Regulatory issues on breath tests and updates of recent advances on [^{13}C]-breath tests

2013 J. Breath Res. 7 037103

(http://iopscience.iop.org/1752-7163/7/3/037103)

J. Breath Res. **7** (2013) 037103 (8pp) doi:10.1088/1752-7155/7/3/037103

Regulatory issues on breath tests and updates of recent advances on [^{13}C]-breath tests

Anil S Modak

Cambridge Isotope Laboratories Inc., 50 Frontage road, Andover, MA 01810, USA

E-mail: anilm@isotope.com

Received 30 January 2013
Accepted for publication 17 April 2013
Published 18 June 2013
Online at stacks.iop.org/JBR/7/037103

Abstract

Over the last decade non invasive diagnostic phenotype [^{13}C]-breath tests as well as tests using endogenous volatile organic compounds (VOCs) in breath have been researched extensively. However, only three breath tests have been approved by the FDA over the last 15 years. Despite the potential benefits of these companion diagnostic tests (CDx) for evaluation of drug metabolizing enzyme activities and standalone diagnostic tests for disease diagnosis to personalize medicine, the clinical and commercial development of breath tests will need to overcome a number of regulatory, financial and scientific hurdles prior to their acceptance into routine clinical practice. The regulatory agencies (FDA and EMEA) need to adapt and harmonize their approval process for companion diagnostic tests as well as standalone diagnostic breath tests for personalized medicine. The Center for Devices and Radiological Health has deemed any breath test that involves a labeled ^{13}C substrate/drug and a device requires a Pre Market Approval (PMA), which is analogous to an approved New Drug Application. A PMA is in effect, a private license granted to the applicant for marketing a particular medical device. Any breath test with endogenous VOCs along with a device can be approved via the 510(k) application. A number of ^{13}C breath tests with clinical applications have been researched recently and results have been published in reputed journals. Diagnostic companies will need to invest the necessary financial resources to develop and get regulatory approval for diagnostic breath tests capable of identifying responders/non responders for FDA approved drugs with narrow therapeutic indices (personalized medicine) or for evaluating the activity of drug metabolizing P450 polymorphic enzymes or for diagnosing diseases at an early stage or for monitoring the efficacy of medications. The financial success of these diagnostic breath tests will then depend entirely on how the test is marketed to physicians, healthcare organizations, payers (reimbursement), insurance companies and most importantly to patients, the eventual beneficiaries.

(Some figures may appear in colour only in the online journal)

Introduction

The use of patient's breath by physicians to diagnose, detect and treat health problems or diseases was the prominent way medicine was practiced for centuries since the days of Hippocrates. However, with the advent of diagnostic procedures like blood/urine/saliva tests, x-rays, MRI, CT scans, ultrasound, biopsies, genetic tests etc breath tests receded into the background. Since 1970s, when Linus Puling discovered that human breath is a complex gas mixture, containing well over 200 different volatile organic compounds (VOCs) in picomolar concentrations [1], breath testing is slowly gaining traction as a rapid, non invasive diagnostic option for both diagnosis/detection of disease or evaluation of drug metabolizing CYP450 enzyme activity. Around 4000 VOCs have now been detected in samples of exhaled human

breath [2] and several have been associated with a specific disease. Breath VOCs have more recently been researched as biomarkers of disease while a number of ^{13}C-labeled substrates have been used as probes for evaluating various polymorphic enzyme activities [3].

Breath biomarkers can be classified into two groups:

- breath metabolites, such as $^{13}CO_2$ or $^{14}CO_2$ after administration of a labeled drug or substrate;
- breath components produced endogenously owing to a particular physiological or disease status.

Although numerous breath tests have been researched, patented and published for various clinical applications to date [4], only three breath tests have been approved by the FDA.

- Nitric oxide (NO) breath test [5] to detect and monitor asthma (Aerocrine, Solna, Sweden).
- Heartsbreath test [6] for heart transplant rejection (Menssana Research, Inc., NJ, USA).
- Urea-^{13}C breath test (UBT) [7] for diagnosing *Helicobacter pylori* infection (Meretek, Inc., MD, USA).

Even with FDA approval of the UBT, Heartsbreath test and the NO test, all three have low acceptance in the medical field due to poor marketing and/or inadequate reimbursement (CPT codes). It is absolutely critical for medical device companies to educate physicians, healthcare organizations, payers (reimbursement), insurance companies and most importantly patients, the benefits of these non invasive breath tests post approval.

In the last decade diagnostic noninvasive phenotype [^{13}C]-breath tests using suitably labeled ^{13}C probes and breath tests using endogenous/exogenous VOCs in breath have been extensively researched. Despite the potential benefits of these companion diagnostic tests and stand-alone diagnostic tests for disease diagnosis and detection of polymorphic drug metabolizing enzyme deficiencies in patients the commercial development and subsequent FDA approval of these breath tests will need to overcome a number of regulatory, financial and scientific hurdles prior to their acceptance into routine clinical practice.

In this paper, I endeavor to shed light on the US regulatory (FDA) procedures in getting these breath tests approved via Pre Market Approval (PMA) and 510(k) applications.

Personalized medicine has benefitted a number of patients with over 70 products currently on the market [8]. However, widespread implementation of personalized medicine, and in particular companion diagnostics, has been hindered in part by unclear regulatory requirements. Center for Devices and Radiological Health (CDRH) has prioritized releasing the final companion diagnostics guidance by 31 March 2013 and plans on issuing the co-development draft guidelines by 30 September 2013.

