Assessing Changes In Biomarkers Of Effect In Smokers Who Switch To A Closed System Electronic Cigarette

Liz Mason | Kunming, China | 26th October 2018

IMPERIAL BRANDS SCIENCE

CONTENT

IMPERIAL BRANDS SCIENCE

VAPOUR PRODUCTS HAVE BEEN SHOWN TO REDUCE BIOMARKERS OF EXPOSURE

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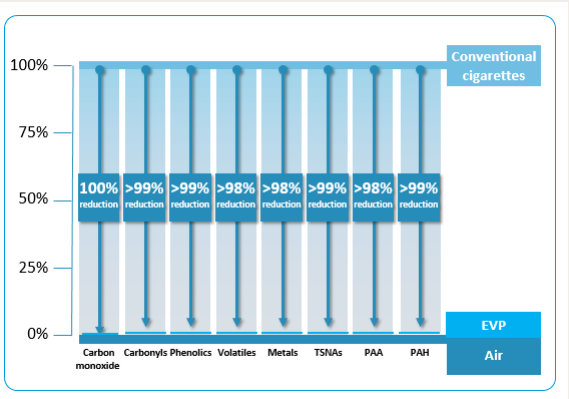
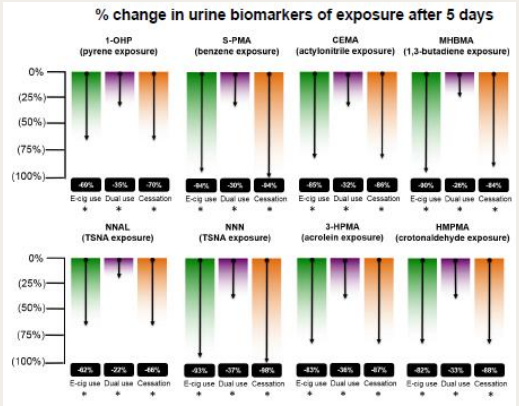
SCIENCE

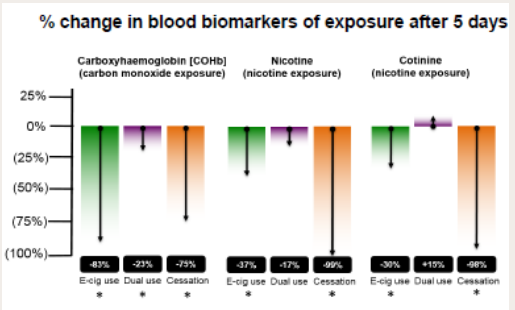
Previous studies have demonstrated that when a smoker switches to a vapour product, they are exposed to significantly lower levels of carcinogens and toxicants in the aerosol...

Reduced formation of toxicants in blu™ e-cigarette aerosol vs. conventional cigarette smoke



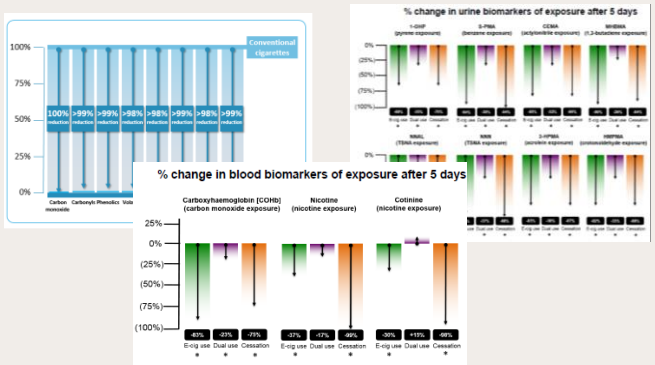
…and significant reductions in biomarkers of exposure…



