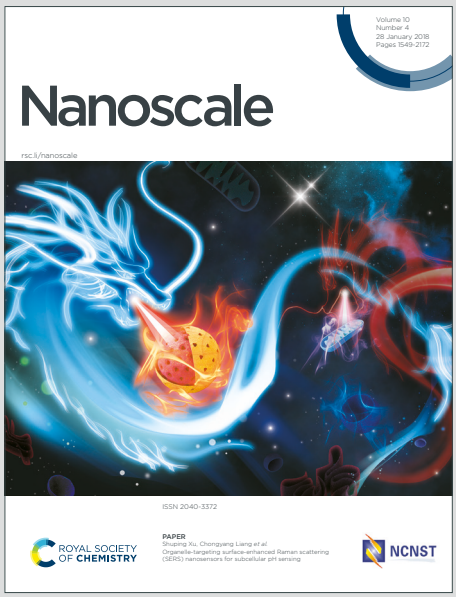
Nanoscale

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: N. Kommineni, N. Kaur, V. G.S. S. Jyothi, N. Narang, M. M. Islam, P. Basim, M. Woollam, L. K. Vora, M. Agarwal, J. Lovell and R. Prasad, *Nanoscale*, 2025, DOI: 10.1039/D5NR01714A.



ROYAL SOCIETY OF CHEMISTRY

rsc.li/nanoscale

View Article Online
DOI: 10.1039/D5NR01714A

Harnessing Volatile Organic Compound Biomarkers for Early Cancer Detection: Molecular to Nanotechnology-based Approaches

Nagavendra Kommineni[1#], Navneet Kaur[2#], Vaskuri G.S. Sainaga Jyothi[3#], Nikesh Narang[4#], Mohammad Mirazul Islam[4], Pratap Basim[1], Mark Woollam[5], Lalitkumar K.Vora[6], Mangilal Agarwal[5,7], Jonathan F. Lovell[8], Rajendra Prasad[9]*

[1]*Thermo Fisher Scientific Inc., Cincinnati, OH 45237, USA.*

[2]*Department of Chemical and Biomedical Engineering, FAMU-FSU College of Engineering, Tallahassee, FL, USA, National High Magnetic Field Laboratory, Tallahassee, FL, 32310, USA.*

[3]*Department of Pharmaceutical Sciences, University of Tennessee Health Science Center (UTHSC), Memphis, TN 38163, USA.*

[4]*Department of Ophthalmology and Visual Sciences, University of New Mexico School of Medicine, Albuquerque, NM 87131, USA.*

[5]*Integrated Nanosystems Development Institute, Indiana University Indianapolis, Indianapolis, Indiana 46202, USA.*

[6]*School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, BT9 7BL, UK.*

[7]*Department of Biomedical Engineering & Informatics, Indiana University Indianapolis, Indianapolis, Indiana 46202, USA.*

[8]*Department of Biomedical Engineering, University at Buffalo, State University of New York, NY 14068, USA.*

[9]*School of Biochemical Engineering, Indian Institute of Technology (BHU) Varanasi, Uttar Pradesh 221005, India.*

View Article Online
DOI: 10.1039/D5NR01714A

Abstract

Early-stage cancer diagnosis is considered a grand challenge, and even though advanced analytical assays have been established through molecular biology techniques, there are still clinical limitations. For example, low concentration of target biomarkers at early stages of cancer, background values from the healthy cells, individual variation, and factors like DNA mutations, remain the limiting factor in early cancer detection. Volatile organic compound (VOC) biomarkers in exhaled breath are produced during cancer cell metabolism, and therefore may present a promising way to diagnose cancer at the early stage since they can be detected both rapidly and non-invasively. However, there are challenges in VOC analysis, especially regarding standardization of sampling, necessity for preconcentration, and cancer-specificity of biomarkers. There are also additional challenges, including the design and development of highly sensitive miniaturized sensors that detect VOC biomarkers at low concentrations with minimum cost efforts. In the present article, we have reviewed the potential impact of VOCs in cancer detection in the context of traditional methods such as liquid biopsies, which are typically employed at advanced stages of cancer progression. Described ultrasensitive technologies such as gas chromatography-mass spectrometry (GC-MS) and electronic noses using a variety of nanomaterials have been considered as technologies for breath-based early cancer detection.

Key words: Volatile Organic Compounds, Cancer, Biomarkers, Early-stage Diagnosis, Translational diagnostics

View Article Online
DOI: 10.1039/D5NR01714A

Introduction

Volatile organic compounds (VOCs) have emerged as promising biomarkers for early disease detection, especially for cancer, over the past decade. The changes in breath odor and VOC profiles are linked to metabolic alterations that occur during disease progression. Although VOC analysis is also viable in urine, sweat, as well as other biological sample types, exhaled breath is of significant interest in the research community since it is highly non-invasive, of virtually limitless supply, and most importantly a rich source of biomarkers. In 1971, the presence of about 250 VOCs was reported in breath,[1] and since then research in this area has expanded significantly. The human "volatilome" comprises hundreds if not thousands of unique analytes across different biological sample types.[2] Notably, there have been reports showing that canines can sniff VOCs for the detection of cancer.[3] Although the FDA has approved some VOC biomarkers like $^{13}CO_2$ for H. Pylori, branched hydrocarbons for organ transplant rejection, and nitric oxide for asthma,[4,5] currently there are no VOC-based tests implemented clinically for early cancer detection.

Cancer is one of the major causes for deaths and is projected to increase to 15.3 million deaths per year by 2040.[6] Amongst cancers, lung, colon, prostate and breast cancer have been the most prominent ones.[7] Early cancer detection is crucial for identifying cancer prior to clinical manifestation, enabling therapeutic intervention well before metastatic progression, which could significantly improve patient outcomes and quality of life. Traditional cancer diagnoses majorly rely on procedures and techniques like biopsies, radioimmunoassays, imaging techniques (positron emission tomography-computed tomography scan CT PET scan), and endoscopic examinations (colonoscopy, mammography, gastroscopy, etc.). However, method sensitivity is limited to the size of the tumor, and therefore these tests generally do not facilitate early detection.[8–12] Several efforts are in progress for detecting cancer-specific biomarkers including cell-free nucleic acids, circulating tumor cells, proteins, and lipids via analysis of blood or other biofluids.[12–14] However, the low detection values, background values from the healthy cells, individual variation, and factors like DNA mutations from non-malignant clonal hematopoiesis of indeterminate potential, remain the limiting factor in early cancer detection.[15] For instance, interleukin-6 (IL-6) is a biomarker associated with cancer progression, but it is also elevated in conditions like lung fibrosis and chronic kidney disease, complicating its use as a cancer-specific marker.[16–18] Similarly, other pro-inflammatory markers (matrix metalloproteinases/interleukins/cytokines, ratios of neutrophils/lymphocytes)[19,20] are not cancer-specific and therefore may lead to ambiguous signals.

View Article Online
DOI: 10.1039/D5NR01714A

In contrast, VOC analysis may be a faster approach for detecting cancers where the specific biomarkers are released through exhaled breath, sweat, or urine, thus providing an accessible means for monitoring metabolic changes associated with cancer.[4,21] The emerging significance of VOCs in early cancer can be understood through recent addition of VOCs in clinical trials involving early ovarian cancer detection (NCT06613230), non-small cell lung cancer detection (NCT06707519) and improving the detection of early lung cancer in a diverse population (NCT06628102).

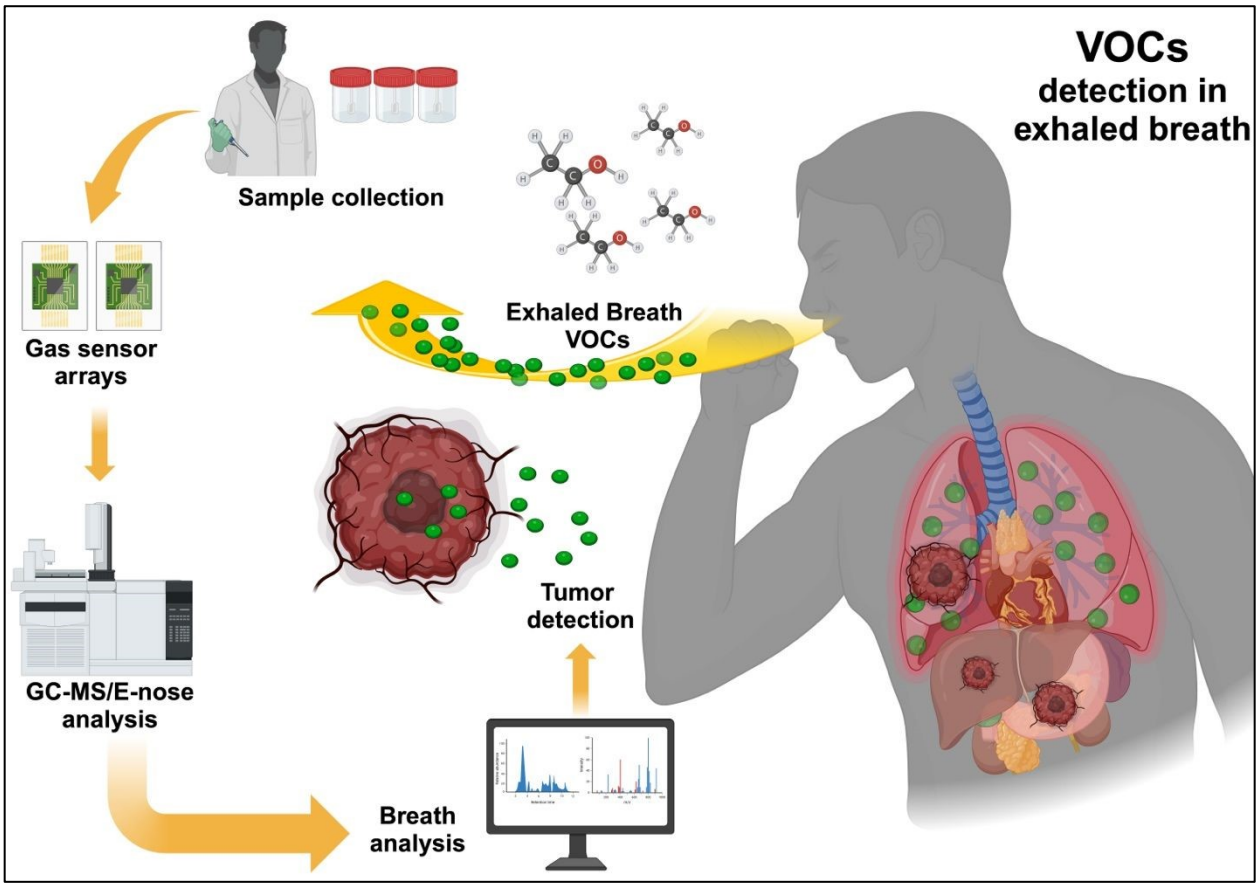


Figure 1. Schematic showing the representation of sample collection to instrumentation about VOC detection in exhaled breath for cancer markers diagnosis. The current figure was prepared using BioRender.

The presence of cancer is often accompanied by hypoxia, hyperproliferation of cells, change in microenvironments, and excessive inflammatory activities, resulting in the release of VOCs both locally and systemically.[21] Consequently, the VOC profile is shifted indicating the metabolic and

View Article Online
DOI: 10.1039/D5NR01714A

genetic changes that may not be present in healthy individuals. Thus, sensing spectral dysregulation amongst the markers that would either not be aggressively released or not released in detectable limits in the healthy subjects, is the translational approach for VOC sensing. In comparison to traditional methods, including low-dose CT (lung cancer), mammography (breast cancer), gastroscopy (gastric cancer), colonoscopy (colorectal cancer), CA125 (cancer antigen 125) and transvaginal ultrasound (ovarian cancer), VOC analysis demonstrates comparable or even superior sensitivity, making them a promising option for cancer screening in the future.[22]

Various techniques has been employed for VOC biomarker detection (Figure 1) including gas chromatography–mass spectrometry (GC-MS), gas chromatography–ion mobility spectrometry (GC-IMS), proton transfer reaction–mass spectrometry (PTR-MS), selected ion flow–tube mass spectrometry (SIFT-MS), and electronic-noses (e-noses).[4,22–26] Mass spectrometry (MS) techniques are the most useful when identification of unknown VOCs is of ultimate importance, and can be broken down into offline (GC-MS, GC-IMS) and online (PTR-MS, SIFT-MS) methodologies. Online methodologies infuse sample VOCs directly into the MS, where analytes are ionized at ambient conditions, and therefore are relatively more rapid. Offline techniques on the other hand have the highest sensitivity/resolution, and therefore are the most well situated for biomarker discovery research although they are time-consuming. Even though MS techniques are well-suited for VOC identification, these instruments tend to occupy a relatively large footprint and require trained personnel to run assays. Therefore, there is a significant interest in designing and developing miniaturized gas sensors (e-noses) for rapid detection of biomarkers at a point-of-care. Further comparative breakdown of detection efficiencies is listed in Table 1[27-38] and several common VOCs (acetone, isoprene, hexanal, limonene, 2-pentanone, acetaldehyde, 2-butanone, and ethylbenzene) reported in literature have been studied for quantification of concentration levels in breath using these techniques.[39-48]

Table 1. Comparisons of techniques used in VOC detection for cancer diagnosis and their detection limits.

