

OVERVIEW OF IN VITRO METHODS TO ASSESS ELECTRONIC NICOTINE DELIVERY SYSTEMS BASED ON 'TOXICITY TESTING IN THE 21ST CENTURY' PRINCIPLES

ENDS 2018

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OVERVIEW



Imperial Brands Harm Reduction Strategy

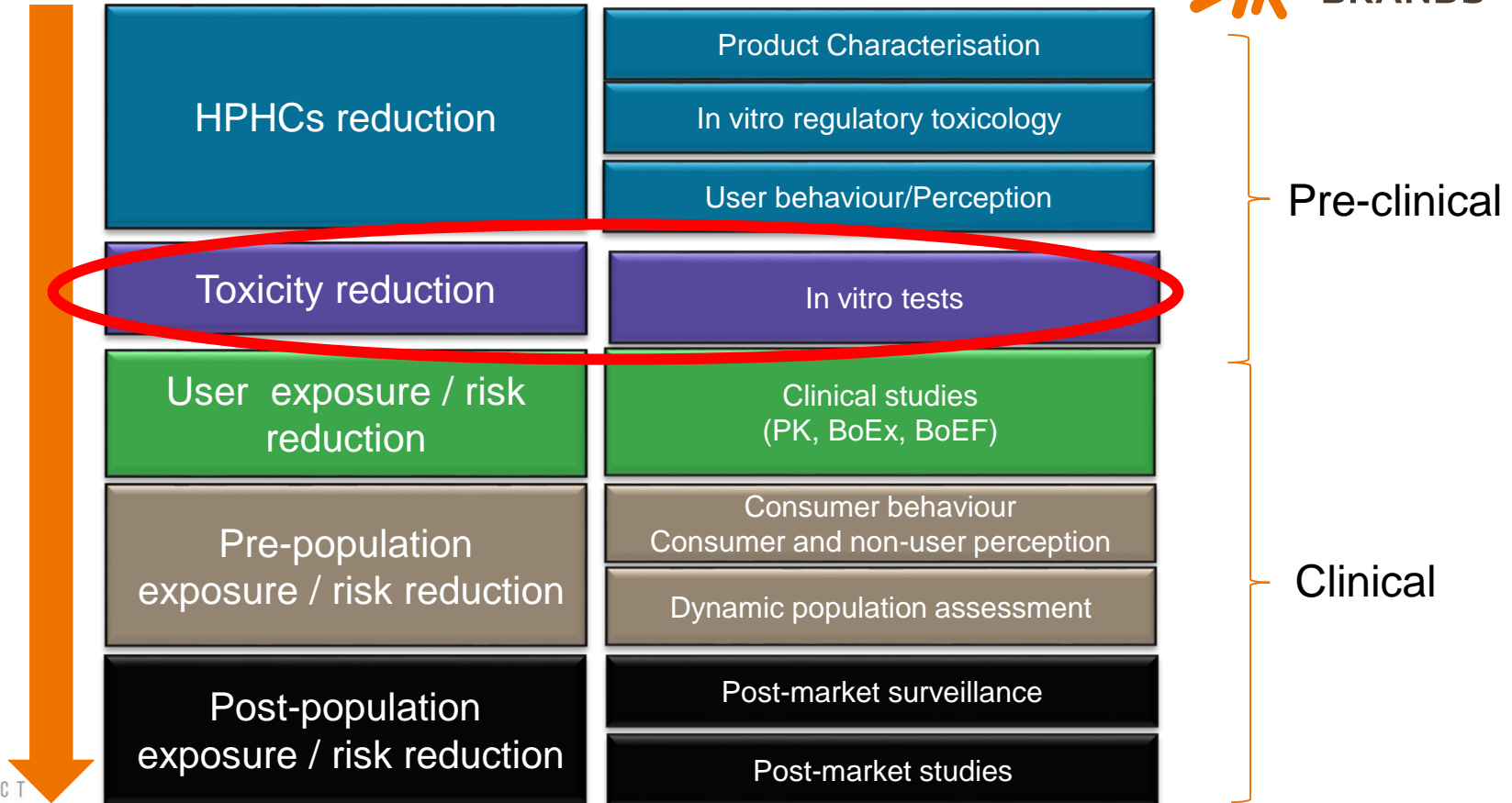
The *in vitro* TT21C Approach

In Vitro Data

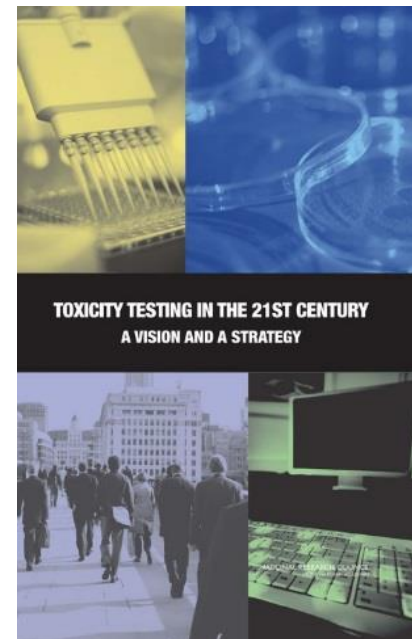
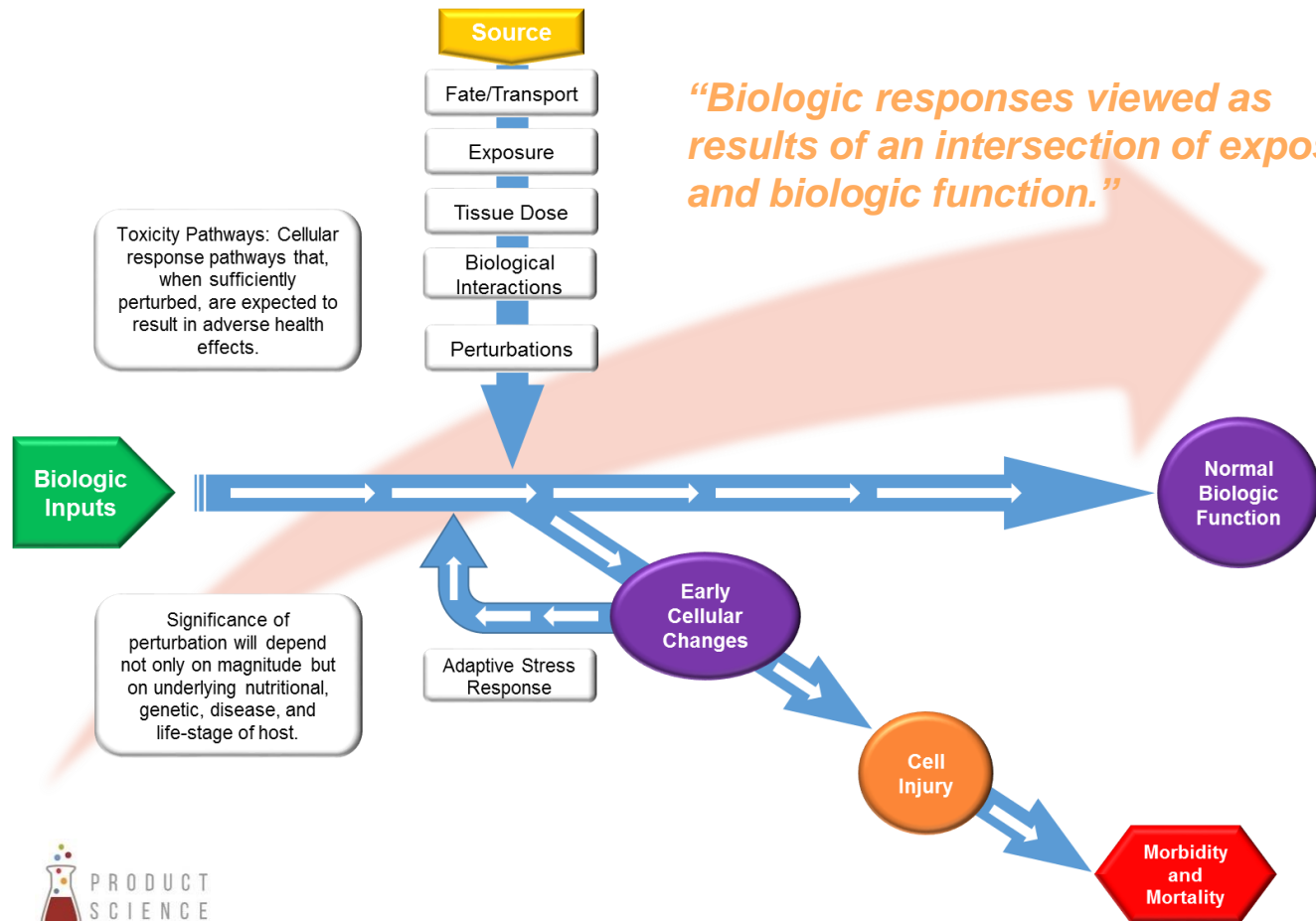
Overview of Systems Biology and AOPs