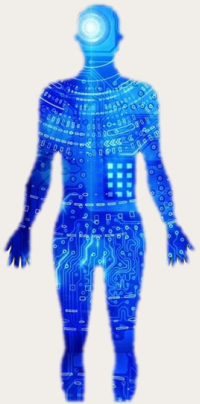


"Chemical Composition of myblu™ Pod-System E-Cigarette Aerosols: A Quantitative Comparison with Conventional Cigarette Smoke", Poster presentation, 1st Scientific Summit Tobacco Harm Reduction (http://www.fontemscience.com/wp-content/uploads/2018/06/2018-04-18-aerosol-chemistry-thr-summit-2018-poster_final.pdf); Tayyarah R and Long GA. "Comparison of select analytes in aerosol from e-cigarettes with smoke from conventional cigarettes and with ambient air." Regulatory toxicology and pharmacology 70 3 (2014). O'Connell et al (2016): Reductions in biomarkers of exposure (BoE) to harmful or potentially harmful constituents (HPHCs) following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers, Toxicology Mechanisms and Methods, DOI: 10.1080/15376516.2016.1196282. http://www.fontemscience.com/wp-content/uploads/2017/05/fontem2research-1.pdf

VAPOUR PRODUCTS HAVE BEEN SHOWN TO REDUCE BIOMARKERS OF EXPOSURE



What is the biological impact when a smoker switches to a vapour product?



Clinical markers:

- can be defined as a measurable biochemical, physiologic, behavioural, or other alteration in an organism.
- can point to sub-clinical changes which, if left unchecked, may lead to a disease state.

Monitoring changes in clinical markers when a smoker switches to a vapour product may provide us with a better understanding of the harm reduction potential of these products.

"Chemical Composition of myblu™ Pod-System E-Cigarette Aerosols: A Quantitative Comparison with Conventional Cigarette Smoke", Poster presentation, 1st Scientific Summit Tobacco Harm Reduction (http://www.fontemscience.com/wp-content/uploads/2018/06/2018-04-18-aerosol-chemistry-thr-summit-2018-poster_final.pdf); Tayyarah R and Long GA. "Comparison of select analytes in aerosol from e-cigarettes with smoke from conventional cigarettes and with ambient air." Regulatory toxicology and pharmacology 70 3 (2014).
O'Connell et al (2016): Reductions in biomarkers of exposure (BoE) to harmful or potentially harmful constituents (HPHCs) following partial or complete substitution of cigarettes with electronic cigarettes in adult smokers, Toxicology Mechanisms and Methods, DOI: 10.1080/15376516.2016.1196282

CLINICAL MARKERS CAN BE DETECTED BY TARGETED OR UNTARGETED ANALYSES

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Targeted

- Defined and specific number of metabolites
- Chemically and biochemically characterised
- Greater selectivity and sensitivity

- Biased by what we already know

Untargeted

- Unbiased
- 100s to 1000s of metabolites can be measured
- May see unexpected changes

