Breath-based biomarker detection

informing drug development and future treatment regimes

The recent approval of the first liquid biopsy test for the diagnosis of non-small cell lung cancer (NSCLC) demonstrates how biomarker-based detection tools are becoming important components of precision medicine-based drug treatment regimes.

Precision medicine allows detailed insights into the disease processes that underlie clinical disease. This field has first started with genetics, and genetic mutation-based models that can predict disease state such as tumour phenotype or likely therapeutic response are gaining much ground in oncology.

A genetic focus to precision medicine is limited, however, as this is a largely static picture that does not capture the biological variability of disease. In recent years there has been increasing focus on downstream molecules through transcriptomics, proteomics and metabolomics. Metabolomics is particularly interesting to stratify patients to the right treatment and inform development pipelines in the future, as analysis can be performed on molecules closely linked to disease activity.

Furthermore, the administration of drugs results in additional metabolites which can be used to evaluate pharmacokinetics and therapy efficacy. This paper reviews the use of the volatile fraction of metabolites, so-called volatile organic compounds (VOCs), as a means to characterise disease processes and link the right patient to the right treatment.

Biomarkers for disease: example of liquid biopsy

Novel cancer assessment techniques such as liquid biopsy are becoming increasingly common. Liquid biopsy is a test done on a sample of blood to look for cancer cells from a tumour that are circulating in the blood, or for pieces of DNA from tumour cells that are in the blood. Results from this type of biomarker test can be returned to the patient in a matter of days, greatly reducing delay and uncertainty from traditional biopsies. Replacing invasive procedures with simple blood tests will also allow clinicians to greatly increase compliance rates. The applications include selection of therapies matched to individual patient mutations as well as screening and early detection.

While liquid biopsy is undoubtedly a useful tool

By Billy Boyle and Dr Mike Murphy

in therapy selection, the fundamental challenge when applied to screening is low sensitivity for early stage cancers. This is not a question of improving the technology; this directly relates to the basic biology of the cancer and limited amounts of circulating tumour DNA for stage 1 cancers. For applications such as early stage screening, or for when effective treatment stratification depends on factors outside of genetic make-up, new modalities for detecting biomarkers need to be explored.

VOCs as a developer's tool
Every time you breathe out there are thousands of volatile metabolites called volatile organic com-

Volatile Organic Compounds (VOCs) in breath provide a real-time snapshot of current medical state

pounds on your breath. The presence and concentration of VOCs directly reflects the current underlying biochemical activity and state of cells and tissues, in contrast to, for example, a genomic analysis which only provides the starting blueprint.

There are typically three origins of VOCs. The first, most directly relevant for pulmonary disease, is metabolic activity in local airways tissue. These VOCs can be particularly useful for profiling disease type compared to genetic methods as there is a concentration effect: a change in a single gene can give rise to hundreds or more of different downstream molecules. Also, these chemicals would not be detectable in blood-based testing unless they first dissolve into the bloodstream.

Second, systemic biomarkers relevant for non-pulmonary disease can also be detected, as the pulmonary system is highly efficient at exchanging chemicals with the circulatory system, including any VOCs present in the early stages of disease. With each exhalation a breath test can trap and store the VOCs derived from blood. It takes approximately one minute for your entire circulating blood flow to pass round the body once. By continuously sampling breath over this time period, in essence the volatile fraction of all the blood in your body can be sampled in a way that is non-invasive and completely pain-free.

The ability to concentrate exhaled chemicals via continuous sampling enables the identification of chemicals that are present at naturally low levels in the blood in a way that is not possible with other sampling modes such as blood tests, and can lead to dramatically improved test sensitivity across a range of diseases. Third, exogenous molecules such as those arising from the microbiome or introduced from the environment, including downstream drug metabolites from therapeutic interventions, will also be present in the breath. By measuring biomarkers from all these sources, a 'Breath biopsy' can provide a rapid and non-invasive means to gain detailed insight in the functioning of the body in health and disease.

Breath biopsy to guide treatment regimes
Modern breath testing started in 1971 with the work of Nobel Prize winner Linus Pauling, who demonstrated that there were hundreds of identifiable VOCs present in breath. Recently there have been significant advancements in the technologies used in breath analysis, and breath diagnostics are now used in the clinical setting, for example using fractional exhaled nitric oxide (FeNO) as a treatment guide for certain types of asthma. Despite this,

there is still little agreement in identified breath biomarkers within a given disease, and breath testing is rarely used routinely in clinical settings.

New improvements in our ability to collect and inexpensively analyse breath opens up a range of new applications for breath biomarker tests. Improvements combining high sensitivity to VOCs and flexibility and reproducibility in sample collection allow breath biomarkers to be incorporated into early clinical trials and also to be used to screen and stratify patients for those new therapies.

