Targeted breath analysis: exogenous volatile organic compounds (EVOC) as targeted metabolic probes in Breath Biopsy



#save100klives
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8th November 2018

owlstonemedical.com

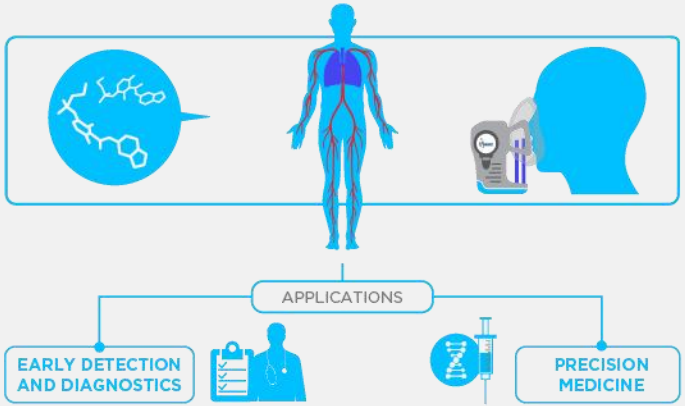
Breath Biopsy® - Reshaping healthcare in the 21st Century

OUR MISSION:

TO SAVE **100,000** LIVES & **1.5B** IN HEALTHCARE COSTS.

OUR VISION:

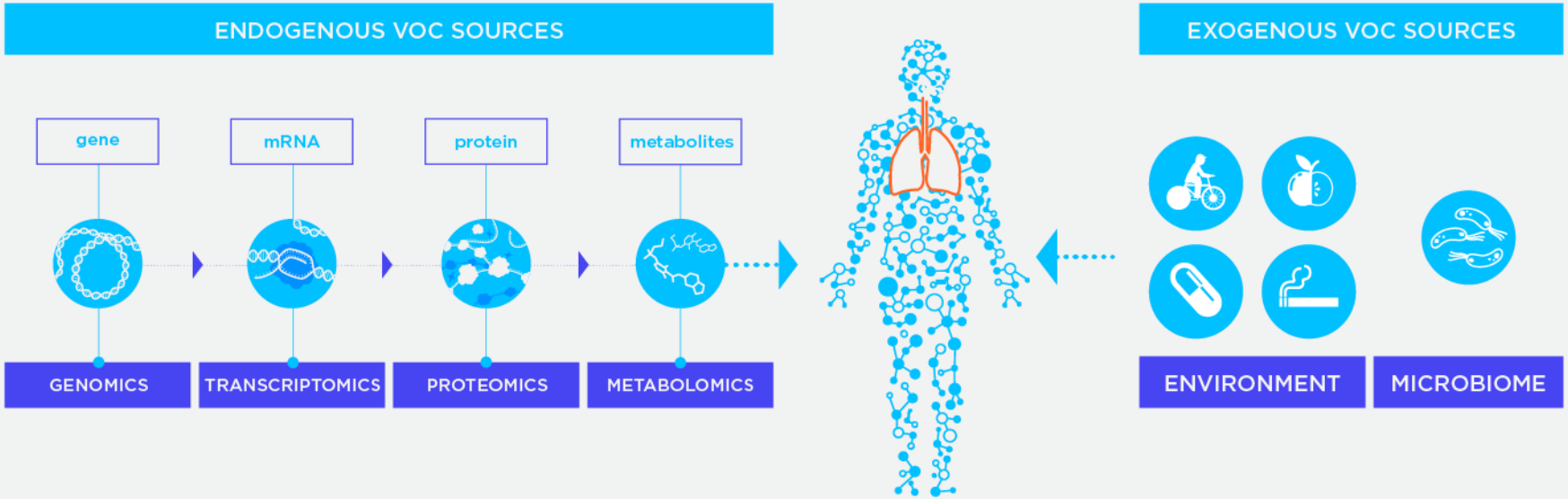
THE GLOBAL LEADER IN BREATH BIOPSY
FOR EARLY DETECTION AND PRECISION MEDICINE.



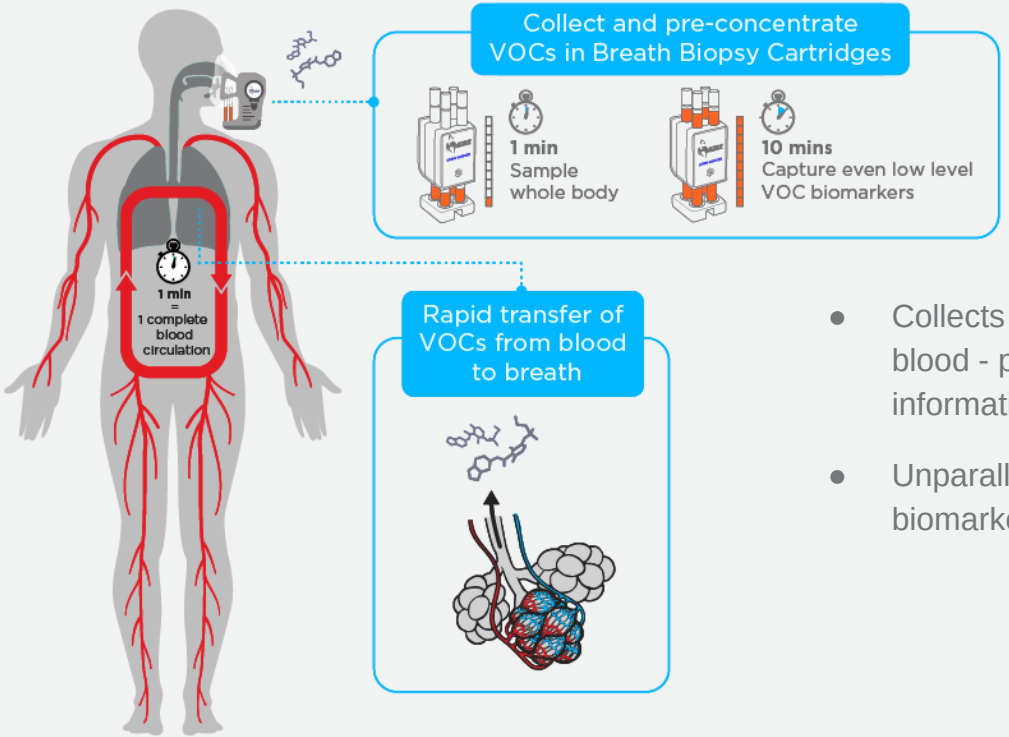
STRATA LuCID nTERCEPT

TARGETING BILLION DOLLAR MARKETS
IN LUNG CANCER, COLON CANCER
AND RESPIRATORY DISEASE

What are VOCs? Endogenous and Exogenous



Breath Biopsy: Whole Body Metabolome Sampling



- Collects VOCs originating from airways tissues and blood - providing both local and systemic disease information
- Unparalleled sensitivity for the detection of disease biomarkers in breath

Where are we in “Breathomics”?

- Modern breath testing commenced in 1971, with the work of Nobel Prize winner Linus Pauling.
- Hundreds of scientific papers published suggesting the presence of VOC biomarkers across a range of diseases.

SOME PUZZLING QUESTIONS

1 Why is there very little agreement in identified biomarkers within a disease?

2 Why is breath testing not used routinely in clinical setting?

SOME HISTORICAL CHALLENGES

- Maturity of breath sampling hardware and protocols for robust, repeatable sampling.

- Study design and size - small patient numbers in pilot studies and lack of blinded validation studies.

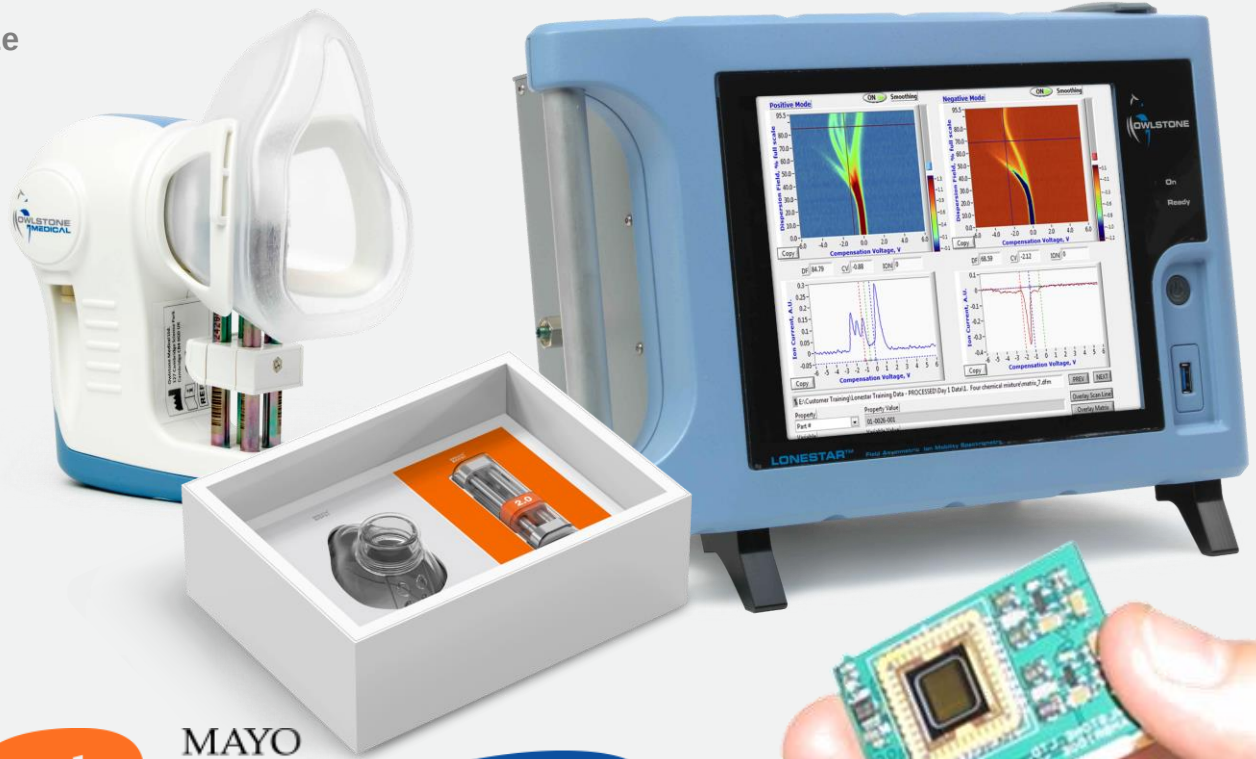
- High end, expensive spectrometer vs low performance enose.
- Different analytical techniques required in biomarker discovery and clinical translation.

WITHOUT SOLVING THESE YOU CAN'T HAVE CONFIDENCE IN INITIAL BIOMARKER DISCOVERY AND VALIDATION

Breath Biopsy Enabling Technologies

Collect samples anywhere and analyze in central lab or near patient

- ReCIVA Breath Sampler for reproducible collection of specific breath fractions
- Breath Biopsy Cartridge captures every VOC from breath and can be shipped without special handling
- Rapid, sensitive and selective VOC analysis based on proven FAIMS sensor technology
- Analysis in central lab or at point of care



NHS Roche gsk MAYO CLINIC SAMSUNG

In development Breath Biopsy hardware - ReCIVA Breath Sampler, CASPER Air Supply, Breath Biopsy kits, mobile sample collection station

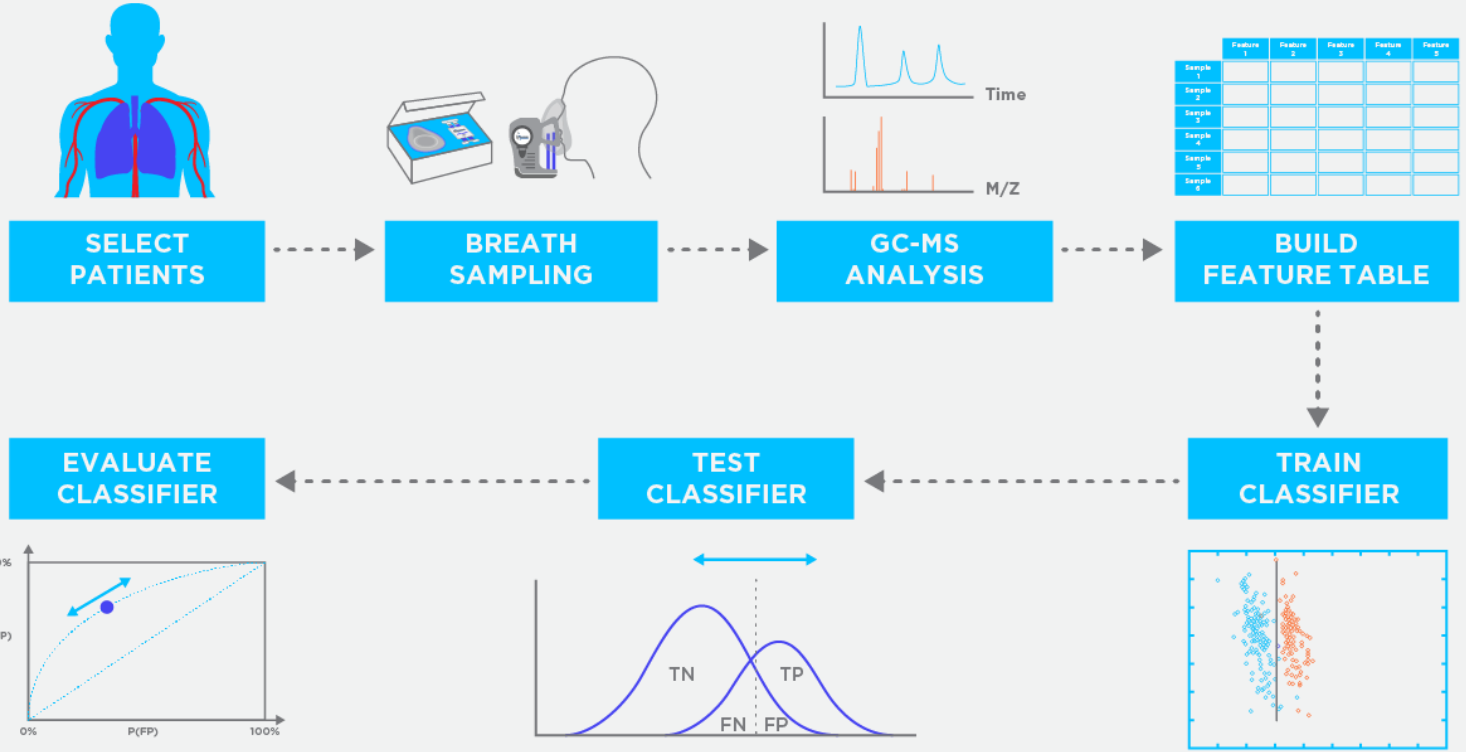
Breath Biopsy Products and Services

Lease **ReCIVA Breath Sampler** and purchase **Breath Biopsy Kits** to collect breath samples

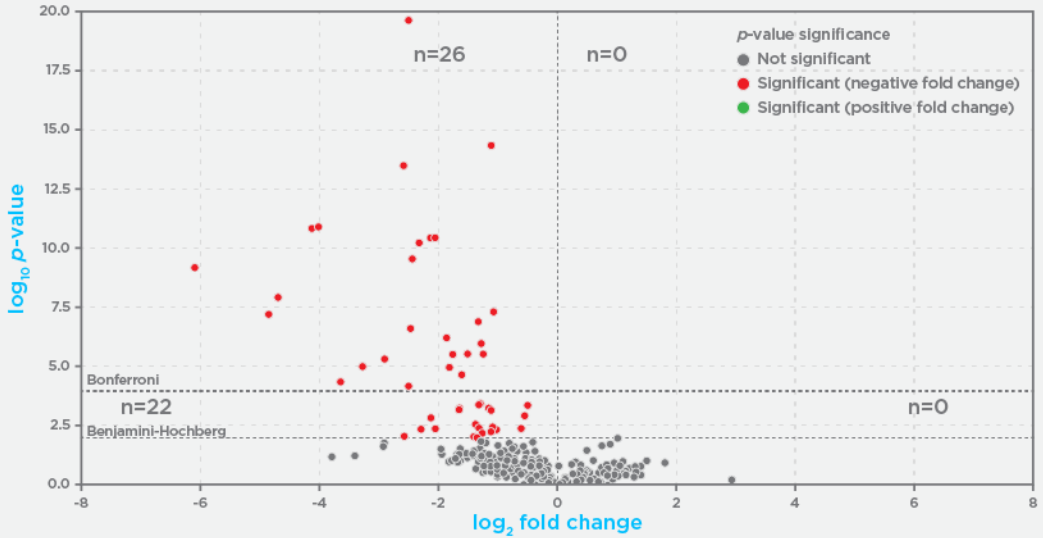
Send Breath Biopsy Cartridges for analysis using **Owlstone Medical's Breath Biopsy Services**

Analyze Breath Biopsy Cartridges in your own lab with the **Lonestar VOC Analyzer**

