



# Breath Analysis and Cancer: The Future of Early Detection and Personalised Medicine

### VOCs and Breath

There are many challenges associated with cancer management, including delayed diagnosis, low efficacy of treatment, and heterogeneity of disease. CT, MRI, and PET scans cannot identify small tumours, meaning it is difficult to achieve early diagnosis for patients. Histological biopsy is considered the gold standard for cancer diagnosis; however, it is invasive, time-consuming, and expensive. There is an urgent need for low cost and accessible diagnostic techniques that would allow for the earlier detection of cancer, personalised therapy, and assessment of treatment efficacy.

picked up, and distributed via the bloodstream, before being exhaled in breath (Figure 1). Exogenous VOCs interact with biological systems and provide valuable health and disease information. The collection, identification, and quantification of these VOCs can provide a window into what is happening inside the body, offering great potential as non-invasive biomarkers to indicate disease onset and progression.

Exhaled breath, enriched with VOCs, offers a promising sample matrix for clinical analysis and diagnosis of disease. By testing breath samples against established reference ranges, clinicians in the future could detect abnormal VOC levels that may signal the presence of disease. Compared to traditional sampling methods such as blood and faeces, breath analysis provides several key benefits; primarily its non-invasiveness, enhancing patient comfort and simplifying the approval process for clinical trials. Breath is an abundant and renewable resource, allowing for preconcentration of its compounds before analysis, which can improve test accuracy. Another important advantage is the flexibility of breath sampling, which can be conducted virtually anywhere. This opens the door to decentralised trial designs and at-home testing, making disease monitoring and diagnosis more accessible and convenient for patients.

Volatile organic compounds (VOCs) are gaseous compounds produced throughout the body by numerous biological processes and released in biological samples such as faeces, urine, blood, and breath, with many advantageous attributes that position them as candidate novel biomarkers for cancer. The VOCs detectable in the breath can originate either from within the body (endogenous VOCs) or from external sources such as diet, medication, and environmental exposure (exogenous VOCs). Endogenous VOCs are produced throughout the body,