The traditional breath collection technique of exhaling directly on to a sensor risks swamping the VOC signal with humidity and volatile chemicals naturally present in the background environment. Breath storage is most commonly carried out by exhaling into Tedlar® bags, which suffer from short breath sample retention times and poor transportability, and are not always suitable for patients with impaired breathing. To address these issues, a consortia of breath scientists (http://www.breathe-free.org/) developed a breath sampler that enables quick and easy breath collection in patients. This sampler allows robust, targeted selection of different portions of breath, allowing a focus on those fractions carrying the highest concentration of target biomarkers. Combining such a sampler with a clean air supply eliminates humidity and background contamination issue, and allows for comfortable sample collection.

VOCs and translational medicine

With improved sample collection, it now becomes feasible to introduce breath biopsy into the clinical setting. To perform the analysis on the captured breath sample, there are already high end analytical instruments such as gas chromotograph mass spectrometers (GC-MS) that are capable of detecting VOCs with high analytical accuracy. A GC-MS is the gold standard in sample identification, where chemicals in the sample are first separated by their chemical properties and then ionised and further distinguished by their mass to charge ratio. While their utility is well-proven, the cost and complexity of these machines restrict translation deployment in the clinic and make them an unlikely solution in the primary care setting.

On the other end of the scale, there are a plethora of simple materials-based sensors (so-called 'Electronic noses', resonant cantilevers, nanotubes, and other devices) that are cost-effective and easy to use. They operate by pattern recognition: this allows them to differentiate between 'disease' and 'control' states but does do not give the identity of
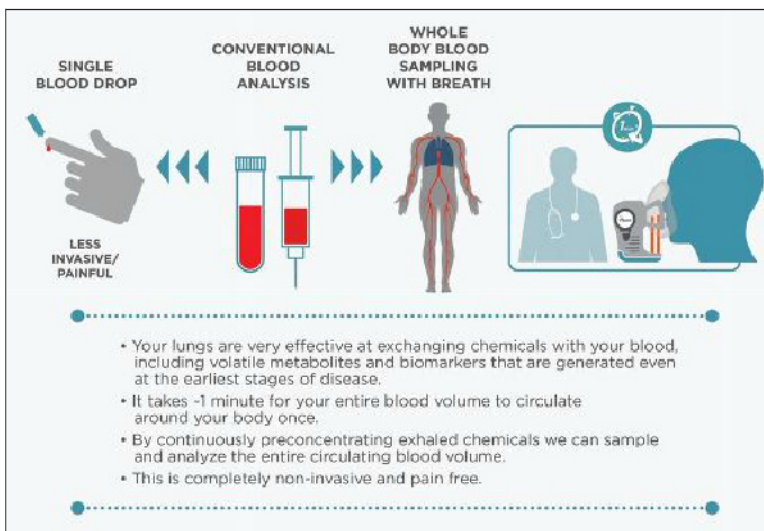
Breath allows us to sample large volumes of blood completely non-invasively, leading to improved test sensitivity

the specific biomarkers that drive the detection signal, and so cannot help identify the mechanism behind the disease state.

A Field Asymmetric Ion Mobility Spectrometry (FAIMS) sensor platform combines the analytical power of a GC-MS with the flexibility and ease-of-use of materials-based sensors. The FAIMS chip can be programmed in software to detect targeted disease VOCs with high sensitivity and selectivity. First, the VOCs are ionised allowing their path to be altered by electric fields. The second stage of the process takes advantage of this by applying an alternating voltage across the channels of the FAIMS chip that the ions are travelling through. This creates an alternating electric field that separates the ions depending on their mobility, which is different for each type of molecule. Finally, to detect the presence of a specific disease biomarker, a 'compensation voltage' is applied that steers the molecules of interest to the detector, where they are counted.

The FAIMS chip is highly sensitive, able to detect at low parts per billion (ppb) and in some cases part per trillion (ppt) levels making it well suited for detecting breath VOCs which are typically present around the tens of ppb range. FAIMS is also highly selective, so even in a typical breath sample containing hundreds of VOCs unrelated to the disease of interest, it can ignore this chemical noise and detect with a low incidence of false positives. In addition the small size and software programmability of the FAIMS chip makes it suitable for near patient or point of care operation.

