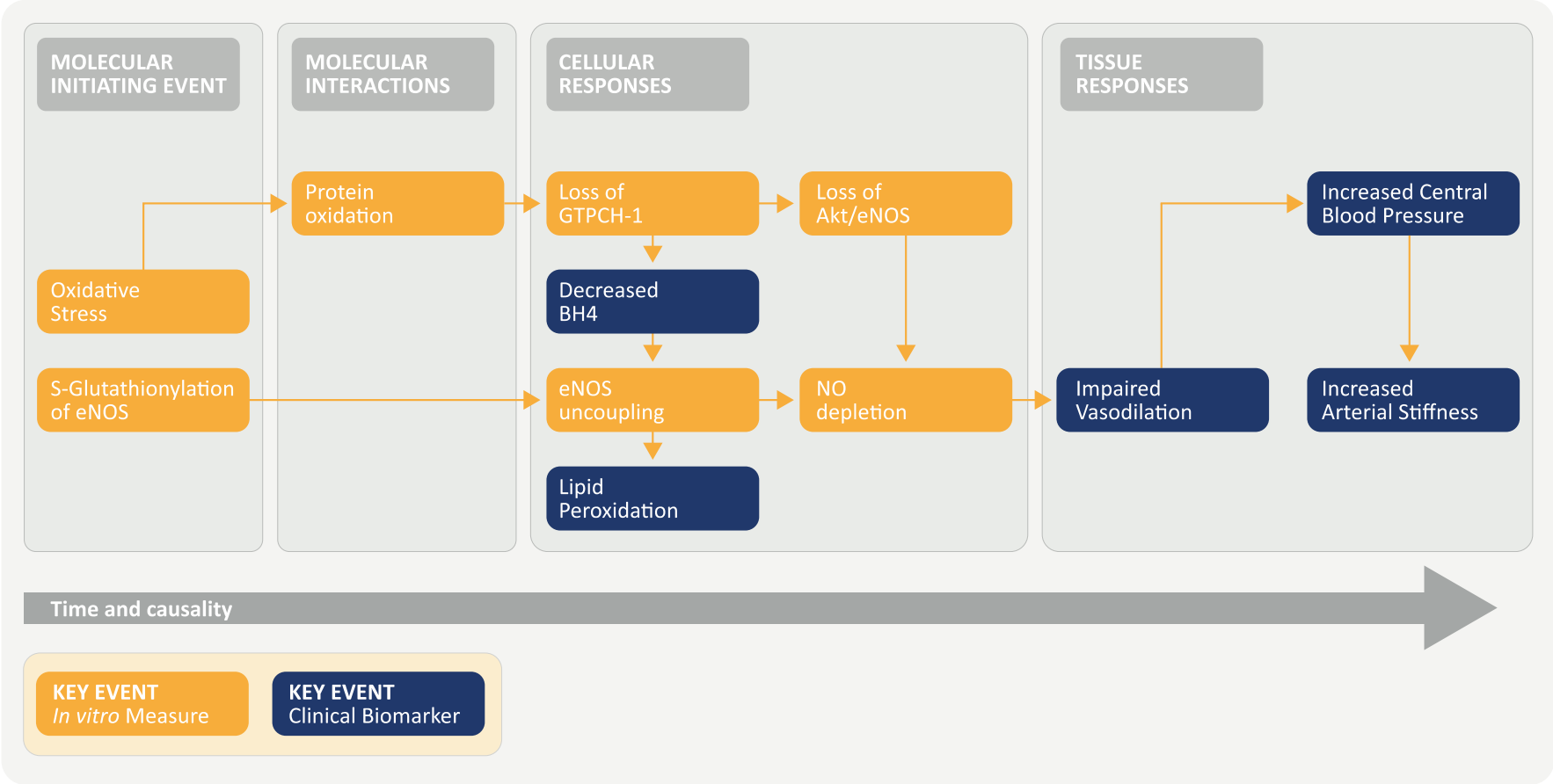
27 significantly differently abundant proteins for 4 above 3-fold change:

- **AL3A1_HUMAN**
- **CATD_HUMAN**
- **CEAM5_HUMAN**
- **K1C16_HUMAN**

27 candidate proteins identified that will be observed in a longitudinal cohort study

- Haswell *et al*. A targeted proteomic comparison of human induced sputum from smokers and non-smokers. Society of Toxicology meeting 2016 (Abstract # 3041)
- Camacho *et al*. A targeted proteomic comparison of human-induced sputum from smokers and non-smokers. Submitted for publication.