- Data processing of untargeted analysis can be challenging

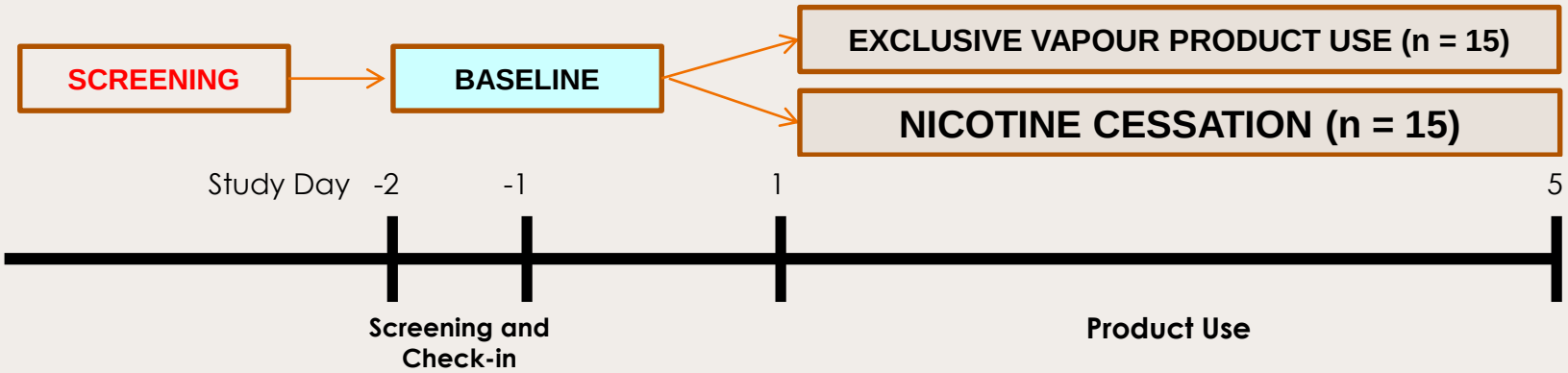
STUDY AIM

- AIM: To evaluate changes in clinical markers when healthy smokers switch from conventional cigarettes to a typical closed system electronic cigarette.

Study	Device	Time points compared	Analytical method
5 day confined clinical study	Blu Tobacco flavour, 2.4% nicotine	BL vs 5 day	- Targeted analysis of potential clinical markers*
Two year 'real-life' ambulatory study	Puritane Tobacco or menthol flavour, 1.6% nicotine	BL vs 24 month	- Targeted analysis of potential clinical markers* - Non-biased untargeted metabolomics approach to find potential clinical markers

*147 potential clinical markers from Myriad RBM's CardioMAP, ImmunoMAP, and InflammationMAP biomarker panels

5 DAY CLINICAL STUDY UNDER CONFINED CONDITIONS – *STUDY DESIGN*



- Compared smokers who switched to a closed system e-cigarettes (blu) against those who quit conventional cigarettes unassisted after 5 days
- Blood samples taken at baseline and Day 5; these underwent targeted biomarker analysis

NONE OF THE TARGETED BIOMARKERS SHOWED SIGNIFICANT CHANGES IN THE ACUTE STUDY

- When comparing the cessation group vs. exclusive vapour product group, between group analysis did not highlight any significant changes in the 147 clinical markers measured in this study following a 5-day product switch.
    - The changes between the groups were small compared to the general donor-to-donor variability.

- After correcting for donor-to-donor variability, ICAM1 showed a non-significant decreased for e-cigarette users compared from the nicotine cessation group but this wasn't statistically significant

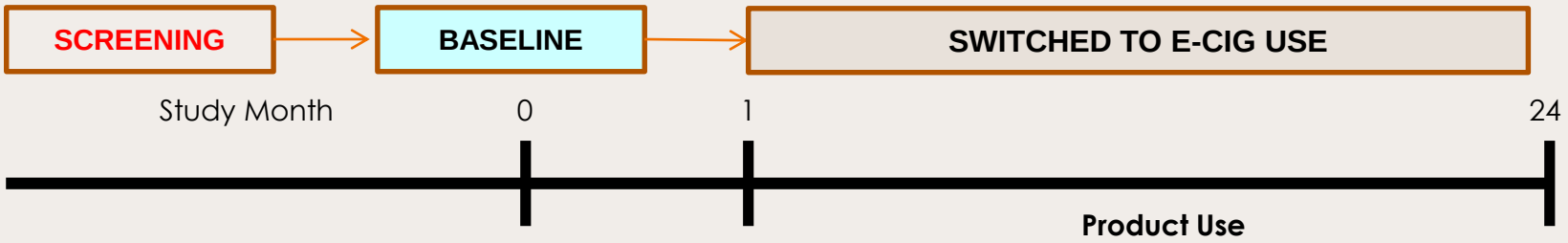
NONE OF THE TARGETED BIOMARKERS SHOWED SIGNIFICANT CHANGES IN THE ACUTE STUDY

- As part of this study, we previously reported* that although significant reductions in biomarkers of exposure were observed, only small but not statistically significant improvements in cardiovascular and pulmonary function were seen after 5 days

Is 5 days enough time to see changes in clinical markers?