View Article Online
DOI: 10.1039/D5NR01714A

Technique	Detection Limit	Key References
GC-MS (Gas Chromatography–Mass Spectrometry)	**10-90 ppt**	**27, 28**
GC-IMS (with Ion Mobility)	**50 ppt – 7 ppb**	**29, 30**
PTR-MS (Proton Transfer Reaction MS)	**60 ppt – 3ppb**	**31, 32**
SIFT-MS (Selected Ion Flow Tube MS)	**500 ppt – 7 ppb**	**33, 34**
Electronic Nose (e-Nose)	**100 ppb – 10 ppm**	**35, 36**
Colorimetric Arrays	**20 ppb – 1 ppm**	**37, 38**

Among VOCs and their respective functionalities, *aldehydes* have been most recognized amongst candidate biomarkers, showing significantly elevated levels in cancer patients compared to healthy individuals. Their relatively high solubility in blood allows for rapid detection through breath analysis. Aldehyde production in cancer cells is linked to cytochrome P450 through the lipid-oxidation of omega-3 and -6 polyunsaturated fatty acids. Cytochrome P450 is overexpressed in some cancers and is one of the reasons for higher release of aldehydes as VOCs in cancer patients. Moreover, endogenous aldehyde production can also be linked to a higher amount of saturated lipids in cancer cell membranes. Thus, aldehyde production is an indicator of overexpression of cytochrome P450, membrane lipid composition, and increased oxidative stress, which is often accompanied in cancer cells.[49-52] In addition to aldehydes which are prevalent in many types of cancer, studies have indicated *alkanes* and *esters* as VOCs produced in breast cancer, *ketones* in breast, colon and stomach cancers, *alcohols* in lung, colon, prostrate and stomach cancers, and *ethers* in lung cancer, further expanding the potential of VOCs as biomarkers for early cancer detection.[53]

Apart from endogenous VOCs, recent studies have also explored the potential of synthetic probes which release VOC reporters in exhaled breath after interacting with a designated cancer target. For example, D_5-ethyl-β-D-glucuronide (synthetic probe) was previously designed to be intravenously administered and readily metabolized to D_5-ethanol (VOC reporter) by β-glucuronidase in tumor-bearing mice. β-glucuronidase is extracellularly accumulated in tumor microenvironments in comparison to healthy cells where it is found intracellularly. The research strategy included capitalizing on the hydrophilic nature of D_5-ethyl-β-D-glucuronide, where D_5-ethanol release is an indicator of β-glucuronidase and tumor activity. This innovative approach has

View Article Online
DOI: 10.1039/D5NR01714A

shown promise in distinguishing between healthy and cancer-bearing subjects and has been extended to lung cancer models and human clinical trials.[54, 55]

As research in VOCs continues to advance, it is essential to standardize collection methods, data analysis, and biomarker validation to facilitate the translation of VOC technology into clinical practice. The potential for VOC-based cancer screening and monitoring represents an exciting frontier in early cancer detection and personalized medicine. In this review, we explain the contemporary techniques for cancer detection as the founding diagnostic stage, emphasize mechanistic insights, and address recent progress and interests in VOCs as potential biomarkers for early cancer detection. We also expand on their characteristics, modes of detection, practical diagnostic examples, and discuss prospects of the methodology and technology.

Single-cell liquid biopsy for cancer detection

Single-cell biopsy is an advanced technique explored for the detection of cancer, where bodily fluids are collected from the patients and the individual cancer cells or cell-free DNA are examined.[56] Liquid biopsies are less invasive than the standard biopsies, involving the simple withdrawal of blood or other fluids rather than the collection of solid tissue samples. Liquid biopsies also possess numerous advantages, including convenient sampling, minimal risk to patients, the ability to assess tumor heterogeneity and the possibility of performing serial sampling to monitor disease progression or treatment response.[57, 58] Circulating extracellular nucleic acid (cell-free DNA; cfDNA) and circulating tumor DNA (ctDNA) can be isolated from the blood. While ctDNA is specifically derived from tumor cells, cfDNA refers to all DNA freely floating in circulation, which may originate from both normal and tumor cells (Figure 2). Liquid biopsies allow for the isolation and analysis of circulating tumor cells (CTCs) from blood samples. These CTCs are intact cancer cells that have detached from the primary tumor and entered the bloodstream, potentially leading to metastasis. CTCs are typically defined as CD45− Cytokeratin (CK)+ cells, which can either actively or passively shed from primary tumors or metastatic lesions into the bloodstream. CTCs passively shed or actively evade the tumor or metastatic lesion into the circulating blood. CTCs may reach distant organs through the vascular network, leading to distant blood-borne metastasis. Next-generation sequencing (NGS) can be used for CTC and ctDNA/cfDNA assays. [56,59] This technology allows for comprehensive genetic profiling, enabling the evaluation of both functional CTC characteristics as well as their protein and RNA content.

View Article Online
DOI: 10.1039/D5NR01714A

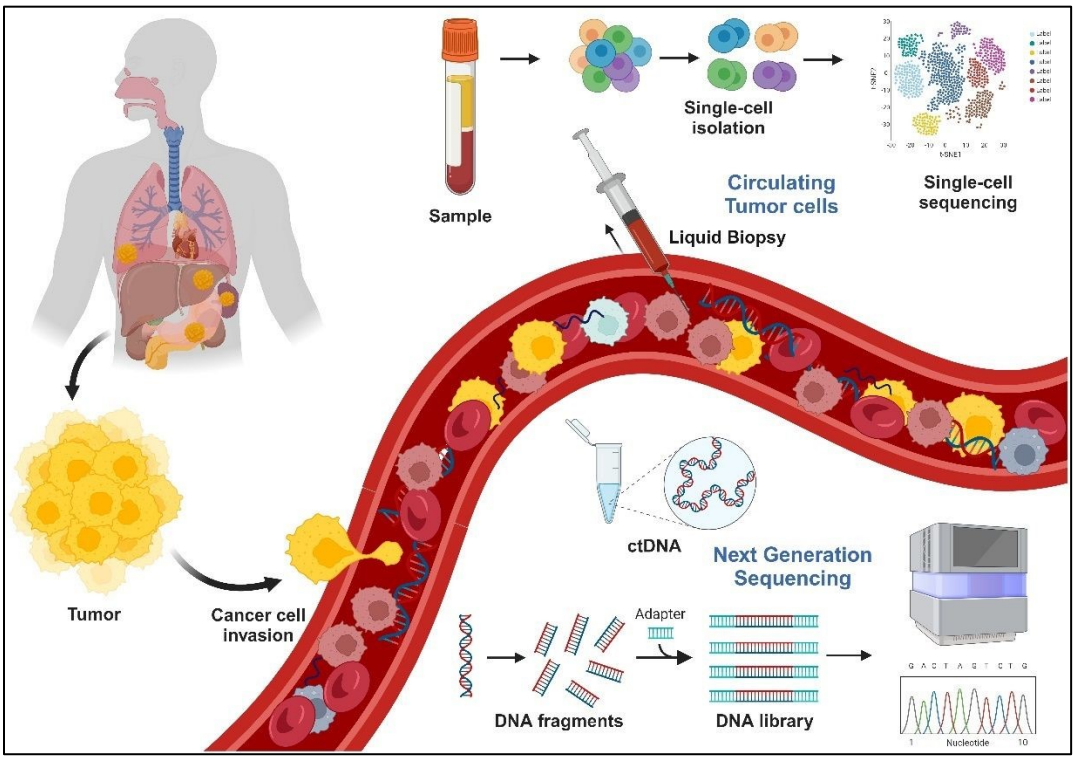


Figure 2. Illustration indicating the liquid biopsy of cancer for early diagnosis by utilizing circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) as biomarkers for early-stage cancer diagnosis. Images were designed by us using BioRender.

The enrichment and retrieval of CTCs from peripheral blood samples represent a major technological challenge for successfully implementing CTCs as a liquid biopsy approach.[60] Most notably, there is substantial diversity in the efficiency and specificity of CTC isolation techniques. Most CTC isolation platforms are still hampered by a low number of detected CTCs in the blood. Moreover, this is a complex procedure with high cost that often leads to low purity of the retrieved CTCs. Numerous techniques for isolation have been developed recently, such as negative selection by the depletion of all unwanted blood cells, positive selection using potential cancer markers, and strategies based on the unique physical characteristics of cancer cells (such as size or deformability). Enrichment of CTCs based on immunogenicity is a commonly employed method for separating CTCs. Certain biomarkers that are expressed on the cell surface are used to trap the cells, which are then attached to a magnetic material or device surface. Since CTCs express a range of surface markers, there isn't a single, universal CTC antigen.[61] There are numerous ways to

View Article Online
DOI: 10.1039/D5NR01714A

positively enrich CTCs. AdnaTest (QIAGEN) employs cancer-specific antibody-coated beads, and the real-time polymerase chain reaction is used to ascertain the expression patterns on the cells.[62] Cells containing magnetic nanoparticles affixed to antibodies are gathered by magnetic-activated cell sorting (MACS). A robotically controlled magnetic rod and antibody-coated magnetic beads are used in MagSweeper (Stanford University, previously developed by Illumina), an immunomagnetic enrichment method utilized to separate CTCs.[63] The only CTC isolation technique with FDA approval, CellSearch® (Menarini Silicon Biosystems), uses epithelial cell adhesion molecule (EpCAM) which is widely utilized for positive selection and the gold standard as a cancer marker. However, EpCAM is often lost during processes such as the epithelial-to-mesenchymal transition (EMT) that take place when cancer cells intravasate into the bloodstream.[64]

Importance of early-stage cancer diagnosis

Early detection of cancer greatly increases the chances of survival and boosts the quality of life after diagnosis. Compared to patients diagnosed at a later stage, those with early cancer diagnoses have a higher chance of survival, less treatment-related morbidity, and an improved quality of life. The progress of early diagnosis is complex and encompasses early detection initiatives like mammography for breast cancer as well as early symptom presentation to primary care physicians. For example, more than 90% of cancer cases in England are discovered outside of the country's screening programs for bowel, breast, and cervical cancer. This emphasizes how crucial it is to visit primary care as soon as possible and when the first symptoms start to appear. ‘Be Clear on Cancer’ is one of several public awareness initiatives that aims to inform the public about early cancer indicators and promote early medical appointments. Research indicates that these programs have raised public awareness and may have contributed to early cancer identification, though there is debate over whether they primarily identify advanced tumors rather than early-stage cancers. Current investigations exploring the connection between symptoms and cancer stage have offered valuable new insights. A previous study supported the relevance of public knowledge in aiding early diagnosis by finding that several symptoms, such as a typical moles and breast lumps, are correlated with a lower likelihood of advanced disease.[65]

ctDNA has gained a lot of attention for its practical abilities to efficiently detect malignancies. Recently, a study found that using ctDNA presents a promising method for early cancer identification.[66] By refining sequencing methods to identify ctDNA without requiring prior

View Article Online
DOI: 10.1039/D5NR01714A

knowledge of tumor mutations, this approach advances the field. Approximately 60–70% of the 200 patients with stage 1 and stage 2 tumors (colon, breast, lung, and ovarian cancers) were recruited for this study and assessed for ctDNA. In asymptomatic individuals, this strategy may have a significant impact on early cancer identification and lead to better patient outcomes. One of the main areas of continuing study is the capacity to use ctDNA to detect tiny, asymptomatic cancers. Empirical evidence suggests that it may not be possible to identify very small tumors, such as those with a diameter of less than 5 mm. Because of the low quantity of ctDNA in the blood, tumors of this size are generally challenging to detect. For instance, 1 mm-diameter tumors are linked to extremely low ctDNA concentrations that may be undetectable with existing techniques. The likelihood of identifying ctDNA increases with tumor size, up to about 5 mm in diameter, although the low quantity of circulating tumor DNA makes detection difficult even in these cases.[67] The sensitivity and specificity of the current approach have limits, notwithstanding its potential. Because the technique depends on finding mutations that are found in a very small percentage of the ctDNA, its sensitivity may be hampered. Additionally, sampling errors may make it difficult to detect extremely small tumors. Furthermore, the existence of somatic mutations unrelated to cancer and technical noise may compromise the excellent specificity reported in previous studies. For example, one of these previous studies reported a sensitivity of 62% and a specificity of >99% in detecting early-stage cancers.[68] Overall, ctDNA exhibits potential, although its current use in identifying very early-stage or asymptomatic malignancies may be restricted. Further developments and integrated diagnostic methods could improve its efficacy in the early diagnosis of cancer.