Concluding remarks

IMPERIAL BRANDS HARM REDUCTION STRATEGY



TT21C APPROACH

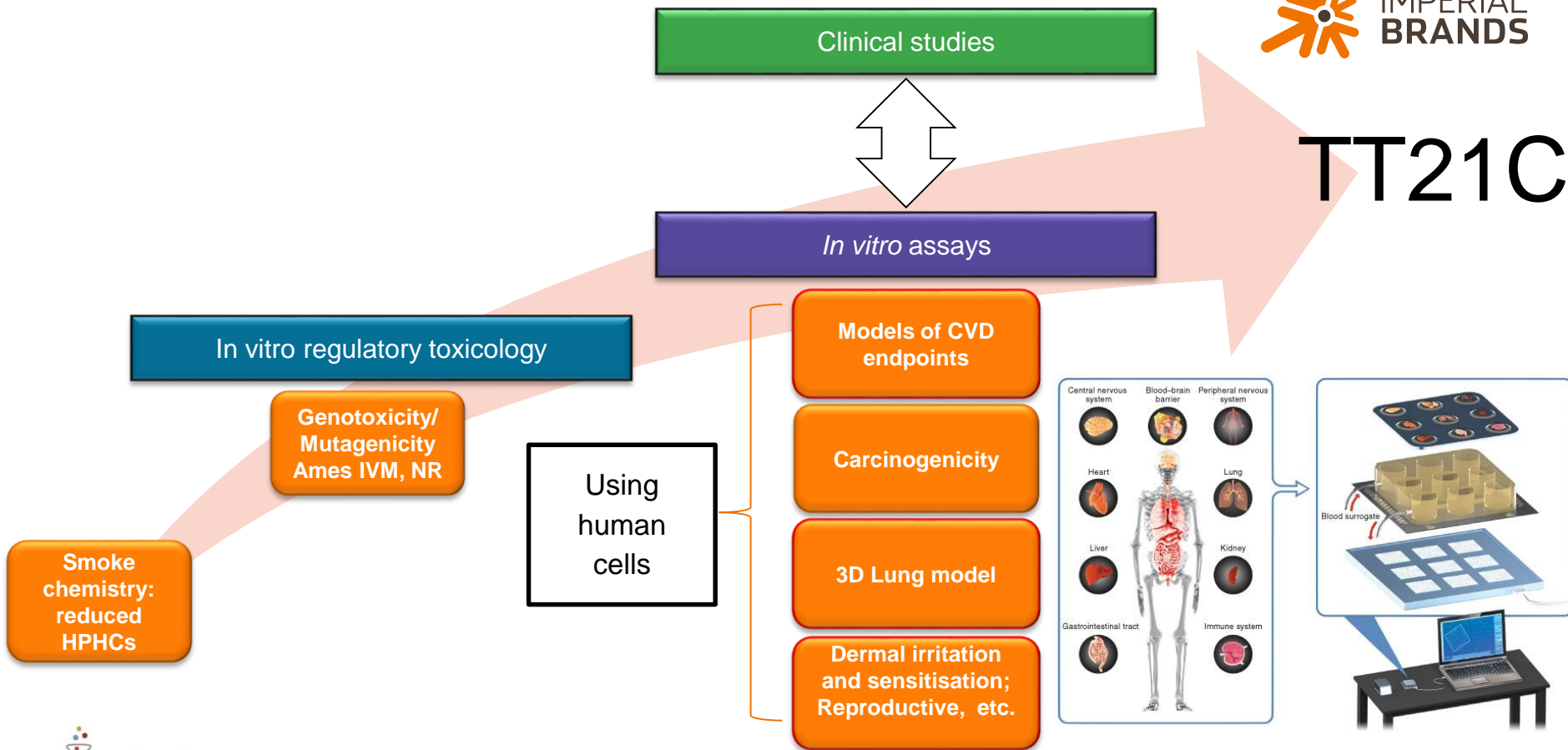


Adopted from National Research Council. (2007). *Toxicity testing in the 21st century: a vision and a strategy*. National Academies Press.