Breath Biopsy Discovery Methodology



Volcano Plot: significant features and fold change

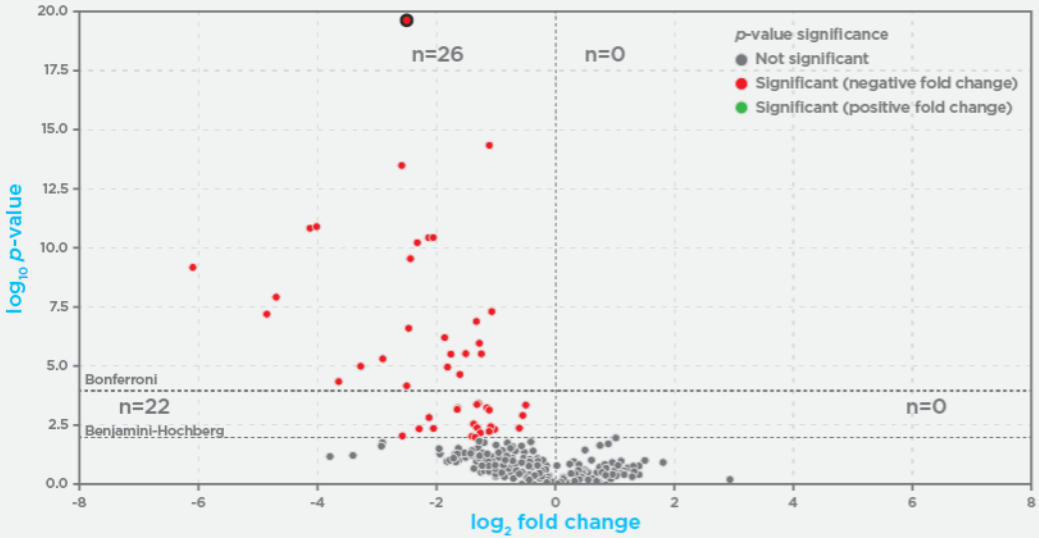


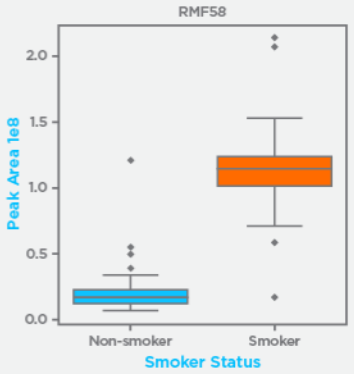
A Volcano plot enables quick visual identification of those features that display large-magnitude fold changes between classes, that are also statistically significant. There are two regions of interest in the volcano plot: those points that are found in the top-left or top-right areas of the plot represent features that display large magnitude fold changes as well as high statistical significance (log_{10} p-value based on Wilcoxon rank sum). Statistical significance can be determined using either Benjamini-Hochberg, or by the calculated Bonferroni correction (0.05 divided by the number of features, in this case 0.05 / 475 = 0.000105). Both cut-offs are shown as horizontal lines in the volcano plot. Features that with a negative fold change between the classes (reduced in Non-smokers compared to Smokers), and significant p-values above the Benjamini-Hochberg cut-off are shown in green. Green dots are features that with a positive fold change between the classes (increased in Non-smokers compared to Smokers), and significant p-values above the Benjamini-Hochberg cut-off. The number of features

Features ranked by p-value

Feature	log2 fold change	*p*-value
RMF58	-2.502	2.57E-20
RMF57	-1.117	4.79E-15
RMF38	-2.587	3.32E-14
RMF44	-4.012	1.24E-11
RMF53	-4.130	1.49E-11
RMF115	-2.045	3.77E-11
RMF19	-2.135	3.81E-11
RMF113	-2.325	5.92E-11
RMF124	-2.437	2.79E-10
RMF93	-6.098	6.65E-10
RMF63	-4.692	1.19E-08
RMF86	-1.072	4.88E-08
RMF75	-4.852	6.06E-08
RMF9	-1.328	1.26E-07
RMF125	-2.468	2.47E-07
RMF142	-1.865	6.06E-07
RMF162	-1.281	1.08E-06
RMF21	-1.507	2.91E-06
RMF31	-1.246	2.95E-06
RMF158	-1.763	3.10E-06
RMF126	-2.901	5.01E-06
RMF146	-3.275	1.01E-05
RMF153	-1.818	1.11E-05
RMF123	-1.606	2.25E-05
RMF43	-3.641	4.47E-05

Box Plot: Feature RMF58





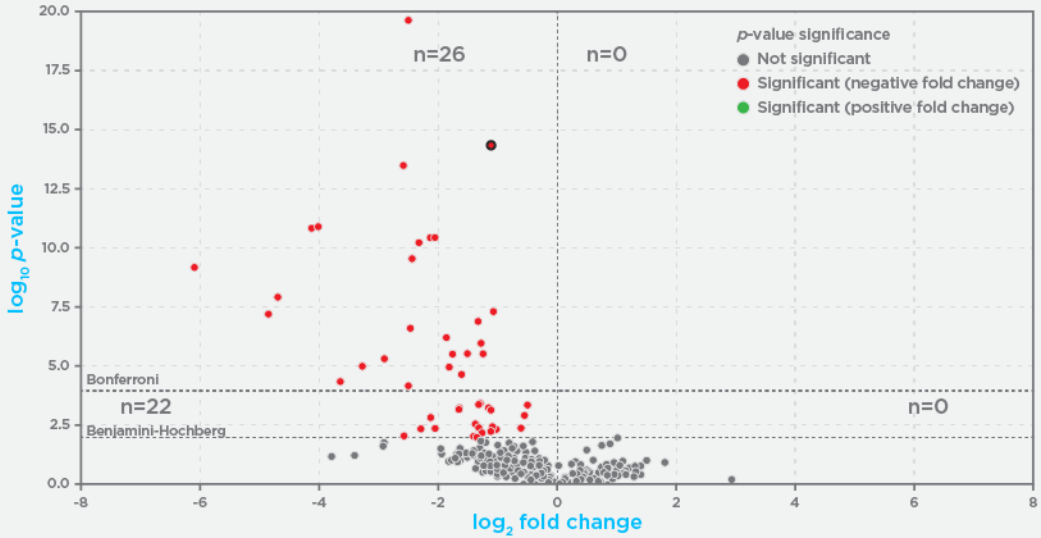
Feature	RMF58
p-value	2.57E-20
Log_2 fold change	-2.502
Tentative ID	Benzene

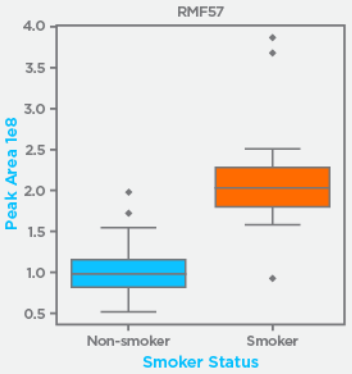
On the volcano plot (left), red dots are features that with a negative fold change between the classes (reduced in Non-smokers compared to Smokers), and significant p-values above the Benjamini-Hochberg cut-off. Green dots are features that with a positive fold change between the classes (increased in Non-smokers compared to Smokers), and significant p-values above the Benjamini-Hochberg cut-off. See here for an explanation of the volcano plot. The red dot with black outline represents feature RMF58.

The box plot (upper right) shows the distribution of peak area measured for feature RMF58 in non-smokers vs. smokers. See here for an explanation of the box plot.

The table shows *p*-value, log_2 fold change between classes (non-smokers *vs.* smokers) and Tentative ID for feature RMF58. Negative fold changes are highlighted in red, positive fold changes in green. Statistically significant p-values below the Benjamini-Hochberg cut-off are shown in yellow. Measured spectra are compared against the NIST unit mass spectral library in order to assign a tentative ID to each feature. Please note the tentative ID is likely to be inaccurate, so additional structural elucidation is required to confirm compound identity (available, subject to additional fee).

Box Plot: Feature RMF57





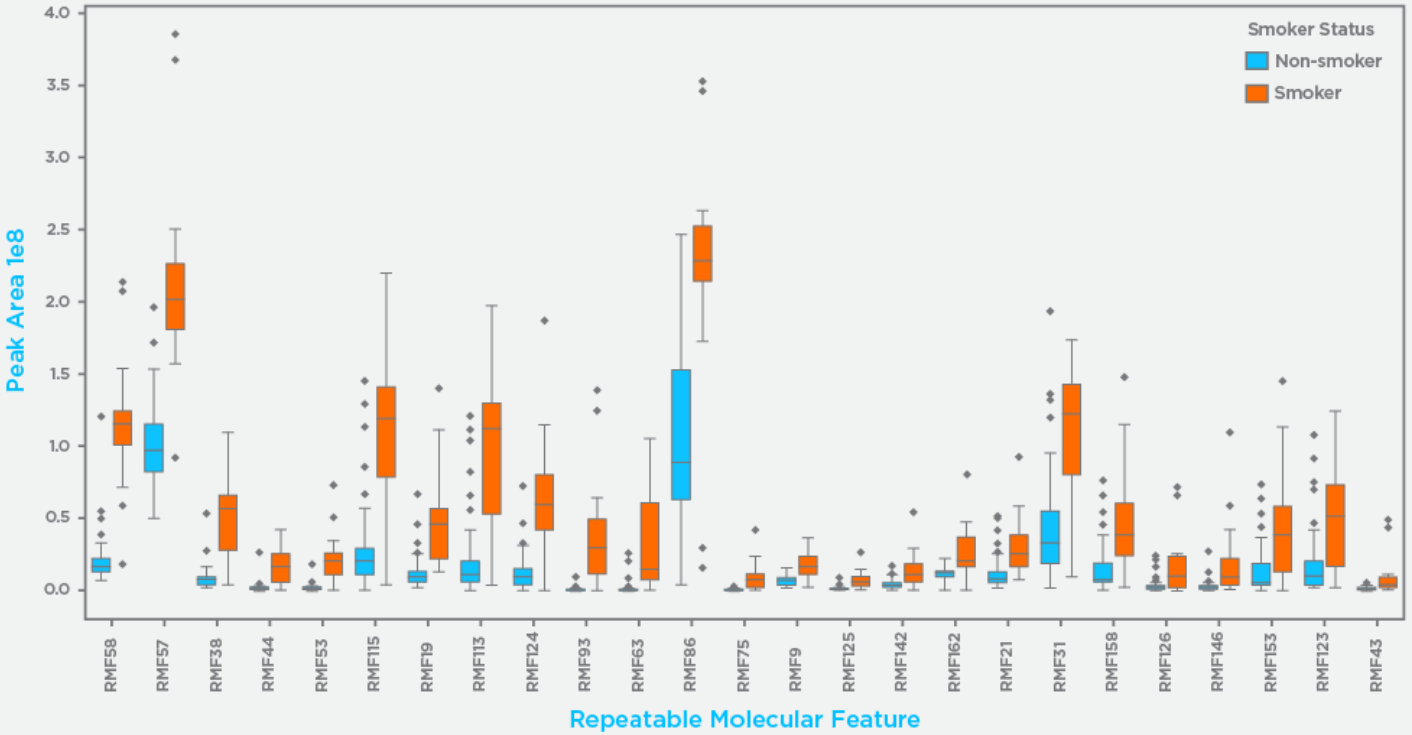
Feature	RMF57
p-value	4.79E-15
Log_2 fold change	-1.117
Tentative ID	Fumaronitrile

On the volcano plot (left), red dots are features that with a negative fold change between the classes (reduced in Non-smokers compared to Smokers), and significant p-values above the Benjamini-Hochberg cut-off. Green dots are features that with a positive fold change between the classes (increased in Non-smokers compared to Smokers), and significant p-values above the Benjamini-Hochberg cut-off. See here for an explanation of the volcano plot. The red dot with black outline represents feature RMF57.

The box plot (upper right) shows the distribution of peak area measured for feature RMF57 in non-smokers vs. smokers. See here for an explanation of the box plot.

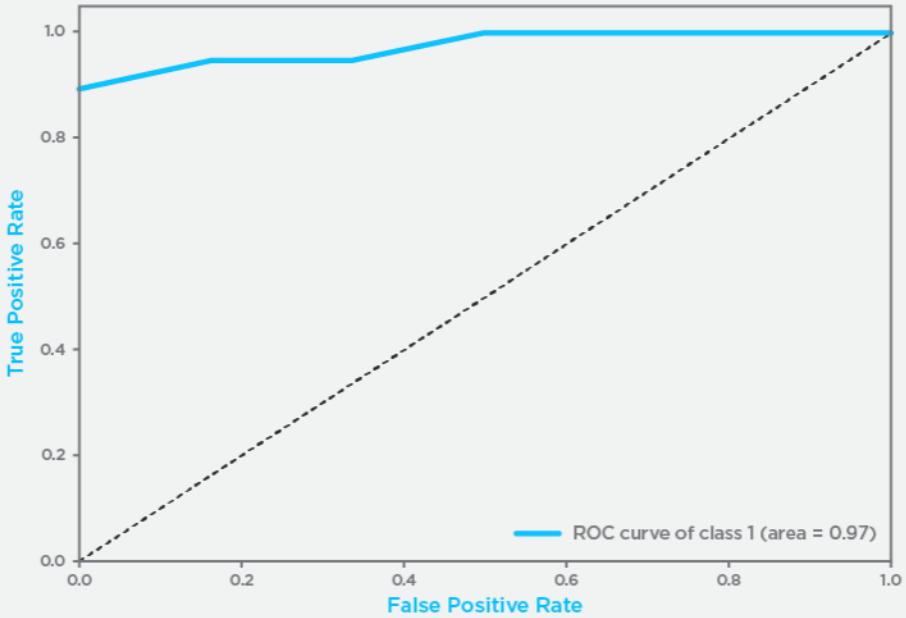
The table shows *p*-value, log_2 fold change between classes (non-smokers *vs.* smokers) and Tentative ID for feature RMF57. Negative fold changes are highlighted in red, positive fold changes in green. Statistically significant p-values below the Benjamini-Hochberg cut-off are shown in yellow. Measured spectra are compared against the NIST unit mass spectral library in order to assign a tentative ID to each feature. Please note the tentative ID is likely to be inaccurate, so additional structural elucidation is required to confirm compound identity (available, subject to additional fee).

Box Plots: Top 25 Features Ranked by P-value



The box plots above visualize the distribution of the peak area of the top 25 features. The top 25 features were selected based on the p-value of the fold change between classes, non-smokers vs. smokers. The horizontal line within the box represents the median peak area of the feature. The box displays the upper and lower quartiles of the data, while the error bars represent the maximum and minimum peak areas (excluding outliers). Outliers are represented by dots.

Random Forest: ROC Curve k Fold Validation



Random Forest is a non-supervised machine learning algorithm used for classifier building. k fold validation is a method of cross-validation used to test the model's ability to predict new data that was not used in estimating it, in order to flag problems like overfitting or selection bias, and to give an insight on how the model will generalize to an independent dataset. Multiple rounds of cross-validation are performed using different partitions, and the validation results are combined over the folds to give an estimate of the model's predictive performance.

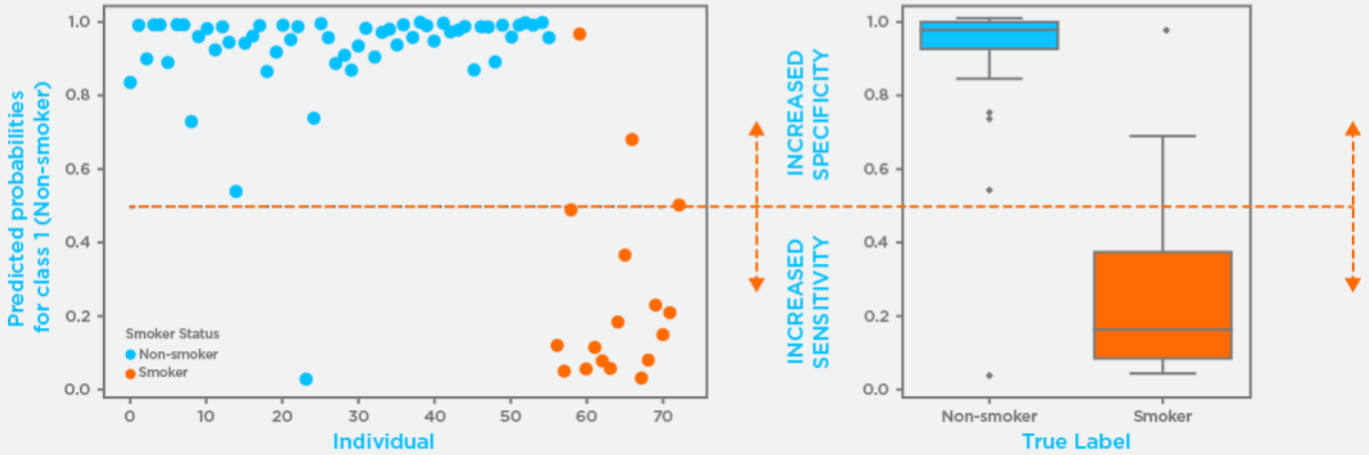
The ROC curve shows the trade-off between sensitivity (or True Positive Rate, TPR) and specificity (1 – False Positive Rate, FPR). Classifiers that give curves closer to the top-left corner indicate a better performance. As a baseline, a random classifier is expected to give points lying along the diagonal (FPR = TPR). The closer the curve comes to the 45-degree diagonal of the ROC space, the less accurate the test.