Example candidate AOP for Arterial Stiffness (CVD)

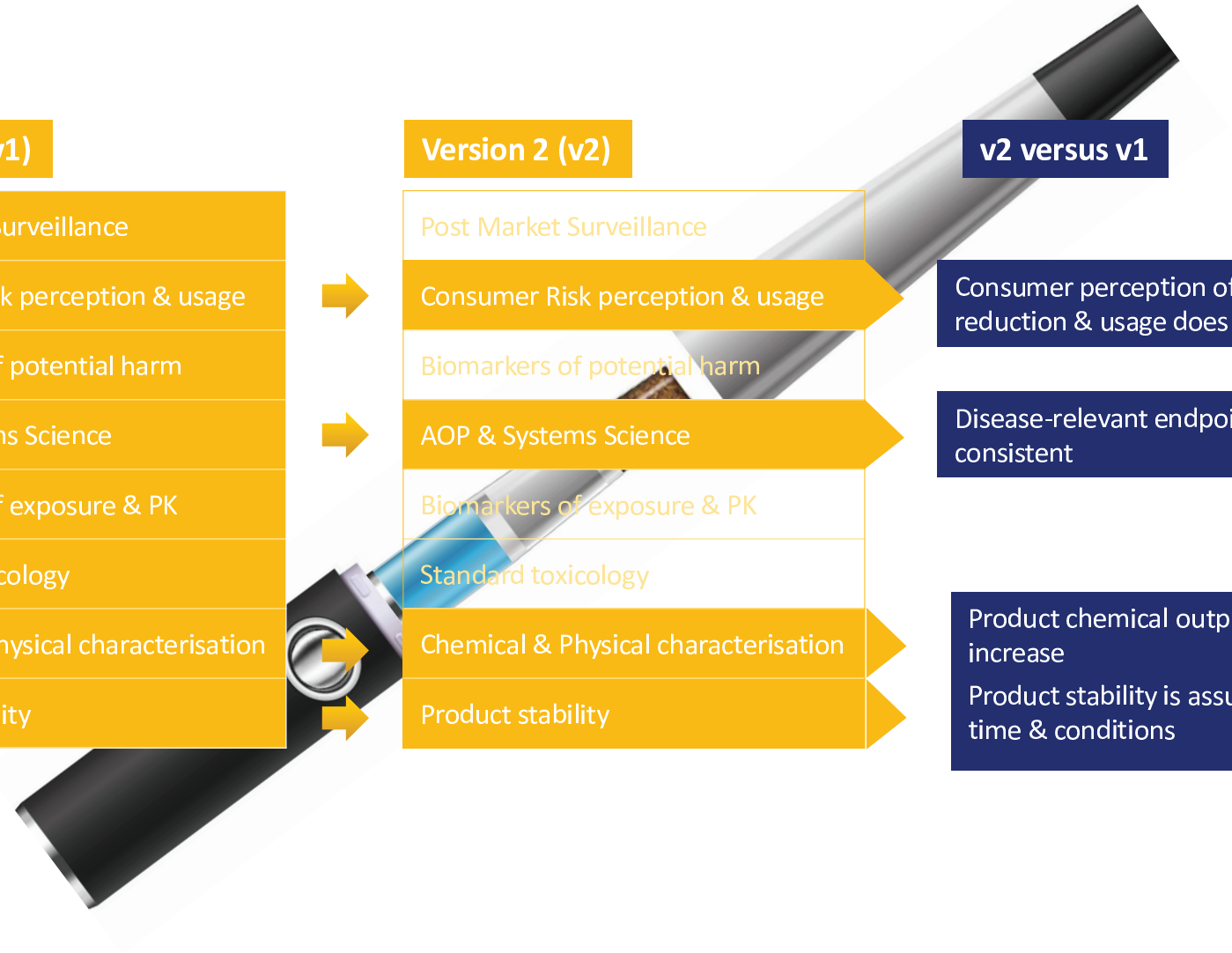


- AOP for arterial stiffness submitted to OECD Nov '15. EXTENDED ADVISORY GROUP ON MOLECULAR SCREENING AND TOXICOGENOMICS meeting minutes: https://community.oecd.org/community/mst
- Chen CA *et al.* Nature. 2010; 23;468(7327):1115-8.
- Laurent S *et al.* Ann Med. 2012;44 Suppl 1:S93-7.
- Abdelghany *et al.* Society of Toxicology meeting 2015 (Abstract # 1818)
- El-Mahdy *et al.* Cigarette Smoke Constituents Cause Endothelial Dysfunction Due To Oxidative Depletion of Tetrahydrobiopterin and Activation of the Ubiquitin Proteasome System. (*Submitted for publication)*

Bridging approach

- Large datasets from multiple non-clinical, clinical and population studies required to substantiate modified risk products
- BoPH is a key foundational dataset to establish the risk profile of a product
- Innovation will proceed at a rapid pace therefore need to bridge between product variants
- One approach to bridging could be the use of subsets of data from the original variant (V1) for assessing subsequent variant (V2)