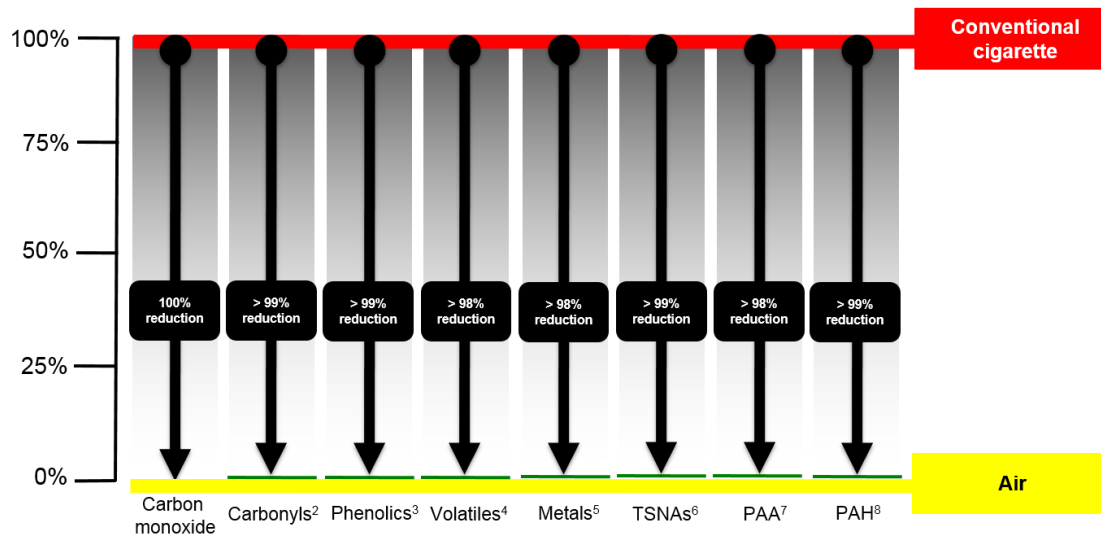
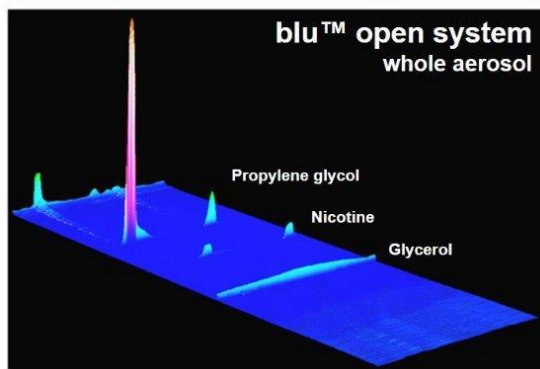
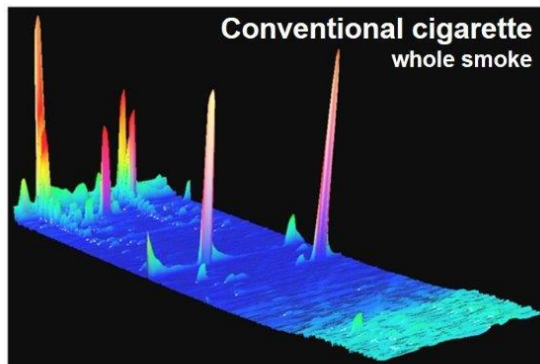
- Weight of Evidence (WoE) approach to:
 - Support the consensus that exposure to EVP aerosol results in less toxicity and harm than conventional cigarettes
 - Consists of regulatory approved assays and a mixture of simple and complex *in vitro* models, with lots of endpoints associated with tobacco related diseases
 - Studies undertaken to internationally agreed standards and to strict quality constraints to ensure reliability and robustness of the results obtained
 - Scientific scrutiny via peer reviewed publications