Disease related VOC chemical markers in bodily fluids

Disease-related chemical markers in bodily fluids have been utilized for diagnostic purposes since ancient times. These biomarkers can be detected in various sample types, including blood, urine, cerebrospinal fluid, saliva, sweat, breath, and tears. VOCs represent a particularly interesting class of potential biomarkers, as they can be detected in multiple bodily fluids and are associated with specific metabolic disorders and diseases. For instance, cadaverine and putrescine are linked to cystinuria, isovaleric acid to isovaleric acidemia, and dimethylsulfide to hypermethioninemia.[69-72] Thus, VOCs released through bodily secretions change the overall odor fingerprint of the individual when compared with the healthy state and are important chemical markers/indicators for disease detection.

View Article Online
DOI: 10.1039/D5NR01714A

In cancer, processes like overexpression of cytochrome P450 enzymes, angiogenesis, oxidative stress, and altered glycolysis lead to the production of specific biomarkers, with aldehydes, alkanes, and alcohols being key VOC classes associated with the disease.[49-51] Aldehydes are produced in many types of cancers due to the above mentioned metabolic processes. As for other functional groups, their prevalence in different types of cancers already been discussed in the introduction (*alkanes* and *esters:* breast cancer, *ketones*: breast, colon and stomach cancers, *alcohols*: lung, colon, prostrate and stomach cancers, and *ethers*: lung cancer). Ethane and pentane, which are saturated alkanes, are generated as byproducts of lipid peroxidation and are widely recognized as potential biomarkers for a host of different medical conditions. Lipid peroxidation is an indicator of several underlying conditions like inflammation, aging, and cancer. Alkanes have very low solubility in the blood, thus preventing further metabolism and ensuring they appear in breath within minutes, making them important VOC markers.[73, 74]

Aldehyde production can also be altered due to the presence of ALDHs (aldehyde dehydrogenase) and ADHs (alcohol dehydrogenase), as ALDHs oxidize aldehydes to acids. [75, 76] It is reported that in ovarian cancer cell lines, the downregulation of 10-formyltetrahydrofolate dehydrogenase leads to increased aldehyde levels.[61] Thus, based on the enzymatic conversion, the levels of volatile aldehydes can be increased. For example, the ADHs that convert alcohol to aldehydes is suggested to play a role in metastasis and ALDH overexpression in lung cancer.[61, 62] Moreover, some aldehydes are consumed by cancer cells (as well as other surrounding cells) which may alter the overall concentration level of aldehydes. Nonetheless, C $\geq$6 aldehydes (hexanal, heptanal, octanal, nonanal) are more stable and are associated with several cancers.[4] Notably, higher amounts of nonanal have also been associated with apoptosis.[75]

Ketone production is hypothesized to occur through beta-oxidation of long chain fatty acids and branched chain fatty acids in the mitochondria. ADHs also catalyze the conversion of alcohols to ketones with a preference for primary alcohols. In liver cancer, the ADHs activity is highly elevated. Therefore in hepatocellular cancer, the ketone production is higher due to the long-chain fatty acid metabolism.[78] Acetone, the simplest ketone, is produced through two main pathways: decarboxylation of acetoacetate and dehydrogenation/ADH metabolism of isopropanol in the body. Despite its distinctive odor, acetone's utility as a biomarker is limited due to its production in various physiological processes. On the contrary, other ketones such as 2-nonanone, 2-butanone, 3-heptanone, 4-heptanone, and cyclohexanone have shown to be potential biomarkers.[78-81]

View Article Online
DOI: 10.1039/D5NR01714A

Alcohols are also important VOCs owing to their diffusion into the blood and appearance in breath from the gastrointestinal tract. Although numerous biomarkers have been reported for various types of cancer, it is important to note that a single marker is generally insufficient for a definitive diagnosis. Instead, a comprehensive profile or "map" of VOCs is essential for accurate early cancer detection. This approach recognizes the complexity of cancer biology and the potential for overlapping biomarkers across different cancer types and other physiological conditions. Furthermore, the interpretation of VOC biomarkers is complicated by their varying blood-breath-fat partition coefficients. These coefficients describe how VOCs distribute between different compartments in the body, affecting their concentrations in various biological samples such as breath, blood, or urine. Currently, corrective measures to account for individual variations in VOC production, metabolism, and distribution are not well-defined. This presents a significant challenge in standardizing VOC-based diagnostic approaches across diverse patient populations.[82]

Disease-specific VOC biomarkers for early cancer detection

An overarching challenge in translating the potential of VOC biomarkers to clinical applications include the fact that they must demonstrate adequate disease-specificity. In other words, it is a critical concern that patients with different forms of cancer will test positive using a VOC-based assay developed for a particular cancer type. Figure 3 presents an overview of VOC functional groups identified in exhaled breath across various cancer types. This highlights the emerging role of breathomics in enabling non-invasive, early cancer detection. For example, Saalberg *et al.* reported that the most frequently observed VOC biomarkers for lung cancer in exhaled breath include 2-butanone, 1-propanol, isoprene, ethylbenzene, and hexanal.[83] Other breath studies have also reported VOCs with diverse functionality to be potential biomarkers of lung cancer.[84] VOC discovery for lung cancer has also been extended to urine, with studies identifying different subsets of biomarkers that in large do not overlap with breath-based studies.[85, 86] Some potential markers in urine for lung cancer in these studies include 2-pentanone, 2-ethyl-1-hexanol, 2-hexenal, 2-heptanone, and many other compounds.[86]

View Article Online
DOI: 10.1039/D5NR01714A

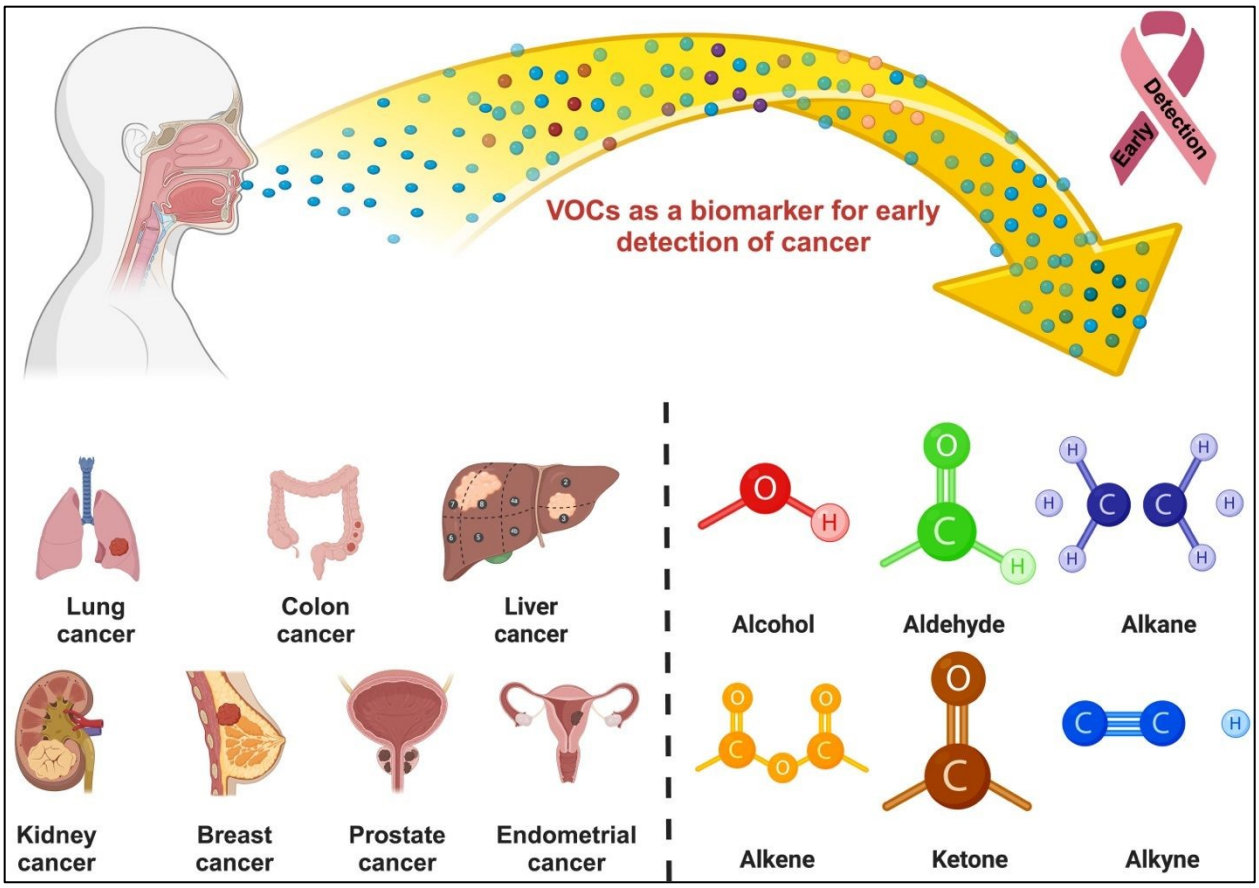


Figure 3. Small molecules "VOC functional groups" in exhaled breath for the early-stage detection of various types of cancer viz., lung, colon, liver, kidney, breast, prostate and endometrial cancer. Whole schematic and all images were designed using BioRender.

