

# The use of *in vitro* human biomarkers from relevant primary cell systems, to assess the effects of experimental and commercial e-liquids

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## 1. Introduction and Objectives

### 1.0 Introduction

As part of the ongoing stewardship of electronic cigarettes, and in line with the National Research Council (NRC) "Toxicity Testing in 21st Century: A Vision and a Strategy" and subsequent documents, Fontem Ventures B.V., have investigated the utility of a new technique offered by DiscoverX.

DiscoverX, is a leading supplier of in-house cell-based assays and services for the drug discovery and development industries. The BioMAP®, Diversity PLUS product was chosen, consisting of 12 primary human cell-based systems from multiple tissues designed to model different aspects of the human body in an *in vitro* format (see Table 1). Cells are cultured either alone or as co-cultures and stimulated with a combination of biological proprietary factors (e.g. cytokines, growth factors, mediators, etc.) to recreate the multi-component signalling networks seen in normal cells. The identification of and disruption of key cellular signalling pathways are one of the main focuses of the NRC report. The Diversity PLUS panel consists of 148 biomarker readouts and has been used as a tool for drug discovery, competitive analysis and as a comparison to clinical standards of care. Each test agent generates a signature BioMAP® profile that is created from the changes in protein biomarker readouts within individual system environments. Each readout is then measured quantitatively by immune-based methods that detect proteins released into the cell media (e.g., ELISA) or functional assays that measure proliferation and cell viability.

### 1.1 Objectives

In the first instance, base e-liquids (without flavours) with and without nicotine were used to determine the suitability of the BioMap® system. Next subsequent testing evaluated the effects of commercial flavoured e-liquids on the system. Additionally, the osmolality of the diluted e-liquids was also measured to examine the potential effects of osmotic stress due to high concentrations of Propylene glycol (PG) and Vegetable glycerine (VG) being added to the systems (Iskandar *et al.*, 2016; Gonzalez-Suarez *et al.*, 2017).

## 2. Overview of BioMap® Plus Panel

### 2.0 BioMap® Plus panel

Various disease states are potentially captured by the panel of 12 human primary cell lines. Vascular biology is modelled in both a Th1 (3C system) and a Th2 (4H system) inflammatory environment, as well as in a Th1 inflammatory state specific to arterial smooth muscle cells (CASM3C system). Additional systems recreate entire aspects of the systemic immune response including monocyte-driven Th1 inflammation (LPS system) or T cell stimulation (SAG system), chronic Th1 inflammation driven by macrophage activation (/Mphg system) and the T cell-dependent activation of B cells that occurs in germinal centres (BT system).

The BE3C system (Th1) and the BF4T system (Th2) represent potential markers of airway inflammation of the lung, whilst the MyoF system models myofibroblast-lung tissue remodelling. Lastly, skin biology is addressed in the KF3CT system modelling Th1 cutaneous inflammation and the HDF3CGF system models potential wound healing. Whilst disease progression is not currently well understood its entirety, inflammation is one of the key process that has been implicated in a number of diseases.

Table 1: Systems in BioMAP PLUS panel

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

## 3. Results: Effects of increasing concentrations of nicotine in base e-liquids and commercial e-liquids on the BioMAP® profile

Chart 1: The effects of Base liquid, ± 2.4 or 4.5% nicotine added to BioMAP at 1% concentration