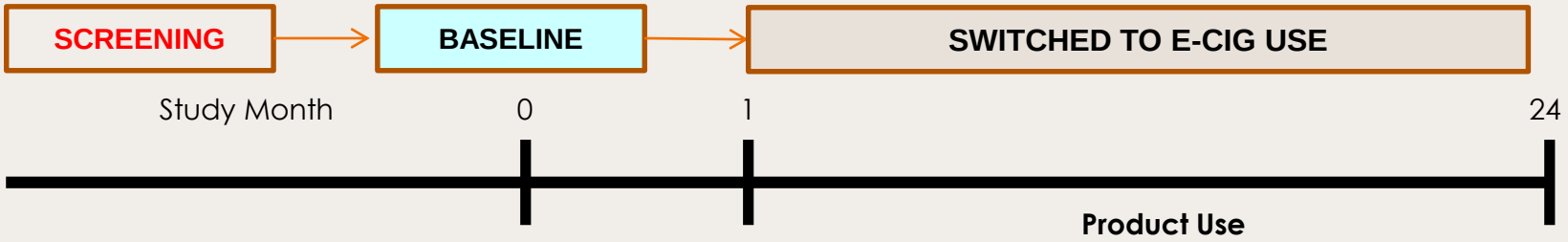
*D'Ruiz et al (2017) Reg Tox & Pharm, 87;36-53

TWO YEAR ‘REAL-LIFE’ AMBULATORY STUDY – *STUDY DESIGN*



- 209 subjects enrolled in the study
- Two flavour choices: tobacco or menthol (subjects were allowed to change flavour over the course of the study).
- The nicotine concentration of the product was 1.6%.
- Ambulatory study design; blood samples taken and protocol compliance checked at regular intervals

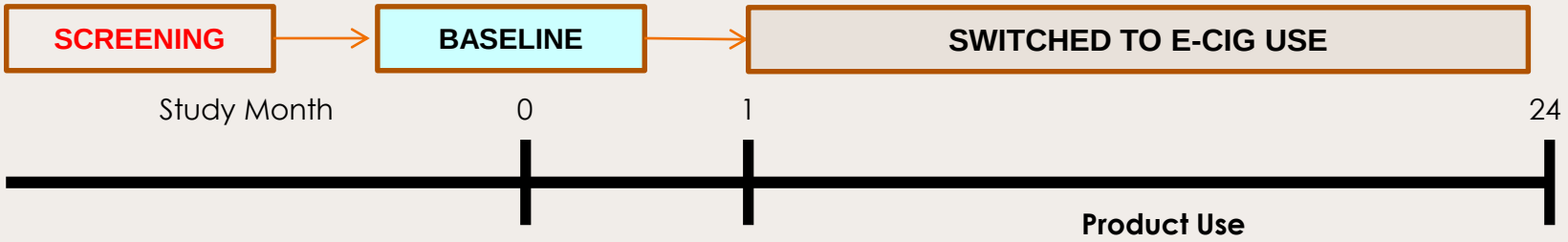
TWO YEAR ‘REAL-LIFE’ AMBULATORY STUDY – *STUDY DESIGN*



- Reported primary outcomes from this clinical trial after 24 months:
  - Significant decreases in biomarkers of exposure
  - Small decreases in lung function were observed but were not clinically relevant
  - Other parameters such as heart rate, blood pressure and body weight stayed stable
  - White blood cell, HDL and LDL, and haemoglobin levels also remained stable.

Walele et al (2018) Evaluation of the safety profile of an electronic vapour product used for two years by smokers in a real-life setting. Regulatory Toxicology and Pharmacology 92 (2018) 226–238.

TWO YEAR ‘REAL-LIFE’ AMBULATORY STUDY – *STUDY DESIGN*



- 70/209 completed the two year trial with 80% compliance (43 males, 27 females)
- Baseline and 24 month samples were analysed:
  - Specific and targeted analysis of potential clinical markers, and
  - Non-biased untargeted metabolomics approach