Aside from lung cancer, many studies have been conducted to discover non-invasive biomarkers for breast cancer, as the current methods for screening/diagnosis often lead to overdiagnosis and overtreatment. Previous work for breast cancer has been done in murine models, with urinary volatile ketones and terpenes/terpenoids implicated as significant candidates.[87] Regarding previous studies focusing on breath-based biomarker discovery, Phillips *et al.* was one of the first research groups to profile breast cancer. Biomarker candidates were tentatively identified as ethylidenecyclopropane, octamethylcyclotetrasiloxane, limonene, 1,2,4,5-tetramethylbenzene, tridecane, 2,7,10-trimethyldodecane, tetradecane, longifolene, 2-ethyl-1-octanol, and 2,6-di-tert-butylbenzoquinone. The sample size for this study was relatively large (n > 250), and the diagnostic results were relatively modest with sensitivity = 75.3% and specificity = 84.8%.[88]

View Article Online
DOI: 10.1039/D5NR01714A

Beyond this study, an array of volatile aldehydes including hexanal, heptanal, octanal, and nonanal have also been implicated as potentially clinically relevant markers.[88, 89] Urinary biomarkers have also been studied, as Kure *et al.* previously built a predictive model based on just two VOCs (2-butanone and 2-propanol), which could distinguish breast cancer with > 90% accuracy.[90] Beyond these compounds, another study conducted by Silva *et al.* identified other biomarkers including but not limited to p-cymene, acetic acid, dimethyl sulfide, and 4-heptanone.[91]

Perhaps the most important cancer type to improve screening technologies for, prostate cancer has also been studied for VOC biomarkers with a large emphasis on urine given its local proximity to the tumor.[92] Current screening methods (i.e., the prostate-specific antigen (PSA) test) have limited accuracy in prostate cancer detection as an array of factors not related to cancer can elevate PSA levels. Limited studies have been undertaken to establish a breath-based profile of prostate cancer biomarkers using technologies beyond electronic nose. Nonetheless, there have been multiple studies aiming to identify prostate cancer biomarkers using human urine samples. In the first study of its kind, in 2015 Khalid *et al.* identified a biosignature of just four VOCs in urine, that when coupled to the results of the PSA test, could distinguish prostate cancer with approximately 70% accuracy (dihyrdromyrcenol, pentanal, 3-octanone, and 2-octanone).[93] Lima *et al.* undertook a similar analysis, which revealed that VOCs (volatile carbonyl compounds (VCCs) in particular) were dysregulated by prostate cancer. A biomarker panel of just 6 analytes (hexanal, 2,5-dimethylbenzaldehyde, 4-methylhexan-3-one, dihydroedulan IA, methylglyoxal, and 3-phenylpropionaldehyde) could distinguish prostate cancer with a sensitivity of 89% and specificity of 83%.[94] Finally, a previous study sought to identify VOC biomarkers that could not only distinguish prostate cancer from healthy controls, but also from patients diagnosed with bladder cancer. These results showed that there were 7 VOCs associated with prostate cancer (toluene, phenol, acetic acid, 2-ethyl-1-hexanol, dimethyl disulfide, among others). Using biomarker panels, prostate cancer could be classified with area under the curve (AUC) of 0.83 – 0.97. Furthermore, the two urinary cancers could be stratified from one another with AUC 0.73 – 1.0.[95]

Colorectal cancer, although not as abundantly studied as some of the other cancer types mentioned above, is also another important carcinoma in which VOCs could aid in developing accurate screening technologies. Research has focused on identifying biomarkers in fecal samples, having identified an array of potential biomarkers including 2-propanol, 2-hexanone, p-xylene, and other compounds.[96] Although feces may be the ideal sample of interest for this malignancy, there is also

View Article Online
DOI: 10.1039/D5NR01714A

research focusing on urine and breath biomarkers. For example, Amal *et al.* previously demonstrated that breath-based biomarkers were statistically significantly dysregulated in patients diagnosed with colon cancer (p-value < 0.017). These compounds included ethanol, acetone, ethyl acetate, and 4-methyloctane.[97] Another published study identified an array of other breath-based compounds beyond these VOCs to be potentially useful in colorectal cancer diagnosis.[98] Some of these VOCs incorporated 2-ethyl-1-hexanol, acetic acid, decanal, benzaldehyde and others including saturated hydrocarbons. VOC biomarkers have also been elucidated in urine samples, with Arasaradnam *et al.* identifying a biosignature that could distinguish colorectal cancer with 88% sensitivity and 60% specificity. These compounds consisted of acetaldehyde, acetone, 2-pentanone, 4-heptanone, allyl isothiocyanate, and other VOCs.[99] An overview of different VOC biomarker candidates associated with some of the most studied cancer types is illustrated in Figure 4. Given exhaled breath and urine are the most common sample types for viable VOC analysis, compounds are listed for these matrices separately.

As demonstrated through compiling results from some of the most widely cited studies, there is little to no agreement regarding the specific VOC biomarkers for different cancer types. For example, different biological sample types will have fundamental differences in VOC profiles and therefore are not directly comparable. For breath-based studies, given there is no gold standard method for sampling/collection, many different techniques are implemented across literature. It should also be noted that not all studies run pure analytical standards to confirm VOC identification, making biomarker comparison among literature difficult.[100] Finally, it should also be noted that many studies report pilot data with relatively small sample sizes. In these studies, a degree of skepticism should be held especially when machine learning or pattern recognition is used to report diagnostic accuracies. In turn, all these factors make it difficult or unachievable to demonstrate VOCs are disease-specific through literature review and qualitative analysis. To combat these challenges, large and/or multi-center studies using comprehensive/standardized methods for collection and analysis should be undertaken on a host of different cancer types to quantitatively demonstrate VOC profiles are cancer specific. Some studies have taken this initiative[99, 101] with Nakhleh *et al.* identifying a subset of 13 VOCs that could distinguish 17 different medical conditions (including different types of cancer) with a clinically relevant accuracy of 86%.[101]

View Article Online
DOI: 10.1039/D5NR01714A

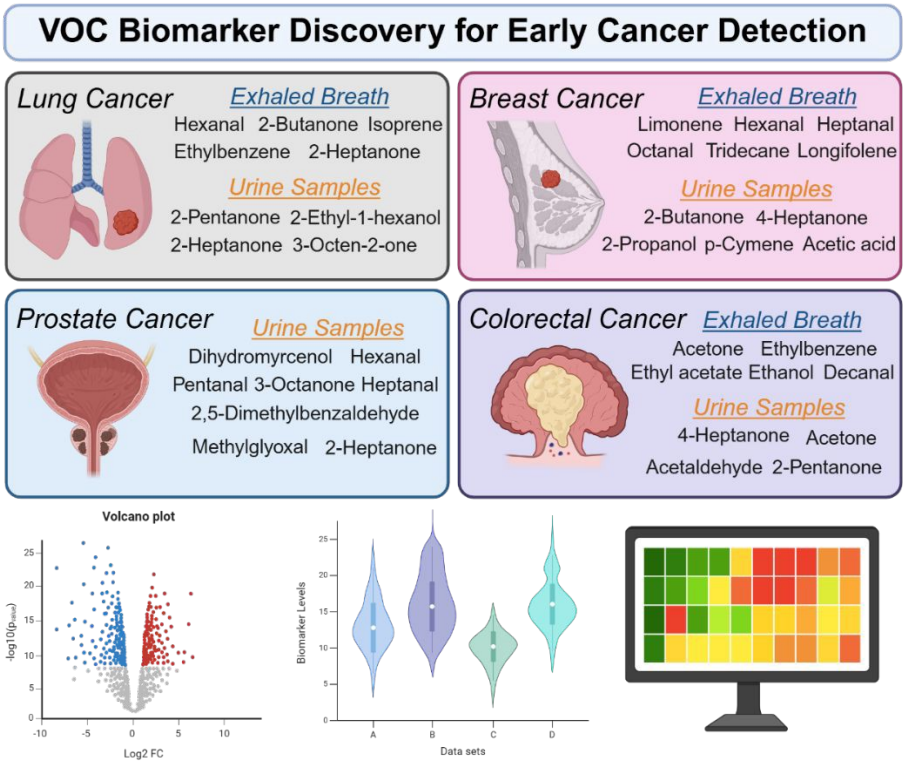


Figure 4. Summary of VOC biomarkers in breath and urine for different cancer types that have been widely studied in the literature.[83-85,88,89,91,93-99,102-106]

Non-invasive diagnosis of cancer from breath volatilome

Cancer presents a significant challenge owing to problems such as heterogeneity in the disease, inconsistent therapeutic success, and delayed detection. Conventional imaging techniques such as positron emission tomography (PET), magnetic resonance imaging (MRI), and low dose computed tomography (CT) have limited sensitivity, rely on tumor size, and may be expensive or entail a large amount of radiation.[107] Furthermore, for precise staging using these techniques, invasive follow-up procedures like bronchoscopy or needle biopsies are usually necessary. Mammography has limits regarding breast density and sensitivity for small tumors, despite its effectiveness in lowering the death rate associated with breast cancer. Although histological biopsies are intrusive, costly, and dangerous, they have long been regarded as the gold standard for diagnosing cancer. As a result, non-invasive diagnostic techniques that can speed up the bedside evaluation of therapy efficacy, help identify cancer earlier and stratify patients for personalized therapies are urgently required. Promising developments have been made recently in biomarker-based diagnoses, notably

View Article Online
DOI: 10.1039/D5NR01714A

in the field of volatilomics, which studies the VOCs released by cancer cells and their surrounding environment.

Breath analysis via miniaturized sensors is an emerging science that uses the distinct chemical profiles of volatile compounds in exhaled breath to potentially diagnose diseases, including cancer, in a non-invasive manner. The noninvasiveness, speed, and potential cost-effectiveness of this approach make it appealing. Previous research[108, 109] has demonstrated a novel method of cancer diagnosis utilizing cutting-edge sensor technology for breath analysis. The goal was to use an ultrasensitive, molecularly modified silicon nanowire field-effect transistor (SiNW FET) to analyze exhaled breath to detect gastric cancer. The trichloro-(phenethyl)-silane (TPS) layer on this sensor improves its capacity to identify VOCs correlated to gastric cancer (2-propenenitrile, furfural). The sensor can distinguish between common environmental VOCs found in breath samples and particular VOCs at incredibly low concentrations (down to 5 parts per billion) by combining components of lock-and-key and cross-reactive sensing. The sensor was tested in a clinical setting on breath samples from 107 participants, including healthy controls and those who had gastric cancer. Over 85% of the time, the sensor was able to accurately distinguish between conditions that were malignant and those that weren't. This accuracy held true even when controlling for gender and other confounding variables like tobacco use. The study's blind analysis demonstrated the sensor's potential as a trustworthy diagnostic tool and confirmed its efficacy. The fact that this technology is non-invasive is one of its main benefits. This breath analysis method provides a more convenient and less invasive option to traditional biopsy methods. Additionally, the diagnostic procedure is made simpler, portable, and cost-effective compared to more complex sensor arrays by using a single molecularly altered SiNW FET sensor.

In another study, [109] SiNW FET was used for the identification and categorization of disease breath prints. Based on the VOCs found in exhaled breath, this technique can diagnose and differentiate between several illnesses, including asthma, chronic obstructive pulmonary disease (COPD), lung cancer, and gastric cancer. The sensors were tuned through a training procedure that linked the sensitivity and selectivity of the SiNW FET sensors to VOCs associated with specific diseases. The optimized sensors were then evaluated in a clinical setting using breath samples from 374 patients. The outcomes showed that, in most situations, the SiNW FETs could reliably identify and distinguish between disease states with over 80% accuracy. This technology provides a direct, non-invasive diagnostic tool without the need for needles, surgery, or radioactive materials, which

View Article Online
DOI: 10.1039/D5NR01714A

gives it a substantial edge over older procedures. The study emphasizes how SiNW FETs can revolutionize diagnostic procedures. These sensors are ideal for widespread clinical and point-of-care application because they are very sensitive, react quickly to changes in VOC concentrations, and are reasonably priced to manufacture. Additionally, they provide the opportunity to track the advancement of the disease, which can be very helpful in treatment planning. To properly evaluate and improve this technique, more multicenter clinical studies with bigger sample sizes are necessary.