Random Forest: Model Evaluation

		Breath Test (Predicted Label)		
		Smoker	Non-smoker	Total
True label	Smoker	14 True Negatives	3 False Positives	17
	Non-smoker	1 False Negatives	55 True Positives	56
Total		15	58	73

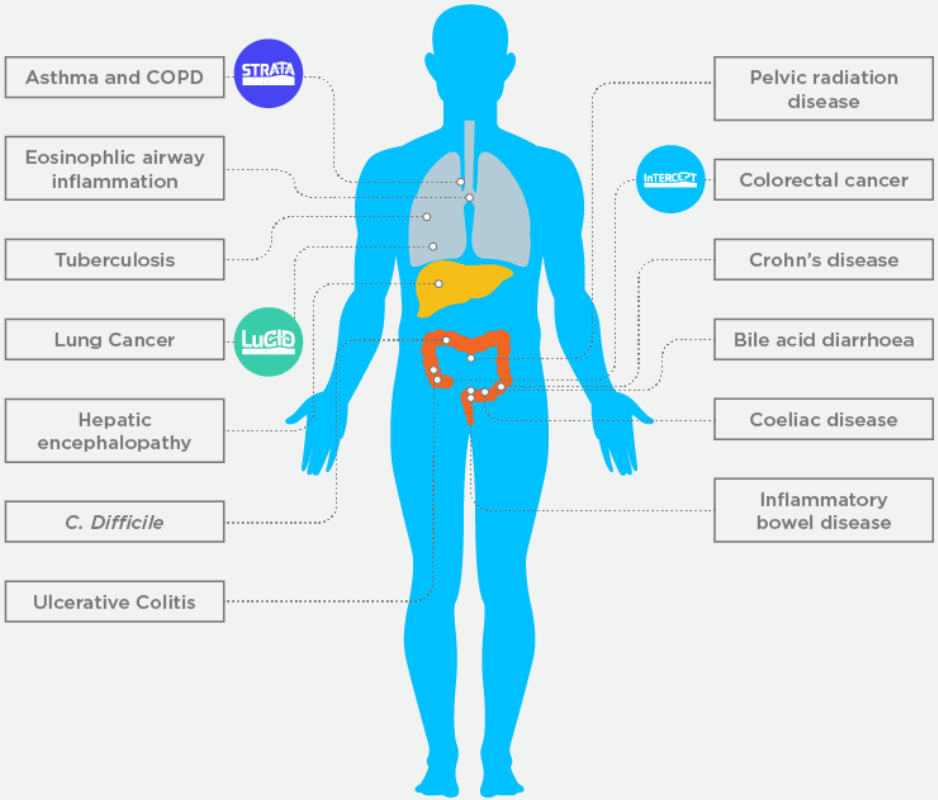
Performance of the Classifier

Sensitivity	98%
Specificity	82%
Positive Predictive Value	95%
Negative Predictive Value	93%



Confusion matrix describing the performance of the Random Forest Classification Model to classify the samples (top). The figures describe the prediction probabilities of individual samples (bottom left), and box plots of each class (bottom right). Dashed line represents the Non-smoker threshold (0.5).

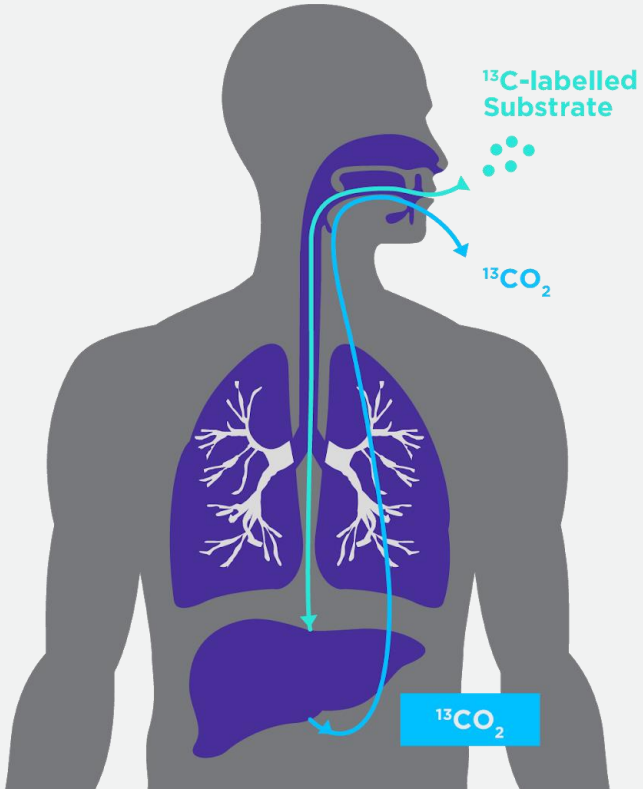
Enabling a broad range of applications across cancer, inflammatory and infectious disease



Application	Patient Numbers	Sens./Spec.
Cancer		
Colorectal cancer	*N*=133; 83 colon 50 controls	88/60
Pelvic radiation disease	*N*=23	90/90
Inflammation		
Inflammatory Bowel	*N*=62; 48 with IBD	74/88
Disease (IBD) Inflammatory Bowel	14 controls *N*=76; 54 with IBD and 22 healthy controls	74/75
Disease (IBD) Coeliac	N = 47; 27 with histological confirmation of coeliac disease 20 controls with irritable bowel syndrome	85/85
Bile acid diarrhoea	*N*=110 23 with bile acid diarrhoea 42 with ulcerative colitis and 45 symptomatic controls	85/90
Eosinophilic airway inflammation	*N*=52; 27 with eosinophilia	Accuracy: 85%
Asthma/COPD	*N*=78	No reported accuracy
Infection		
Clostridium difficile	*N*=213 –71 with *C. diff* positive by microbiological analysis	92/86
Hepatic encephalopathy (HE)	*N*=42; 22 with HE and 20 healthy controls	88/68

>100 peer reviewed papers and scientific posters - link

Measurement of liver enzymatic activity using ^{13}C-labelled substrate



- ^{13}C labelled substrate is administered (e.g. orally, intravenously) and metabolised by the liver
- Leads to production of $^{13}CO_2$ secreted through the lungs via breath

^{13}C-labelled substrate Breath Biopsy tests for measuring liver function

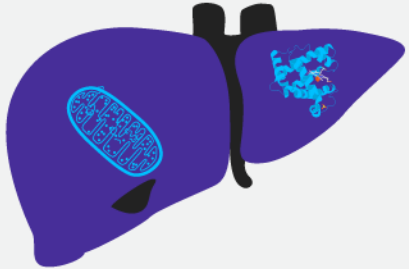
Breath tests determine the rate of labeled carbon dioxide to estimate enzyme activity, organ function or presence of disease.

Mitochondrial:

- **1-^{13}C-Methionine**
  - Exclusively metabolised by hepatic mitochondria
  - Concentration of $^{13}CO_2$ exhaled correlates with liver disease severity, traces appear within 20 mins.
- **Sodium 1-13C-octanoate**
  - Undergoes liver mitochondrial beta-oxidation
  - Impairment of mitochondrial beta-oxidation has been reported with several liver diseases such as nonalcoholic steatohepatitis (NASH)

Cytosol:

- **L-[1-^{13}C]phenylalanine**
  - Quantitatively monitors the rate of hepatic phenylalanine metabolism
  - Abnormal elevation of the plasma concentrations of the aromatic amino acids, phenylalanine and tyrosine are seen in the liver disease



CYP450:

- **^{13}C-Methacetin**
  - Completed within 60 minutes
  - Undergoes extensive liver first pass clearance
  - Cytochrome P450 IA2 converts methacetin cia O-dealkylation to acetaminophen and 1-$^{13}CO_2$
- **[^{13}C]Caffeine**
  - Highly specific for P4501A2 isoenzyme activity
  - Samples collected over 60 mins ratio of $^{13}CO_2$ to $^{12}CO_2$ indicates enzyme activity

Breath Biopsy tests for measuring liver function: ^{13}C-Methacetin Breath Test (MBT)

Aliment Pharmacol Ther 2005; 21: 179–185. doi: 10.1111/j.1365-2036.2005.02317.x

^{13}C-methacetin breath test as liver function test in patients with chronic hepatitis C virus infection

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Accepted for publication 21 October 2004

SUMMARY

Background: The ^{13}C-methacetin breath test enables the quantitative evaluation of the cytochrome P450-dependent liver function.
Aim: To find out whether this breath test is sensitive in noncirrhotic patients also with chronic hepatitis C in early stages of fibrosis.
Methods: Sixty-one healthy controls and 81 patients with chronic hepatitis C underwent a ^{13}C-methacetin breath test. In all patients, a liver biopsy was performed. The liver histology was classified according to the histology activity index–Knodell score.
Results: Delta over baseline values of the patients at 15 min significantly differed from controls (19.2 ± 9.2‰ vs. 24.1 ± 5.7‰; $P < 0.003$). The cumulative recovery after 30 min in patients was 11.4 ± 4.8% and in healthy controls 13.8 ± 2.8% ($P < 0.002$). However, patients with early fibrosis (histology activity index IVB) did not differ in delta over baseline values of the patients at 15 min (23.2 ± 7.9‰ vs. 22.6 ± 7.2‰: $P = 0.61$) or cumulative recovery (13.6 ± 3.7% vs. 13.2 ± 3.8%; $P = 0.45$) from patients with more advanced fibrosis (histology activity index IVC). Patients with clinically nonsymptomatic cirrhosis (histology activity index IVD; Child A) metabolized ^{13}C-methacetin to a significantly lesser extent (delta over baseline values of the patients at 15 min: 8.3 ± 4.9‰: $P < 0.005$ and cumulative recovery after 30 min: 5.6 ± 3.2%; $P < 0.003$). The ^{13}C-methacetin breath test identified cirrhotic patients with 95.0% sensitivity and 96.7% specificity.
Conclusion: The non-invasive ^{13}C-methacetin breath test reliably distinguishes between early cirrhotic (Child A) and noncirrhotic patients, but fails to detect early stages of fibrosis in patients with chronic hepatitis C.

INTRODUCTION

Several ^{13}C breath tests are used for the non-invasive assessment of hepatocellular function. The ^{13}C-methacetin breath test (MBT) enables the quantitative evaluation of the cytochrome P450-dependent liver function,[1–3] whereas the results of the ^{13}C-MBT show a good correlation to the severity of liver cirrhosis according to the Child–Pugh score. ^{13}C-methacetin is rapidly metabolized by healthy liver cells (cytochrome P450 1A2) into acetaminophen and $^{13}CO_2$ by a single dealkylation. The increase of $^{13}CO_2$ in breath samples can be quantified by isotope ratio mass spectrometry or nondispersive isotope-selective infrared spectroscopy.[4–6] Like the ^{13}C-aminopyrine breath test and the monoethylglycinexylidide (MEGX) test, the ^{13}C-MBT is a microsomal liver function test.

To determine the degree of fibrosis in patients with chronic liver disease, liver biopsy is still considered as the gold standard. Non-invasive serum tests to classify patients as having mild or advanced liver fibrosis allow correct determination in approximately 40–70% of the cases only.[7–11] Most liver function tests, including the ^{13}C breath tests, together with the clinical judge-

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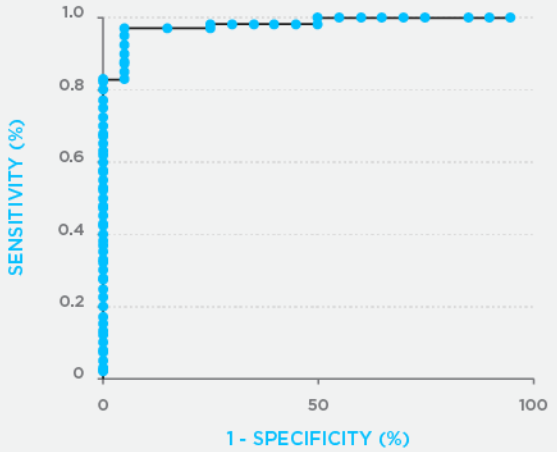
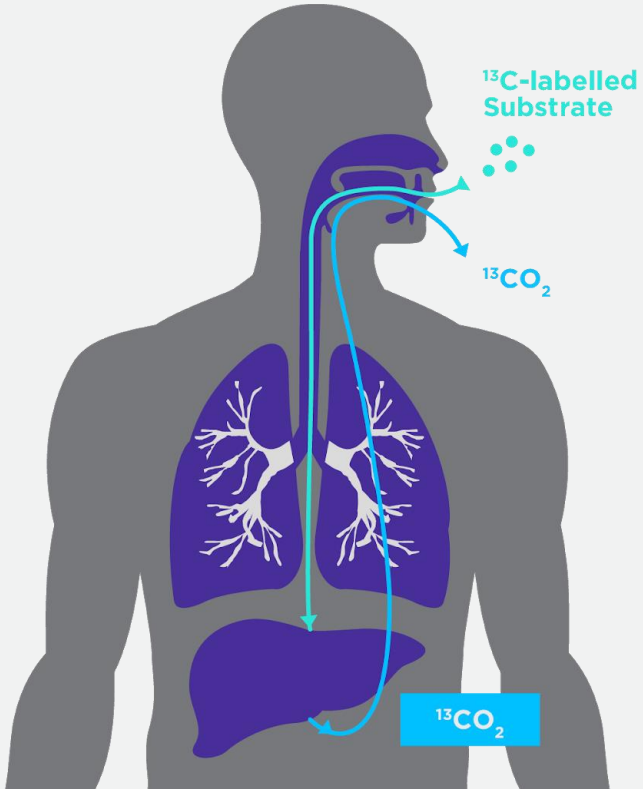
- Safe, simple and accurate test for diagnosing chronic liver disease in patients.
- Enables differentiation between different stages of chronic liver diseases.
- MBT can reliably distinguish between early cirrhotic and non-cirrhotic patients with **95%** sensitivity and **97%** specificity.



Measurement of liver enzymatic activity using ^{13}C-labelled substrate



- ^{13}C labelled substrate is administered (e.g. orally, intravenously) and metabolised by the liver
- Leads to production of $^{13}CO_2$ secreted through the lungs via breath

\+ Powerful strategy for assessing metabolic phenotypes / organ function

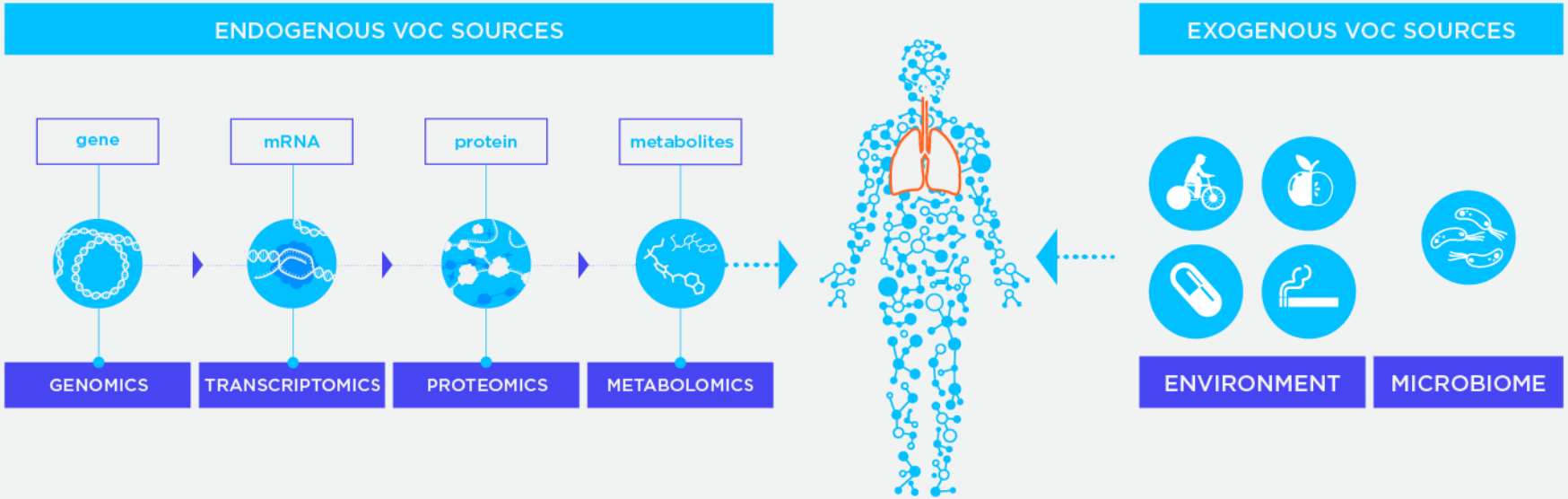
\- Test is **invasive** if intravenous administration required, and needs to take place in clinic

\- **Cannot administer multiple probes** - only one enzyme can be assessed at a time

\- Labelled isotope probes **require regulatory approval**

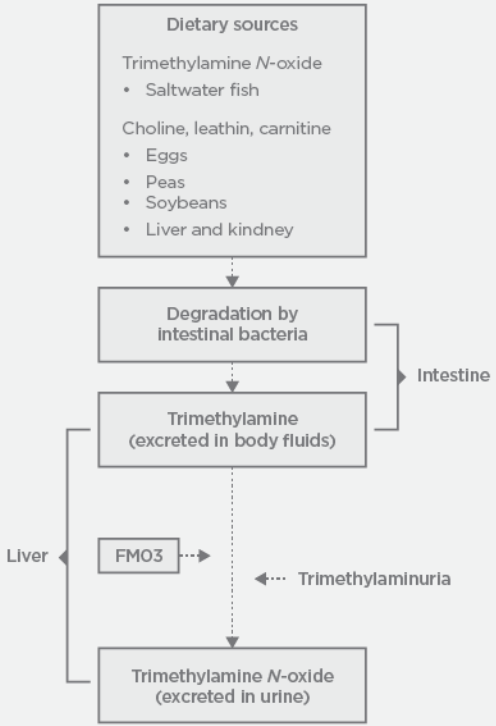
\- Labelled isotope is **very expensive**