Regulatory approvals of ^{13}C breath tests

The US regulatory agency (FDA) in 1976 established three regulatory classes for medical devices. The three classes are based on the degree of regulatory control necessary to assure that the various types of devices are safe and effective. In 2002, the FDA founded the Office of Combination products to cover the regulatory life cycle of drug-device, drug-biologic, and device-biologic medical devices now known as combination products. Combination products are therapeutic and diagnostic products that combine drugs, devices, and/or biological products [9]. Breath tests which use ^{13}C labeled substrates as probes, for evaluating drug metabolizing enzyme activities or detection and diagnosis of medical disorders, and a device to measure the biomarker $^{13}CO_2$ in breath, are designated as class III drug-device combination products. Combination products will be reviewed for FDA approval primarily by the CDRH as the lead agency since the device is considered to be primary mode of action. The ^{13}C labeled substrate in the combination product is considered to be a new drug entity, with the Center for Drug Evaluation and Research (CDER) responsible for review. The device used for measuring the biomarker $^{13}CO_2$ in breath could be a mass, IR or laser spectrometer. Two IR spectrometers have been approved by the FDA in the US for the UBT–UBiT IR spectrometer in 2002 and POCone spectrometer in 2004 (figure 1). Other IR spectrometers are available from German, Chinese and Israeli companies but not approved for sale in the US.

Figure 1. POCone IR spectrometer for measuring the change in the carbon isotope ratio ($^{13}CO_2/^{12}CO_2$) in carbon dioxide in human breath.

To initiate a clinical study with a drug-device combination product (medical device) with significant risk (class III) in humans an investigative device exemption (IDE) application needs to be submitted to the FDA. A pre IDE meeting can be set up if deemed necessary by the study sponsor to discuss details of the IDE application. The IDE application needs the following necessary components [9]:

(1) investigational plan with the detailed clinical study protocol,
(2) a CMC section with a description of the methods, facilities, and controls used for the manufacture, processing, packing and storage of the ^{13}C labeled probe.

The CDRH and CDER will review the IDE application and notify the sponsors of the IDE application of their decision

within 30 days of receipt. The results of the IDE application can then be submitted in a PMA application. The PMA application submission fee is $248 000 ($62 000 for small business). The FDA has 180 days to review the PMA application. An approved PMA—like an approved New Drug Application (NDA)—is, in effect, a private license granted to the applicant/sponsor for marketing a particular medical device.

If the ^{13}C breath tests are to be used as diagnostic medical devices for identifying responders/non-responders for new or approved FDA drugs they will be labeled as companion diagnostics (CDx). In November 2011, the FDA released a draft guidance for *in vitro* companion diagnostics [10]. However, there is still no guidance document that covers *in vivo* companion diagnostics. The FDA is also expected to issue final guidance on research-use only diagnostics as well as a series of draft guidances for laboratory-developed tests, diagnostics co-development and pre-investigational device exemption. Despite some additional clarity from the FDA, drug developers still will have to navigate their way forward in developing companion diagnostics.

Regulatory approvals for breath tests using endogenous VOCs

Section 510(k) of the Food, Drug and Cosmetic Act requires device manufacturers who must register, to notify FDA of their intent to market a medical device at least 90 days in advance. This is known as premarket notification—also called PMN or 510(k). This allows FDA to determine whether the device is equivalent to a device already placed into one of the three classification categories. Breath tests using endogenous VOCs are considered to be low risk and do not require an IDE application or a PMA application for regulatory approval. These breath tests can go through the regulatory approval process via the 510(k) clearance. A 510(k) is a premarketing submission made to FDA to demonstrate that the device to be marketed is as safe and effective, that is, substantially equivalent, to a legally marketed device that is not subject to PMA. 510(k) (PMN) to FDA is required at least 90 days before marketing unless the device is exempt from 510(k) requirements [11].

FDA will rely only upon valid scientific evidence to determine whether there is reasonable assurance of the safety and effectiveness of the device. Valid scientific evidence, from well-controlled human clinical investigations or partially controlled studies, will be reviewed by CDRH. If the data submitted in a 510(k) application can fairly and responsibly be concluded by qualified experts to have reasonable assurance of the safety and effectiveness of a device under its conditions of use it would be approved. The amount and degree of evidence required may vary according to the characteristics of the device, its conditions of use, the existence and adequacy of warnings and other restrictions, and the extent of experience with its use.

For the measurement of NO content in breath a special controls guidance was developed to support the classification of the breath nitric oxide test system into class II (special controls). A breath nitric oxide test system is a device intended to measure fractional nitric oxide in human breath. Measurement of changes in the fractional nitric oxide concentration in expired breath aids in evaluating an asthma patient's response to anti-inflammatory therapy, as an adjunct to established clinical and laboratory assessments of asthma. A breath nitric oxide test system combines chemiluminescence detection of nitric oxide with a pneumotachograph, display, and dedicated software. In 2008, the NIOX MINO® Nitric Oxide Monitoring System (K072816) by Aerocrine AB was approved by the FDA [12]. Multi-center clinical studies with 62 patients were performed to validate the intended use and verify substantial equivalence to the predicate device NIOX®. The NIOX® a class II device was originally approved in 2003 with a 510(k) application (K021133). A clinical study was performed together with *in vitro* testing to obtain clearance. Exhaled NO levels were measured in unstable steroid-naive adult and pediatric asthmatic subjects and again after a two-week treatment with inhaled corticosteroids. Exhaled NO levels decreased highly significantly, with 95% confidence limits for the decrease of −40% to −60% accompanied by clinical improvement. This trial, together with extensive *in vitro* testing, led to the clearance of NIOX by the US Food and Drug Administration.