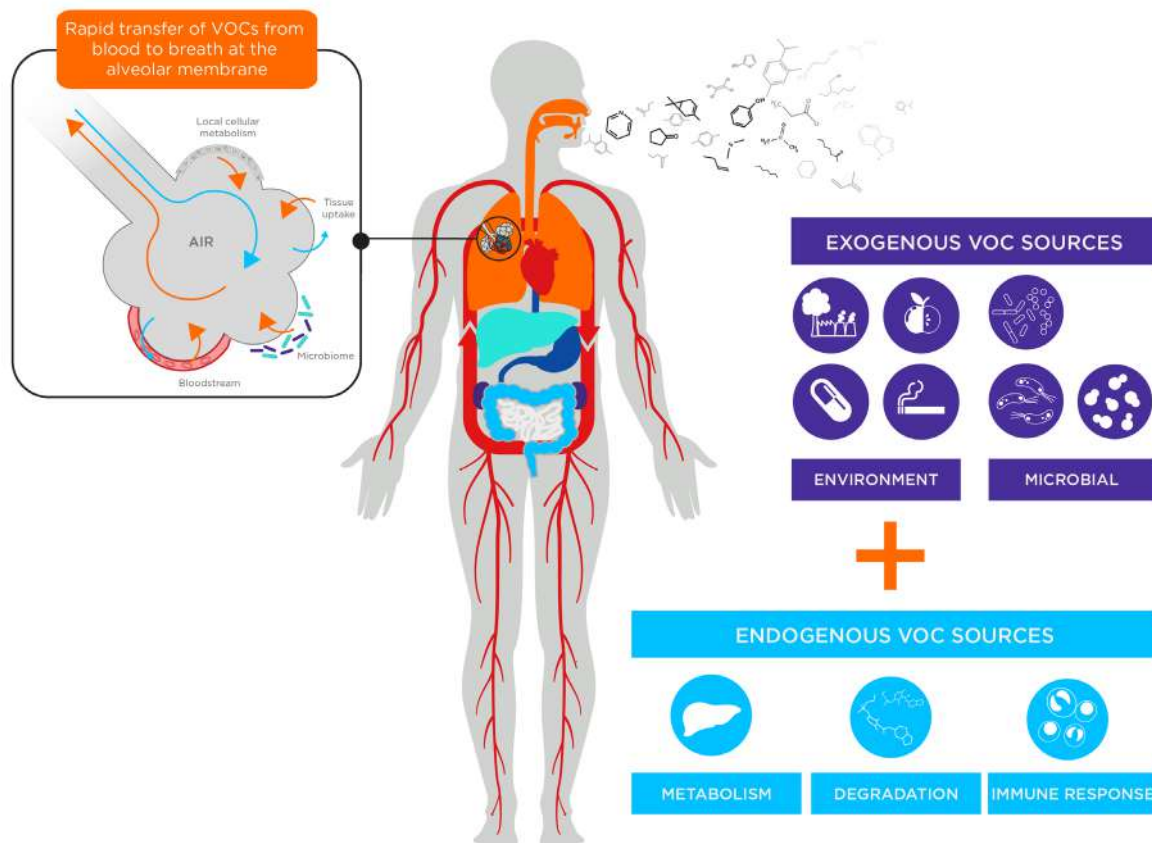


Figure 1. Pathways of volatile organic compounds (VOCs) from exogenous (environmental and microbial) and endogenous (metabolic, degradation, and immune response) sources to exhaled breath



### Measuring VOCs in Breath

Breath testing presents significant challenges due to the complex nature of breath itself, as well as the technical difficulties in the collection and analysis of exhaled breath. The VOCs are often found in very low concentrations in breath, frequently at trace levels, making them hard to detect without highly sensitive equipment. Researchers must also ensure that breath samples are uncontaminated with VOCs from background sources. Without proper collection and storage protocols, contaminants such as ambient air, bacteria, or particles from the collection apparatus can alter results. There are then often challenges associated with analysing the breath samples due to the complexity of breath and the thousands of compounds that are found in breath, many of which may not be characterised, as they require advanced analytical tools which can be time-consuming and costly. The entire breath collection process needs to be rigorously controlled to ensure reliable and reproducible results.<sup>1</sup>

To overcome these challenges, instruments such as the ReCIVA® Breath Sampler have been developed by multi-disciplinary leaders in the breath research field as an optimised tool for robust and reliable breath sample collection whilst ensuring safety and comfort during use.<sup>2</sup> This device enables the collection of replicate breath samples by directly capturing and pre-concentrating VOCs from breath onto sorbent tubes. The ReCIVA is connected to a source of clean air for users to breathe into, which minimises the contribution of contaminating VOCs entering a breath sample from the outside air, and therefore VOCs present in the breath can be distinguished more easily. Studies often utilise gas chromatography-mass spectrometry (GC-MS) due to its heightened sensitivity and capacity to handle a wide range of VOCs at low concentrations to detect and analyse VOCs accurately in the breath.

### Metabolic Processes

Understanding the mechanistic origin of VOCs detectable in the

breath and identifying those compounds involved in cancer-related pathophysiology and metabolic pathways is important in assessing whether they could serve as biomarkers for cancer. In normal cells, energy is produced through aerobic respiration, which includes glycolysis, the tricarboxylic acid cycle (TCA) cycle, and oxidative phosphorylation. When oxygen is limited, pyruvate, the end-product of glycolysis, no longer enters the TCA cycle but is converted into lactate. However, cancer cells require vast amounts of energy to support their rapid growth. The Warburg effect refers to the phenomenon of increased aerobic glycolysis and lactate production, along with the disruption of energy production in the TCA cycle, which cancer cells require for rapid proliferation. This altered metabolism results in increased production of acetyl-CoA, which in turn elevates the levels of ketones that can be detected in exhaled breath (Figure 2).