UNTARGETED ANALYSIS SUGGESTS SEVERAL BIOCHEMICAL PATHWAYS MAY BE AFFECTED BY VAPOUR PRODUCT USE

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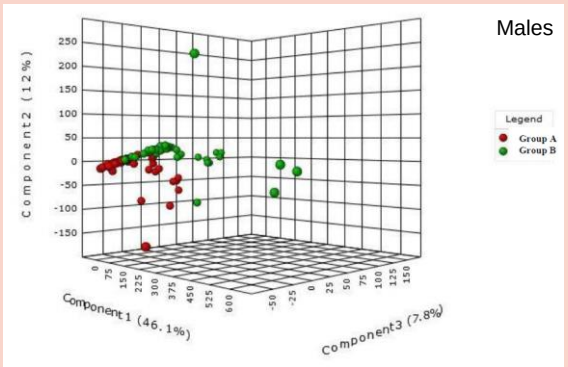
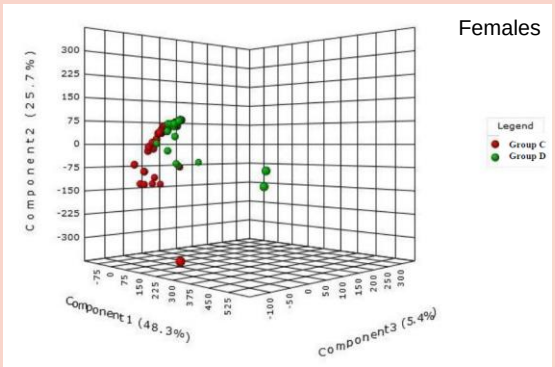
Targeted	Untargeted
No significant changes in the 147 measured clinical markers	Moderate separation between baseline and 24 month samples by PCA   Significant alteration in metabolites involved in: • Amino acid metabolism • Sertonin/tryptophan metabolism • Arachidonic acid metabolism

IMPLICATED BIOCHEMICAL PATHWAYS
AMINO ACID METABOLISM

- Several amino acids showed a significant alterations following a two year switch from smoking to a Vapour Product

Males and Females ↓ alanine, asparagine ↑ lysine

Males ↓ alanine, leucine, serine, phenylalanine, lanthionine, cysteine

Females ↑ proline ↓ glycine

- Similar changes in amino acid metabolism were observed in a previous study when looking at changes in untargeted biomarkers in smokers who quit nicotine unassisted.

Goettel et al, (2017) Metabolomic fingerprinting in various body fluids of a diet-controlled clinical smoking cessation study using a validated GC-TOF-MS metabolomics platform. 16(10):3491-3503.

IMPLICATED BIOCHEMICAL PATHWAYS
SEROTONIN/TRYPTOPHAN METABOLISM

Metabolomic changes in the serotonin biosynthesis in plasma and urine of smokers after having quit for 3 months

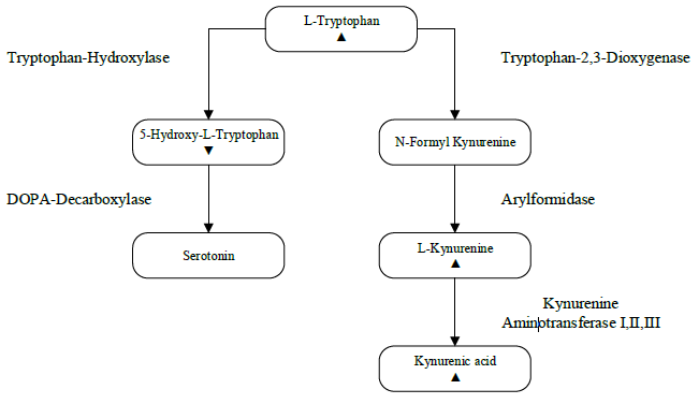


Figure from Goettel et al, (2017) Metabolomic fingerprinting in various body fluids of a diet-controlled clinical smoking cessation study using a validated GC-TOF-MS metabolomics platform. 16(10):3491-3503.