Adding to that, breast cancer is another major area for which early detection using VOC detection must be addressed. Breast cancer is still a major oncology concern because of its intricacy and its need for accurate, non-invasive diagnostic techniques. A unique method for identifying molecular subtypes of breast cancer based on VOCs found in exhaled breath has been made possible. This method seeks to overcome the drawbacks of conventional diagnostic techniques, including the high expense and technical challenges of biopsies and gene expression profiling. The breath samples collected from 276 female volunteers were divided into three groups (bearing benign conditions, ductal carcinoma in situ (DCIS), and malignant lesions) and analyzed using GC-MS and artificially intelligent nano sensor arrays. GC-MS revealed a strong association between the existence of cancer and 23 VOCs with notable variations in concentration across BC patients with different molecular subtypes. By utilizing artificial intelligence, the nanoarray was able to attain an astounding 83% accuracy rate in differentiating between molecular subtypes of breast cancer and carcinogenic versus non-cancerous cases. In cross-validation experiments, the method produced accuracies between 82% and 87%, sensitivities between 81% and 88%, and specificities between 76% and 96%. This volatilomic approach has the benefit of offering a non-invasive, economical, and time-efficient method for the diagnosis and subtype classification of breast cancer. Conventional techniques, such as gene expression profiling, can be impeded by sample degradation and the intricacy of post-translational modifications. They are also restricted by the requirement for large and superior tissue samples. By identifying VOC patterns connected to genetic and protein alterations linked to cancer, breath analysis provides a means of molecular subtype profiling. This technique can distinguish between several BC subtypes, including Triple Negative, Luminal A, Luminal B, and HER2+. It may also help with early identification, patient risk assessment, and the choice of targeted therapy.[110]

View Article Online
DOI: 10.1039/D5NR01714A

Colorectal cancer is a major global health concern, with forecasts of 2.2 million new cases and 1.1 million deaths by 2030.[111, 112] Fecal immunochemical assays are commonly used for non-invasive screening; however, their sensitivity and specificity might vary. This results in false positives and needless treatments, even though colonoscopy is the gold standard for diagnosis.[113] Investigating the utilization of VOCs in fecal samples (which should be an ideal sample for studying colorectal cancers) as biomarkers for CRC detection is one way. There were eighty individuals in all, twenty-four of whom had adenomatous polyps, twenty-four of whom had adenocarcinomas, and thirty-two of whom had no lymphadenopathy. For individuals without cancer, feces were taken 48 hours prior to the colonoscopy; for those with colorectal cancer, they were taken 2-4 weeks after the procedure. Using magnetic graphene oxide as the extractant, magnetic headspace adsorptive extraction (Mag-HSAE) was used to isolate the VOCs. This was followed by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS). This technology provides a reliable approach for VOC identification in fecal samples by utilizing magnetic graphene oxide, which has a high capacity to extract aromatic chemicals at a low cost. Significant biomarkers for colorectal cancer (CRC) were found to include p-cresol, 3(4H)-dibenzofuranone, and 4a,9b-dihydro-8,9b-dimethyl-3(4H)-dibenzofuranone. Cancer samples had much higher concentrations of p-cresol, which had an area under the curve of 0.85, 83% sensitivity, and 82% specificity. Comparably, 3(4H)-dibenzofuranone,4a,9b-dihydro-8,9b-dimethyl- 3(4H)-DBZ displayed 78% sensitivity and 75% specificity with an AUC of 0.77. The AUC increased to 0.86 when both biomarkers were combined, increasing sensitivity to 87% and specificity to 79%. Additionally, p-cresol showed promise in identifying pre-malignant lesions, with 83% sensitivity, 63% specificity, and an AUC of 0.69. These results suggest that Mag-HSAE-TD-GC-MS is a sensitive and non-invasive technique for early CRC detection and differentiation from cases of pre-malignancy.[114]

In a different study, a novel method of cancer detection based on flexible sensors fabricated using molecularly modified gold nanoparticles (GNPs) is presented. These sensors are intended to function as a component of a dynamic cross-reactive diagnostic array that can identify and categorize VOCs associated with disease in exhaled breath. The sensors can offer a rich dataset from each device by bending GNPs, which changes their spatial structure and improves diagnostic accuracy. The flexible GNP-based sensors are designed to react to VOCs at parts per billion (ppb) concentrations, which have been linked to ovarian cancer. Owing to this technology, the sensors

View Article Online
DOI: 10.1039/D5NR01714A

can distinguish between VOCs that are associated with ovarian cancer and those that come from unrelated environmental sources. The sensors have the potential to be utilized for reliable, non-invasive cancer diagnosis because of their great accuracy in detecting these VOCs. The sensors were even able to identify breath samples from women with ovarian cancer (82% accuracy). Using GNP sensors has several benefits, including portability, low cost, and the ability to function without intrusive procedures or sophisticated equipment. These sensors' dynamic bending states improve their diagnostic capabilities by allowing them to record a broad variety of VOC interactions. The study also emphasizes how these sensors could be used as a non-invasive, extra alternative for early disease identification and monitoring in addition to current ovarian cancer screening techniques. Breath-based diagnostics have advanced significantly because of the incorporation of molecularly altered GNPs into flexible sensor arrays. This strategy could revolutionize cancer screening and follow-up by providing a straightforward, affordable, and non-invasive diagnostic instrument that overcomes some of the present obstacles in the detection and management of diseases. [115]

Nature inspired approaches for volatile organic compounds detection

An innovative development in non-invasive diagnostics is the incorporation of nature-inspired methods for the detection of VOCs for the purpose of cancer diagnosis and other medical applications.[116] The natural olfactory systems of many different organisms provide important information for the development of biosensors that can detect biomarkers at trace levels in breath samples. These systems, in particular the wide range of G-protein coupled receptors (GPCRs), which include the vomeronasal receptors (V1R and V2R), olfactory receptors (ORs), tracing amine-associated receptors (TAARs), and formyl peptide receptors (FPRs), shed light on the complex processes by which biological organisms perceive and interpret smells.[117]

Biosensing using these natural systems has the potential to revolutionize diagnostic procedures by providing extremely specific, sensitive, and non-invasive testing techniques. Nevertheless, there are several difficulties in converting these concepts from biology into useful biosensor technology. Traditional diagnostic methods, such as those for disorders of the respiratory system, frequently need expensive equipment, centralized laboratories, and labor-intensive procedures. Modern biosensors, on the other hand, take advantage of developments in micro- and nanotechnology to enhance performance indicators including sensitivity, specificity, and reaction time.[118] Even with these developments, the stability and functionality of biological components such as ORs outside

View Article Online
DOI: 10.1039/D5NR01714A

of their native membranes remain a major challenge for biosensor integration. The use of synthetic peptides and molecularly imprinted polymers (MIPs), which replicate the features of receptors while improving stability and performance, are examples of recent advancements in the production of biosensors that offer promising solutions.[119, 120]

The development of bioelectronic noses (B-ENs) and electronic noses (e-noses) is a significant step toward the practical use of artificial olfaction. These devices can assess complicated gas mixtures without the requirement for separation by utilizing an array of chemical sensors and highly developed computing systems.[101] VOCs may be analyzed qualitatively and quantitatively using e-noses, which offer a "fingerprint" of the compounds. These systems' designs frequently take inspirations from the neurophysiology of the human olfactory system to mimic its capacity for pattern recognition. Even though the e-noses that are currently in use are efficient in some applications, research is constantly being conducted to improve them so that they can more accurately detect and identify a larger variety of VOCs. Simultaneously, the field is progressing in the application of biological components in biosensors, like ORs and odorant binding proteins (OBPs). Peptides that can imitate the action of these biological receptors have been developed because of advancements in synthetic biology and genetic engineering. These peptides provide several benefits, such as increased reusability, simpler immobilization, and increased stability. Additionally, these peptides are being designed and optimized using computational technologies like molecular dynamics and virtual docking, which improves their specificity and sensitivity for VOC detection. Biosensors that are more useful and efficient are being made possible by the convergence of biological knowledge and technological innovation. There is great evidence that this field will continue to progress with future research efforts to enhance sensor performance and develop biomimetic materials. [116]

Functional hybrid nanostructures for volatile organic compound detection

The use of functional hybrid nanostructures for the detection of VOCs is an innovative field of study with important applications in industrial safety, environmental monitoring, and health diagnostics.[121] Chemical sensors have become one of the most promising techniques for tracking VOCs, which can be used as biomarker for disease. Among these, nanostructured zinc oxide (ZnO)-based gas sensors have attracted a lot of interest because of their improved gas sensing capabilities. A facile and rapid method has been reported for the synthesis of ZnO nanosheets and their use in developing a resistive gas sensor for smartphone-based VOC detection that is sensitive

View Article Online
DOI: 10.1039/D5NR01714A

enough to target biomarkers linked to lung cancer.[122] The study reports an easy-to-scale fabrication of ZnO nanosheets by anodizing zinc foil in a KNO_3 solution. Using the resulting material, a smartphone-based chemiresistive sensor was developed and it showed remarkable limits of detection for VOCs linked to lung cancer, such as isopropanol (11 ppb), acetone (4 ppb), and diethyl ketone (0.9 ppb). The large surface area and porosity of the ZnO nanosheets, which were verified by scanning electron microscopy (SEM) and Brunauer-Emmett-Teller (BET) tests, are responsible for the sensor's great sensitivity and quick response. By connecting the sensor to an Arduino Uno with a Bluetooth module, data transmission to a smartphone for real-time analysis was made possible, substantially improving the sensor's functionality. In a different study, researchers developed an artificial intelligence(AI)-powered nanoarray using gold nanoparticles and carbon nanotubes to detect and classify 17 diseases from exhaled breath with 86% accuracy (Figure 5). Each disease had a unique "breathprint," unaffected by confounding factors. The results were validated using gas chromatography–mass spectrometry, identifying 13 key volatile compounds. This approach offers a low-cost, non-invasive tool for personalized disease screening and diagnosis.[101]

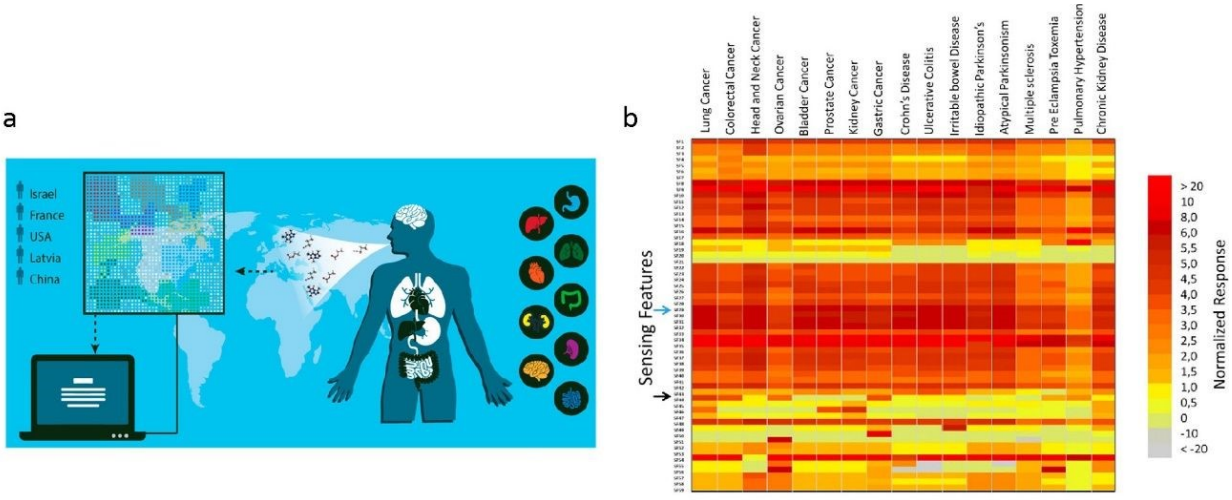


Figure 5. (a) shows the study design, involving breath sample collection from 1404 subjects across five countries for disease diagnosis, (b) presents a heat map of sensor responses to 17 diseases using nanomaterial-based sensors.[101]

Low concentrations of VOCs linked to lung cancer significantly affected the signal obtained on the smartphone-based chemiresistor device. Notably, excessive humidity and the presence of

View Article Online
DOI: 10.1039/D5NR01714A

chemicals that interfere with signal transmission, like ammonia, carbon dioxide, and hexane, had no effect on the sensor's function. This robustness suggests that breath analysis could be used to diagnose lung cancer non-invasively using this technology. Another study developed a different high-performance chemiresistive sensor[123] to detect VOCs, which may be lung cancer biomarkers. This sensor makes use of core-shell hybrid nanostructures made of conducting polymer poly(3,4-ethylenedioxythiophene) (PEDOT) and Fe_3O_4 magnetic nanoparticles (MNPs). In the presence of polymerized ionic liquids (PILs), which served as a stabilizing agent and a linker to bind the MNPs with PEDOT, the MNPs were synthesized using a microwave-assisted method. The resultant Fe_3O_4 hybrids modified via PEDOT–PIL were assessed as the active sensing component in a chemiresistive sensor, exhibiting remarkable sensitivity and low noise levels for the detection of VOCs, such as acetone, a potential marker in the breath of lung cancer patients. With a 38.8% increase in sensitivity and an 11% decrease in noise, the developed PEDOT–PIL–Fe_3O_4 sensor demonstrated significant gains over its PEDOT–PIL–counterpart. The benefits of embedding MNPs in conducting polymers for VOC detection are shown by this improved performance. The sensor exhibited high sensitivity towards VOCs at about 1 ppm, thus indicating its promise in lung cancer diagnosis.