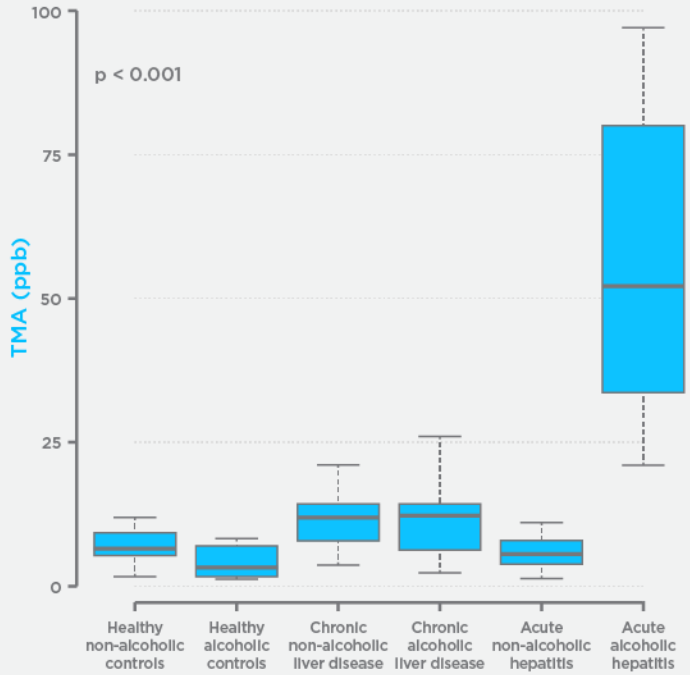
Exogenous VOCs on breath



Dietary sources of Trimethylamine (TMA) and acute alcoholic hepatitis

Hepatic flavin monooxygenase (FMO) family of enzymes, FMO3 convert trimethylamine (TMA), a volatile organic compound which smells like rotting fish, into trimethylamine N-oxide (TMAO), an odorless stable oxidation product which contributes to the atherosclerosis in humans. Subjects with chronic liver disease, in general, have impaired capacity to convert TMA into TMAO. Furthermore, alcohol consumption in patients with alcoholic liver disease induces bacterial overgrowth and increases gut permeability and the translocation of bacteria-derived lipopolysaccharides from the gut to the liver. It therefore may be desirable to determine whether the amounts or concentration of volatile compounds in a biological sample, for example, a breath sample correlate with the diagnosis of liver disease.





High Concentration of Exogenous VOC Limonene Associated with Liver disease

EBioMedicine 2 (2015) 1243–1250

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Volatile Biomarkers in Breath Associated With Liver Cirrhosis — Comparisons of Pre- and Post-liver Transplant Breath Samples

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ARTICLE INFO

Article history:
Received 9 June 2015
Received in revised form 17 July 2015
Accepted 20 July 2015
Available online 26 July 2015

Keywords:
Breath analysis
Cirrhosis
Diagnosis limonene
Liver transplant
PTR-MS
Volatile organic compounds

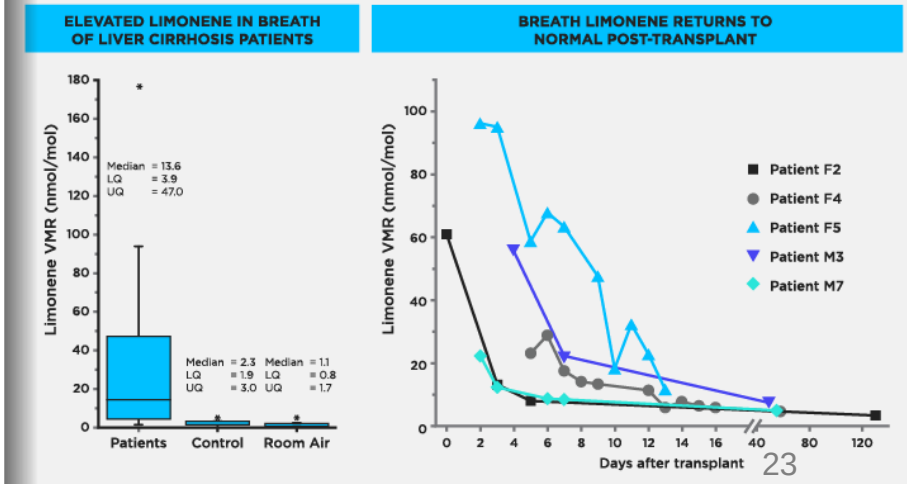
ABSTRACT

Background: The burden of liver disease in the UK has risen dramatically and there is a need for improved diagnostics.
Aims: To determine which breath volatiles are associated with the cirrhotic liver and hence diagnostically useful.
Methods: A two-stage biomarker discovery procedure was used. Alveolar breath samples of 31 patients with cirrhosis and 30 healthy controls were mass spectrometrically analysed and compared (stage 1). 12 of these patients had their breath analysed after liver transplant (stage 2). Five patients were followed longitudinally as in-patients in the post-transplant period.
Results: Seven volatiles were elevated in the breath of patients versus controls. Of these, five showed statistically significant decrease post-transplant: limonene, methanol, 2-pentanone, 2-butanone and carbon disulfide. On an individual basis limonene has the best diagnostic capability (the area under a receiver operating characteristic curve (AUROC) is 0.91), but this is improved by combining methanol, 2-pentanone and limonene (AUROC curve 0.95). Following transplant, limonene shows wash-out characteristics.
Conclusions: Limonene, methanol and 2-pentanone are breath markers for a cirrhotic liver. This study raises the potential to investigate these volatiles as markers for early-stage liver disease. By monitoring the wash-out of limonene following transplant, graft liver function can be non-invasively assessed.

1. Introduction

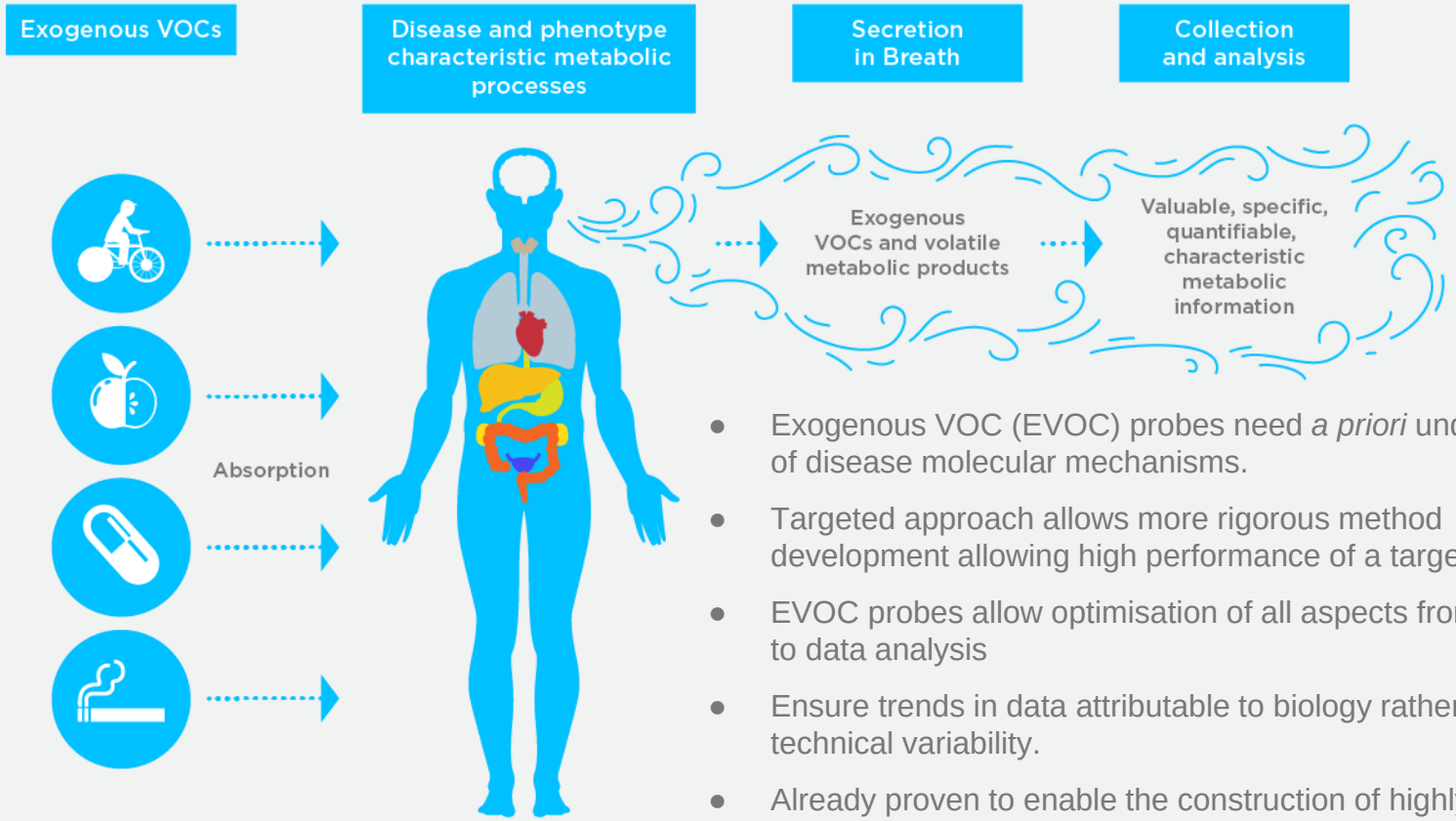
The publication of the 2014 Lancet Commission on liver disease has highlighted how the burden of liver disease in the UK has risen sharply over the past few decades and that it poses a major public health issue for 83% of deaths (Davies, 2012). It is the third biggest cause of premature mortality, with three quarters of liver deaths due to alcohol (Williams et al., 2014). Liver disease has a widespread effect not only to the patient, encompassing physical and psychological morbidity and mortality, but also incurring significant societal costs. One of the

- Patients suffering from liver cirrhosis have raised levels of limonene in their breath due to the liver failing to produce enzymes for metabolism.
- After liver transplant, limonene levels in exhaled breath returned to normal as the metabolism resumed
- Shows VOCs in breath can be used to monitor a patient's response to therapeutic intervention.



Fernández del Río R et al., EBioMedicine (2015); 2(9); 1243–1250

Administer Exogenous VOC (EVOC) Probes



- Exogenous VOC (EVOC) probes need *a priori* understanding of disease molecular mechanisms.
- Targeted approach allows more rigorous method development allowing high performance of a target analyte.
- EVOC probes allow optimisation of all aspects from sampling to data analysis
- Ensure trends in data attributable to biology rather than technical variability.
- Already proven to enable the construction of highly accurate tests.

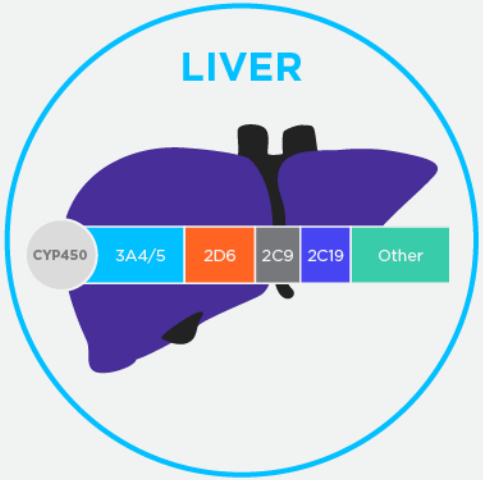
Breath Biopsy EVOC Probes to Determine Metabolizer Phenotype: Matching the Right Patient to the Right Drug and Dose

The majority of small molecule drugs are metabolized by a class of enzymes called **CYP450**[1]

These drugs include anticoagulants, antidepressants, pain medication, statins and oncology drugs with a combined total of billions of prescriptions per year

Product Name	Main Therapeutic Indication	Metabolised by	Prescriptions (m, US, 2015)
Lipitor	Cardiovascular	3A4/5	93.9
Zoloft	Mental Health	2D6, 2C9, 2B6, 2C19, 3A4/5	38.9
Prozac	Mental Health	2D6	28.3
Tramadol	Pain	2B6, 2D6, 3A4/5	24.9
Crestor	Cardiovascular	2C9	22.7
Warfarin	Cardiovascular	1A2, 3A4/5	20.8
Nexium	Gastrointestinal	3A4/5, 2C9	13.2
Spiriva Handihaler	Respiratory	3A4/5, 2D6	9.4
Januvia	Diabetes	3A4/5	8.9
Eliquis	Cardiovascular	3A4/5, 2C19	3.3
Tamoxifen	Oncology	2D6, 3A4/5	2.2

http://clincalc.com/DrugStats/Drugs/

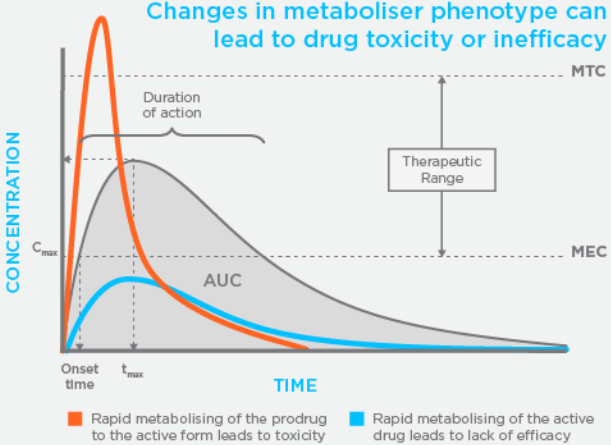


70%

of drugs are metabolised by only four enzymes

Pharmacogenomic tests can determine metaboliser genotype, but actual phenotype can be changed by a number of factors including other drugs and diet

Changes in metaboliser phenotype can lead to drug toxicity or inefficacy



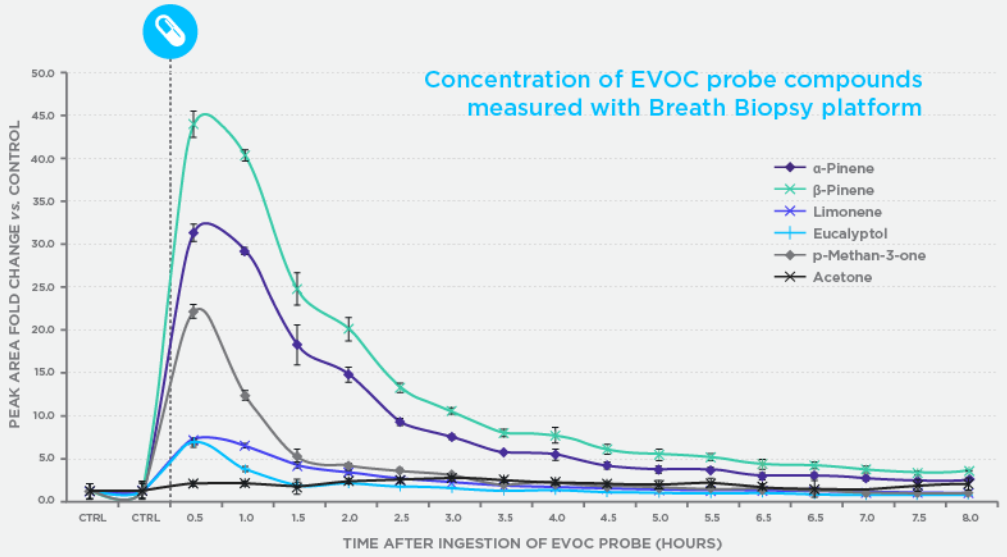
In **2018**, over **2.2 million Adverse Drug Reactions**[2] and more than **230 thousand** deaths are expected in the United States alone

1) https://ghr.nlm.nih.gov/primer/genefamily/cytochromep450;
2) https://www.sciencedirect.com/science/article/pii/S0163725813000065

Breath Biopsy EVOC Probes to Determine Metabolizer Phenotype: Matching the Right Patient to the Right Drug and Dose

Exogenous VOC (EVOC) probes, comprised of GRAS (generally recognised as safe) compounds, are metabolised by the same **CYP450** enzymes as drugs. By measuring the kinetics of a mix of EVOC probe substrates and metabolites on breath, we can determine metaboliser phenotype.