In 2008, the FDA cleared for marketing a non-invasive Heartsbreath test (H0300004) [13] by Menssana Research, Inc. *under the Humanitarian Device Exemption (HDE) program* which consists of : a breath collection apparatus for collection of VOCs in alveolar breath followed by analysis of the VOCs in alveolar breath and room air by gas chromatography and mass spectroscopy. The interpretation of the VOCs with a proprietary algorithm predicted the probability of grade 3 heart transplant rejection. The clinical study for approval used 1061 breath VOC samples collected from 539 heart transplant recipients prior to scheduled endomyocardial biopsy. Breath VOCs were analyzed by gas chromatography and mass spectroscopy, and the *breath methylated alkane contour* (BMAC) was derived from the abundance of C4–C20 alkanes and monomethylalkanes. The gold standard of rejection (predicate device) was the concordant set of International Society for Heart and Lung Transplantation (ISHLT) grades in biopsies read by two cardiac pathologists.

Updates on recent advances in ^{13}C breath tests

Interindividual differences in drug disposition are critical reasons for adverse drug reactions (toxicity) and lack of drug response (efficacy). The majority of phase I and phase II drug-metabolizing enzymes are polymorphic and constitute essential factors for the clinical outcome of drug therapy.

CYP450 enzymes in the liver catalyze the initial step in the biotransformation of xenobiotics. More than 50 CYP450 isozymes are known to exist in humans [14] and they have been classified into 17 families and 39 subfamilies based on amino acid sequence similarities. The bulk of drug metabolisms are carried out by a few members of the CYP1, 2, and 3 families and occurs primarily in the liver, which contains the highest concentration of CYP450 in the body. On average, 70% of the P450s expressed in adult human liver consist of the following

isozymes: 1A2, 2A6, 2B6, the 2C subfamily (2C8, 2C9, 2C18, and 2C19), 2D6, 2E1, and the 3A subfamily (3A4 and 3A5) [15, 16].

^{13}C-Breath tests with several clinical applications have been published over the last three decades [17, 18]. More recently, emphasis has been placed on utilizing a suitably ^{13}C labeled *in vivo* breath test probe for a specific CYP450 isozyme to detect enzyme deficiencies or evaluate the rate of metabolism of a drug or the inhibition of probe substrate or the level of enzyme activity. These phenotype breath tests are rapid (less than 1 h), non invasive and utilize a single breath collection post ingestion of the ^{13}C probe.

Pantoprazole-[^{13}]C breath test (Ptz-BT)

Cytochrome P450 (CYP) 2C19 ranks among the most important drug metabolizing enzymes in humans. Several clinically important drugs including proton pump inhibitors (omeprazole, esomeprazole, lansoprazole, rabeprazole and pantoprazole), clopidogrel, cyclophosphamide and voriconazole are primarily cleared by metabolism via the CYP2C19 system. The activity of this enzyme, however, is highly variable among individuals, in part due to polymorphisms in the CYP2C19 gene, as several variant alleles that influence enzymatic activity have been identified, and due to exposure to drugs, diet and environmental chemicals that may inhibit or induce its activity. Certain disease conditions also appear to affect its activity. The potential clinical relevance of the large interindividual differences in CYP2C19 activity to treatment outcome of its substrates has been demonstrated in recent years. For example, eradication of *Helicobacter pylori* in peptic ulcer patients is much greater in poor metabolizers (PMs) of CYP2C19 than extensive metabolizers (EMs). Evidence also exist that clinical response to clopidogrel and cyclophosphamide, two drugs that require metabolic activation by CYP2C19 to exert pharmacological activity, might be substantially reduced in patients with reduced CYP2C19 activity. A predictive test that was capable of evaluating CYP2C19 activity in humans would be a valuable tool to optimize therapy and avoid adverse effects of drugs primarily metabolized by CYP2C19 enzyme. A few clinical studies have demonstrated that the pantoprazole-^{13}C breath test (Ptz-BT) can be a useful diagnostic test to rapidly evaluate CYP2C19 enzyme activity [19–21] and potentially personalize PPI [22] and clopidogrel [23, 24] therapy for the individual patient. The Ptz-BT offers greater practical clinical utility over existing genotype and phenotype approaches in predicting or assessing CYP2C19 activity, particularly in effectively distinguishing (PMs) from intermediate metabolizers (IMs) and EMs of CYP2C19. This is because the Ptz-BT can be noninvasively performed at a single time-point breath collection at 30 min post ingestion of Ptz-^{13}C. The Ptz-BT, a rapid *in vivo* phenotype test, captures variability of CYP2C19 enzyme activity owing to both genetic and epigenetic factors, especially drug–drug interactions.

Dextromethorphan-[^{13}C] breath test (DM-BT)

Cytochrome P450 2D6 (CYP2D6) is an important example of a clinically relevant drug-metabolizing enzyme for which genotyping and phenotyping information has the potential to improve drug safety and efficacy [25–27]. CYP2D6 is involved in the biotransformation of more than 40 therapeutic entities, including several beta-receptor antagonists, antiarrhythmics, antidepressants, antipsychotics, and morphine derivatives (for an updated list, see http://medicine.iupui.edu/flockhart/). Considerable variability in *CYP2D6* gene expression and activity has also been attributed to well-known genetic polymorphisms. To date, more than 60 allelic variants plus additional subvariants of CYP2D6 have been identified (http://www.cypalleles.ki.se). Inheritance of two recessive loss-of-function alleles results in a PM phenotype, whereas combinations of full- or reduced-function alleles result in a wide spectrum of phenotypes, including intermediate (IM), extensive (EM), and ultra-rapid metabolizers (UM). There are considerable differences in allele frequencies among populations, giving rise to variable percentages of UM, EM, IM, and PM subjects within a given population or ethnic group. The Dextromethorphan-^{13}C breath test (DM-BT) for the evaluation of CYP2D6 enzyme activity [28] has the potential to become a companion diagnostic test (CDx) for several drugs metabolized by the enzyme including Tamoxifen the endocrine therapy for breast cancer patients [29].