With the progress made in detecting and quantifying breath VOCs over the past decades, a recently published systemic review performed a meta-analysis of breath (and urinary) VOCs to assess their diagnostic potential in cancer detection.<sup>3</sup> Based on the analysis of 85 publications, the authors reported that VOCs have a sensitivity of 0.89 and a specificity of 0.88 for cancer screening. The authors then focused on metabolic pathways, identifying several microbiota-related VOCs in lung, colorectal, breast, and liver cancers that enriched various metabolic pathways.<sup>3</sup> Notably, butanoate metabolism was enriched across all four cancer types and was the most enriched pathway in lung, breast, and liver cancers.

These findings highlight the importance of one specific metabolite, butyrate, which is generated by gut microbial fermentation. Butyrate serves as a transport substrate in butanoate metabolism and plays a critical role in regulating host energy homeostasis. Since altered levels of butyrate have been associated with several diseases, including cancer, it holds potential as a biomarker.<sup>4,5,6</sup> While butyrate can be detected

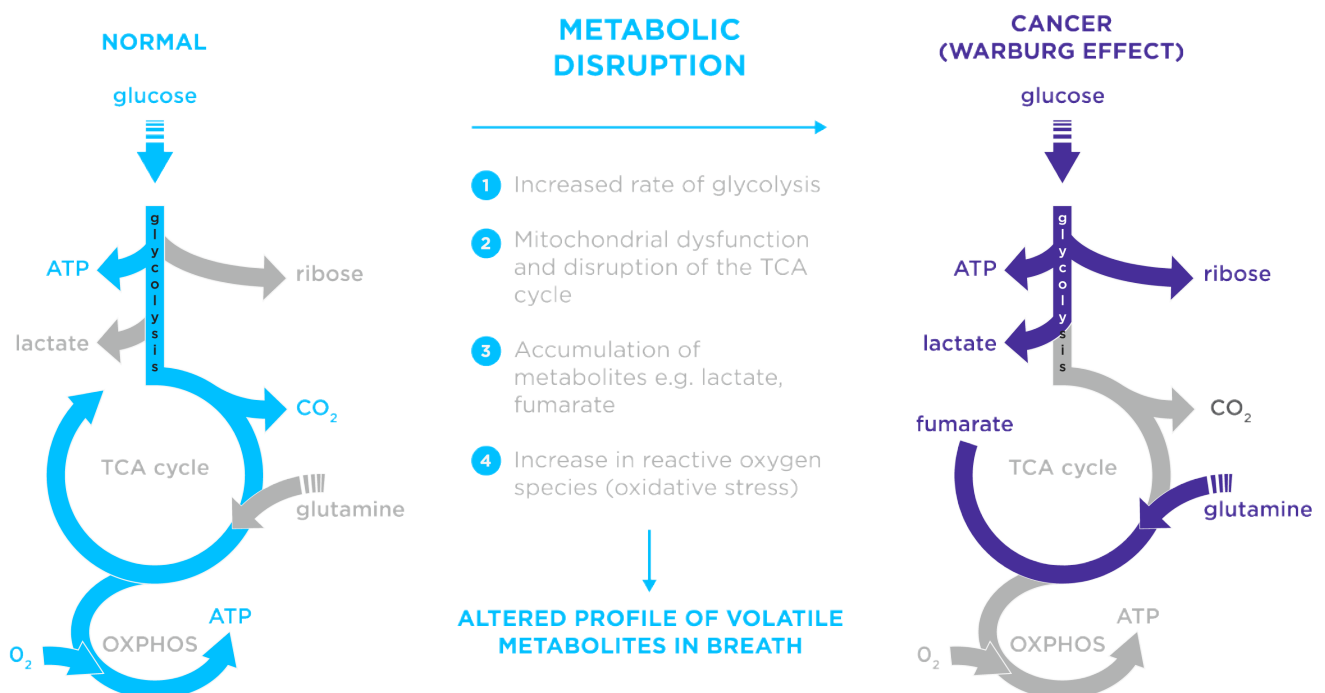


Figure 2. Comparison of normal metabolism and cancer metabolism (Warburg effect)