In a 2023 study by Cai *et al*.,[124] a non-invasive diagnostic method for gastric cancer using a paper based colorimetric assay with integrated smartphone sensing was reported. The method was tested on breath samples from 40 gastric cancer patients and 40 healthy subjects. The sensor array was designed using porous nylon filter paper incorporated with functionalized nanoplasmonic materials and chemically responsive organic dyes. The sensors changed color when exposed with the VOCs which was analyzed using a smartphone camera and orthogonal partial least squares discriminant analysis (OPLS-DA). The sensing efficiency was confirmed using GC-MS which identified 25 VOCs differentiating gastric cancer patients from the healthy subjects. The key biomarkers detected in gastric cancer patients included acetone, furfural, benzaldehyde, isoprene, and phenyl acetate. The sensor demonstrated a detection limit in the ppb range (acetone ~ 33 ppb using 2-pyridinethiol modified GNPs, furfural ~ 228 ppb using 2-phenylethanethiol modified GNPs, benzaldehyde ~ 69 ppb using 2-naphthalenethiol modified GNPs, isoprene ~ 78 ppb using 2-aminoethanethiol modified GNPs, phenyl acetate ~ 67 ppb using 11-mercaptoundecanol modified GNPs) and differentiated the gastric cancer subjects with an accuracy of 90%, showing high promise of the breath analysis in cancer detection. Another example of a colorimetric sensor

View Article Online
DOI: 10.1039/D5NR01714A

used chemically reactive dyes immobilized on a nanoporous matrix of organically modified siloxanes to study VOCs in the breath of 229 study subjects (92 with lung cancer and 137 controls). The nanoporous structure increased surface area, improving sensitivity to VOCs. The results were printed onto a disposable cartridge capturing the color changes used for determining VOC changes to identify unique breath biosignatures. The sensor exhibited a sensitivity of 70-91% and specificity of 73-86% amongst different lung cancer types (lung cancer, adenocarcinoma, squamous cell carcinoma, small cell lung cancer).[125]

A novel method was presented [126] which combined genetically modified M13 bacteriophages with metallic nanostructures to create a gap plasmonic color sensor system. Because their structural protein capsids can be genetically altered to preferentially bind to target analytes, M13 bacteriophages were chosen for this application. Based on their functional groups, this alteration makes it possible to precisely identify VOCs. These genetically modified bacteriophages (engineered to display specific peptides on its surface which can interact with VOCs of interest) enable the sensor system to distinguish between distinct VOCs with more efficiency, increasing selectivity and overall performance. By employing hierarchical cluster analysis, the multiarray biosensor, which made use of the gap plasmonic color film, was able to identify VOCs in breath samples. A great degree of discrimination between various chemicals was made possible by this method. The method was used to categorize breath samples taken from 50 lung cancer patients and 70 healthy individuals during clinical trials. The technology identified lung cancer breath samples with an accuracy rate higher than 89%, which was a promising outcome. Machine learning analysis, which improved the sensor's capacity to discern between malignant and healthy breath samples, further corroborated this high rate of classification accuracy. Through non-invasive breath analysis, this hybrid approach not only increases the sensor's sensitivity and selectivity but also offers a strong foundation for the early identification of lung cancer. The technology is promising in clinical diagnostics and for the development of sophisticated, portable sensing devices for disease detection. This is highlighted by the effective classification of VOCs and its high accuracy in identifying samples that are malignant.

Future remarks

Detecting VOCs through breath analysis offers enormous promise for rapid and non-invasive detection of disease so that ultimately patients can avoid health complications at an early stage. The ideal biomarker for disease detection should be of the VOCs of endogenous origin. However,

View Article Online
DOI: 10.1039/D5NR01714A

as discussed in the above sections, engineered breath biomarkers may be a more practical and reliable approach. Hence, newer approaches can be designed for induced VOC production. Recent advances in targeted drug delivery and disease progression have allowed scientists to achieve long circulating formulations, and have a better understanding of barrier functions, enzymatic/cell membrane changes in diseased cells and microenvironments, etc. Thus, leveraging those understandings together can be used for designing an encoded (or conjugated) molecular probe that have (1) targeted delivery and/or tumor targeted vectors, (2) long-circulating time, and (3) specific enzyme-cleavable VOC reporters. The design can be undertaken such that the blood solubility after cleavage is significantly diminished to facilitate spontaneous breath detection. Also, the background value of the released VOC should be minimal, meaning that it should not be endogenous nor be present in healthy breath samples.

Exemplary approaches include using D_5-ethyl-β-D-glucuronide which is intravenously administered in mice and readily metabolized to D_5-ethanol (VOC) by β-glucuronidase.[54] Therefore, the release of free D_5-ethanol is an indicator of tumor microenvironments' activity using this assay. In another example, Chan *et al.* devised intrapulmonary delivery of hydrofluoroamine (VOC reporter)-conjugated activity based nano sensors which are susceptible to serine protease neutrophil elastase as an indicator of lung disease.[127] Thus, VOC detection using exogenous probes producing non-endogenous disease related breath markers can be the future of the technology in producing robust and reproducible disease signals. Additionally, the improvements in sample collection measures and instrumentation will accelerate the translation of the technology. For endogenous VOCs, further research in disease biology and understanding of VOC origins will facilitate the identification of robust biomarkers, including early indicators of aggressive cancers, which can be accepted by both the clinical and scientific community.

More hope can also be seen from newer techniques like cavity ring-down spectroscopy. Larracy *et al.* demonstrated non-small cell lung cancer detection with 51.61%–66.13% sensitivity and 73.96%–97.92% specificity.[128] As the research further progresses to find unique molecules for different cancer types, tunable diode laser absorption spectroscopy can be a highly effective approach. Further, as an extension to nature-inspired approaches, neuron-based VOC biosensors can be highly promising, employing olfactory receptor neurons (ORNs) or reconstituted receptor proteins enabling ultrasensitive detection of disease specific VOCs. These approaches are very promising, as ORNs are naturally sensitive to VOCs at trace concentration levels. They also have

View Article Online
DOI: 10.1039/D5NR01714A

high selectivity and therefore may be able to be tuned for the discrimination of structurally similar targets in which traditional e-Noses cannot. It should be noted that for portable sensor technologies, unknown VOC interferents during collection/sampling may bias results in a manner that cannot be normalized. Nonetheless, this can be overcome through ensuring that sensors are cross-sensitive and selective to the VOC targets of interest and implementing analytically validated breath sampling methodology that does not introduce interfering compounds.

Conclusion

Molecular biology techniques in combination with advanced assays and technologies such as next-generation sequencing have indeed strengthened the understandings of cancer, surface markers, vasculature, microenvironments, ctDNA, CTC isolation strategies, and cytokine release. However, translating these findings at a large-scale screening level using conventional methods like liquid biopsies is still challenging. Additionally, sample collection for gold-standard methodologies typically occurs at later stages, such as during tumor progression. To circumvent this challenge, VOCs offer an efficient approach, as outlined in this review with ultrasensitive techniques like nanotechnology-based sensors and GC-MS demonstrating notable differences in the breath fingerprints of cancer patients with high accuracy in clinical settings. Although the present strategies are majorly focused on endogenously originated VOCs, there are also reasonable advances and growing interests in designing synthetic breath-based biomarkers, both of which hold significant potential as diagnostic tools. Taking ahead from there, the FDA's role is crucial in the approval and translation of VOC sensor technologies to accelerate non-invasive detection of tumors and other related markers at a very early stage. The current challenges which need to be overcome for FDA approval are 1) lack of methods for standardized breath sample collection, 2) lack of analytical standards for biomarker identification and results validation, 3) overlapping biomarker models, 4) lack of reproducibility of results amongst different population sets and 5) the lack of biological understanding. Accelerating the development of these tools to overcome these challenges could significantly improve early-stage cancer diagnostics and patient outcomes.

Corresponding Authors

- Rajendra Prasad -*School of Biochemical Engineering, Indian Institute of Technology (BHU) Varanasi, Uttar Pradesh 221005, India*

View Article Online
DOI: 10.1039/D5NR01714A

Email: rajendra.bce@iitbhu.ac.in
ORCID: https://orcid.org/0000-0001-9851-8630

Dedication: We dedicate this article to the memory of the late Professor Sanjiv Sam Gambhir, a molecular imaging scientist.

Contribution: #These authors have contributed equally to this work.

Conflict of Interest: Authors don't have any conflicts or agreements associated with is manuscript.

Data availability: No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

List of Abbreviations

VOC	Volatile organic compound
GC-MS	Gas chromatography-mass spectrometry
IL-6	interleukin-6
GC-IMS	gas chromatography–ion mobility spectrometry
SIFT-MS	selected ion flow–tube mass spectrometry
PTR-MS	proton transfer reaction–mass spectrometry
ctDNA	circulating tumor DNA
CTCs	circulating tumor cells
NGS	Next-generation sequencing
MACS	magnetic-activated cell sorting
EpCAM	epithelial cell adhesion molecule
EMT	epithelial-to-mesenchymal transition
ALDHs	aldehyde dehydrogenase
PSA	prostate-specific antigen
VCCs	volatile carbonyl compounds
PET	positron emission tomography

View Article Online
DOI: 10.1039/D5NR01714A

MRI	magnetic resonance imaging
CT	computed tomography
SiNW FET	silicon nanowire field-effect transistor
TPS	trichloro-(phenethyl)-silane
COPD	chronic obstructive pulmonary disease
DCIS	ductal carcinoma in situ
Mag-HSAE	Magnetic Headspace Adsorptive Extraction
TD-GC-MS	thermal desorption-gas chromatography-mass spectrometry
GNPs	gold nanoparticles
TAARs	tracing amine-associated receptors
GPCRs	G-protein coupled receptors
FPRs	formyl peptide receptors
OBPs	odorant binding proteins
BET	Brunauer-Emmett-Teller
SEM	scanning electron microscopy
PEDOT	polymer poly(3,4-ethylenedioxythiophene)
MIPs	molecularly imprinted polymers
OPLS-DA	orthogonal partial least squares discriminant analysis

References:

1
H. Haick, Y. Y. Broza, P. Mochalski, V. Ruzsanyi and A. Amann, *Chem. Soc. Rev.*, 2014, **43(5)**, 1423–1449.
2
M. Aydin and C. N. Harvey-Woodworth, *British Dental Journal*, 2014, **217**, E1.
3
L. Pauling, A. B. Robinson, R. Teranishi and P. Cary, *Proceedings of the National Academy of Sciences*, 1971, **68(10)**, 2374–2376.
4
Biomarkers. [(accessed on 3 October 2023)]; Available online: https://www.fda.gov/science-research/focus-areas-regulatory-science-report/focus-area-biomarkers
5
V. Vassilenko, Pedro Catalão Moura and M. Raposo, *Biomedicines*, 2023, **11**, 3029–3029.
6

View Article Online
DOI: 10.1039/D5NR01714A

Cancer Statistics – NCI, https://www.cancer.gov/about-cancer/understanding/statistics.Accessed: 2025-06-20
7
SEER Cancer Statistics Factsheets: Common Cancer Sites. National Cancer Institute. Bethesda, MD, https://seer.cancer.gov/statfacts/html/common.html. Last accessed May 10, 2024.
8
A. Bleyer and H. G. Welch, *New England Journal of Medicine*, 2012, **367**, 1998–2005.
9
A. Shaukat, M. Shyne, J. S. Mandel, D. Snover and T. R. Church, *Gastroenterology*, 2021, **160**, 1397-1399.e3.
10
K.-L. Huang, S.-Y. Wang, W.-C. Lu, Y.-H. Chang, J. Su and Y.-T. Lu, *BMC Pulmonary Medicine*, DOI:https://doi.org/10.1186/s12890-019-0883-x.
11
M Gangwani, A. Aziz, D S Dahiya, M Nawras, M. Aziz and S. Inamdar, *Translational Gastroenterology and Hepatology*, DOI:https://doi.org/10.21037/tgh-23-4.
12
A. Pulumati, A. Pulumati, B. S. Dwarakanath, A. Verma and R. V. L. Papineni, *Cancer Reports*, DOI:https://doi.org/10.1002/cnr2.1764.
13
V. A. Adalsteinsson, G. Ha, S. S. Freeman, A. D. Choudhury, D. G. Stover, H. A. Parsons, G. Gydush, S. C. Reed, D. Rotem, J. Rhoades, D. Loginov, D. Livitz, D. Rosebrock, I. Leshchiner, J. Kim, C. Stewart, M. Rosenberg, J. M. Francis, C.-Z. Zhang and O. Cohen, *Nature Communications*, 2017, **8**, 1–13.
14
E. Heitzer, I. S. Haque, C. E. S. Roberts and M. R. Speicher, *Nature Reviews Genetics*, 2018, **20**, 71–88.
15
D. Crosby, S. Bhatia, K. M. Brindle, L. M. Coussens, C. Dive, M. Emberton, S. Esener, R. C. Fitzgerald, S. S. Gambhir, P. Kuhn, T. R. Rebbeck and S. Balasubramanian, *Science*, DOI:https://doi.org/10.1126/science.aay9040.
16
N. Kumari, B. S. Dwarakanath, A. Das and A. N. Bhatt, *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine*, 2016, **37**, 11553–11572.
17
J. Chen, Y. Wei, W. Yang, Q. Huang, Y. Chen, K. Zeng and J. Chen, *Frontiers in Oncology*, 2022, **12**, 903800.
18
X. Wang, G. Tang, Y. Liu, L. Zhang, B. Chen, Y. Han, Z. Fu, L. Wang, G. Hu, Q. Ma, S. Sheng, J. Wang, X. Hu and S. Shao, *Frontiers in Pharmacology*, 2022, **13**, 1033674.
19
S. Mustafa, S. Koran and L. AlOmair, *Frontiers in Molecular Biosciences*, DOI:https://doi.org/10.3389/fmolb.2022.896099.
20
D. Ravindranathan, V. A. Master and M. A. Bilen, *Biology*, 2021, **10**, 325.
21