Methacetin-[^{13}]C breath test (MBT)

The importance of CYP1A2 for drug interactions has risen over the past decade due to the growing number of drugs metabolized by this enzyme [30]. Some of the drugs that warrant particular attention are theophylline, clozapine, olanzapine, and tizanidine. Among CYP1A2 inducers, smoking is probably the most significant, but the usual enzyme inducers rifampin and barbiturates can also substantially increase CYP1A2 activity. Two indoleamines and neurotransmitters serotonin and tryptamine showed an inhibitory effect on the activity of phenacetin O-deethylase [31]. Cimetidine, ciprofloxacin, enoxacin, and fluvoxamine are potent inhibitors of CYP1A2. Other substances, which were either poor or partial inhibitors of CYP1A2 were dopamine (DA), L-tyrosine, adrenaline, indole-3-acetic acid, L-tryptophan, and 5-hydroxyindole acetic acid [31].

The [^{13}C]-MBT has been extensively researched for evaluating CYP1A2 enzyme activity and to evaluate quantitative liver function [32–42]. Methacetin is primarily O-demethylated by the CYP1A2 enzyme in the liver to acetaminophen and the breath biomarker $^{13}CO_2$.

Sodium 1-[^{13}]-C-propionate breath test (BBT)

Vitamin B12 deficiency or hypocobalaminemia is a low blood level of vitamin B_{12} which is becoming a emergent health problem. It can cause permanent damage to nervous tissue if left untreated. The most commonly used diagnostic tests are limited in accuracy, sensitivity, and are non-specific for B12 deficiency. The B12 breath test (BBT) for detection of vitamin B12 deficiency is based on the metabolism of the substrate sodium 1-[^{13}]-C-propionate to the biomarker $^{13}CO_2$ in breath with vitamin B12 as a cofactor [43]. Statistical analysis revealed that two breath collection times (10 and

Table 1. VOCs in breath identified as potential diagnostic markers of oxidative stress in various diseases.

Disorders	VOC's detected	Study
Asthma	NO, pentane, ethane, 8-isoprostane	Dweik [53], Olopade [54], Paredi [55], Montuschi [56]
Allograft rejection	Carbonyl sulfide	Studer [57]
Breast cancer	Alkanes, monomethylated alkanes	Phillips [58]
Chronic obstructive pulmonary disease (COPD)	Isoprene, C16 hydrocarbon, 4,7-dimethyl-undecane, 2,6-dimethyl-heptane, 4-methyl-octane, hexadecane, aldehydes	Schooten [59], Phillips [60], Basanta [61]
Colorectal cancer	1,3-dimethylbenzene, 1,2-pentadiene, cyclohexane, methylcyclohexane, 4-methyloctane	Altomare [62]
Cystic fibrosis	Carbonyl sulfide, alkanes, C5–C16 hydrocarbons, N-methyl-2-methylpropylamine	Robroeks [63] Barker [64]
Diabetes	Acetone, ethanol, methyl nitrate, C4-C20 n-alkanes and their monomethylated derivatives, ethylbenzene, m/p-xylene	Novak [65], Gallassetti [66, 67], Phillips [68]
Inflamatory bowel syndrome	Ethane, propane, pentane	Pelli [69]
Angina, heart transplant rejection, ischemic heart disease	Alkanes, 22 methylated alkanes	Phillips [70]
Hepatic coma	Methyl mercaptan, dimethylsulfide	Hisamura [71], Kaji [72]
Liver disease	Dimethyl sulfide, acetone, 2-butanone, 2-pentanone, indole, dimethyl selenide, ethanol, ethane	Van den Velde [73], Sehnert [74], Solga [75]
Lung cancer	Aldehydes, 2-ethyl-1-hexanol, 2-methylpentane, dimethyl succinate, 2-pentanone, phenol, 2-methylpyrazine, 2 hexanone, 2-butanone and acetophenone	Fuchs [76], Amann [77], Hanai [78], Horvath [79]
Rheumatoid arthritis	Pentane	Humad [80]
Schizophrenia	Pentane, ethane, carbon disulfide	Phillips [81], Puri [82]
Tuberculosis	Derivatives of naphthalene, benzene and alkanes	Phillips [83]

20 min) post ingestion of the labeled probe can accurately, reliably, and reproducibly diagnose vitamin B12 deficiency.

Levodopa-[^{13}C] breath test (LD-BT)

Peripheral carbidopa (CD) levels directly impact on central DA production in Parkinson disease (PD) through extracerebral inhibition of dopa decarboxylase (AADC) resulting in an increase in levodopa (LD) bioavailability. Recent data suggests that higher CD doses than those presently used in PD treatment may result in improved clinical response. Optimizing CD doses in individual patients may, therefore, result in ideal individualized treatment. The Levodopa-[^{13}C] breath test can be a useful noninvasive diagnostic tool for evaluation of aromatic amino acid decarboxylase (AADC) enzyme activity using the biomarker $^{13}CO_2$ in breath, a first step in personalizing CD doses for PD patients [44].

Uracil-2-[^{13}C] breath test (Ura-BT)

5-Fluorouracil (5-FU) is one of the most commonly administered cancer chemotherapeutic agents for the treatment of solid tumors, including colorectal and breast cancers. In the USA, approximately 250 000–300 000 patients are treated with 5-FU chemotherapy annually [45]. Owing to its narrow therapeutic index, severe dose-related 5-FU toxicity remains a serious clinical problem. Approximately 31% of cancer patients receiving bolus 5-FU treatment experience grade III–IV hematologic toxicity [46], and 40–60% of those cancer patients are dihydropyrimidine dehydrogenase deficient [47, 48]. The uracil-2–^{13}C breath test (Ura-BT) [49, 50] can rapidly evaluate pyrimidine metabolic disorder prior to initiating 5-FU therapy.