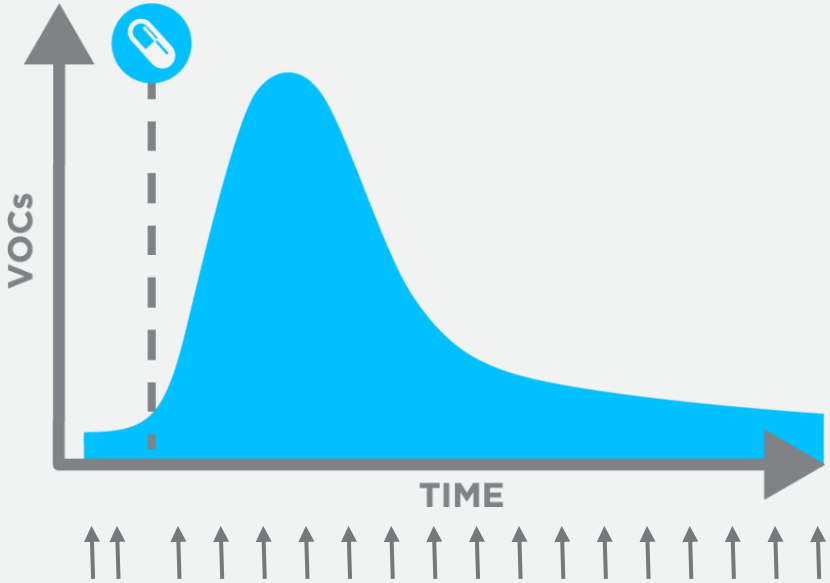
Example EVOC probe	CYP450
Limonene	2C9,2C19
Eucalyptol	3A4
Linalool	2C19,2D6



Over **$1.1b global pharmacogenomics diagnostics market**[1] by 2021, growing at >**20%**

[1] Companion Diagnostics: Technologies and Markets, BCC Research Report code BIO077C March 2017

Terpene EVOC probe washout curve experiment

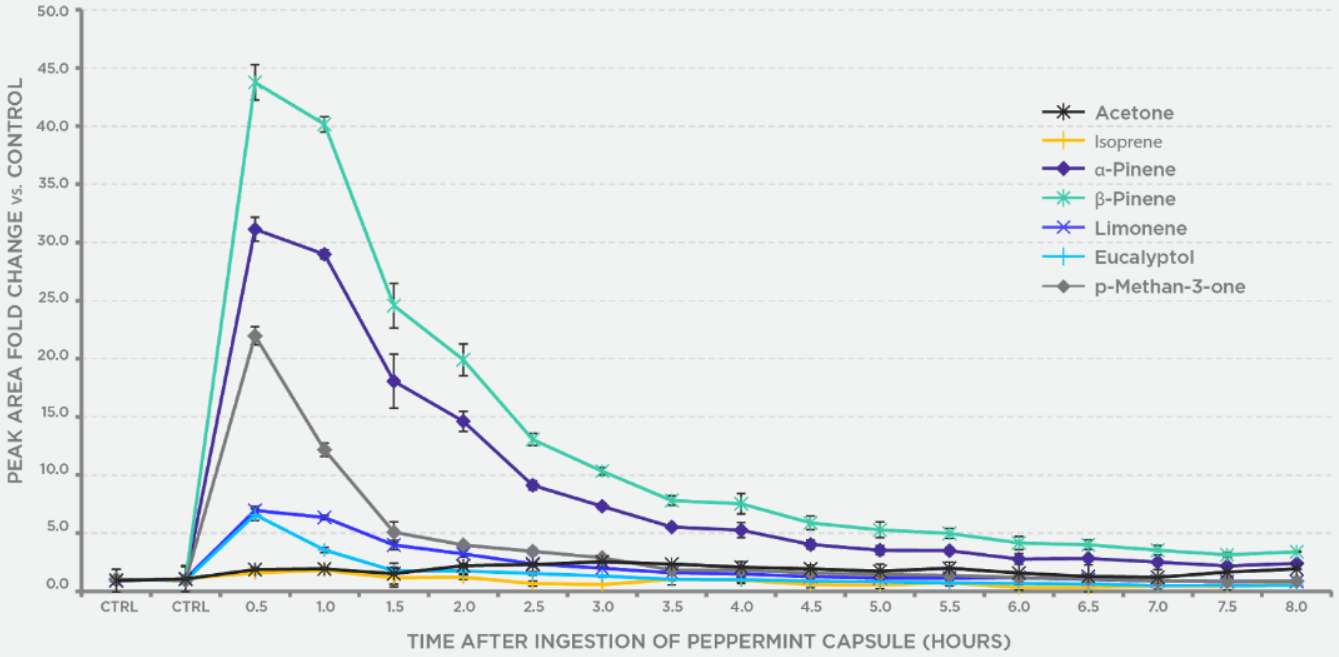


2 pre-ingestion controls

Repeated breath collects from one individual over 8 hour time period (16 timepoints)

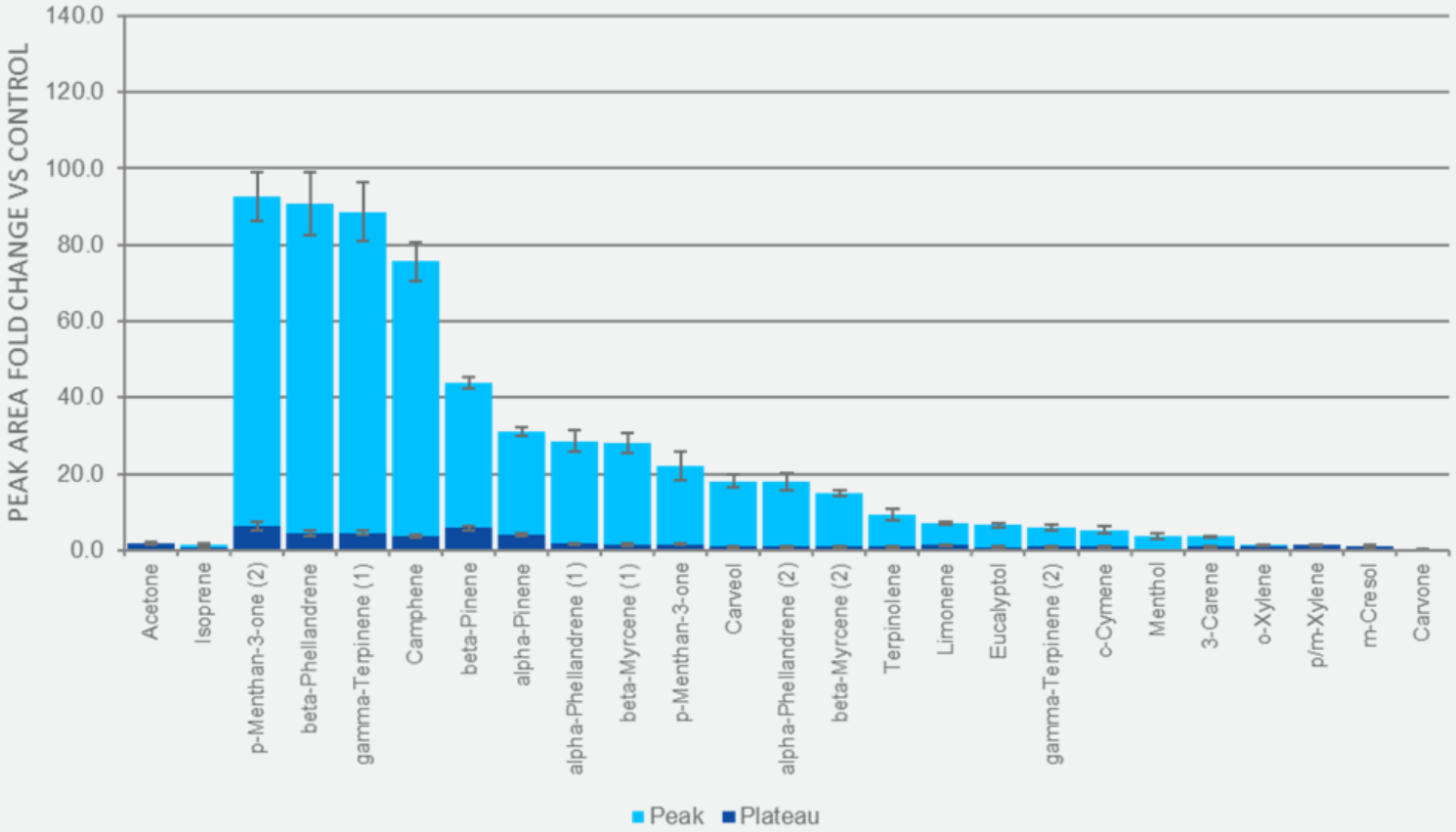
Four replicate VOC sample tubes collected and analyzed at each timepoint

Terpene EVOC probe washout curve - fold change vs control



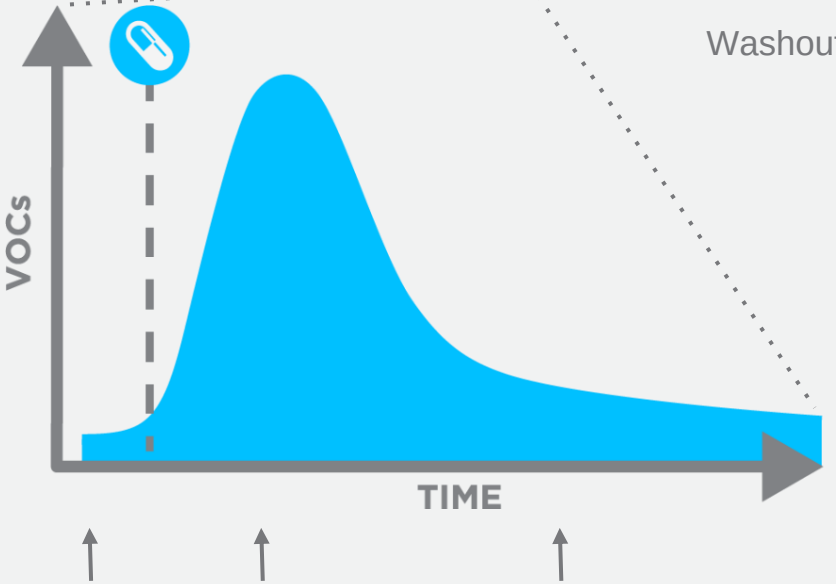
- Concentration of EVOC probe in breath **rises rapidly after administration**.
- Rate of decrease of probe concentration in breath as it is metabolized **relates directly to metabolizer phenotype**

EVOC probe fold change for multiple substrates



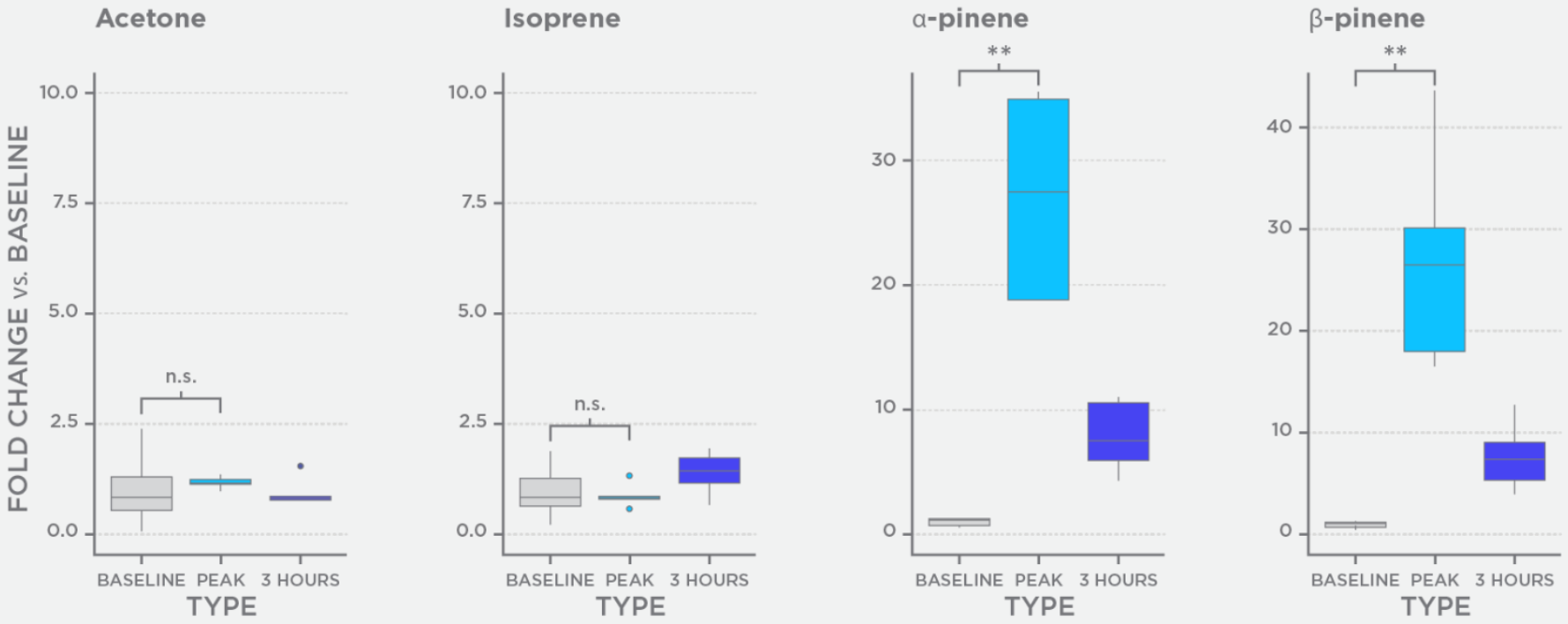
Repeat sampling over 5 weeks

Washout experiment repeated 9 times on the same individual over 5 weeks

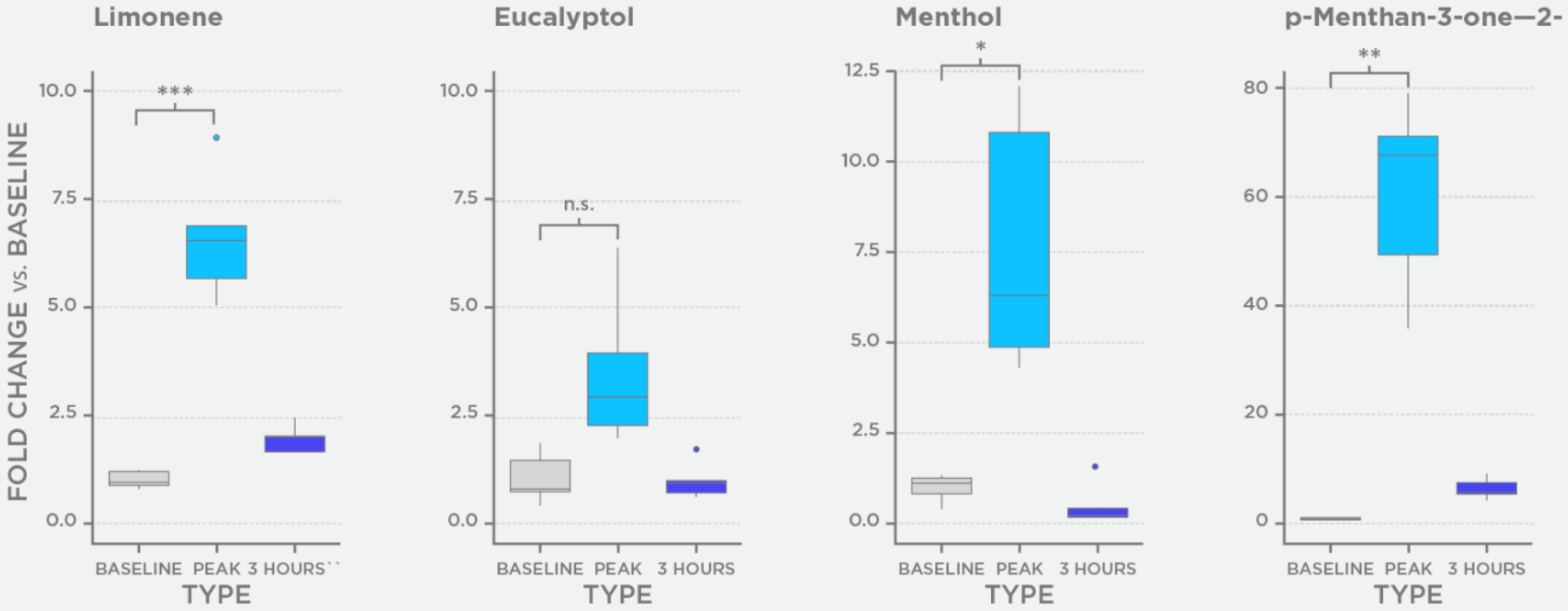


Two replicate VOC sample tubes analyzed at each timepoint

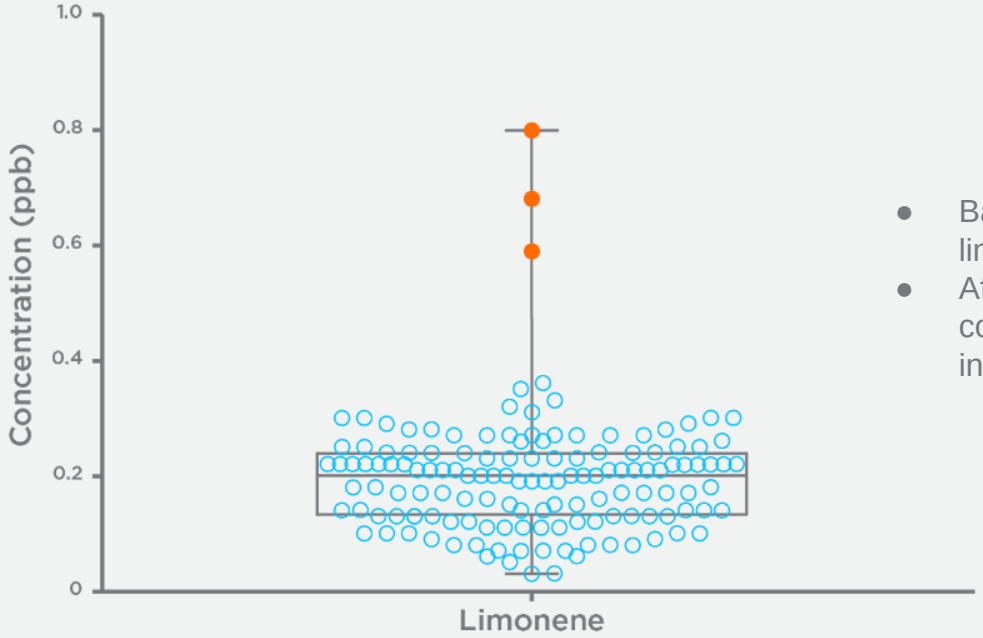
Baseline, peak and post 3hr EVOC probe concentrations