PRE-CLINICAL APPROACH

TT21C

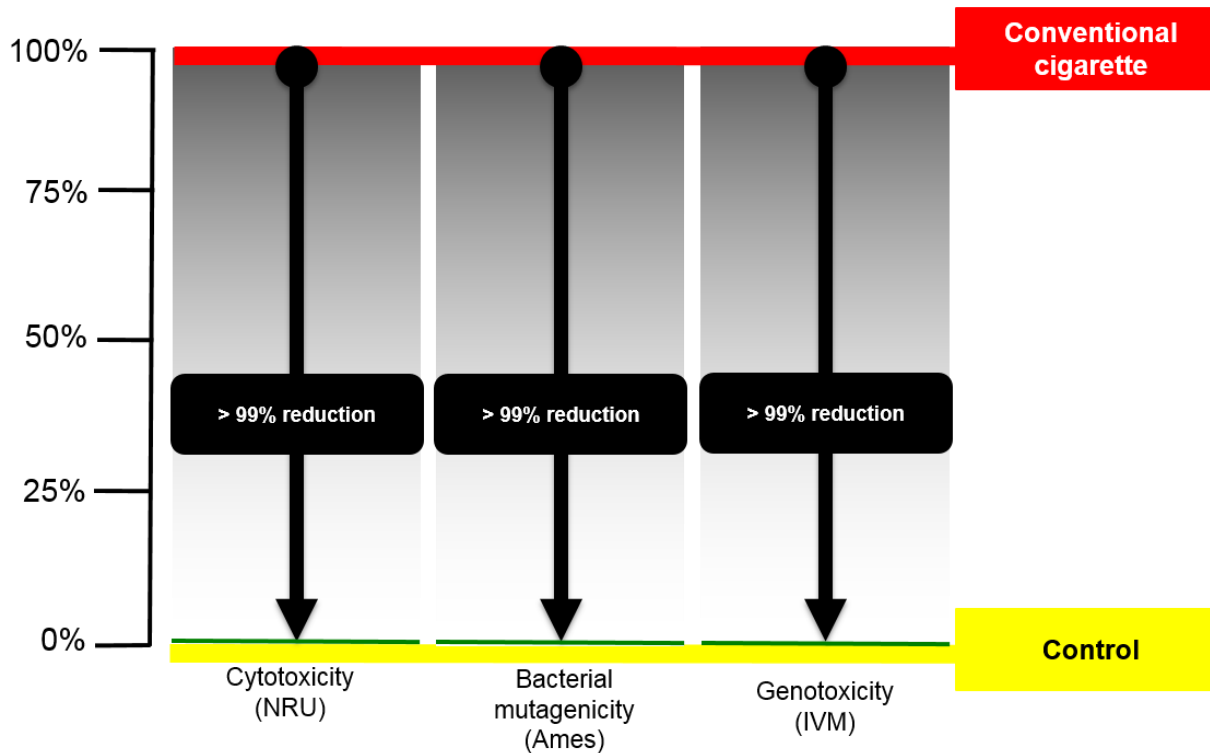


REDUCED FORMATION OF CHEMICAL ANALYTES IN EVP AEROSOL...



98-100% Reduction in number and levels of toxicants
and carcinogens in ENDS aerosols

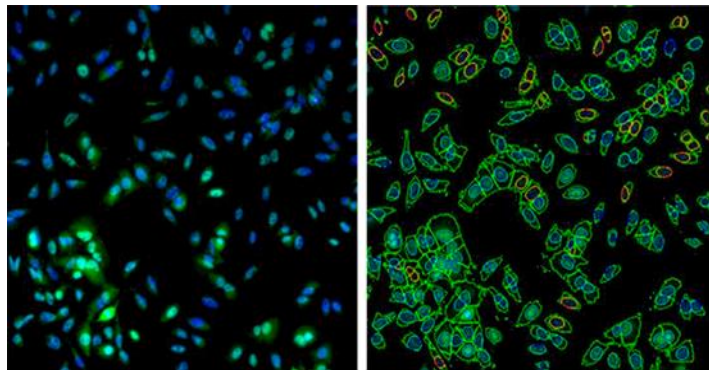
...LEADS TO REDUCED TOXICITY IN STANDARD TOXICOLOGY ASSAYS



RESULTS OF HIGH CONTENT SCREENING (HCS) ANALYSIS



HCS End point	Exposure time (4 & 24 hours)
Cell health	
Cell count	✓
Nuclear size	✓
DNA structure	✓
Mitochondrial mass	✓
Mitochondrial Membrane Potential	✓
Cell cycle arrest	✓
Cellular ATP	✓
Apoptosis	✓
Necrosis	✓
Transcriptional activation	
NFK β translocation	✓
Oxidative stress	
Glutathione content (GSH)	✓
oxidative stress (ROS)	✓



Test concentrations:

- 0.0002-0.5% e-liquid with NHBE* cells exposed for 4 and 24 hours. **No effects were seen.**
- 0.5, 1, 3, 4, 5, 7, 8, 10% e-liquid with NHBE cells exposed for 24 hours. Effects above 3% due to osmolarity effects.

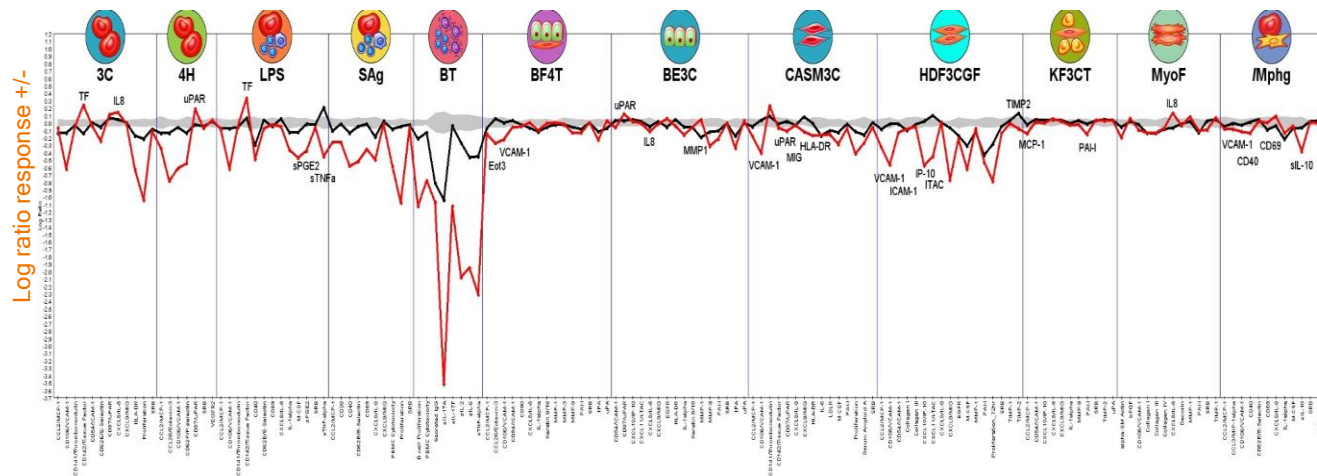
*NHBE Normal human bronchial epithelial cells