Urea-[^{13}C] breath test (UBT)

Tuberculosis (TB) is a disease of the lungs caused by the bacteria *Mycobacterium tuberculosis*. The sputum smears test is the most popular and least expensive tool used to detect the presence of the TB bacteria via a microscope. However, the test's results are not always accurate, and the test cannot determine if the bacteria will be resistant to standard TB treatments. The most sensitive test currently available is the sputum culture test, which takes at least a month to generate accurate results, and requires expensive equipment that is not available in developing countries where *M. tuberculosis* is predominantly found.

The FDA approved UBT has been successfully implemented to detect urease activity due to *Helicobacter pylori* infection in the gastrointestinal tract for over 15 years. The *M. tuberculosis* urease enzyme encoded by ureA, ureB and ureC (Rv1848, Rv1849 and Rv1850) hydrolyzes urea into carbon dioxide and ammonia [51]. Clinical trials with the nebulized delivery of ^{13}C-urea dry powder formulations directly to the lungs will need to be carried out to prove the clinical utility of the UBT in detection of TB and monitoring the efficacy of treatment. The conversion rate per minute showed that the UBT may rapidly and reliably detect pulmonary TB associated with a variety of pathologic presentations [52]. UBT may provide a useful diagnostic and biomarker assay for TB and for treatment response.

Future of breath tests

Diagnosis of disease at an early stage can make the disease much more treatable, improve survival and reduce the costs of healthcare by minimizing hospitalization for adverse events.

Table 2. ^{13}C Breath tests for personalizing existing drugs.

Breath tests	Enzyme	Drugs
DM-BT	CYP2D6	Tamoxifen, nudexta, abilify, venaflaxine, psychiatric medications, analgesic medications
Ptz-BT	CYP2C19	Clopidogrel, PPI's, Cyclophosphamide, thalidomide
MBT	CYP1A2	Fluvoxamine, ciproflaxin
Ura-BT	DPD, DPHD	5-fluorouracil
L-DOPA-BT	AAAD	Carbidopa-levodopa

Breath tests which utilize endogenous VOCs as biomarkers for the diagnosis of an ailment/disease must ensure that the endogenous compound or set of compounds detected in breath due to oxidative stress originates exclusively from the afflicted organ or tissues to make them highly selective and accurate as diagnostic tests. This is critical as the same VOCs could be present in breath of patients with either different diseases or ailments of different organs. Some examples of endogenous VOCs present in breath due to specific disease or ailment are listed in table 1.

Stable isotope-labeled xenobiotics can be used as probes to provide rapid *in vivo* phenotype assessment of phase I enzymes (CYPP450). The DM-BT (CYP2D6), MBT (CYP1A2) and Ptz-BT (CYP2C19) offer promise as rapid, point of care and non invasive phenotyping assays or CDx for evaluating enzyme activity thereby enabling physicians to personalize medication for existing drugs table 2. These breath tests can also be used as CDx to identify non-responders to new medications in clinical trials metabolized primarily by specific enzymes.

Diagnostic breath tests, a promising new field in medicine, will potentially offer noninvasive, real-time, POC disease diagnostics and metabolic status monitoring to enable physicians personalize therapy.

Conclusions

Regulatory boards (FDA and EMEA) need to provide clear, concise and consistent guidelines for approval of breath tests as companion diagnostic tests (CDx) or as standalone tests for personalizing medication. Breath tests both with endogenous VOCs or ^{13}C substrates can be integrated into routine clinical practice for personalized medicine provided diagnostic companies are willing to invest in getting regulatory approval for these unique promising non invasive diagnostic breath tests.

References

[1] Pauling L, Robinson A, Teranishi R and Cary P 1971 Quantitative analysis of urine vapor and breath by gas–liquid partition chromatography *Proc. Natl Acad. Sci. USA* **68** 2374–6

[2] Phillips N, Herrera J, Krishnan S, Zain M, Greenberg J and Cataneo R 1999 Variation in volatile organic compounds in the breath of normal humans *J. Chromatogr.* B **729** 75–88

[3] Modak A 2010 Breath biomarkers for personalized medicine *Personal. Med.* **7** 643–53

[4] Modak A 2007 Stable isotope breath tests in clinical medicine: a review *J. Breath Res.* **1** 014003

[5] Silkoff P E, Carlson M, Bourke T, Katial R, Ogren E and Szefler S 2004 The aerocrine exhaled nitric oxide monitoring system NIOX is cleared by the US food and drug administration for monitoring therapy in asthma *Allergy Clin. Immunol.* **114** 1241–56

[6] Phillips M *et al* 2004 Heart allograft rejection: detection with breath alkanes in low levels (the HARDBALL study) *J. Heart Lung Transplant.* **23** 701–8

[7] Graham D *et al* 1987 *Campylobacter pylori*detected non-invasively by the ^{13}C-urea breath *Lancet* **1** 1174–7

[8] Personalized Medicine Coalition *'The Case for Personalized Medicine'* 3rd edn www.personalizedmedicinecoalition.org/sites/default/files/files/Case_for_PM_3rd_edition.pdf

[9] www.fda.gov/CombinationProducts/AboutCombinationProducts/default.htm

[10] www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/HowtoMarketYourDevice/InvestigationalDeviceExemptionIDE/ucm046706.htm#reqele

[11] www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327.pdf
www.fda.gov/medicaldevices/productsandmedicalprocedures/deviceapprovalsandclearances/510kclearances/default.htm