Baseline, peak and post 3hr EVOC probe concentrations

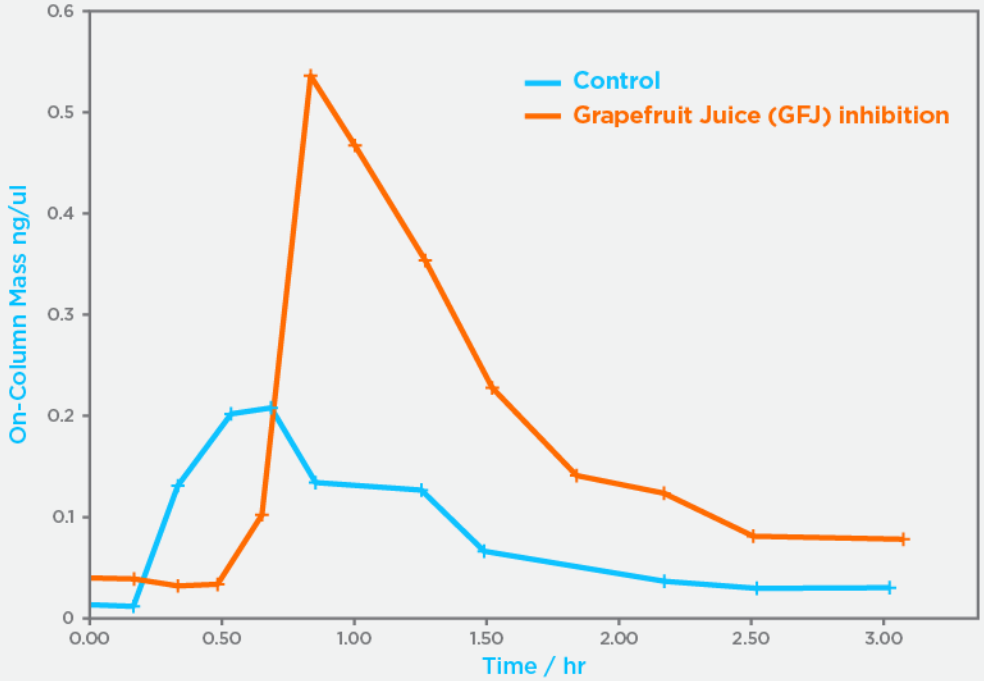


Limonene breath concentration after EVOC administration

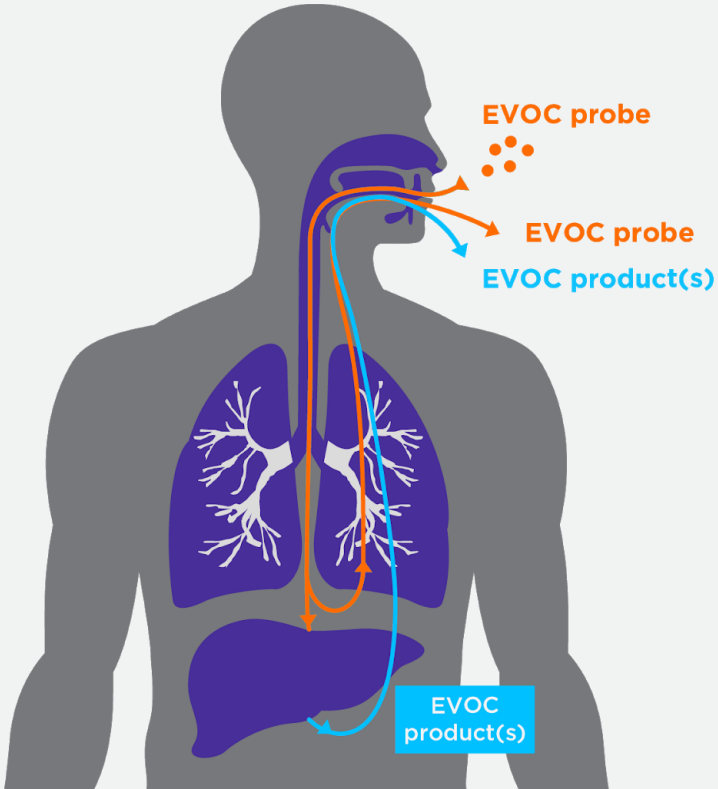


- Background level of EVOC probe compound limonene measured in 136 people (blue circles).
- After administration of EVOC probe breath concentration of limonene (orange circles) increased sharply.

Volunteer A - Eucalyptol washout before and after GFJ CYP3A4 inhibitor



Advantages of EVOC Probes



- Exogenous VOCs can also be used to assess metabolic function *in vivo*
- Probe and any volatile metabolic products are rapidly secreted in breath
- Assess enzymatic activity by monitoring clearance of the EVOC probe from the system **and** the secretion of metabolic product(s) generated.

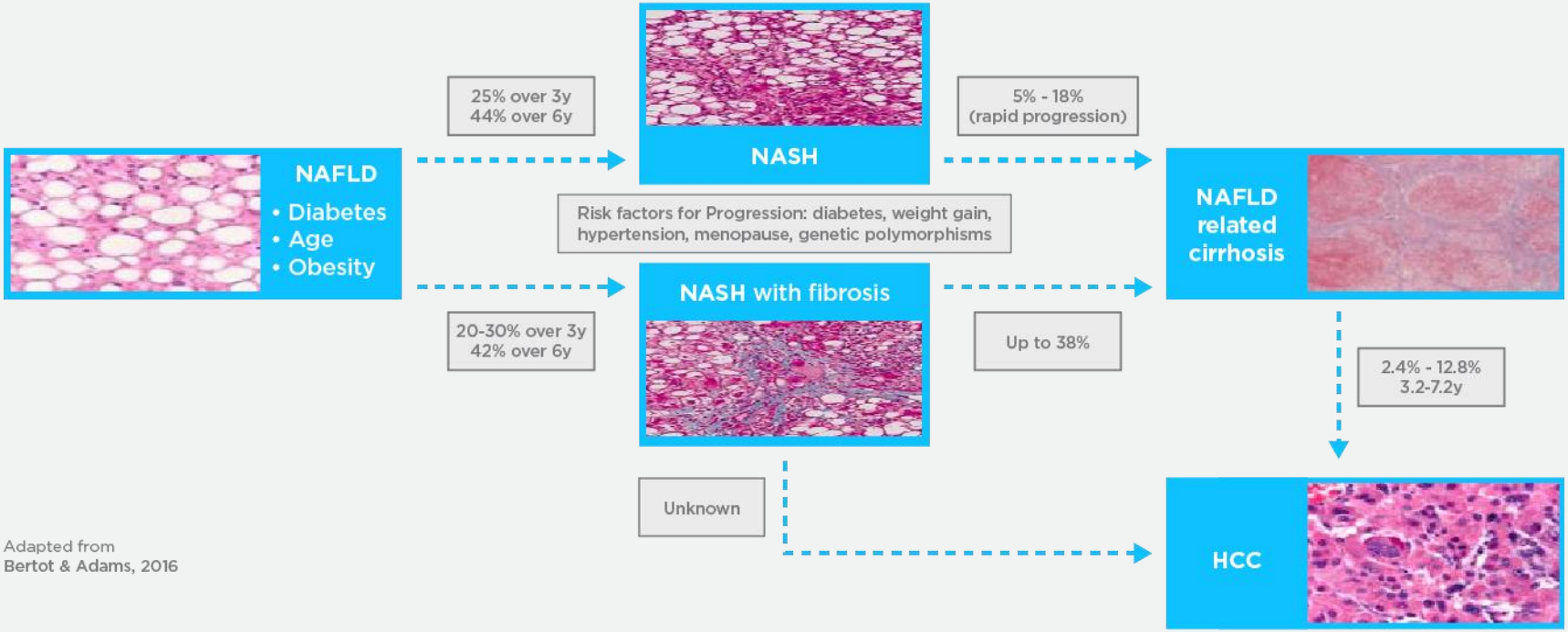
\+ **Completely non-invasive**

\+ **Can administer cocktail of probes** - test multiple targets

\+ **Safe probes simplify regulatory requirements**

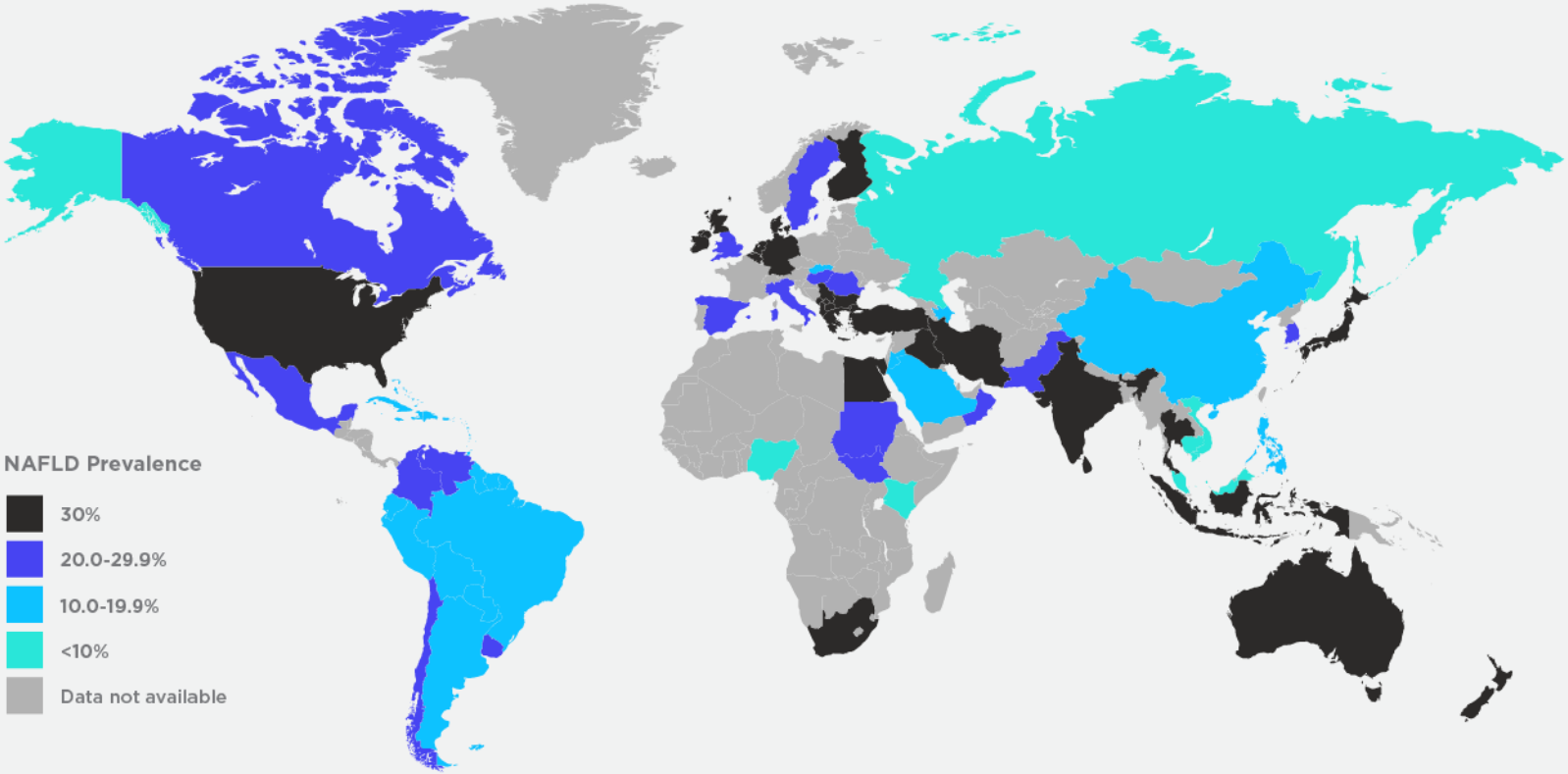
\+ **EVOC probe substrates are very low cost**

NAFLD and NASH

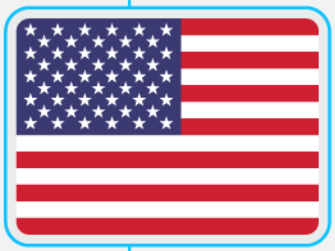


Adapted from
Bertot & Adams, 2016

Global Prevalence of NAFLD



Economic Burden / Cost of Disease



- Over 64 million people projected to have NAFLD
- Estimated annual direct medical costs of $103bn ($1,613 per patient)
- Estimated annual societal costs of $189bn ($2,947 per patient)
- Totalling $292bn per year ($4,560 per patient)

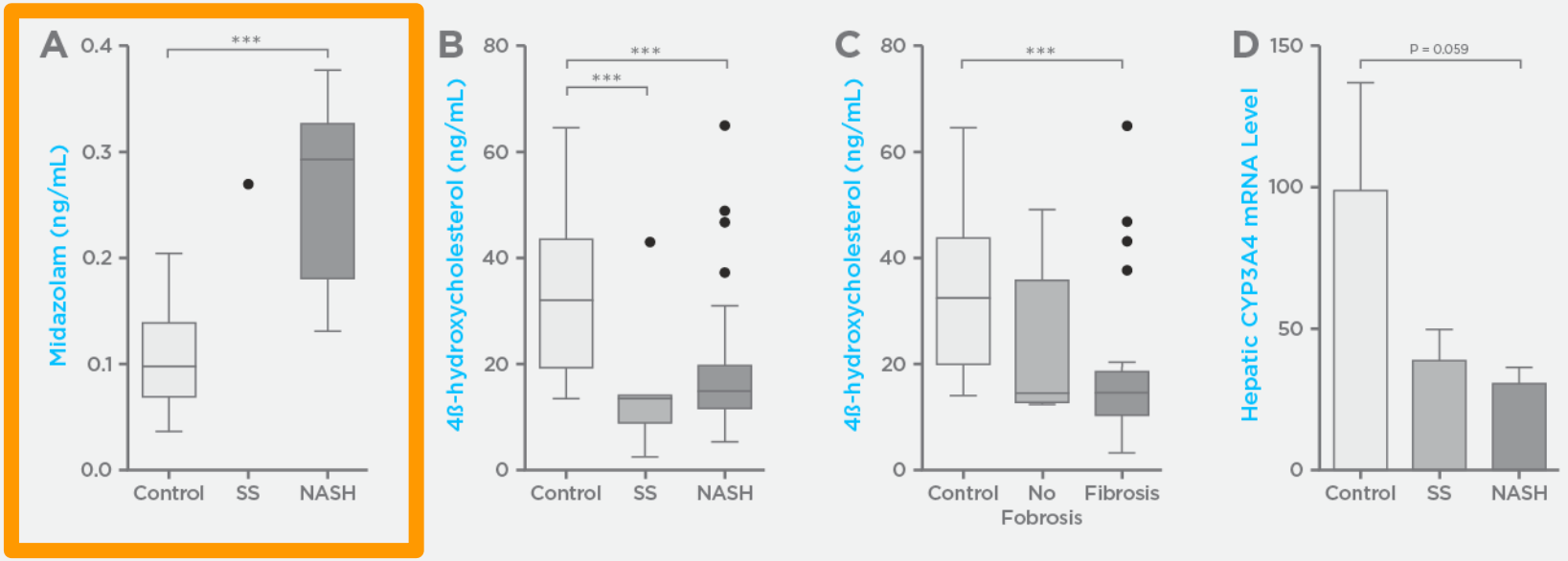
Direct Medical Cost
Annual hospitalisation costs for NAFLD (without cirrhosis), applied to all non-cirrhosis states

Societal Costs
Monetary value assigned to QALYs lost due to NAFLD

- Approximately 52 million people with NAFLD
- Estimated annual direct medical costs of approx. €35bn (from €354 to €4,421 per patient)
- Estimated annual societal costs of approx. €200bn (from ~€2,500 to €4,421 per patient)
- Totalling ~€235bn per year (from ~€2,975 to €5,460 per patient)

- Highest total costs in patients age 45-65 due to increased prevalence in this group
- Highest individual patient costs in group aged 65+, reflecting individual in more advance state of disease
- This is likely to increase as the incidence of NAFLD rises

CYP3A4 Activity and Expression in Nonalcoholic Fatty Liver Disease- Woolsey et al