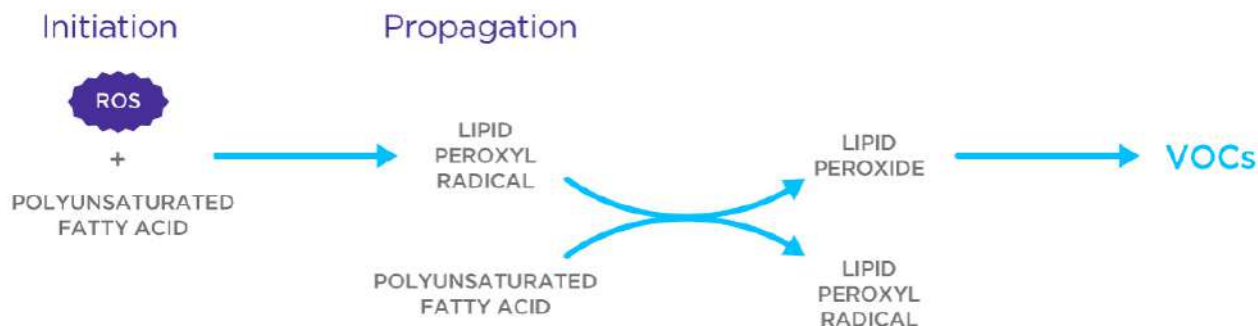


Figure 3. Lipid peroxidation pathway leading to the formation of volatile organic compounds (VOCs)

from blood samples, butyrate is volatile and detectable in exhaled breath, and so could support future clinical applications, including disease management and exploring therapeutic targets.

### Cancer and Inflammation-Associated VOCs.

Chronic inflammation in the body can have many negative impacts on health. Specifically, chronic inflammation has been associated with the development and progression of various types of cancer.<sup>7</sup> The mechanisms that link cancer and inflammation are complex and include interactions between immune cells and signalling molecules that can promote tumour growth and metastasis. Being able to detect signs of chronic inflammation in the body would, therefore, be useful for diagnosing and monitoring patients as well as in clinical research. As certain VOCs are byproducts of the metabolic processes altered by inflammation and cancer, breath analysis can offer a non-invasive way of detecting cancer earlier, as well as monitoring disease progression through the detection of inflammatory markers.

Studies have found that nearly all neoplastic lesions (abnormal masses of tissue that form when cells divide and grow more than they should) contain immune cells at densities ranging from subtle infiltrations to gross inflammation.<sup>8</sup> Research on the link between inflammation and cancer pathogenesis continues to produce results demonstrating the tumour-promoting effects that immune cells (mainly from the innate immune system) have on cancer progression. For example, inflammation can supply the tumour microenvironment with bioactive molecules. These molecules can include growth factors that increase proliferative signalling, factors that limit cell death, and enzymes that facilitate angiogenesis and metastasis.<sup>9</sup> Angiogenesis plays a role in the growth of cancer as tumours need a consistent blood supply if they are to grow and metastasize. Tumours can cause this blood supply to occur by giving off chemical signals that stimulate angiogenesis and resulting blood vessels feed tumours with nutrients and oxygen, allowing tumours to metastasize.<sup>10</sup>

When inflammation occurs, the increased production of reactive oxygen species (ROS) can lead to oxidative stress, including lipid peroxidation. Lipid peroxidation is the oxidative degradation of lipids in cell membranes which generates VOCs such as alkanes and aldehydes. Oxidative stress and lipid peroxidation have been associated with cancer progression, specifically in lung carcinogenesis, as oxidative stress in lung tissue lipids can cause the presence of ROS in the lungs (Figure 3).<sup>11</sup> Studies have been conducted to compare the

VOCs produced by lung cancer cells *in vitro* to the ones found in exhaled breath from lung cancer patients.