[12] www.accessdata.fda.gov/cdrh_docs/pdf7/K072816.pdf

[13] www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm081213.htm

[14] Nelson D R 1999 Cytochrome P450 and the individuality of species *Arch. Biochem. Biophys.* **369** 1–10

[15] Guengerich F P 1995 *Cytochrome P450: Structure, Mechanism, and Biochemistry* 2nd edn ed Paul R Ortiz de Montellano (New York: Plenum) Chapter 14

[16] Shimada T, Yamazaki H, Mimura M, Inui Y and Guengerich F P 1994 Interindividual variations in human liver CYP-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians *J. Pharmacol. Exp. Ther.* **270** 414–23

[17] Modak A S 2005 ^{13}C breath tests : transition from research to clinical practice *Breath Analysis for Clinical Diagnosis and Therapeutic Monitoring* ed A Amann and D Smith (Singapore: World Scientific) pp 457–78

[18] Modak A S 2013 An update on ^{13}C-breath tests: the transition to acceptability into clinical practice *Volatile Biomarkers: Non-Invasive Diagnosis in Physiology and Medicine* ed A Amann and D Smith (Amsterdam: Elsevier) pp 263–80

[19] Desta Z, Modak A, Nguyen P D, Lemler S M, Kurogi Y, Li L and Flockhart D A 2009 Rapid identification of the hepatic cytochrome P450 2C19 activity using a novel and noninvasive [^{13}C]-pantoprazole breath test *J. Pharmacol. Exp. Ther.* **329** 297–305

[20] Thacker D L, Modak A, Nguyen P D, Flockhart D A and Desta Z 2011 Stereoselective pharmacokinetics of stable isotope (±)-[^{13}C]-pantoprazole: implications for a rapid screening phenotype test of CYP2C19 activity *Chirality* **23** 904–9

[21] Thacker D L, Modak A, Flockhart D A and Desta Z 2013 Is (+)-[^{13}C]-pantoprazole better than (±)-[^{13}C]-pantoprazole for the breath test to evaluate CYP2C19 enzyme activity? *J. Breath Res.* **7** 016001

[22] Furuta T, Kodaira C, Nishino M, Yamade M, Sugimoto M, Ikuma M, Hishida A, Watanabe H and Umemura K 2009 [^{13}C]-pantoprazole breath test to predict CYP2C19

phenotype and efficacy of a proton pump inhibitor, lansoprazole *Aliment. Pharmacol. Ther.* **30** 294–300

[23] Furuta T, Iwaki T and Umemura K 2010 [^{13}C]-Pantoprazole breath test as a predictor of the anti-platelet function of clopidogrel *Eur. J. Clin. Pharmacol.* **66** 457–63

[24] Tazaki J *et al* 2012 Prediction of clopidogrel low responders by a rapid CYP2C19 activity test *J. Atheroscler. Thromb.* **19** 186–93

[25] Kirchheiner J *et al* 2004 Pharmacogenetics of antidepressants and antipsychotics: the contribution of allelic variations to the phenotype of drug response *Mol. Psychiatr.* **9** 442–73

[26] Gardiner S J and Begg E J 2006 Pharmacogenetics, drug-metabolizing enzymes, and clinical practice *Pharmacol. Rev.* **58** 521–90

[27] Goetz M P *et al* 2007 The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen *Breast Cancer Res. Treat.* **101** 113–21

[28] Leeder J S, Pearce R E, Gaedigk A, Modak A and Rosen D I 2008 Evaluation of a [^{13}C]-dextromethorphan breath test to assess CYP2D6 phenotype *J. Clin. Pharmacol.* **48** 1041–51

[29] Opdam F L, Dezentje V O, den Hartigh J, Modak A S, Vree R, Batman E, Smorenburg C H, Nortier J W, Gelderblom H and Guchelaar H J 2013 The use of the [^{13}]-C-dextromethorphan breath test for phenotyping CYP2D6 in breast cancer patients using tamoxifen: association with CYP2D6 genotype and serum endoxifen levels *Cancer Chemother. Pharmacol.* **71** 593–601

[30] Faber M S, Jetter A and Fuhr U 2005 Assessment of CYP1A2 activity in clinical practice: Why, how, and when? *Basic Clin. Pharmacol. Toxicol.* **97** 125–34

[31] Agúndez J A, Gallardo L, Martínez C, Gervasini G and Benítez J 1998 Modulation of CYP1A2 enzyme activity by indoleamines: inhibition by serotonin and tryptamine *Pharmacogenetics* **8** 251–8

[32] Schneider A *et al* 2007 ^{13}C-methacetin breath test shortened: 2-point-measurements after 15 minutes reliably indicate the presence of liver cirrhosis *J. Clin. Gastroenterol.* **41** 33–7

[33] Dinesen L, Caspary W F, Chapman R W, Dietrich C F, Sarrazin C and Braden B 2008 ^{13}C-methacetin-breath test compared to also noninvasive biochemical blood tests in predicting hepatic fibrosis and cirrhosis in chronic hepatitis C *Dig. Liver Dis.* **40** 743–8

[34] Lalazar G *et al* 2008 A continuous ^{13}C- methacetin breath test for noninvasive assessment of intrahepatic inflammation and fibrosis in patients with chronic HCV infection and normal ALT *J. Viral Hepat.* **15** 716–28

[35] Razlan H, Marzuki N M, Tai M L, Shamsul A S, Ong T Z and Mahadeva S 2011 Diagnostic value of the ^{13}C methacetin breath test in various stages of chronic liver disease *Gastroenterol. Res. Pract.* **2011** 235796

[36] Rayes N, Pilarski T, Stockmann M, Bengmark S, Neuhaus P and Seehofer D 2012 Effect of pre- and probiotics on liver regeneration after resection: a randomised, double-blind pilot study *Benef. Microbes* **3** 237–44