THE USE OF A MULTI-CELL LINE PANEL TO ASSESS FLAVOUR AND NICOTINE



System Name	Icon	Cell	Disease
3C		Venular endothelial cells	Cardiovascular Disease, Chronic Inflammation
4H		Venular endothelial cells	Allergy, Asthma, Autoimmunity
LPS		Peripheral blood mononuclear cells, Venular endothelial cells	Cardiovascular Disease, Chronic Inflammation
SAG		Peripheral blood mononuclear cells, Venular endothelial cells	Autoimmune Disease, Chronic Inflammation
BT		B cells, Peripheral blood mononuclear cells	Allergy, Asthma, Autoimmunity, Oncology
BF4T		Bronchial epithelial cells, Dermal fibroblasts	Allergy, Asthma, Fibrosis, Lung Inflammation
BE3C		Bronchial epithelial cells	COPD, Lung Inflammation
CASM3C		Coronary artery smooth muscle cells	Cardiovascular Inflammation, Restenosis
HDF3CGF		Dermal fibroblasts	Chronic Inflammation, Fibrosis
KF3CT		Dermal fibroblasts, Keratinocytes	Dermatitis, Psoriasis
MyoF		Lung fibroblasts	Chronic Inflammation, Fibrosis, Matrix Remodeling, Wound Healing
/Mphg		Macrophages, Venular endothelial cells	Cardiovascular Disease, Chronic Inflammation, Restenosis