A study by Buszewki *et al.* quantitatively measured VOCs in the headspace of healthy and lung cancer tissues and compared these results to VOCs obtained from breath samples of healthy individuals and lung cancer patients.<sup>12</sup> A total of 22 compounds were found in both the headspace of cancerous tissues and the breath of lung cancer patients, including alcohols, aldehydes, ketones, and hydrocarbons. Compounds such as acetone, ethanol, 1-propanol, and carbon disulfide were found in higher levels in the headspace of cancerous tissues compared to healthy tissues. These same compounds were also found in increased concentrations in the breath samples of patients with lung cancer and could, therefore, be used as biomarkers of lung cancer. Aldehydes and alkanes are products of lipid peroxidation from inflammation, highlighting that the compounds found in exhaled breath in this study could be markers of inflammation caused by cancer and be used to identify and diagnose cancer at an earlier stage through exhaled breath analysis.

### EVOC Probes and Lung Cancer

Understanding cancer-related metabolic pathways opens the door to developing powerful dynamic breath tests with boosted diagnostic accuracy, such as those that use an EVOC (exogenous VOCs) probe-based approach. EVOC probes are designed to target specific enzymes that are part of the affected metabolic processes in a disease state. Ingestion of an EVOC probe and measuring the probe and its metabolic products through a breath test has shown safety and great success in liver disease, where a longitudinal study showed that levels of the administered EVOC probe returned to baseline more slowly in cirrhotic patients than in controls after ingestion, with the best-performing timepoint observed at 60 minutes post-administration (AUC = 0.91).<sup>13</sup>

With the current understanding of cancer-related metabolic pathways, EVOC probes targeting the Warburg effect or lipid peroxidation pathways with high sensitivity and specificity could greatly benefit early detection in a non-invasive manner. In many cancer types, increased oxidative stress can lead to the overproduction of aldehydes, and the enzyme aldo-keto reductase (AKR) is suggested to effectively remove aldehydes by converting them into alcohols.<sup>14</sup> Also, in healthy cells, certain enzymes are present inside the cells, whereas in cancer cell types these enzymes are found to leak out the cell and be present in the tumour microenvironment.<sup>15</sup> Leveraging this concept, researchers have strategically designed an EVOC probe to amplify altered metabolic signals and measure