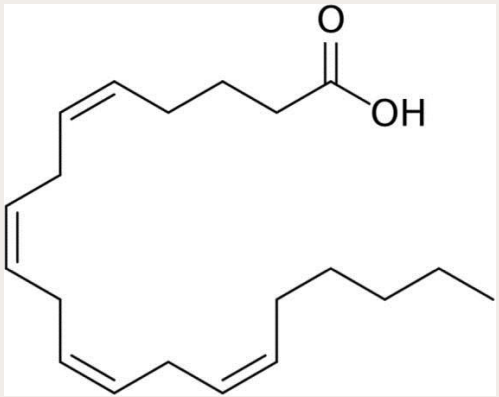
In this study:

- Females showed significant decreases in serotonin after 24 months Vapour Product use ($p<0.001$).
- Males and females showed a significant increase in 5-hydroxyindoleacetic acid at 24 months ($p<0.001$).
- Indicates a shift towards serotonin in smokers and away from serotonin in vapers, in agreement with the results from the smoking cessation study.

IMPLICATED BIOCHEMICAL PATHWAYS
ARACHIDONIC ACID METABOLISM

- Arachidonic acid is a precursor of several eicosanoid species
    - Eicosanoids are well-established clinical markers indicating oxidative stress and inflammation
- Arachidonic acid significantly increased after 24 months in smokers who switched to vaping
- These findings are in agreement with the results from the smoking cessation study, showing increased levels of arachidonic acid after 3 months of smoking cessation

Arachidonic acid hairpin conformation



Hanna and Hafez (2018) J Adv Res. 2018 May; 11: 23–32.

OVERALL CONCLUSIONS

- Minimal changes in targeted clinical markers were observed in the acute or longer-term study
- Untargeted clinical markers were identified with small but statistically significant changes upon switching from a tobacco cigarette to a Vapour Product after 24 months
- These biomarkers belonged to similar biochemical pathways that have been implicated during a smoking cessation study
- Further long-term studies with stricter compliance thresholds may demonstrate more substantial effects of product switching

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Ana-Maria Cravo
Simon Craige
Carl D'Ruiz

celerion

ABF

COVANCE

PROTAVIO

MYRIAD RBM

Thank you.