[37] Afolabi P, Wright M, Wootton S A and Jackson A A 2013 Clinical utility of [^{13}]-C-liver-function breath tests for assessment of hepatic function *Dig. Dis. Sci.* **58** 33–41

[38] Kasicka-Jonderko A, Jonderko K, Budniok M, Krusiec-Świdergo B and Bońska-Fajfrowska B 2013 Comparison of two dosage regimens of the substrate for the [^{13}C]-methacetin breath test *Isotopes Environ. Health Stud.* **49** 109–21

[39] Shteyer E, Lalazar G, Hemed N, Pappo O, Granot E, Yerushalmi B and Gross E 2013 Continuous [^{13}C]-methacetin breath test differentiates biliary atresia from other causes of neonatal cholestasis: results of a pilot study *J. Pediatr. Gastroenterol. Nutr.* **56** 60–5

[40] Ishii Y, Suzuki S, Asai S and Murai I 2012 Liver function assessment with three [^{13}C] breath tests by two-point measurements *Isotopes Environ. Health Stud.* **48** 543–57

[41] Kasicka-Jonderko A, Nita A, Jonderko K, Kamińska M and Błońska-Fajfrowska B 2012 [^{13}C]-methacetin breath test reproducibility study reveals persistent CYP1A2 stimulation on repeat examinations *World J. Gastroenterol.* **17** 4979–86

[42] Stibbe K J, Verveer C, Francke J, Hansen B E, Zondervan P E, Kuipers E J, de Knegt R J and van Vuuren A J 2011 Comparison of non-invasive assessment to diagnose liver fibrosis in chronic hepatitis B and C patients *Scand. J. Gastroenterol.* **46** 962–72

[43] Wagner D A, Schatz R, Coston R, Curington C, Bolt D and Toskes P P 2011 A new [^{13}C] breath test to detect vitamin B12 deficiency: a prevalent and poorly diagnosed health *Probl. J. Breath Res.* **5** 046001

[44] Modak A, Durso R, Joseph E and Rosen D 2012 A rapid non invasive L-DOPA-^{13}C breath test for optimally suppressing extracerebral AADC enzyme activity—toward individualizing carbidopa therapy in Parkinson's disease *J. Park. Dis.* **2** 349–56

[45] Raida M, Schwabe W and Häusler P 2001 Prevalence of a common point mutation in the dihydropyrimidine dehydrogenase (*DPD*) gene within the 5′-splice donor site of intron 14 in patients with severe 5-fluorouracil (5-FU)-related toxicity compared with controls *Clin. Cancer Res.* **7** 2832–9

[46] Meta-Analysis Group 1998 Toxicity of fluorouracil in patients with advanced colorectal cancer: effect of administration schedule and prognostic factors *Cancer J. Clin. Oncol.* **16** 3537–41

[47] Johnson M R and Diasio R B 2001 Importance of dihydropyrimidine dehydrogenase (DPD) deficiency in patients exhibiting toxicity following treatment with 5-fluorouracil *Adv. Enzyme Regul.* **41** 151–7

[48] Van Kuilenburg A B, Meinsma R, Zoetekouw L and Van Gennip A H 2002 High prevalence of the IVS14 +1G→A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity *Pharmacogenetics* **12** 555–8

[49] Mattison L, Ezzeldin H, Carpenter M, Johnson M and Diasio R 2004 Rapid identification of dihydropyrimidine dehydrogenase deficiency by using a novel 2–^{13}C-uracil breath test *Clin. Cancer Res.* **10** 2652–9

[50] Mattison L, Fourie J, Renee A, Modak A, Saif M and Diasio R 2006 Racial differences in dihydropyrimidine dehydrogenase activity: increased prevalence of DPD deficiency in African Americans compared to Caucasians *Clin. Cancer Res.* **12** 5491–5

[51] Habel J E *et al* 2010 Structure of Rv1848 (UreA), the Mycobacterium tuberculosis urease gamma subunit *Acta Crystallogr. Sect. F* **66** 781–6

[52] Jassal M S, Nedeltchev G G, Lee J H, Choi S W, Atudorei V, Sharp Z D, Deretic V, Timmins G S and Bishai W R 2010 ^{13}[C]-urea breath test as a novel point-of-care biomarker for tuberculosis treatment and diagnosis *PLoS One* **5** e12451

[53] Dweik R A, Boggs P B, Erzurum S C, Irvin C G, Leigh M W, Lundberg J O, Olin A C, Plummer A L and Taylor D R 2011 On behalf of the American thoracic society committee on interpretation of exhaled nitric oxide levels (FENO) for clinical applications An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications *Am. J. Respir. Crit. Care Med.* **184** 602–15

[54] Olopade C O, Zakkar M, Swedler W I and Rubinstein I 1997 Exhaled pentane levels in acute asthma *Chest* **111** 862–5