CYP3A4 activity and expression in NAFLD. (A) Plasma MDZ concentrations 3 hours after oral MDZ microdose (100 mg) in healthy control (n = 20) and biopsy proven NAFLD subjects (SS, n = 1; NASH, n = 9). Shown as Tukey box plots with median (line), 25 to 75 percentiles (box), and minimum/maximum values (whiskers). Statistical analysis by two-tailed t test (control versus NASH). (B) Fasting, plasma 4b-OHC concentrations in control (n = 20) and NAFLD subjects (SS, n = 7; NASH, n = 23). Statistical analysis by one-way ANOVA followed by the Dunnett test. (C) Plasma 4b-OHC concentrations in healthy controls (n = 20) and NAFLD subjects according to histologic assessment of fibrosis (no fibrosis, n = 6; fibrosis, n = 24). Statistical analysis by one-way ANOVA followed by the Dunnett test. (D) CYP3A4 mRNA expression in archived normal liver tissue (n = 9) and NAFLD liver biopsy samples (SS, n = 3; NASH, n = 14) compared using one-way ANOVA followed by the Dunnett test. Bars represent means with S.E.M. Gene expression was normalized to a commercial normal pooled human liver RNA sample. **P , 0.001; ***P , 0.0001. ANOVA, analysis of variance

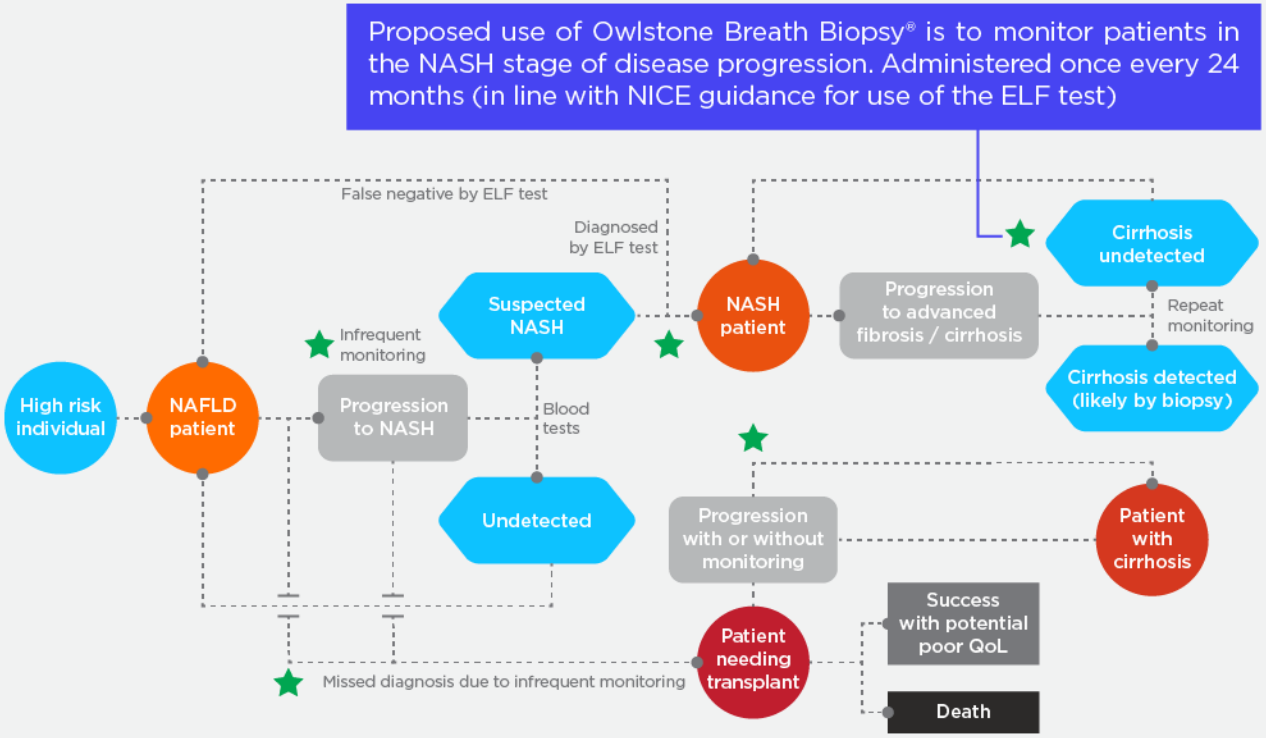
Opportunities for Breath Biopsy Test

Each green star represents an opportunity for a reliable and frequent non-invasive test to be conducted, reducing the likelihood of progression

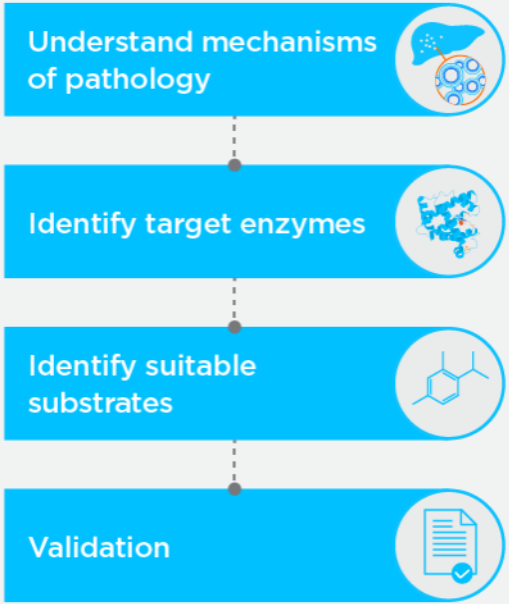
Although costs would increase at each of these timepoints, cost burden could be reduced through:

- Earlier intervention
- Less progression to severe disease
- Less need for transplant
- Less death from disease
- Less survivors with poor QoL

*It should be noted that these opportunities are related to the development of an effective treatment, but the pipeline in this area is rich.



Designing EVOC probes



1.Optimise pairing of substrate to enzyme(s): different enzymes have different substrate-specificity, and this might also be affected by disease conditions. Moreover, it is unlikely that an EVOC probe is metabolised by a single enzyme, as usually multiple enzymes contribute to metabolism of exogenous compounds. While this aspect can be harnessed for the development of multiplex approaches aimed at assessing multiple enzyme activities at once, this is a critical issue when building assays for specific enzymes. Screening of different EVOC probes, and analysis of the specificity of different enzymes for the same probe, can finally lead to an optimised match between EVOC probe and enzyme of interest.

2. Identify viable route of administration: several routes of administration (oral, intravenous, sublingual, inhalation, transdermal, etc) can be envisioned based on the enzyme activity of interest. Organ/tissue distribution of the target enzyme will dictate the choice of the route of administration. For instance, if the target enzyme is present in the gastrointestinal tract, oral administration is preferable, while in case of hepatic expression either oral or intravenous administration could constitute viable options. Of note, the route of administration can drastically affect assay kinetics, with oral administration usually being slower than intravenous injection due to first-pass metabolism.

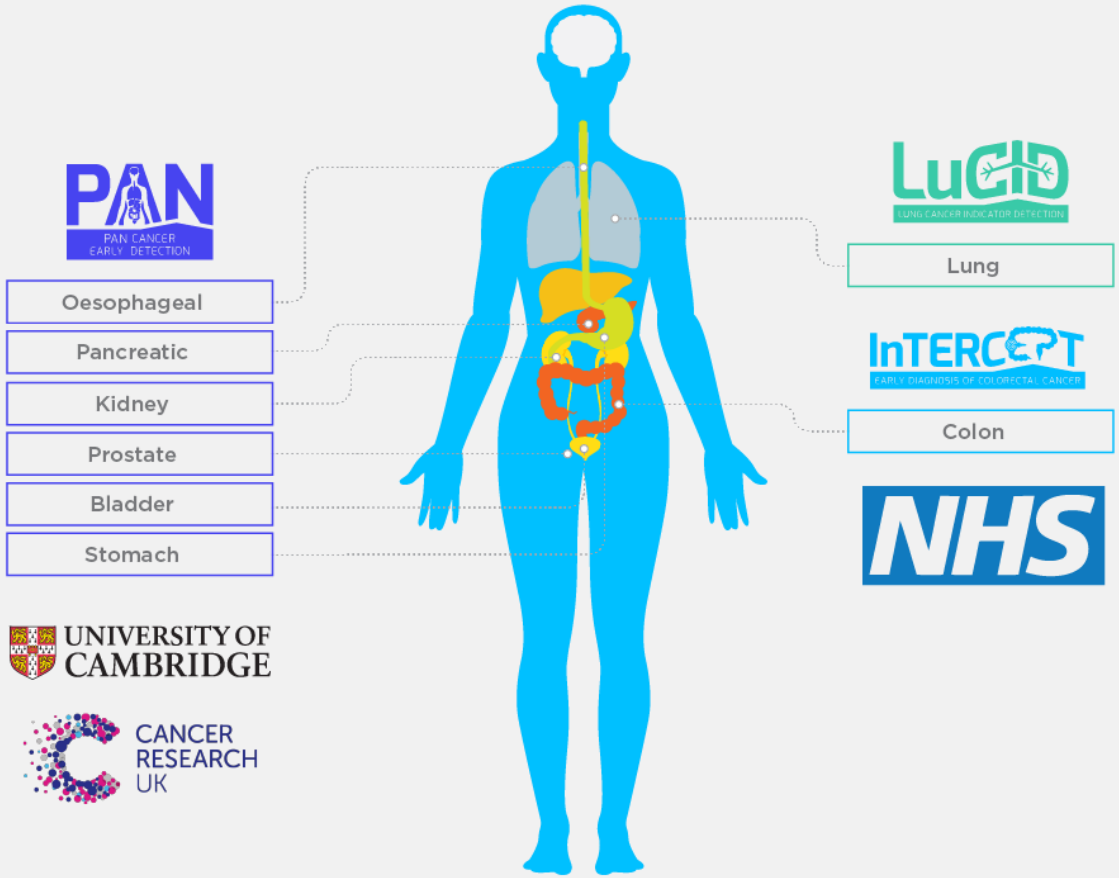
3. Distribution kinetics: distribution of different compounds in the body is affected by route of administration, metabolism kinetics, as well as by physicochemical properties of the EVOC probe itself. For instance, lipophilic compounds will be retained in fat deposits, and excreted via breath, for longer time than hydrophilic compounds. These considerations have to be taken into account when selecting EVOC probes.

4. Likelihood of secretion in breath: this aspect will depend on the EVOC probes, or derived metabolites, and is based on volatility of the compounds of interest. This depends on physical properties of the compounds, such as boiling point and water/air partition coefficient. Selection of EVOC probes that, not only are metabolised by the enzyme of interest, but also are secreted in breath at high proportions, is fundamental for the development of EVOC probe strategies.

5. Dosage of EVOC probes: the amounts of EVOC probe that reach the enzyme of interest will determine the ability of the assay to reveal differences in enzymatic activity. Indeed, evaluation of enzymatic activity is usually measured as a function of substrate concentration [85]. Defined ranges of substrate concentrations are needed to assess differences in enzyme Vmax (the maximal catalytic rate with saturating concentrations of substrate) or Km (enzyme affinity for the substrate). Appropriate dosage of EVOC probe will change according to the enzyme of interest.

6. Kinetics of metabolism and breath excretion of the EVOC probe itself, and/or of product metabolites, in healthy subjects have to be determined in order to measure intra- and inter-individual variability, as well as to assess contribution of potential confounding factors such as diet, lifestyle, age, gender, current medication, etc. Breath values from healthy subjects can

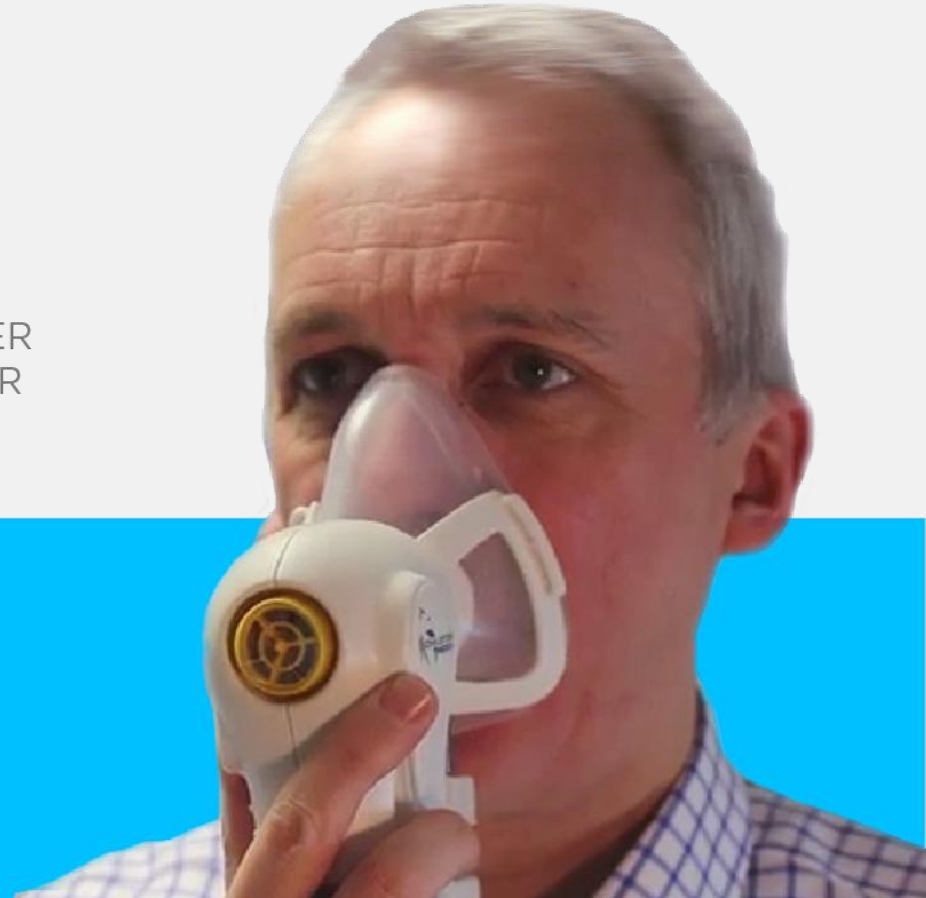
Owlstone Medical Cancer Clinical Trials



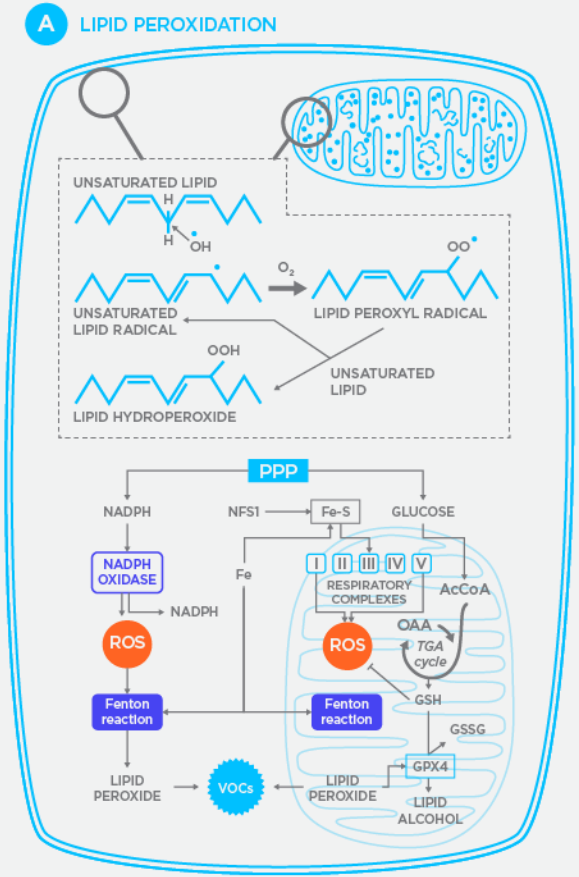
LuCID: Lung Cancer Indicator Detection

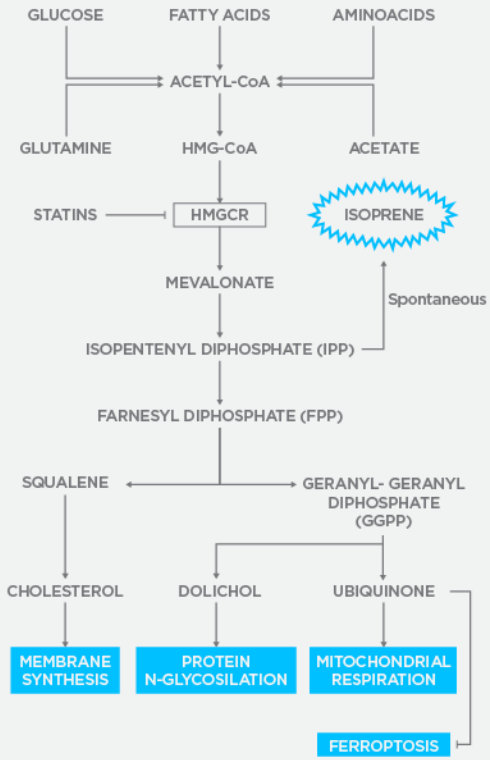
- FUNDED BY THE NHS
- LARGEST BREATH BIOMARKER TRIAL EVER UNDERTAKEN IN THE WORLD RECRUITING UP TO **4,000** PATIENTS
- EVALUATING UTILITY OF BREATH BIOMARKER DETECTION IN DETECTION OF LUNG CANCER

