illnesses and may also provide an accurate means to diagnose these conditions. Human brain gene expression studies of alcoholics and cocaine abusers suggest that specific patterns of gene expression may underlie addiction-related phenotypes and provides evidence that a molecular classification of alcoholism may be feasible in the future (Liu *et al*, 2006; Mash *et al*, 2007). However, in order for expression profiling to be useful in the clinical screening of dependence and consumption, the tissues or cells under investigation need to be readily accessible. A wide variety of screening tests are available that relies upon peripheral blood samples for assaying biological markers. For example, blood tests allow early detection, and in some cases prevention, of conditions such as prostate cancer, diabetes, thyroid dysfunction, and heart disease. Blood samples offer advantages over procedures such as tissue biopsy as they are fast, non-invasive, and can be repeated many times on the same individual. Blood biomarkers also offer the potential to predict disease before any detrimental symptoms are manifested. Genomic profiling of peripheral blood samples could be of great value in identifying biomarkers for complex diseases including addiction. This idea is supported by studies utilizing white blood cells to identify discrete patterns of expression associated with modeled complex disease states in animals (Tang *et al*, 2001). In addition, patterns of gene expression have been identified from blood samples obtained from a large number of healthy individuals, revealing surprising consistency in expression of genes associated with age, gender, and blood composition (Tang *et al*, 2001). Thus, it's feasible that nucleated blood cells of alcohol-dependent individuals could show changes in gene expression that will provide a 'signature' of the disease.

Discovery of reliable blood-based molecular markers of alcohol dependence and use would mark a milestone for addiction research and offer a great benefit for predicting the disease even without knowing the role of the markers in the disease process. Once biomarkers are discovered, the opportunity for early detection and intervention as well as personalized therapeutics should lead to new treatments for the disease.

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DISCLOSURE/CONFLICT OF INTEREST
The authors declare that, except for income received from my primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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From rapid *In Vitro* screening to rapid *In Vivo* screening in the drug discovery process

Synthetic combinatorial methods, combined with rapid assays, have fundamentally advanced the ability to synthesize and screen large numbers of compounds. Using a range of combinatorial approaches, libraries composed of tens of thousands to millions of different compounds have been produced. Combinatorial chemistry and high-throughput screening is now a universally utilized tool for drug discovery and development, but the harsh reality is that the drug discovery process remains extremely slow and enormously expensive. Drug candidates resulting from many combinatorial approaches have also often tended not to have drug-like properties and thus have a high inherent rate of attrition in the later stages of drug development because of poor physicochemical properties. Although unrelated to the advances in combinatorial approaches, it is worth noting that increased regulatory issues and unrealistic public expectations have reduced the number of approved drug entities over the past 20 years from approximately 35 per year to 10 or less.

One approach to circumvent this high attrition rate would be to use *in vivo* models directly in the discovery phase to identify candidates with desired biological profiles while simultaneously eliminating those compounds with poor absorption, distribution, metabolism, and elimination (ADME)/pharmacokinetic (PK) properties.

It is clearly unrealistic to use discovery *in vivo* models to screen the large collections of hundreds of thousands of the individual compounds currently available. A potential solution that shows promise is the use of mixture-based combinatorial libraries directly for *in vivo* testing. This offers a unique opportunity to carry out successful preliminary studies in which tens to hundreds of thousands of compounds would be screened directly in translational *in vivo* assays. This has been accomplished in early studies carried out in rats and dogs to monitor blood pressure and heart rate (Houghten, 1994) using 400 separate mixtures each of 132 000 hexapeptides. Immunological modulation by large mixtures has also been accomplished (Shukaliak Quandt *et al*, 2004). Recent studies have involved research into pain therapeutics utilizing *in vivo*

models with a tetrapeptide library made up of a total of 6 250 000 peptides (200 mixtures made up of 125 000 tetrapeptides each) (Dooley *et al*, 1998; Houghten *et al*, 2006, 2008). Mixtures ranging from 2500 to 125 000 tetrapeptides have yielded clear *in vivo* activity that is not necessarily related to classic *in vitro* target-based screening. For mixture-based small molecule libraries the process can be improved by careful selection of those libraries guided by theoretical calculation of their drug-like properties. Over the past 10 years a process termed cassette testing (Liu *et al*, 2008, and references cited therein) has been used to study *in vivo* ADME with small mixture sets (typically 5–10 related compounds) to facilitate the early elimination of compounds with poor drug-like profiling in PK profiling.

The concept of using large, highly diverse mixture-based libraries for the identification of inherently more advanced 'hits' by the direct *in vivo* testing is both exciting and promising. It remains to be seen if these recent early preliminary successes will fulfill their current potential promise.

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Targeting the serotonin 2C receptor for the treatment of obesity and type 2 diabetes

The neurotransmitter serotonin (5-hydroxytryptamine; 5-HT) has a well-established role in energy homeostasis. The clinical potential of beneficially manipulating the 5-HT system is best illustrated by the efficacy of compounds such as fenfluramine and sibutramine, both of which increase 5-HT bioavailability, in the pharmacological treatment of obesity. The success of these compounds in reducing food intake and body weight has stimulated interest in dissecting the mechanisms whereby 5-HT influences energy balance. Early pharmacological studies implicated the 5-HT_{2C} receptor (5-HT_{2C}R; previously classified as the 5-HT_{1C} receptor) in these effects, a suggestion supported by the later observation that genetic inactivation of the 5-HT_{2C}R, but not other 5-HT receptors, produces hyperphagia and obesity in the mouse (Tecott *et al*, 1995). 5-HT_{2C}R knockout mice also display blunted responses to fenfluramine, indicating that action at these receptors is required for the anorectic effect of this compound (Vickers *et al*, 1999). Collectively, these findings motivated attempts to develop selective 5-HT_{2C}R agonists for the treatment of obesity.

Unfortunately, the high degree of sequence homology between the 5-HT_{2C}R and 5-HT_{2B}R has proved a major challenge in efforts to generate a truly specific high affinity 5-HT_{2C}R agonist that does not stimulate 5-HT_{2B}Rs. Activity at the 5-HT_{2B}R is a particular concern because action at these receptors is thought to contribute to the valvular heart disease reported in some patients following fenfluramine-phentermine use (Fitzgerald *et al*, 2000). Nevertheless, recent studies using combined pharmacological and genetic approaches in murine models have recently rekindled pharmaceutical interest in drug discovery programs focusing on generating more selective high affinity 5-HT_{2C}R agonists; for example, it has been shown that fenfluramine influences appetite through the melanocortin system (Heisler *et al*, 2002). This brain pathway, specifically acting through the melanocortin 4 receptor, is critical for the normal regulation of energy balance, and integrates inputs from many other neuropeptides and neurotransmitters.

More recently, a distinct role for the 5-HT_{2C}Rs in glucose homeostasis has also been reported in rodents. Specifically, both a classic 5-HT_{2C}R agonist (with binding affinity for other 5-HT receptors) and a more selective and high affinity 5-HT_{2C}R agonist were demonstrated to reduce elevated insulin levels and improve glucose tolerance and insulin sensitivity in both genetically obese mice and in mice with diet-induced obesity, both with impaired glucose tolerance and insulin resistance (Zhou *et al*, 2007). Importantly, these effects were achieved at concentrations of the compounds which were too low to influence food intake, energy expenditure, locomotor activity, or body weight. These findings indicate that the 5-HT_{2C}R may be a mechanistically novel target for the treatment of type 2 diabetes. This has been corroborated by genetic inactivation of the 5-HT_{2C}R in mice, which, either alone or in combination with leptin deficiency, impairs glucose homeostasis (Wade