[55] Paredi P, Kharitonov S A and Barnes P J 2000 Elevation of exhaled ethane concentration in asthma *Am. J. Respir. Crit. Care Med.* **162** 1450–4
[56] Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov S A and Barnes P J 1999 Increased 8-isoprostane, a marker of oxidative stress, in exhaled condensate of asthma patients *Am. J. Respir. Crit. Care Med.* **160** 216–20
[57] Studer S M *et al* 2001 Patterns and significance of exhaled-breath biomarkers in lung transplant recipients with acute allograft rejection *J. Heart Lung Transplant.* **20** 1158–66
[58] Phillips M, Cataneo R N, Saunders C, Hope P, Schmitt P and Wai J Volatile biomarkers in the breath of women with breast cancer *J. Breath Res.* **4** 026003
[59] Van Berkel J J, Dallinga J W, Möller G M, Godschalk R W, Moonen E J, Wouters E F and Van Schooten F J 2009 A profile of volatile organic compounds in breath discriminates COPD patients from controls *Respir. Med.* **104** 557–63
[60] Phillips C O, Syed Y, MacParthal′ain N, Zwiggelaar R, Claypole T C and Lewis K E 2012 Machine learning methods on exhaled volatile organic compounds for distinguishing COPD patients from healthy controls *J. Breath Res.* **6** 036003
[61] Basanta M, Ibrahim B, Dockry R, Douce D, Morris M, Singh D, Woodcock A and Fowler S J 2012 Exhaled volatile organic compounds for phenotyping chronic obstructive pulmonary disease: a cross-sectional study *Respir. Res.* **13** 72
[62] Altomare D F, Di Lena M, Porcelli F, Trizio L, Travaglio E, Tutino M, Dragonieri S, Memeo V and Gennaro G 2012 Exhaled volatile organic compounds identify patients with colorectal cancer *Br. J. Surg.* **100** 144–50
[63] Robroeks C M *et al* 2010 Metabolomics of volatile organic compounds in cystic fibrosis patients and controls *Pediatr. Res.* **68** 75–80
[64] Barker M, Hengst M, Schmid J, Buers H J, Mittermaier B, Klemp D and Koppmann R 2006 Volatile organic compounds in the exhaled breath of young patients with cystic fibrosis *Eur. Respir. J.* **27** 929–36
[65] Novak B J *et al* 2007 Exhaled methyl nitrate as a noninvasive marker of hyperglycemia in type 1 diabetes *Proc. Natl Acad. Sci. USA* **104** 15613–8
[66] Galasetti P R, Novak B, Nemet D, Rose-Gottron C, Cooper D M, Meinardi S, Newcomb R, Zaldivar F and Blake D R 2005 Breath ethanol and acetone as indicators of serum glucose levels: an initial report *Diabetes Technol. Ther.* **7** 115–23
[67] Lee J, Ngo J, Blake D, Meinardi S, Pontello A M, Newcomb R and Galassetti P R 2009 Improved predictive models for plasma glucose estimation from multi-linear regression analysis of exhaled volatile organic compounds *J. Appl. Physiol.* **107** 155–60
[68] Phillips M, Cataneo R N, Cheema T and Greenberg J 2004 Increased breath biomarkers of oxidative stress in diabetes mellitus *Clin. Chim. Acta* **344** 189–94
[69] Pelli M A, Trovarelli G, Capodicasa E, De Medio G E and Bassotti G 1999 Breath alkanes determination in ulcerative colitis and Crohn's disease *Dis. Colon Rectum* **42** 71–6
[70] Phillips M *et al* 2004 Heart allograft rejection: detection with breath alkanes in low levels (the HARDBALL study) *J. Heart Lung Transplant.* **23** 701–8
[71] Hisamura M 1979 Quantitative analysis of methyl mercaptan and dimethyl sulfide in human expired alveolar gas and its clinical application: study in normal subjects and patients with liver diseases *Nippon Naika Gakkai Zasshi* **68** 1284–92
[72] Kaji H, Hisamura M, Saito N and Murao M 1978 Evaluation of volatile sulfur compounds in the expired alveolar gas in patients with liver cirrhosis *Clin. Chim. Acta* **85** 279–84
[73] Van den Velde S, Nevens F, Van Hee P, van Steenberghe D and Quirynen M 2008 GC-MS analysis of breath odor compounds in liver patients *J. Chromatogr.* B **875** 344–8
[74] Sehnert S S, Jiang L, Burdick J F and Risby T H 2002 Breath biomarkers for detection of human liver diseases: preliminary study *Biomarkers* **7** 174–87
[75] Solga S F, Alkhuraishe A, Cope K, Tabesh A, Clark J M, Torbenson M, Schwartz P, Magnuson T, Diehl A M and Risby T H 2006 Breath biomarkers and non-alcoholic fatty liver disease: preliminary observations *Biomarkers* **11** 174–83
[76] Fuchs P, Loeseken C, Schubert J K and Miekisch W 2010 Breath gas aldehydes as biomarkers of lung cancer *Int. J. Cancer* **126** 2663–70
[77] Sponring A, Filipiak W, Mikoviny T, Ager C, Schubert J, Miekisch W, Amann A and Troppmair J 2009 Release of volatile organic compounds from the lung cancer cell line NCI-H2087 *In Vitro Anticancer Res.* **29** 419–26
[78] Hanai Y, Ken Shimono K, Oka H, Baba Y, Yamazaki K and Beauchamp G K 2012 Analysis of VOCs released from human lung cancer cells and from the urine of tumor bearing mice *Cancer Cell Int.* **12** 13
[79] Horvath I, Lazar Z, Gyulai N, Kollai M and Lozonczy G 2009 *Eur. Respir. J.* **34** 261–75
[80] Humad S, Zarling E, Clapper M and Skosey J L 1988 Breath pentane excretion as a marker of disease activity in rheumatoid arthritis *Free Radic. Res. Commun.* **5** 101–6
[81] Phillips M, Sabas M and Greenberg J 1993 Increased pentane and carbon disulfide in the breath of patients with schizophrenia *J. Clin. Pathol.* **46** 861–4
[82] Puri B K, Ross B M and Treasaden I H 2010 Increased levels of ethane, a non-invasive, quantitative, direct marker of n-3 lipid peroxidation, in the breath of patients with schizophrenia *Ann. Genet. Psychiatry* **9** S150
[83] Phillips M *et al* 2012 Point-of-care breath test for biomarkers of active pulmonary tuberculosis *Tuberculosis* **92** 314–20