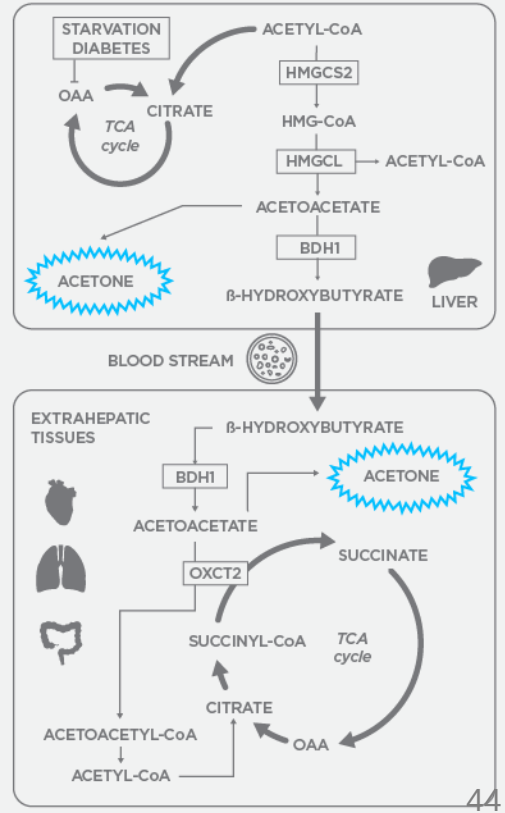
- CHIEF INVESTIGATOR
  - **DR ROBERT RINTOUL**,
  PAPWORTH HOSPITAL NHS
  FOUNDATION TRUST CANCER
  RESEARCH UK CAMBRIDGE INSTITUTE



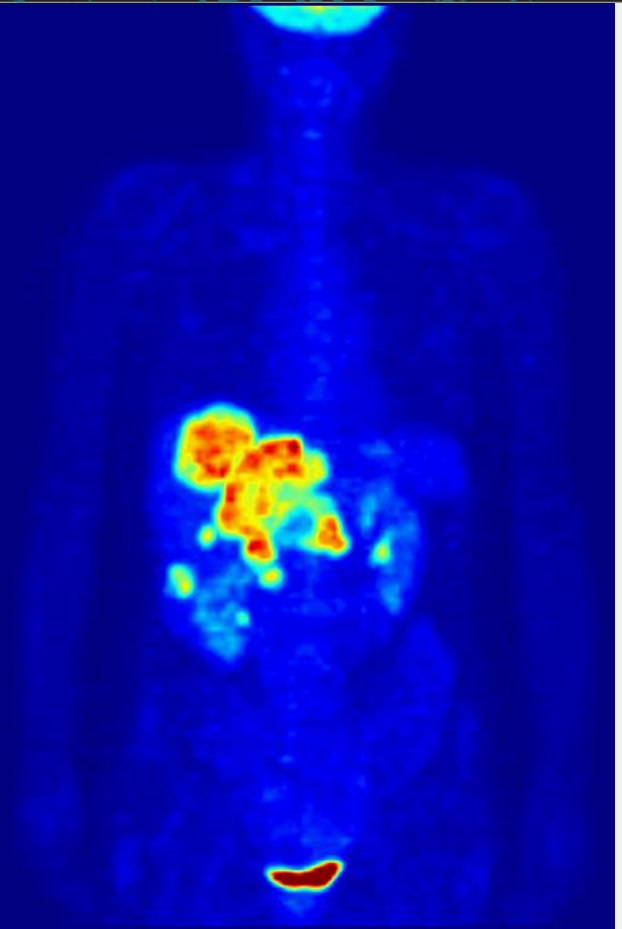
Understanding of endogenous VOC pathways

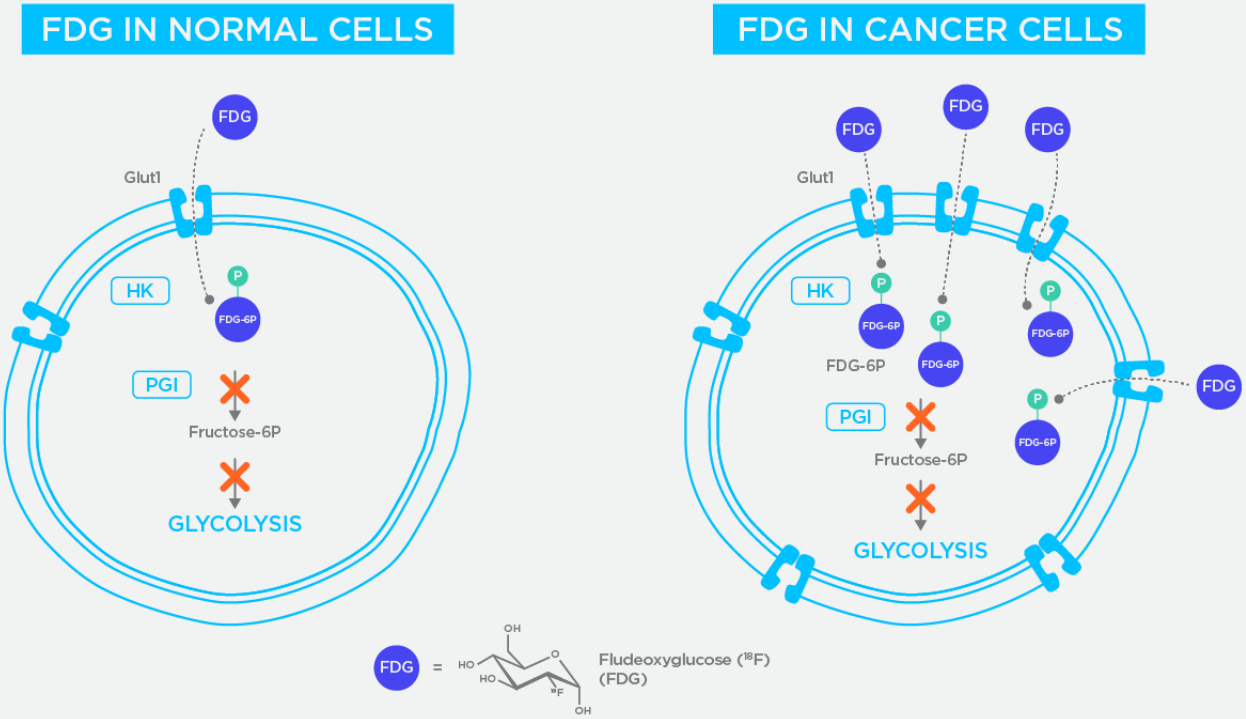




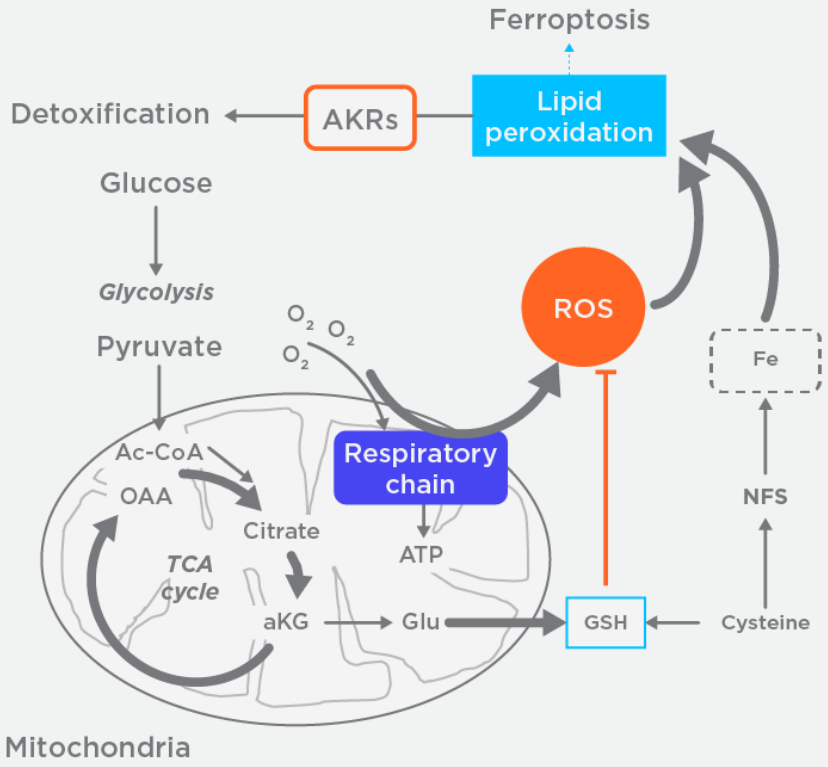


Exogenous probes are the basis of PET scans

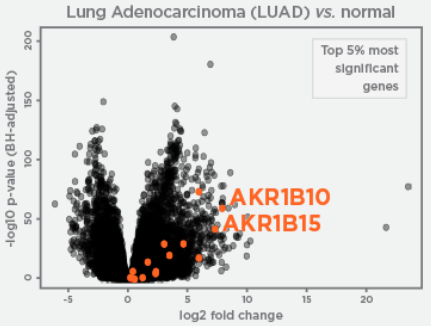


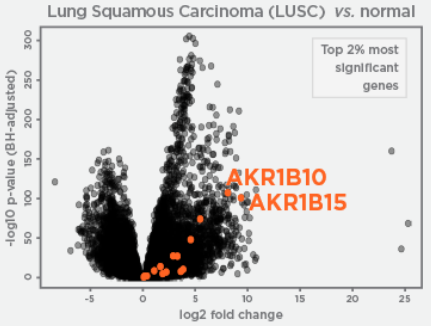


Aldo-keto reductases (AKRs) control lipid peroxidation in lung cancer

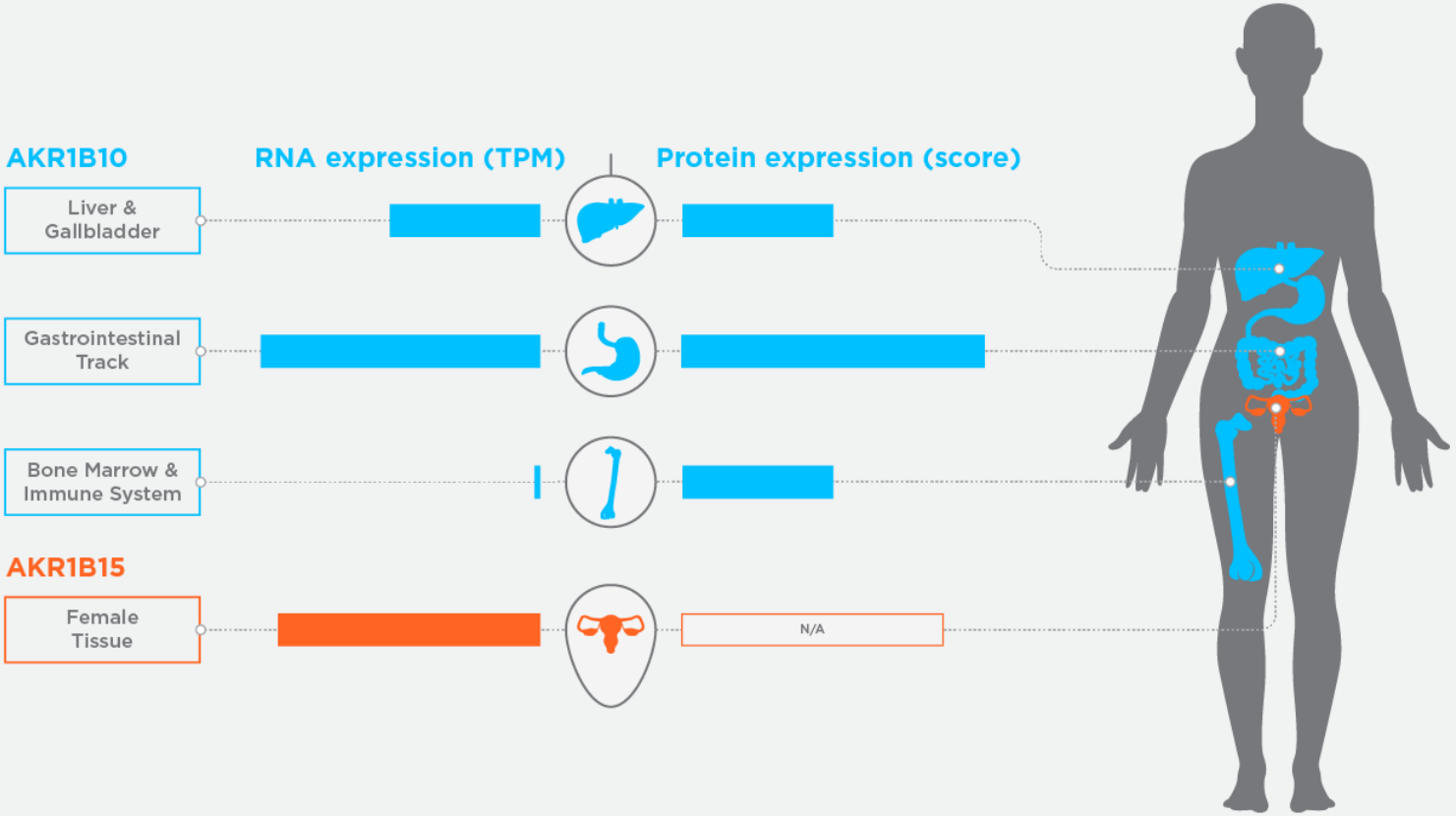


AKR1B10 and AKR1B15 expressed significantly more in lung cancer patients



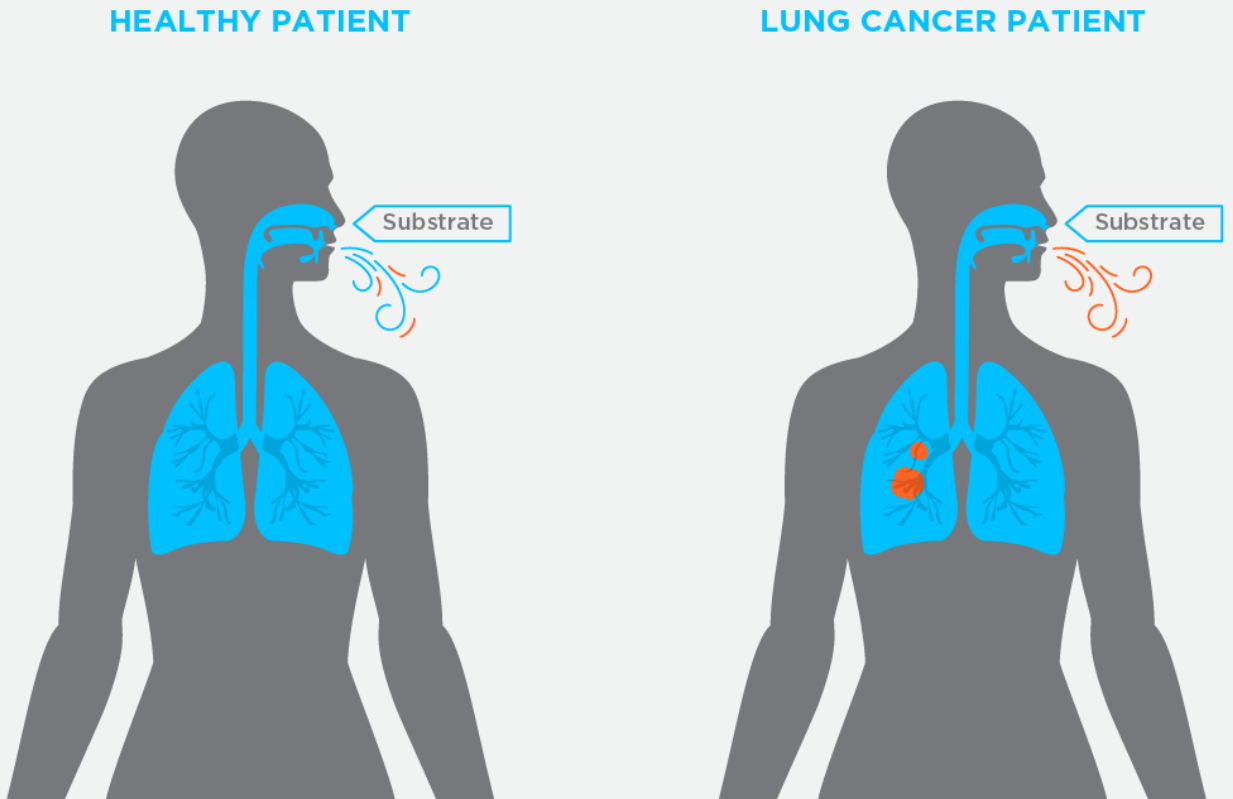


AKR1B10-B15 are not normally expressed in the lungs



Administration of EVOC probe in lung cancer

Assessing presence of cancer through altered metabolic **ACTIVITY**



Acknowledgements

Thanks to the all Owlstone Team, in particular:

- Max Allsworth
- Edoardo Gaude
- Stefano Patassini
- Jasper Boschmans
- Morad Nakhleh
- Marc van der Schee