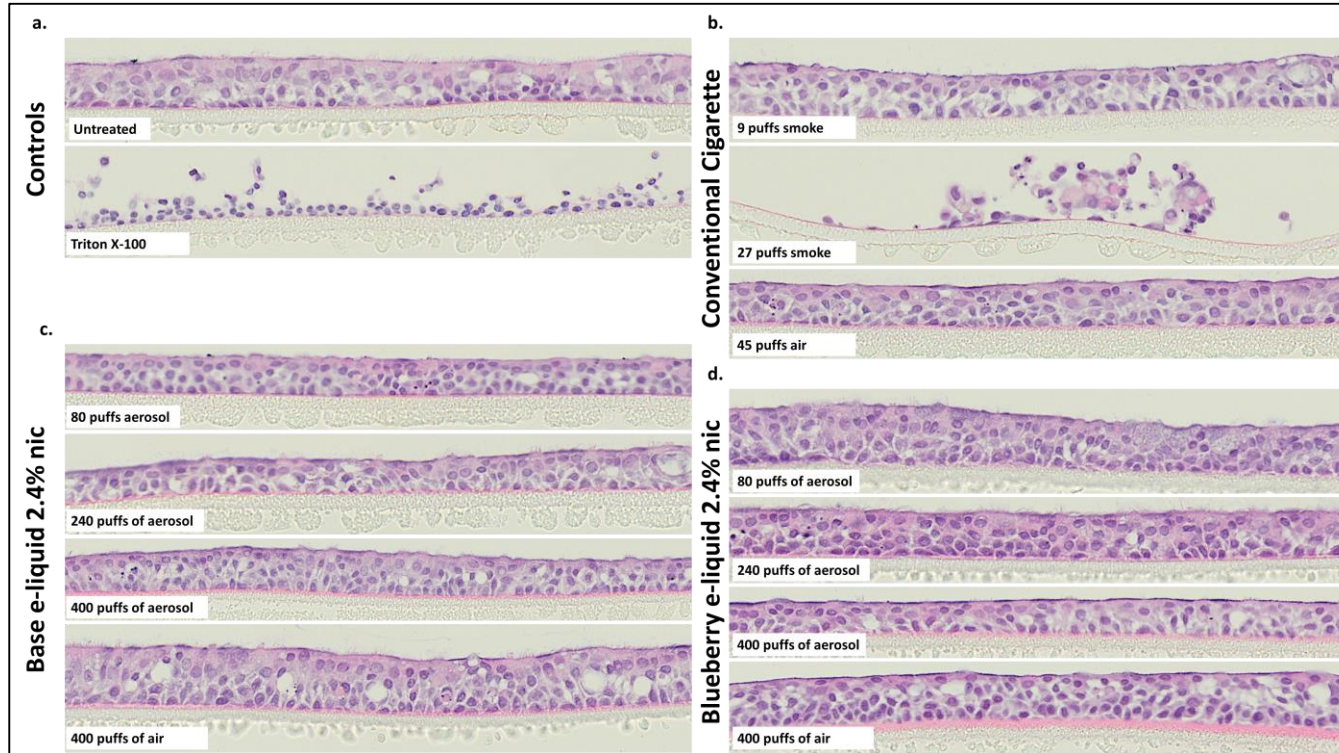


— Base Liquid 2.4% Nicotine

— Base Liquid 2.4% Nicotine + Flavour



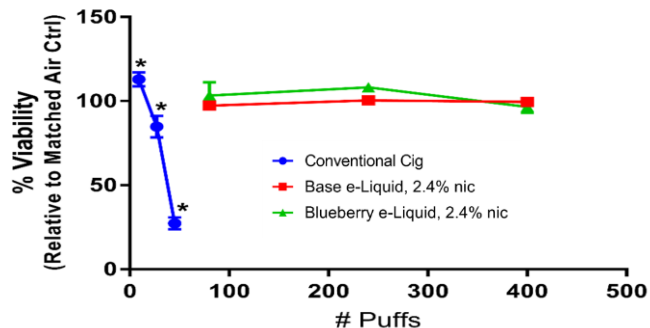
NO IMPACT OF EVP AEROSOL ON HUMAN 3D LUNG MODEL UP TO 400 PUFFS...



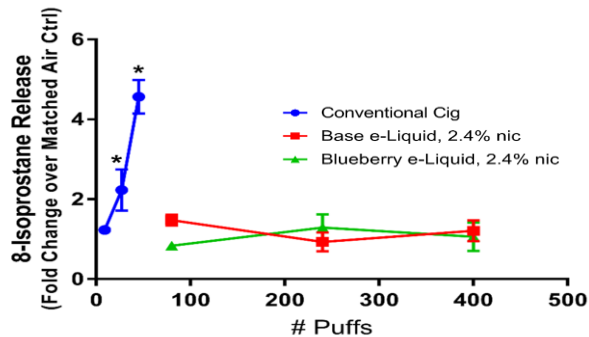
....AND ON A WIDE RANGE OF CELLULAR ENDPOINTS



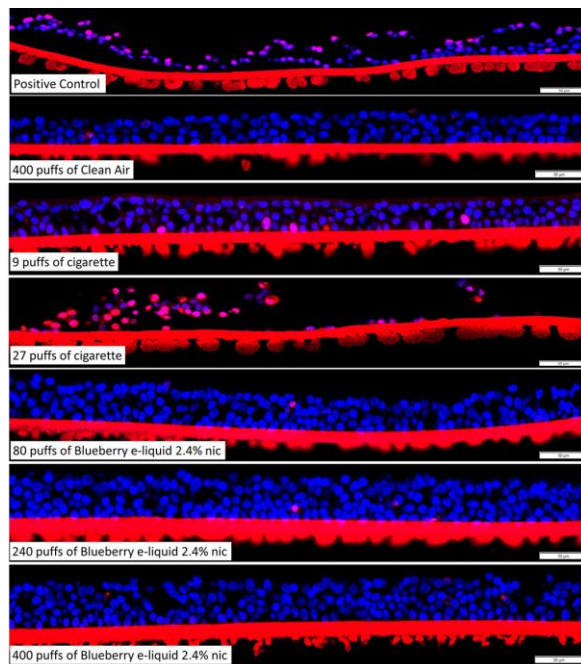
Tissue viability



The oxidative stress response

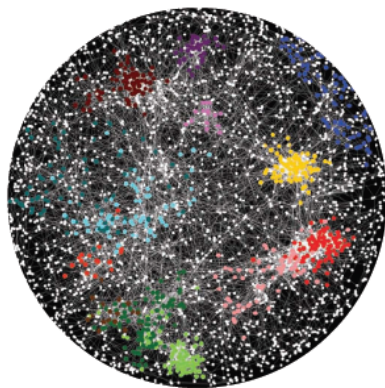


γ -H2AX staining and quantification as a marker of DNA double-strand breaks



SYSTEMS BIOLOGY (TOXICOLOGY)

Biology



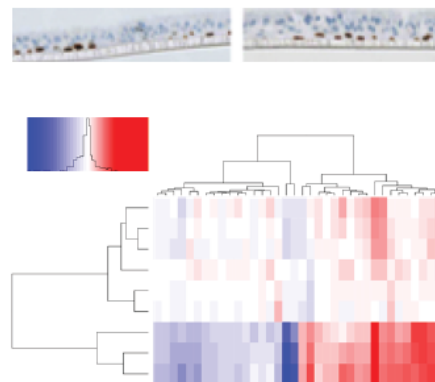
A biological system is a network of genes, proteins, lipids, metabolites and cells in an equilibrium