View Article Online
DOI: 10.1039/D5NR01714A

F. Diehl, K. Schmidt, M. A. Choti, K. Romans, S. Goodman, M. Li, K. Thornton, N. Agrawal, L. Sokoll, S. A. Szabo, K. W. Kinzler, B. Vogelstein and L. A. Diaz Jr, *Nature Medicine*, 2008, **14**, 985–990.
22
R. E. Amor, M. K. Nakhleh, O. Barash and H. Haick, *European Respiratory Review*, DOI:https://doi.org/10.1183/16000617.0002-2019.
23
C. Di Natale, A. Macagnano, E. Martinelli, R. Paolesse, G. D'Arcangelo, C. Roscioni, A. Finazzi-Agrò and A. D'Amico, *Biosensors and Bioelectronics*, 2003, **18**, 1209–1218.
24
C. Turner, P. Španěl and D. Smith, *Physiological Measurement*, 2005, **27**, 13–22.
25
M. E. O'Hara, T. H. Clutton-Brock, S. Green and C. A. Mayhew, *Journal of Breath Research*, 2009, **3**, 027005.
26
S. Janfaza, B. Khorsand, M. Nikkhah and J. Zahiri, *Biology Methods and Protocols*, DOI:https://doi.org/10.1093/biomethods/bpz014.
27
J. García-Bellido, L. Freije-Carrelo, M. Redondo-Velasco, M. Piparo, M. Zoccali, L. Mondello, M. Moldovan, B. Bouyssiere, P. Giusti and J. R. Encinar, *Analytical Chemistry*, 2023, **95**, 11761–11768.
28
Q. Xu, X. Hu, L. Zhong, Z. Wang, H. Zhao, J. Fu, D. Cao, L. Liu, Y. Zhang and J. Lang, *ACS Omega*, DOI:https://doi.org/10.1021/acsomega.5c01166.
29
A. Ahrens and S. Zimmermann, *Analytical and Bioanalytical Chemistry*, 2020, **413**, 1009–1016.
30
P Mochalski, H Wiesenhofer, M. Allers, S. Zimmermann, A. Güntner, N. J. Pineau, W. Lederer, Agapios Agapiou, C. N. Mayhew and V. Ruzsanyi, *Journal of Chromatography B*, 2018, **1076**, 29–34.
31
K. B. Haase, W. C. Keene, P. Pszenny, H. R. Mayne, R. W. Talbot and B. C. Sive, *Atmospheric measurement techniques*, 2012, **5**, 2739–2750.
32
H. Schwoebel, R. Schubert, M. Sklorz, S. Kischkel, R. Zimmermann, J. K. Schubert and Wolfram Miekisch, *Analytical and Bioanalytical Chemistry*, 2011, **401**, 2079–2091.
33
A. G. Zogka, M. N. Romanias and F. Thevenet, *Atmospheric measurement techniques*, 2022, **15**, 2001–2019.
34
S.-T. Chin, A. Romano, S. L. F. Doran and G. B. Hanna, *Scientific Reports*, DOI:https://doi.org/10.1038/s41598-018-22890-w.
35
C.-C. Wu, S.-C. Liu, S.-W. Chiu and K.-T. Tang, *Sensors*, 2016, **16**, 1777–1777.
36

View Article Online
DOI: 10.1039/D5NR01714A

E. J. Sunnucks, B. Thurn, A. O. Brown, W. Zhang, T. Liu, S. L. Forbes, S. Su and M. Ueland, *Sensors*, 2024, **24**, 5918.
37
Z. Li and K. S. Suslick, *Analytical Chemistry*, 2018, **91**, 797–802.
38
S. H. Nah, J. B. Kim, T. Chui, Y. Suh and S. Yang, *Advanced Materials*, DOI:https://doi.org/10.1002/adma.202409297.
39
J. C. Anderson, *Obesity*, 2015, **23**, 2327–2334.
40
Jan, Paweł Mochalski, Karsten Königstein, Wang Chang Ting, K. Unterkofler, Arno Schmidt-Trucksäss, C. A. Mayhew, A. T. Güntner and S. E. Pratsinis, *Sensors and Actuators B: Chemical*, 2022, **357**, 131444–131444.
41
P. Sukul, A. Richter, C. Junghanss, J. K. Schubert and W. Miekisch, *Communications biology*, 2023, **6**, 999.
42
J. Huang, S. Kumar and G. B. Hanna, *Journal of Breath Research*, 2014, **8**, 037104.
43
R. Fernández del Río, M. E. O'Hara, A. Holt, P. Pemberton, T. Shah, T. Whitehouse and C. A. Mayhew, *EBioMedicine*, 2015, **2**, 1243–1250.
44
P. Mochalski and K. Unterkofler, *The Analyst*, 2016, **141**, 4796–4803.
45
C. Turner, P. Španěl and D. Smith, *Rapid Communications in Mass Spectrometry*, 2005, **20**, 61–68.
46
M. E. O'Hara, R. Fernández del Río, A. Holt, P. Pemberton, T. Shah, T. Whitehouse and C. A. Mayhew, *Journal of Breath Research*, 2016, **10**, 046010.
47
X.-A. Fu, M. Li, R. J. Knipp, M. H. Nantz and M. Bousamra, *Cancer Medicine*, 2013, **3**, 174–181.
48
M. Koureas, P. Kirgou, G. Amoutzias, C. Hadjichristodoulou, K. Gourgoulianis and A. Tsakalof, *Metabolites*, 2020, **10**, 317.
49
F O F O Alshammari, A. M. M. Youssef, Y. M. Al-Sarayra and H. M. Alrawashdeh, *Interact J Med Res*, 2021, **10**, e31150.
50
H. Esterbauer, R. J. Schaur and H. Zollner, *Free Radical Biology and Medicine*, 1991, **11**, 81–128.
51
M. J. Coon, *Annual Review of Pharmacology and Toxicology*, 2005, **45**, 1–25.
52
S. Singh, R. Rajendran, K. Kuroda, E. Isogai, M. Krstic-Demonacos and C. Demonacos, *Journal of Cancer Metastasis and Treatment*, 2016, **2**, 268.

View Article Online
DOI: 10.1039/D5NR01714A

53
J. Chung, S. Akter, S.-H. Han, Y. Shin, Tae Hyun Choi, I. Kang and Sung Soo Kim, *International Journal of Molecular Sciences*, 2022, **24**, 129–129.
54
J. Lange, Balkis Eddhif, Mehrad Tarighi, Théa Garandeau, Elodie Péraudeau, J. Clarhaut, B. Renoux, Sébastien Papot and P. Poinot, *Angewandte Chemie*, 2019, **58**, 17563–17566.
55
C. F. Labuschagne, R. Smith, N. Kumar, M. Allsworth, B. Boyle, S. Janes, P. Crosbie and R. Rintoul, *Journal of Clinical Oncology*, 2022, **40**, 2569–2569.
56
Kuttiappan Anitha, Bhargavi Posinasetty, K. Naveen Kumari, Santenna Chenchula, R. Padmavathi, S. Prakash and Chikatipalli Radhika, *Clinica Chimica Acta*, 2024, **554**, 117746–117746.
57
D. Di Capua, D. Bracken-Clarke, K. Ronan, A.-M. Baird and S. Finn, *Cancers*, 2021, **13**, 3923.
58
Y. Zhao, J. Tang, K. Jiang, S.-Y. Liu, A. Aicher and C. Heeschen, *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*, 2023, **1878**, 188868.
59
M. Chen and H. Zhao, *Human Genomics*, DOI:https://doi.org/10.1186/s40246-019-0220-8.
60
A. Barradas and L. Terstappen, *Cancers*, 2013, **5**, 1619–1642.
61
Frank, M. Doggen, G. Attard, Johann and Leon W.M.M. Terstappen, *Annals of Oncology* , 2010, **21**, 1851–1857.
62
H. Pei, L. Li, Z. Han, Y. Wang and B. Tang, *Lab on a Chip*, 2020, **20**, 3854–3875.
63
P. Bankó, S. Y. Lee, V. Nagygyörgy, M. Zrínyi, C. H. Chae, D. H. Cho and A. Telekes, *Journal of Hematology & Oncology*, DOI:https://doi.org/10.1186/s13045-019-0735-4.
64
M. M. Ferreira, V. C. Ramani and S. S. Jeffrey, *Molecular Oncology*, 2016, **10**, 374–394.
65
L. Lin, A. Soni, L. M. Sabik and C. Drake, *American Journal of Preventive Medicine*, 2021, **60**, 104–109.
66
J. Phallen, M. Sausen, V. Adleff, A. Leal, C. Hruban, J. White, V. Anagnostou, J. Fiksel, S. Cristiano, E. Papp, S. Speir, T. Reinert, M.-B. W. Orntoft, B. D. Woodward, D. Murphy, S. Parpart-Li, D. Riley, M. Nesselbush, N. Sengamalay and A. Georgiadis, *Science Translational Medicine*, DOI:https://doi.org/10.1126/scitranslmed.aan2415.
67
C. Bettegowda, M. Sausen, R. J. Leary, I. Kinde, Y. Wang, N. Agrawal, B. R. Bartlett, H. Wang, B. Luber, R. M. Alani, E. S. Antonarakis, N. S. Azad, A. Bardelli, H. Brem, J. L. Cameron, C. C. Lee, L. A. Fecher, G. L. Gallia, P. Gibbs and D. Le, *Science translational medicine*, 2014, **6**, 224ra24.
68