www.imperialbrandsscience.com

Supplementary Information

TARGETED BIOMARKERS

CUSTOM MAP USING MYRIAD RBM CARDIOMAP, IMMUNOMAP, AND INFLAMMATIONMAP BIOMARKER PANELS

Cardio	Adiponectin	Adiponectin		Inflammation	Alpha-1-Antitrypsin	AAT		Immuno	Angiopoietin-2	ANG-2
Cardio	Apolipoprotein A-I	Apo A-I		Inflammation	Alpha-2-Macroglobulin	A2Macro		Immuno	AXL Receptor Tyrosine Kinase	AXL
Cardio	Apolipoprotein A-II	Apo A-II		Inflammation	Beta-2-Microglobulin	B2M		Immuno	B Lymphocyte Chemoattractant	BLC
Cardio	Apolipoprotein B	Apo B		Inflammation	Brain-Derived Neurotrophic Factor	BDNF		Immuno	CD40 Ligand	CD40-L
Cardio	Apolipoprotein C-I	Apo C-I		Inflammation	C-Reactive Protein	CRP		Immuno	Epithelial-Derived Neutrophil-Activating Protein 78	ENA-78
Cardio	Apolipoprotein C-III	Apo C-III		Inflammation	Complement C3	C3		Immuno	Eotaxin-3	Eotaxin-3
Cardio	Apolipoprotein D	Apo D		Inflammation	Eotaxin-1	Eotaxin-1		Immuno	Erythropoietin	EPO
Cardio	Apolipoprotein E	Apo E		Inflammation	Factor VII	Factor VII		Immuno	E-Selectin	E-Selectin
Cardio	Apolipoprotein H	Apo H		Inflammation	Ferritin	FRTN		Immuno	FASLG Receptor	FAS
Cardio	N-terminal prohormone of brain natriuretic peptide	NT proBNP		Inflammation	Fibrinogen	Fibrinogen		Immuno	Monokine Induced by Gamma Interferon	MIG
Cardio	C-Reactive Protein	CRP		Inflammation	Granulocyte-Macrophage Colony-Stimulating Factor	GM-CSF		Immuno	Granulocyte Colony-Stimulating Factor	G-CSF
Cardio	CD40 Ligand	CD40-L		Inflammation	Haptoglobin	Haptoglobin		Immuno	Chemokine CC-4	HCC-4
Cardio	Chromogranin-A	CgA		Inflammation	Intercellular Adhesion Molecule 1	ICAM-1		Immuno	Hepatocyte Growth Factor	HGF
Cardio	Clusterin	CLU		Inflammation	Interferon gamma	IFN-gamma		Immuno	Immunoglobulin E	IgE
Cardio	Cystatin-C	Cystatin-C		Inflammation	Interleukin-10	IL-10		Immuno	Interleukin-13	IL-13
Cardio	EN-RAGE	EN-RAGE		Inflammation	Interleukin-12 Subunit p40	IL-12p40		Immuno	Interleukin-16	IL-16
Cardio	E-Selectin	E-Selectin		Inflammation	Interleukin-12 Subunit p70	IL-12p70		Immuno	Interleukin-6 receptor	IL-6r
Cardio	Factor VII	Factor VII		Inflammation	Interleukin-15	IL-15		Immuno	Interferon gamma Induced Protein 10	IP-10
Cardio	Fatty Acid-Binding Protein, heart	FABP, heart		Inflammation	Interleukin-17	IL-17		Immuno	Monocyte Chemotactic Protein 2	MCP-2
Cardio	Ferritin	FRTN		Inflammation	Interleukin-18	IL-18		Immuno	Monocyte Chemotactic Protein 4	MCP-4
Cardio	Fetuin-A	Fetuin-A		Inflammation	Interleukin-1 alpha	IL-1 alpha		Immuno	Macrophage-Derived Chemokine	MDC
Cardio	Fibrinogen	Fibrinogen		Inflammation	Interleukin-1 beta	IL-1 beta		Immuno	Macrophage Migration Inhibitory Factor	MIF
Cardio	Granulocyte Colony-Stimulating Factor	G-CSF		Inflammation	Interleukin-1 receptor antagonist	IL-1ra		Immuno	Macrophage Inflammatory Protein-3 alpha	MIP-3 alpha
Cardio	Granulocyte-Macrophage Colony-Stimulating Factor	GM-CSF		Inflammation	Interleukin-2	IL-2		Immuno	Matrix Metalloproteinase-1	MMP-1
Cardio	Haptoglobin	Haptoglobin		Inflammation	Interleukin-23	IL-23		Immuno	Matrix Metalloproteinase-10	MMP-10
Cardio	Intercellular Adhesion Molecule 1	ICAM-1		Inflammation	Interleukin-3	IL-3		Immuno	Matrix Metalloproteinase-7	MMP-7
Cardio	Interferon gamma	IFN-gamma		Inflammation	Interleukin-4	IL-4		Immuno	Matrix Metalloproteinase-9, total	MMP-9, total
Cardio	Immunoglobulin M	IgM		Inflammation	Interleukin-5	IL-5		Immuno	Myeloid Progenitor Inhibitory Factor 1	MPIF-1