Bridging approach



Version 1 (v1)	Version 2 (v2)	v2 versus v1
Post Market Surveillance	Post Market Surveillance	
Consumer Risk perception & usage	Consumer Risk perception & usage	Consumer perception of risk reduction & usage does not change
Biomarkers of potential harm	Biomarkers of potential harm	
AOP & Systems Science	AOP & Systems Science	Disease-relevant endpoints are consistent
Biomarkers of exposure & PK	Biomarkers of exposure & PK	
Standard toxicology	Standard toxicology	
Chemical & Physical characterisation	Chemical & Physical characterisation	Product chemical output doesn't increase
Product stability	Product stability	Product stability is assured over time & conditions

Summary

- Scientists and regulatory bodies propose that Biomarkers have a key role in the substantiation of modified risk products
- From our RTP cigarette studies we have identified a shortlist of candidate BoPHs
- New *-omic* approaches show promise for identification of additional BoPHs
- AOPs present an opportunity to assess disease-relevant risk factors through the integration of *in vitro* and BoPH endpoints

Acknowledgements

British American Tobacco
M McEwan, F Lowe, E Minet, J Shepperd, N Newland, A Eldridge, G Errington, M Gaça, D Breheny, O Camacho, L Haswell

Celerion
D Graff, K Newland, I Mayer

ABF
G Scherer

Ohio Smoking Research
J Zweier, M El-Mahady, R Ismail, T Abdelghany

Metabometrix
J. Lindon. I. Garcia-Perez, M. Kaluarachchi, C. Boulage

Caprion
L Cortes, P Croteau, L McIntosh, D Chelsky

Selventa
J Park, M Maria

RJ Reynolds
W Fields

Philip Morris International
J Hoeng, K Luettich, M Talikka, J Szostak

References

Abdelghany *et al.* Society of Toxicology meeting 2015, Abstract # 1818.

Abel *et al. Mayo Clin Proc.* 2005, **80**:1022-8

Ankley *et* al. *Environ Toxicol Chem.* 2010, **29(3)**:730-41.

Banerjee *et al.* Differential Gene Expression Using RNA-seq Profiling in a Reconstituted Airway Epithelium, Mucilair™, Exposed to Conventional or Electronic Cigarettes Aerosols. Society of Toxicology 2016, Abstract #3037

Bonaterra *et al. Curr. Mol. Med.* 2010, **10**;180-205

Camacho et al. A targeted proteomic comparison of human-induced sputum from smokers and non-smokers. Submitted for publication.

Chelland Campbell *et al. Atherosclerosis.* 2008, **201**:225-35

Chen CA *et al. Nature*. 2010, 23;468(7327):1115-8.

Daloee *et al. Am J Mens Health.* 2015, pii: 1557988315601724. [Epub ahead of print]

Deo *et al. J. Am. Coll. Cardiol.* 2004, **44**:1812-88

Edwards *et al. J Pharmacol Exp Ther.* 2016, **356(1)**:170-81.

El-Mahdy et al. Cigarette Smoke Constituents Cause Endothelial Dysfunction Due To Oxidative Depletion of Tetrahydrobiopterin and Activation of the Ubiquitin Proteasome System (Submitted for publication)

Frost-Pineda *et al. Nic. Tob. Res.* 2011, **13**:182-93

Garcia-Perez *et al. Bioanalysis.* 2014, **6(20)**:2733-49.

Gross *et al. Clin. Chem.* 2012, **58**:411-20

Haswell *et al.* The effect of cigarette smoke exposure on the proteomic composition of human bronchial epithelial cell air surface liquid. Society of Toxicology 2014, Abstract # 1530.

Haswell *et al.* A targeted proteomic comparison of human induced sputum from smokers and non-smokers. Society of Toxicology 2016, Abstract # 3041.

Jensen *et al. Thorax.* 1998, **53**:784-9

Kaluarachchi *et al.* A Multiplatform Metabolic Phenotyping Approach Integrated with Pathway Mapping to Identify Biochemical Differences between Healthy Smokers and Non-smokers. SOT 2016, Abstract #1107

Krämer *et al. Bioinformatics.* 2014, **30(4)**:523-30.

Laurent S *et al. Ann Med.* 2012;44 Suppl 1:S93-7.

Maeda *et al. Prev. Med.*2003, **37**:283-90

Milne *et al. Biomarkers*, 2005, **10** (Suppl): S10-23

Moffat, *Atherosclerosis* 1988, **75**:85-9

Oguogho *et al. Vasa* 2000, **29**:103-5

Palmer *et al. Eur J Clin Invest.* 2002, **32**:852-7

Pilz *et al. Thrombosis Research* 2000, 99:209-21

Rahman, *Cell Biochem Biophys.* 2005, **43**:167-88

Rångemark *et al. Arterioscler Thromb.* 1993, **13**: 777-82;

Saareks *et al. Naunyn Schmiedebergs Arch Pharmacol,* 2001, **363**:556-61

Shepperd CJ *et al. Regul. Toxicol. Pharmacol.* 2015, **72(2)**:273-91.

bat-science.com

@BAT_Sci

For more information