View Article Online
DOI: 10.1039/D5NR01714A

J. D. Cohen, L. Li, Y. Wang, C. Thoburn, B. Afsari, L. Danilova, C. Douville, A. A. Javed, F. Wong, A. Mattox, R. H. Hruban, C. L. Wolfgang, M. G. Goggins, M. Dal Molin, T.-L. Wang, R. Roden, A. P. Klein, J. Ptak, L. Dobbyn and J. Schaefer, *Science*, 2018, **359**, 926–930.
69
D. G. Burke, B. Halpern, D Malegan, E McCairns, D. Danks, P. Schlesinger and B. Wilken, *Clinical Chemistry*, 1983, **29**, 1834–1838.
70
A. N. Adhikari, R. C. Gallagher, Y. Wang, R. J. Currier, G. Amatuni, L. Bassaganyas, F. Chen, K. Kundu, M. Kvale, S. D. Mooney, R. L. Nussbaum, S. S. Randi, J. Sanford, J. T. Shieh, R. Srinivasan, U. Sunderam, H. Tang, D. Vaka, Y. Zou and B. A. Koenig, *Nature Medicine*, 2020, **26**, 1392–1397.
71
M. E. Chamberlin, T. Ubagai, S. H. Mudd, W. G. Wilson, J. V. Leonard and J. Y. Chou, *Journal of Clinical Investigation*, 1996, **98**, 1021–1027.
72
H. M. Liebich, *Journal of High Resolution Chromatography*, 1983, **6**, 640–650.
73
M. Phillips, R. N. Cataneo, J. Greenberg, R. Gunawardena, A. Naidu and F. Rahbari-Oskoui, *Journal of Laboratory and Clinical Medicine*, 2000, **136**, 243–249.
74
Kneepkens Cm, Ferreira C, Lepage G and R. Cc, *PubMed*, 1992, **15**, 163–86.
75
J. S. Pyo, H. K. Ju, J. H. Park and S. W. Kwon, *Journal of Chromatography B*, 2008, **876**, 170–174.
76
M. Hakim, Y. Y. Broza, O. Barash, N. Peled, M. Phillips, A. Amann and H. Haick, *Chemical Reviews*, 2012, **112**, 5949–5966.
77
Krupenko, Sergey A and N. V. Oleinik, *Cell Growth & Differentiation*, 2002, **13**, 227–236.
78
P. Mochalski, A. Sponring, J. King, K. Unterkofler, J. Troppmair and A. Amann, *Cancer Cell International*, 2013, **13**, 72.
79
J. E. Edwards, R. L. Rose and E. Hodgson, *Chemico-Biological Interactions*, 2005, **151**, 203–211.
80
K. Schallschmidt, R. Becker, C. Jung, W. Bremser, T. Walles, J. Neudecker, G. Leschber, S. Frese and I. Nehls, *Journal of Breath Research*, 2016, **10**, 046007.
81
A. Jiménez-Pacheco, María Salinero-Bachiller, B. Peinado, A. López-Luque, José Luis Miján-Ortiz and J. M. Peinado, *Urologic Oncology: Seminars and Original Investigations*, 2018, **36**, 243.e21–243.e27.
82
T. Issitt, L. Wiggins, M. Veysey, S. T. Sweeney, W. J. Brackenbury and K. Redeker, *Journal of Breath Research*, 2022, **16**, 024001.
83

View Article Online
DOI: 10.1039/D5NR01714A

Y. Saalberg and M. Wolff, *Clinica Chimica Acta*, 2016, **459**, 5–9.
84
S. N. Rai, S. Das, J. Pan, D. C. Mishra and X.-A. Fu, *PLOS ONE*, 2022, **17**, e0277431.
85
R. Gasparri, R. Capuano, A. Guaglio, V. Caminiti, F. Canini, A. Catini, G. Sedda, R. Paolesse, C. Di Natale and L. Spaggiari, *Journal of Breath Research*, 2022, **16**, 046008.
86
Yosuke Hanai, K. Shimono, K. Matsumura, Anil Vachani, S. M. Albelda, K. Yamazaki, G. K. Beauchamp and H. Oka, *Bioscience, Biotechnology, and Biochemistry*, 2012, **76**, 679–684.
87
M. Woollam, L. Wang, P. Grocki, S. Liu, A. P. Siegel, M. Kalra, J. V. Goodpaster, H. Yokota and M. Agarwal, *Cancers*, 2021, **13**, 1462.
88
M. Phillips, R. N. Cataneo, C. Saunders, P. Hope, P. Schmitt and J. Wai, *Journal of Breath Research*, 2010, **4**, 026003.
89
J. Li, Y. Peng, Y. Liu, W. Li, Y. Jin, Z. Tang and Y. Duan, *Clinica Chimica Acta*, 2014, **436**, 59–67.
90
S. Kure, Sera Satoi, T. Kitayama, Y. Nagase, N. Nakano, M. Yamada, N. Uchiyama, S. Miyashita, S. Iida, H. Takei and M. Miyashita, *Scientific Reports*, DOI:https://doi.org/10.1038/s41598-021-99396-5.
91
C. L. Silva, R. Perestrelo, P. Silva and H. Tomás, *Metabolomics*, DOI:https://doi.org/10.1007/s11306-019-1525-2.
92
M. Woollam, A. P. Siegel, A. Munshi, S. Liu, Sunil Tholpady, T. Gardner, B.-Y. Li, H. Yokota and M. Agarwal, *Cancers*, 2023, **15**, 1352–1352.
93
T. Khalid, R. Aggio, P. White, B. De Lacy Costello, R. Persad, H. Al-Kateb, P. Jones, C. S. Probert and N. Ratcliffe, *PLOS ONE*, 2015, **10**, e0143283.
94
A. R. Lima, J. Pinto, A. I. Azevedo, D. Barros-Silva, C. Jerónimo, R. Henrique, M. de Lourdes Bastos, P. Guedes de Pinho and M. Carvalho, *British Journal of Cancer*, 2019, **121**, 857–868.
95
H. Tyagi, E. Daulton, A. S. Bannaga, R. P. Arasaradnam and J. A. Covington, *Biosensors*, 2021, **11**, 437.
96
A. Bond, R. Greenwood, S. J. Lewis, B. M. Corfe, S. Sarkar, P O'Toole, P. Rooney, M. D. Burkitt, G. L. Hold and C. Probert, 2019, **49**, 1005–1012.
97
H. Amal, M. Leja, K. Funka, I. Lasina, R. Skapars, A. Sivins, G. Ancans, I. Kikuste, A. Vanags, I. Tolmanis, A. Kirsners, L. Kupcinskas and H. Haick, *International Journal of Cancer*, 2015, **138**, 229–236.
98

View Article Online
DOI: 10.1039/D5NR01714A

D. F. Altomare, A. Picciariello, M. T. Rotelli, M. De Fazio, A. Aresta, C. G. Zambonin, L. Vincenti, P. Trerotoli and N. De Vietro, *BJS Open*, 2020, **4**, 1189–1199.
99
R. P. Arasaradnam, M. J. McFarlane, C. Ryan-Fisher, E. Westenbrink, P. Hodges, M. G. Thomas, S. Chambers, N. O'Connell, C. Bailey, C. Harmston, C. U. Nwokolo, K. D. Bardhan and J. A. Covington, *PloS one*, 2014, **9**, e108750.
100
W. Ibrahim, L. Carr, R. Cordell, M. J. Wilde, D. Salman, P. S. Monks, P. Thomas, C. E. Brightling, S. Siddiqui and N. J. Greening, *Thorax*, 2021, **76**, 514–521.
101
M. K. Nakhleh, H. Amal, R. Jeries, Y. Y. Broza, M. Aboud, A. Gharra, H. Ivgi, S. Khatib, S. Badarneh, L. Har-Shai, L. Glass-Marmor, I. Lejbkowicz, A. Miller, S. Badarny, R. Winer, J. Finberg, S. Cohen-Kaminsky, F. Perros, D. Montani and B. Girerd, *ACS Nano*, 2016, **11**, 112–125.
102
S. Kumar, J. Huang, N. Abbassi-Ghadi, H. A. Mackenzie, K. A. Veselkov, J. M. Hoare, L. B. Lovat, P. Španěl, D. Smith and G. B. Hanna, *Annals of Surgery*, 2015, **262**, 981–990.
103
A. R. Lima, J. Pinto, C. Carvalho-Maia, C. Jerónimo, R. Henrique, M. de L. Bastos, M. Carvalho and P. Guedes de Pinho, *Cancers*, 2020, **12**, 2017.
104
K. Matsumura, M. Opiekun, H. Oka, A. Vachani, S. M. Albelda, K. Yamazaki and G. K. Beauchamp, *PLoS ONE*, 2010, **5**, e8819.
105
H. Tong, Y. Wang, Y. Li, S. Liu, C. Chi, D. Liu, L. Guo, E. Li and C. Wang, *Cancer Cell International*, DOI:https://doi.org/10.1186/s12935-017-0475-x.
106
J. Chen, Y. Ji, Y. Liu, Z. Cen, Y. Chen, Y. Zhang, X. Li and X. Li, *Cancer Letters*, 2024, **590**, 216881.
107
Q.-P. Zhou, Y.-H. Zhao and L. Gao, *World Journal of Clinical Cases*, 2022, **10**, 249–253.
108
N. Shehada, J. C. Cancilla, J. S. Torrecilla, E. S. Pariente, G. Brönstrup, S. Christiansen, D. W. Johnson, M. Leja, M. P. A. Davies, O. Liran, N. Peled and H. Haick, *ACS Nano*, 2016, **10**, 7047–7057.
109
N Shehada, G. Brönstrup, K Funka, S. Christiansen, M. Leja and H. Haick, *Nano Letters*, 2015, **15**, 1288–1295.
110
O. Barash, W. Zhang, Jeffrey Mark Halpern, Q. Hua, Y. Pan, H. Kayal, K. Khoury, H. Liu, M. Davies and H. Haick, *Oncotarget*, 2015, **6**, 44864–44876.
111
M. Arnold, M. S. Sierra, M. Laversanne, I. Soerjomataram, A. Jemal and F. Bray, *Gut*, 2016, **66**, 683–691.
112

View Article Online
DOI: 10.1039/D5NR01714A

Mouadh Barbirou, H. G. Woldu, Ikram Sghaier, S. A. Bedoui, A. Mokrani, Radhia Aami, Amel Mézlini, Besma Yacoubi-Loueslati, P. J. Tonellato and Balkiss Bouhaouala-Zahar, *BMC Cancer*, DOI:https://doi.org/10.1186/s12885-020-07605-7.
113
N. Pin-Vieito, M. Puga, D. Fernández-de-Castro and J. Cubiella, *World Journal of Gastroenterology*, 2021, **27**, 6415–6429.
114
M. Alustiza, L. Ripoll, A. Canals, O. Murcia, A. Martínez-Roca, A. García-Heredia, M. Giner-Calabuig, R. Jover and L. Vidal, *Clinica Chimica Acta*, 2023, 117273.
115
N. Kahn, O. Lavie, M. Paz, Y. Segev and H. Haick, *Nano Letters*, 2015, **15**, 7023–7028.
116
T. Wasilewski, N. F. Brito, B. Szulczyński, M. Wojciechowski, N. Buda, A. C. A. Melo, W. Kamysz and J. Gębicki, *TrAC Trends in Analytical Chemistry*, 2022, **150**, 116599.
117
J. Fleischer, *Frontiers in Cellular Neuroscience*, DOI:https://doi.org/10.3389/neuro.03.009.2009.
118
M. Ramesh, R. Janani, C. Deepa and L. Rajeshkumar, *Biosensors*, 2022, **13**, 40.
119
O. I. Parisi, F. Francomano, M. Dattilo, F. Patitucci, S. Prete, F. Amone and F. Puoci, *Journal of Functional Biomaterials*, 2022, **13**, 12.
120
Y. Saylan, Ö. Erdem, F. Inci and A. Denizli, *Biomimetics*, 2020, **5**, 20.
121
M. Khatib and H. Haick, *ACS Nano*, 2022, **16**, 7080–7115.
122
M. Salimi and S. M. R. Milani Hosseini, *Sensors and Actuators B: Chemical*, 2021, **344**, 130127.
123
T. T. Tung, D. Losic, S. J. Park, J.-F. Feller, T. Kim and S. An, *International Journal of Nanomedicine*, 2015, 203.
124
Y. Cai, L. Huang, W. Sun, C. Xu, X. Ren, Y. Ye, Y. Zhu, L. Sun, D. Jiang, Q. Zhang and Y. Wang, *Sensors and Actuators B: Chemical*, 2023, **381**, 133411.
125
P. J. Mazzone, X.-F. Wang, Y. Xu, T. Mekhail, M. C. Beukemann, J. Na, J. W. Kemling, K. S. Suslick and M. Sasidhar, *Journal of Thoracic Oncology*, 2012, **7**, 137–142.
126
T M Nguyen, J H Chung, G.-H. Bak, You Hwan Kim, M. Kim, Y.-J. Kim, Ryuk Jun Kwon, E.-J. Choi, Kwang Ho Kim, Yun Seong Kim and J.-W. Oh, *ACS Sensors*, 2022, **8**, 167–175.
127
L. W. Chan, M. N. Anahtar, T.-H. Ong, K. E. Hern, R. R. Kunz and S. N. Bhatia, *Nature Nanotechnology*, 2020, **15**, 792–800.
128
R. Larracy, A. Phinyomark and E. Scheme, *Journal of Breath Research*, 2022, **16**, 026008.

View Article Online
DOI: 10.1039/D5NR01714A

Data availability: No primary research results, software or code have been included and no new data were generated or analysed as part of this review.