Phenotype Breath Tests as Companion Diagnostic Tests (CDx) in Clinical Trials of Drugs

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The emerging field of personalized medicine will require a change in the traditional paradigm of “one drug fits all” in prescribing drugs. The commercial success of new drugs coming to the healthcare field will depend on utilizing differential diagnostic tests and/or companion biomarker assays to identify patients who are responders or non-responders. This major market trend will force pharmaceutical and biotechnology companies to adopt the use of biomarker diagnostic tests in the drug development process.

Companion diagnostic tests (CDx) are assays intended to enable physicians in the selection of right therapy for the patient at the optimal dose for the highest efficacy and minimal toxicity thereby lowering healthcare costs.

Biomarkers for CDx need to be identified in the early stages of drug development and co-developed with the drug to establish their clinical utility. They need to be validated as a diagnostic test in the context of their intended use during phase II and III of clinical trials. The FDA issued guidance to the industry on companion diagnostics in July 2011, including its preference for having the test ready for approval at the same time as the drug.

For pharmaceutical companies, the risk is that CDx could potentially lower sales of their drugs by restricting use to a fraction of potential patients, those who respond well to the drug. However, the flipside is that the FDA can approve the drug for a select group of patients (responders) instead of rejecting the NDA. Developing CDx for drugs that have been withdrawn from the market can also rescue the drugs for a specific population by identifying patients who would have toxicity.

The use of molecular diagnostics for detecting genetic variations, such as mutations or amplifications of specific genes, in order to target therapies to patients who are most likely to benefit, is becoming increasingly common practice in drug development. Breath biomarkers can potentially be used for speeding up drug development by incorporating them as companion diagnostic tools for faster approvals of drugs. Breath biomarkers can also be used to personalize existing FDA-approved drugs, to identify responders/non-responders, to prevent unnecessary ADRs and improve clinical outcomes.

There are two main groups of CDx:

• Tests for existing FDA approved drugs
• Tests that are being co-developed as a companion to a drug in clinical trials

Stable isotope $^{13}$C-labeled compounds have been widely used as diagnostic probes in research laboratories for over 30 years [1]. The metabolism of ingested xenobiotics is clinically significant to optimize therapeutic benefits and minimize risk of toxic side effects. A majority of the drugs approved by the FDA are metabolized by phase I enzymes. Genetic polymorphisms contribute considerably to interindividual variations in the metabolism of numerous drugs and xenobiotics resulting in a significant number of ADRs and variability in drug response [2,3].

Stable isotope-labeled xenobiotics can be used to provide rapid in vivo phenotype assessment of phase I enzymes (CYP P450). The use of suitably labeled stable isotope substrates to induce the generation of biomarker $^{13}$CO$_2$ in breath can lead to diagnostic tests to identify non-responders to medications metabolized primarily by specific enzymes.

CDx for Existing Drugs

Approximately 20-25% of all drugs in clinical use are metabolized, at least in part by CYP2D6 [4]. These drugs include several β-receptor antagonists, antiarrhythmics, antidepressants, antipsychotics, tamoxifen and morphine derivatives [5]. More recently, the consequence of low or no CYP2D6 activity on the formation of the highly estrogenic tamoxifen metabolite, endoxifen, and thus treatment response in breast cancer patients has heightened attention on the potential importance of determining the CYP2D6 phenotype prior to initiation of treatment. The Dextromethorphan-[13C]-breath test (DM-BT) [6] offers promise as a rapid, office-based, and minimally invasive phenotyping assay for CYP2D6 activity and personalizing medication for existing drugs like Tamoxifen [7].

Emerging evidence suggests that interindividual and interethnic differences in CYP2C19 activity influence therapeutic response to drugs, such as proton pump inhibitors (PPI), clopidogrel, fluoxetine, cyclophosphamide and thalidomide. The Pantoprazole-[13C]-breath test (Ptz-BT) [8,9] can be useful diagnostic test to evaluate CYP2C19 enzyme activity and personalize PPI [10] and clopidogrel [11,12] therapy for the individual patient.

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 [13]. 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 hematoxicity toxicity [14], and 40-60% of those cancer patients...
are dihydroprymidine dehydrogenase (DPD) deficient [15,16]. The Uracl-2-\(^{13}\)C breath test (Ura-BT) [17,18] can rapidly evaluate pyrimidine metabolic disorder prior to initiating 5-FU therapy.

The methacetin-\(^{13}\)C breath test (MBT) reflects the hepatic microsomal function of CYP1A2. MBT is also an accurate tool for measuring the degree of inflammation and fibrosis in patients with chronic HCV infection and chronic liver disease [19,20].

**CDx in Clinical Trials for Polymorphic P450 Enzymes**

CYP2D6 enzyme activity is highly variable in the human population and the consequences of phenotypic extremes are generally considered to include an increased risk of concentration dependent side effects at the poor metabolizer (PM) end of the spectrum and the possibility of treatment failure at the ultrarapid metabolizer extreme. The DM-BT can be used as a rapid phenotype CDx in clinical trials of drugs metabolized by CYP2D6. It will help in selection of patients that would respond well to the drug.

The Ptz-BT offers greater practical clinical utility over existing genotypic and phenotype approaches in predicting or assessing CYP2C19 enzyme activity, particularly in effectively distinguishing PMs from IMs and extensive metabolizers of CYP2C19, because it can be noninvasively performed at a single time-point breath collection at 30min. 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.

The MBT has been optimized into a single time point breath collection test for evaluating CYP1A2 enzyme activity.

These rapid, single time-point breath collection, minimally invasive office-based in vivo phenotype assays for CYP1A2, CYP2C19 & CYP2D6 activity could potentially be used as a CDx in clinical trials of new drugs.

The integration of diagnostic breath tests during different phases of drug development or targeting existing drugs can result in safer drugs with enhanced therapeutic efficacy and lowered toxicity in a cost-effective way. The breath tests can potentially help pharmaceutical companies in drug discovery and to design clinical trials in a more efficient and effective way by being able to identify responders and non-responders to the drug. Breath tests can be potentially useful for personalized medicine by early identification of enzyme deficiencies.

**References**

5. http://medicine.iupui.edu/flockhart/