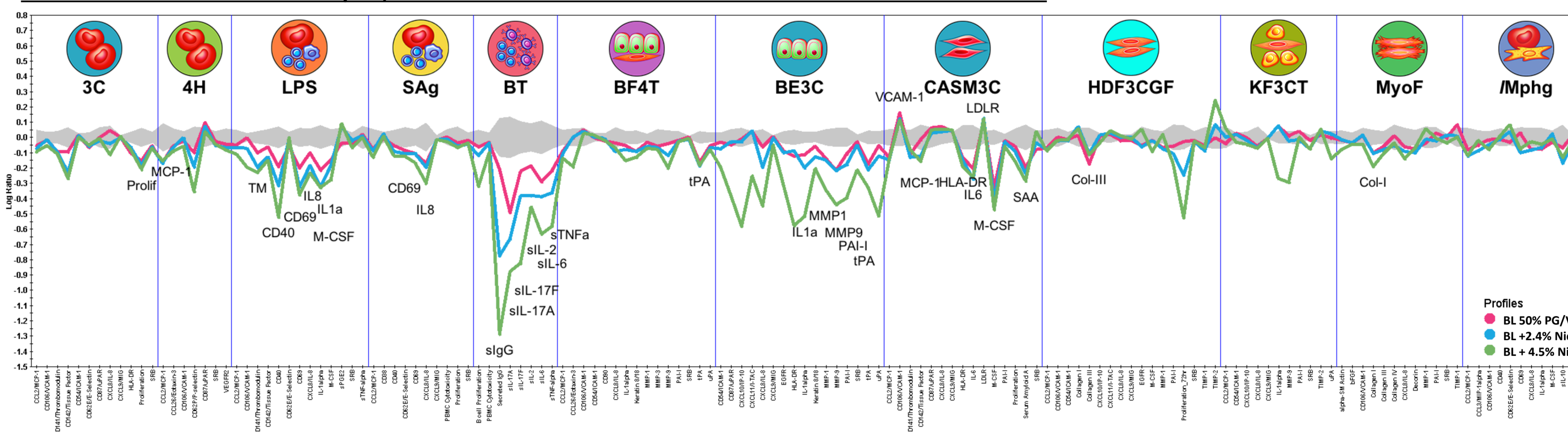


Chart 1: Results of a base e-liquid (BL 1 containing only 50:50 PG and VG ); BL 2 containing 2.4% nicotine and BL 3 with a nicotine content of 4.5% were tested at eight concentrations ranging between 0.031 to 4% added directly to the cell media. The grey area in the middle of the chart represents the historical control values (95% confidence interval). The Y-axis represents a log-transformed ratio of the biomarker readouts for the test agent-treated sample (n = 1) over vehicle controls (average of ≥ 6 vehicle controls from the same plate), cell variability, with values outside of this grey area (positive or negative) being significant.

Chart 2: The effects of flavoured commercial e-liquid plus nicotine added to BioMAP at 0.5% concentration

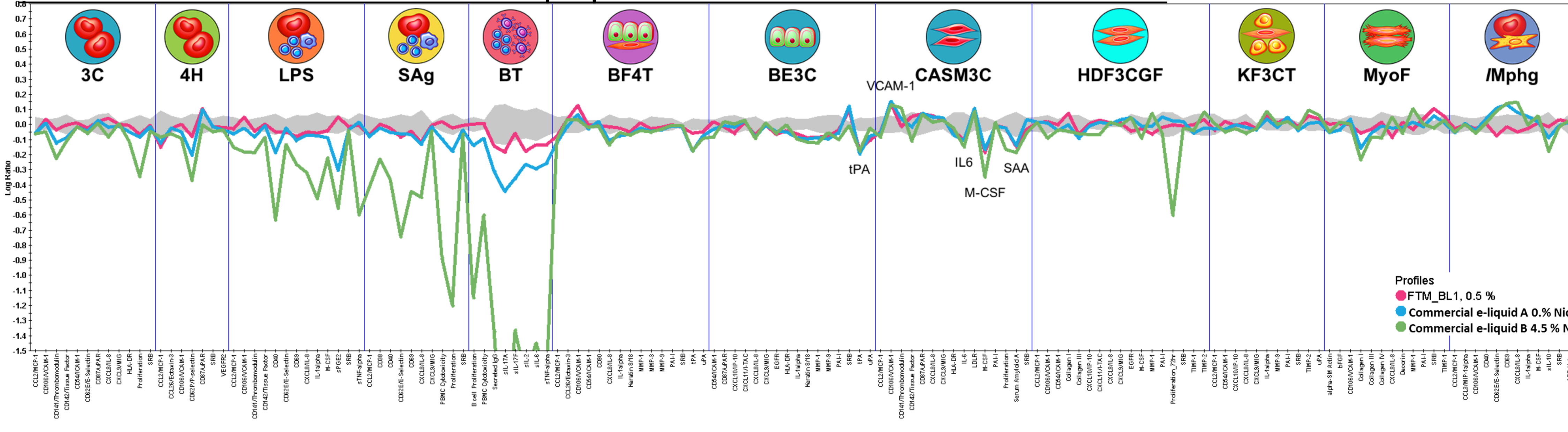


Chart 2: There is an effect of adding commercial e-liquid A above that of the base liquid BL1 in the cell lines LPS, SAG, BT, Mphg at 0.5% with a considerably more marked effect of adding nicotine at 4.5% w/w to the commercial e-liquid B in the following cell lines 3C, LPS, SAG, BT; HDF3CGF. Concentrations were chosen to be neither cytotoxic or anti-proliferative. At both of the concentrations used osmolality was not considered to be an issue being in the range of 300-400 milliosmoles/litre.

## 4. Conclusions

- The addition of nicotine to base liquid gave rise to a significant decrease in biomarkers which were concentration related, in addition to those observed by PG/VG base liquid itself. Certain cell lines relating to BT (peripheral blood mononuclear cells), BE3C (Bronchial epithelial cells) and HDF3CGF/KF3CT (dermal fibroblasts) and LPS peripheral blood cells and endothelial cells were most affected.
- The effects of adding a commercial flavour to the base liquid appear to be minor compared to the effects of adding nicotine
- Some limitations of the assay include a reduced metabolic capacity of the cells and no liver model.
- The use of human primary cells are highly relevant model for the assessment of potential effects in humans.
- The assay appears to potentially be a useful tool for both comparisons between products and adds significantly to a weight of evidence risk assessment approach.

### Future work

- To use e-vapour aerosol extracts in the assay and use of a comparator product (i.e. cigarette) to see relative changes.
- To refine the exposures to physiologically relevant concentrations.

## References

- NRC (2007) Toxicity testing in 21<sup>st</sup> Century- A vision and a strategy <https://www.nap.edu/catalog/11970/toxicity-testing-in-the-21st-century-a-vision-and-a-strategy>
- Iskander *et al.*, (2016) Toxicol Mech Methods 26(6) 389-413.
- Gonzalez-Suarez *et al.*, (2017) App In vitro Toxicol 3(1): 41-55.