Cardio	Interleukin-10	IL-10		Inflammation	Interleukin-6	IL-6		Immuno	Myeloperoxidase	MPO
Cardio	Interleukin-18	IL-18		Inflammation	Interleukin-7	IL-7		Immuno	Thrombospondin-1	Thrombospondin-1
Cardio	Interleukin-1 alpha	IL-1 alpha		Inflammation	Interleukin-8	IL-8		Immuno	TNF-Related Apoptosis-Inducing Ligand Receptor 3	TRAIL-R3
Cardio	Interleukin-1 beta	IL-1 beta		Inflammation	Monocyte Chemotactic Protein 1	MCP-1		Immuno	Interferon alpha	IFN-alpha
Cardio	Interleukin-6	IL-6		Inflammation	Macrophage Inflammatory Protein-1 alpha	MIP-1 alpha		Immuno	Interleukin-22	IL-22
Cardio	Interleukin-6 receptor	IL-6r		Inflammation	Macrophage Inflammatory Protein-1 beta	MIP-1 beta		Immuno	Stromal cell-derived factor-1	SDF-1
Cardio	Interleukin-8	IL-8		Inflammation	Matrix Metalloproteinase-3	MMP-3		Immuno	Macrophage inflammatory protein 3 beta	MIP-3 beta
Cardio	Insulin	Insulin		Inflammation	Matrix Metalloproteinase-9	MMP-9		Immuno	B cell-activating factor	BAFF
Cardio	Interferon gamma Induced Protein 10	IP-10		Inflammation	T-Cell-Specific Protein RANTES	RANTES		Immuno	Interferon-inducible T-cell alpha chemoattractant	ITAC
Cardio	Leptin	Leptin		Inflammation	Stem Cell Factor	SCF		Immuno	6Ckine	6Ckine
Cardio	Apolipoprotein(a)	Lp(a)		Inflammation	Tissue Inhibitor of Metalloproteinases 1	TIMP-1		Immuno	Thymus and activation-regulated chemokine	TARC
Cardio	Lectin-Like Oxidized LDL Receptor 1	LOX-1		Inflammation	Tumor necrosis factor receptor 2	TNFR2		Immuno	Tumor necrosis factor ligand superfamily member 13	APRIL
Cardio	Monocyte Chemotactic Protein 1	MCP-1		Inflammation	Tumor Necrosis Factor alpha	TNF-alpha		Immuno	Dickkopf-related protein 1	DKK-1
Cardio	Macrophage Inflammatory Protein-1 alpha	MIP-1 alpha		Inflammation	Tumor Necrosis Factor beta	TNF-beta		Immuno	Interleukin-31	IL-31
Cardio	Macrophage Inflammatory Protein-1 beta	MIP-1 beta		Inflammation	Vascular Endothelial Growth Factor	VEGF		Immuno	Tumor necrosis factor ligand superfamily member 12	Tweak
Cardio	Matrix Metalloproteinase-1	MMP-1		Inflammation	Vascular Cell Adhesion Molecule-1	VCAM-1		Cardio	P-Selectin	P-Selectin
Cardio	Matrix Metalloproteinase-10	MMP-10		Inflammation	Vitamin D-Binding Protein	VDBP		Cardio	Neuropilin-1	Neuropilin-1
Cardio	Matrix Metalloproteinase-3	MMP-3		Inflammation	von Willebrand Factor	vWF		Cardio	Neutrophil Activating Peptide 2	NAP-2
Cardio	Matrix Metalloproteinase-7	MMP-7		Cardio	Serum Amyloid A Protein	SAA		Cardio	Pigment Epithelium Derived Factor	PEDF
Cardio	Matrix Metalloproteinase-9	MMP-9		Cardio	Serum Amyloid P-Component	SAP		Cardio	Leucine-rich alpha-2-glycoprotein	LRG1
Cardio	Matrix Metalloproteinase-9, total	MMP-9, total		Cardio	Sex Hormone-Binding Globulin	SHBG		Cardio	Thrombin-Activatable Fibrinolysis	TAFI
Cardio	Myeloperoxidase	MPO		Cardio	Receptor for advanced glycosylation end products	RAGE		Cardio	Antithrombin-III	AT-III
Cardio	Myoglobin	Myoglobin		Cardio	Thrombomodulin	TM		Cardio	Ficolin-3	Ficolin-3
Cardio	Plasminogen Activator Inhibitor 1	PAI-1		Cardio	Thyroxine-Binding Globulin	TBG				
Cardio	Vitamin K-Dependent Protein S	VKDPS		Cardio	Tissue Inhibitor of Metalloproteinases 1	TIMP-1				
Cardio	Pulmonary and Activation-Regulated Chemokine	PARC		Cardio	Tumor necrosis factor receptor 2	TNFR2				
Cardio	T-Cell-Specific Protein RANTES	RANTES		Cardio	Tumor Necrosis Factor alpha	TNF-alpha				
				Cardio	Vascular Endothelial Growth Factor	VEGF				
				Cardio	Vascular Cell Adhesion Molecule-1	VCAM-1				
				Cardio	von Willebrand Factor	vWF				