Early detection offers the best chance of survival in lung cancer
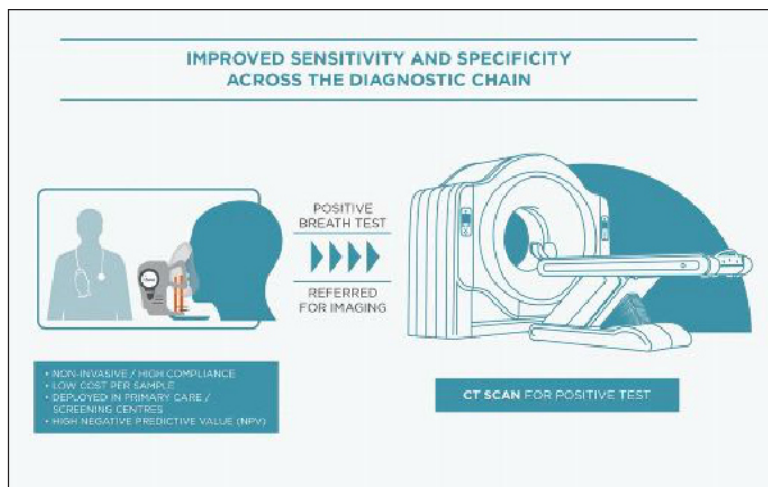
## Lung cancer: enabling earlier intervention

These recent advances, both in breath sample collection and also in VOC analytical tools, open up new opportunities for widespread use of breath biopsy for early, non-invasive detection of disease, particularly for diseases that are currently poorly served. For example, lung cancer is the most common cancer in the world with an estimated 1.8 million cases and 1.6 million deaths in 2012[1]. It is thought that in 2016 there will be more than 221,000 new cases of lung cancer in the United States, and with more than 158,000 deaths predicted, it is the number one cause of cancer deaths. Breast cancer, in comparison, has had a screening programme in place for some time, and the number of diagnoses is estimated to be 250,000, with but only 40,000 associated deaths[2].

The gold standard for lung cancer screening is low-dose computed tomography (LDCT), an imaging procedure that uses special low-dose x-ray equipment to create detailed scans of areas inside the body. In 2012, the National Lung Screening Trial (NLST) reported a mortality reduction of 20% compared to chest x-ray for lung cancer screening. The performance of the trial, however, has raised concerns regarding the high levels of false positives. For every 100,000 people screened for three years about 35,600 (35.6%) will have a false positive result, of these 1,800 would have an invasive procedure (5.1%) and 40 (1.1%) would have a major complication such as bleeding in the lung, a collapsed lung or an infection.

A breath biopsy test performed on patients who show positive for the LDCT test could greatly improve this; a breath test with 80% specificity would result in more than 13,500 fewer follow-up procedures for every 100,000 patients having a positive LDCT result. In addition, the fast turn around (days) of a breath test would greatly reduce the time patients would otherwise spend worrying about indeterminate LDCT test results, which under the current standards could take up to two years to obtain a definitive diagnosis. Alternatively, pre-screening the LDCT test population with a lung cancer breath biopsy test would similarly improve sensitivity and specificity while reducing patient discomfort.

The NHS and Innovate UK are currently funding the largest breath-based clinical trial in the world in order to investigate early stage lung cancer detection. Under chief investigator Robert Rintoul, the multi-centre Lung Cancer Indicator Detection (LuCID) study will sample up to 3,000 patients across the UK, Europe and the US referred under suspicion of lung cancer. Of this group 45% are expected to have lung cancer, about 7% of which are expected to be stage 1.

Besides early cancer detection, this study will also generate evidence to help guide downstream treatment decisions. Firstly, breath analysis could provide a non-invasive means to discern different tumour types, as the detected biomarkers strongly relate to the primary cell type and driving mutations. This could be particularly valuable in the
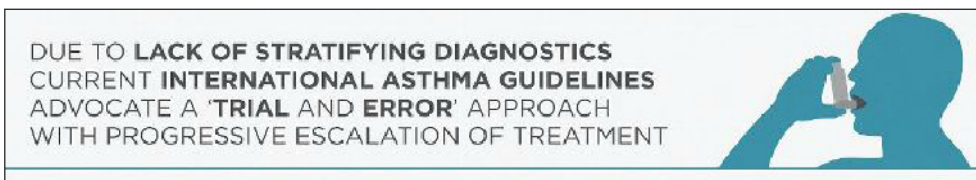
substantial fraction of patients with difficult to biopsy tumours. Secondly, breath biomarkers could potentially predict response to therapy, as has been suggested by more invasive metabolic profiling approaches. In that case ineffective treatment could be either prevented or discontinued quickly after identifying a lack of response. Finally, this approach could help to monitor patients after being treated with curative intent for a recurrence of their tumour.

This approach has benefits compared to CT-based follow-up as it can be done frequently without incurring significant healthcare costs or radiation exposure to patients. Taken together, non-invasive metabolomics through a breath biopsy has the potential to provide detailed insight into tumour biology impacting a wide range of aspects of diagnosis, treatment and monitoring.