Bioinformatics



The networks of genes, proteins, metabolites interactions can be modelled with computer-based tools

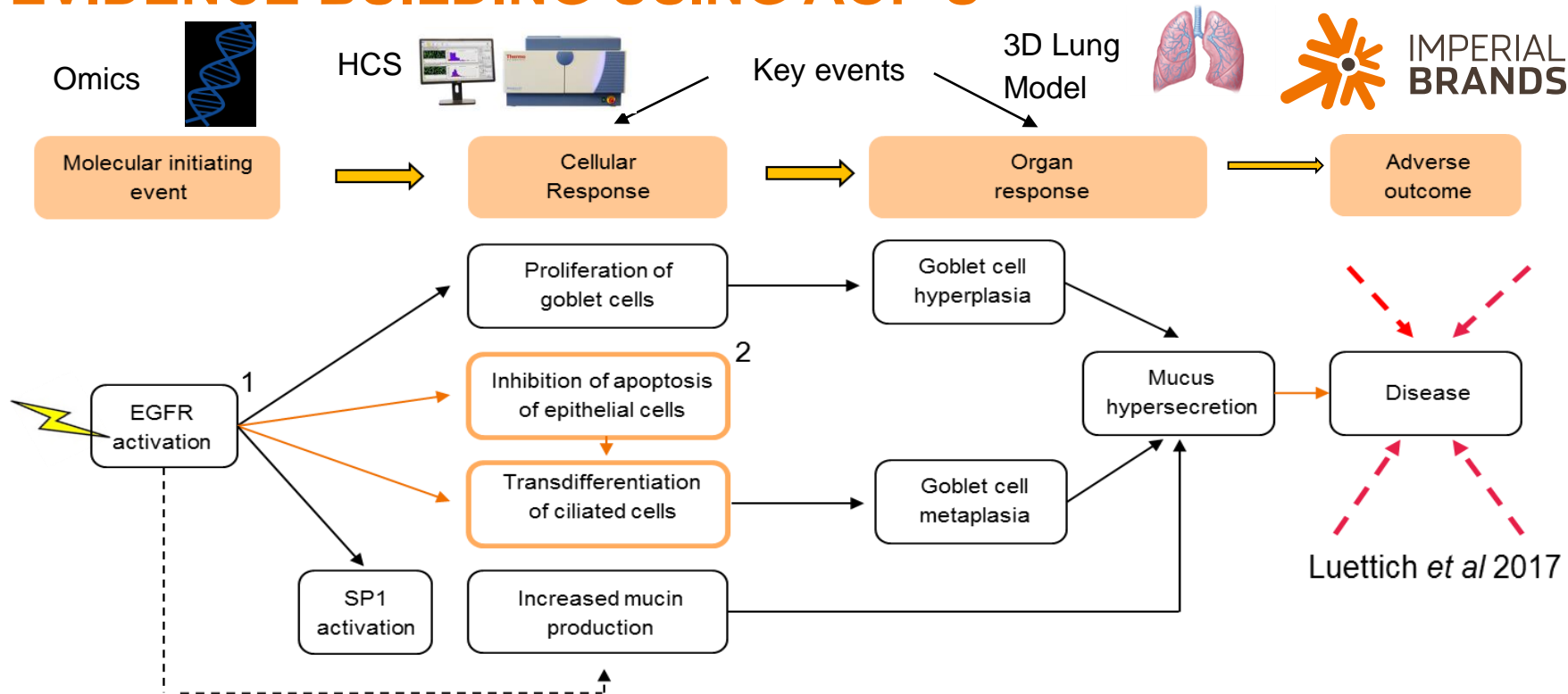
Perturbations (Tox)



The equilibrium of a system is perturbed by a toxic stress and can be measured by combining tox end points, OMICs and bioinformatics

Predictive pathway based disease information

EVIDENCE BUILDING USING AOP'S



Adverse outcome pathways (AOPs) are a way to organise data from different assays such as HCS, Omics and 3D Models to try to understand mechanisms of disease.

CONCLUSIONS



- Human relevant *in vitro* studies include HCS, HTS and Systems Toxicology (-omic technologies) to support a TT21C approach.
- These assays provide mechanistic and disease relevant based information and can be integrated with clinical studies.
- Imperial Brands are currently developing, publishing and utilising TT21C in conjunction with other approaches and technologies, as a weight of evidence approach in the assessment of ENDS.

ACKNOWLEDGEMENTS



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