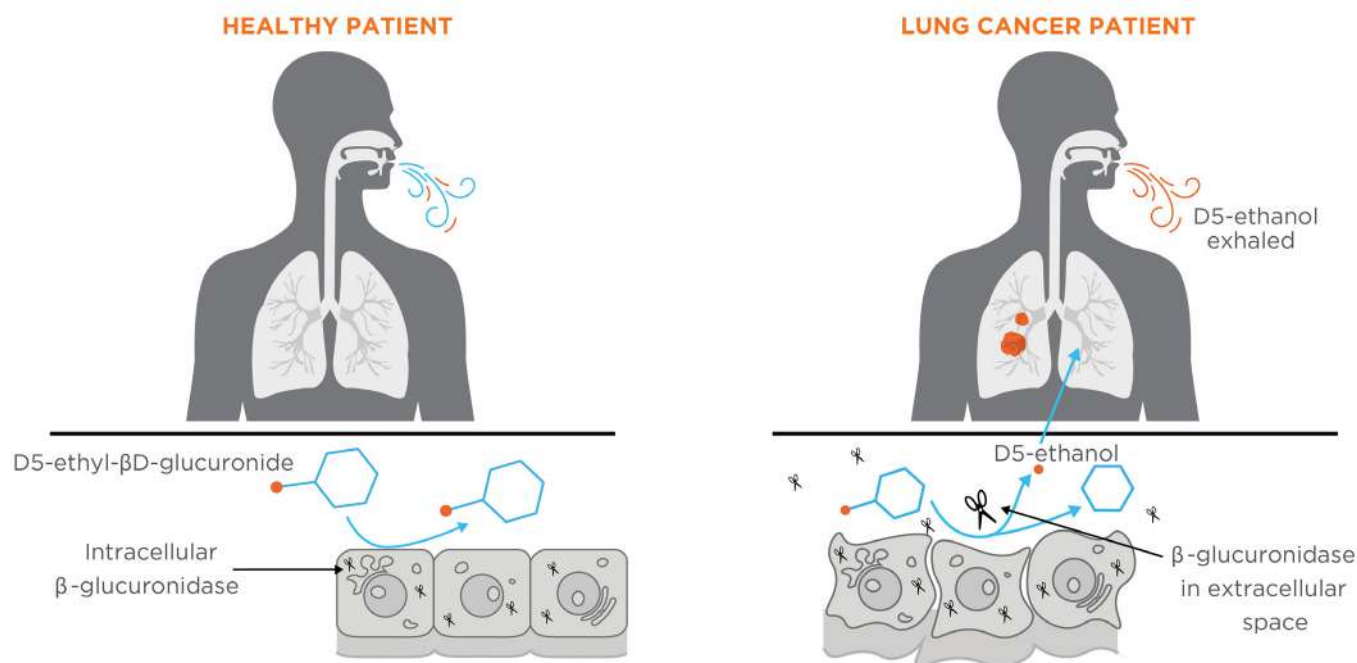


Figure 4. A schematic indicating how  $\beta$ -glucuronidase in the extracellular space around a tumor in the lungs can metabolize the D5-ethyl- $\beta$ D-glucuronide probe into D5-ethanol

VOC production in exhaled breath for lung cancer detection. The probe uses D5-ethyl- $\beta$ D-glucuronide as a substrate and targets  $\beta$ -glucuronidase, an enzyme with elevated levels in the extracellular space of cancer cells. The enzymatic reaction cleaves D5-ethanol, which can be measured in exhaled breath as a reporter for lung cancer screening (Figure 4).

The D5-ethyl- $\beta$ D-glucuronide EVOC probe has demonstrated its safety, mechanism of action, and dosing range in the Phase I study, and a Phase II study is currently underway to confirm the robustness and specificity of this EVOC probe for lung cancer detection.<sup>16</sup> As an innovative approach to breath testing, the use of EVOC probes is expected to expand rapidly in breath science over the coming years, highlighting the rapid evolution of the breath research field, and promising a future where early cancer detection and monitoring can be conducted non-invasively through a simple breath integrated into clinical practice. Other future applications of breath tests for cancer include the development of at-home devices for real-time sample analysis which utilize sensor technologies to detect concentration changes of targeted compounds. When developing portable breath analysis devices, it is crucial to focus not only on disease-relevant biomarker selection, but also on the concentration range of targeted compounds in healthy versus diseased individuals and the detection limits of the chosen sensors.

#### Artificial Intelligence in Breath-based Diagnostics

With the breakthroughs in artificial intelligence (AI) in recent years, it is anticipated that AI could facilitate the processing of large and complex breath datasets, while machine learning algorithms for breath pattern recognition could enhance diagnostic capabilities.<sup>17</sup> The future of breath analysis technology holds great promise for revolutionising disease diagnosis, management, and treatment monitoring, with breath tests for early cancer screening potentially being integrated into routine physical check-ups at clinics. With the right biomarkers, home-based breath testing devices could offer a convenient

solution to monitor health status after treatment, providing longitudinal data that could help physicians notice potential recurrences early. The non-invasive nature of breath tests holds the potential to reduce healthcare costs while improving, and even saving, millions of lives.

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## Dr. Hsuan Chou

Dr. Hsuan Chou is a senior biomarker scientist at Owlstone Medical, where she ensures the successful delivery of customer project results and contributes to manuscript writing. She also supports the biological aspects of internal product development and plays an active role in creating scientific content for the company's technical sales and marketing efforts. Dr. Chou holds a PhD in Plant Science from the University of Connecticut and has several years of postdoctoral experience working with omics data in the broader biology field at North Carolina State University before joining Owlstone in late 2021.

Email: [hsuan.chou@owlstone.co.uk](mailto:hsuan.chou@owlstone.co.uk)



## Lucy Godbeer

Lucy Godbeer is a scientific marketing associate at Owlstone Medical, assisting with scientific content writing for the company. Lucy graduated with a BSc in Biology and has an MSc in Biomedical Science.

Email: [lucy.godbeer@owlstone.co.uk](mailto:lucy.godbeer@owlstone.co.uk)