Asthma: improving drug treatment regimes

The ability to measure disease activity by detecting downstream metabolites on the patient's breath has many applications to treatment stratification as well. For example, asthma is the most common chronic airway disease in the world. It is characterised by a combination of recurrent reversible airflow limitation and characteristic symptoms as a consequence of chronic inflammation in the small airways. There are many different underlying mechanisms responsible for this inflammation, each requiring a different treatment. To date there is no test available that matches a patient to the treatment they respond to optimally other than a trial and error approach with progressive escalation towards higher dose treatment. Furthermore, up to one in five cases of asthma are classified as 'problematic' due to either symptom severity, control or both.

The difference in cell activity related to the different subtypes of asthma is also reflected by the metabolites these cells produce. As mentioned above, biomarkers such as fractional exhaled Nitric Oxide (FeNO) are being used to help guide treatment decisions: FeNO reflects a protective biochemical pathway of the lungs but lacks specificity as it is a single biomarker affected by many processes other than asthma. In spite of this is has been used to measure corticosteroid responsiveness in severe asthma patients, and has been shown to help assess treatment adherence for these patients, as FeNO levels are responsive to the correct application of steroid inhalers.

To address the 4-10% of asthma patients that are not able to achieve control over their symp-

toms due to poor responsiveness to current treatment options, pharmaceutical companies are currently undergoing clinical trials for novel biologic asthma therapies. To date finding the correct patient population for these new therapies has been extremely difficult, and currently available biomarkers such as FeNO, blood eosinophils and periostin have not adequately stratified these severe asthma patients.

Data from the U-BIOPRED study presented at the European Respiratory Society meeting in September 2015 showed that a FAIMS sensor was up to 63% more accurate in stratifying asthma patients in a head-to-head comparison with other gas sensors and classic laboratory techniques such as GC-MS, with an 81% accuracy in stratifying asthma patients responsive to anti-IgE treatment (XOLAIR®/Omalizumab). Being able to guide asthma patients to their most effective therapy will reduce patient discomfort and improve mortality, while reducing emergency care and hospitalisation costs.

The NHS and Innovate UK are currently funding the early stage clinical trial Stratification of Asthma Treatment by Breath Analysis (STRATA) to further explore the opportunity for breath biopsy among patients with problematic asthma. Being able to reliably match precision therapies to the correct patient population will drive further drug discovery. Embedding breath biopsy testing into early stage clinical trials would allow pharmaceutical companies to perform biomarker discovery early on in the drug development process. This gives them the opportunity to develop companion diagnostics that correctly target these therapies, thereby both improving treatment efficacy within the intended population while also creating an easily deployable test for identifying that population. Doing this maximises the chance of achieving regulatory approval for therapies that would otherwise fail due to health economic pressures.

Future possibilities for chemical biomarkers

Building on these advances in disease detection and stratification by therapeutic response, there are further possibilities for using breath biopsy for treatment monitoring and validation. Comparing breath tests with those performed pre-treatment could provide confirmation of the treatment's efficacy by detecting changes in disease-related biochemical activity, or even by detecting the metabolite signatures of the drug itself. Monitoring response in these activity signatures can help confirm the initial diagnosis. Also, tracking these

Precision Medicine

References
1 World Health Organization. International Agency for Research on Cancer. GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. Lung Cancer.
2 US SEER Cancer Statistics Factsheets: Lung and Bronchus Cancer. National Cancer Institute. Bethesda, MD.

changes over time could provide an early warning to changes in the underlying disease state and provide immediate feedback on treatment efficacy.

Miniaturised, yet highly sensitive and selective, breath analysers such as the FAIMS chip open up the possibility for future widespread adoption of breath biomarkers to not only aid in the development of precision therapies and define the relevant patient populations, but also to manage the effective application of those therapies. **DDW**

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VOC analysis
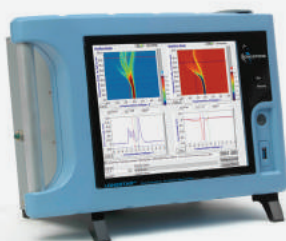
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Overview

Owlstone Medical's Biomarker Services enable academics, clinicians and pharmaceutical companies to identify novel breath biomarkers for diagnostics as well as precision medicine applications including therapeutic response, patient stratification and outcome prediction.

We offer a complete set of services for analysis of volatile organic compound (VOC) biomarkers in breath and other biospecimens. Our services include everything from consultation on optimal study design to biomarker discovery and external blinded validation of the test in the context of the clinical use case.

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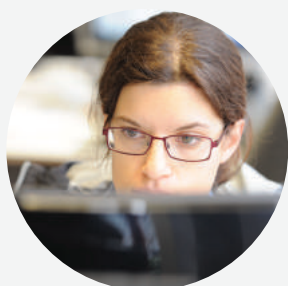
In silico verification and validation

Classification algorithms are verified *in silico* using our proprietary database containing thousands of patient VOC profiles, to provide early evidence of likely clinical performance. Finally, an external blinded test validation is performed in patient groups representative of the intended use population.

Biomarker Services
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