For reprint orders, please contact reprints@expert-reviews.com

Genomic and metabolomic advances in the identification of disease and adverse event biomarkers

Incomplete knowledge of tissue pathogenesis is hampering the identification of biomarkers for the appropriate therapeutic targets to prevent or inhibit disease processes, and the prediction and diagnosis of injury due to disease and adverse events of drug therapy. The revolution in genomics and metabolomics, combined with advanced bioinformatics and computational methods for mining such large, complex data sets, are beginning to provide critical insights into tissue injury. Such results will move us closer to the promise of personalized medicine.

KEYWORDS: biomarker ■ genomic ■ metabolomic ■ metabonomic ■ personalized medicine ■ pharmacogenomic

**Donna L Mendrick[†]
& Laura Schnackenberg**

[†]Author for correspondence:

Division of Systems Toxicology,
HFT-230, National Center for
Toxicological Research, US FDA,
3900 NCTR Road, Jefferson,
AR 72079-4502, USA
Tel.: +1 870 543 7718;
Fax: +1 870 543 7686;
donna.mendrick@fda.hhs.gov

The need for new biomarkers was identified as the highest priority in the US FDA Critical Path Report and List issued several years ago [101]. These documents provide specific examples of where improvement is required; for example, biomarkers of disease activity, safety and individual responses to therapies. Some companies have been hesitant to embrace the concept of personalized medicine and, instead, continue to focus on the blockbuster drug business model. However, the 'one-drug-fits-all' paradigm does not exist, as was illustrated by a review of published studies performed by Spear and colleagues in 2001, which demonstrated that efficacies of marketed drugs ranged from 25 to 80% [1]. Although the pharmaceutical industry may be hesitant to divide the patient population, medical advancements will lead to stratification of disease entities as their pathogenesis becomes understood [2]. The failure of recent drugs to attain their projected market share and success of other drugs coupled with biomarker tests, for example, Genentech's Herceptin®, are beginning to alter the pharmaceutical and biotechnology landscape [3]. Use of genomics and metabolomics promises to provide new insight into the pathogenesis of disease, mechanisms of toxicity and an understanding of individual patients' susceptibility to treatment and adverse events, thus bringing us closer to the age of personalized medicine. (Genomics here is being used pursuant to the definition in the International Conference on Harmonization topic E15 [102]. See Box 1 for additional definitions.) FIGURE 1 describes how these 'omic' technologies, in combination with bioinformatics methods, can discover potential biomarkers. Biomarkers are

qualified in the terms of context of use and can then be used in animal models and/or the stratification of patient populations.

Genomics has been employed for over a decade, so why has it failed to make major inroads into patient treatment and selection of safer drugs? Reasons may include a lack of general acceptance by the medical and pharmaceutical fields, early overenthusiasm and a lack of established processes. As noted earlier, some companies have been hesitant to utilize approaches that stratify patients. New scientific endeavors usually generate exaggerated levels of enthusiasm, and some individuals become disheartened when they do not immediately deliver such measurable benefits of success. However, there is hope, as described in a recent review paper that noted modern tools, such as transcriptomics coupled with bioinformatics, will advance the identification of new biomarkers more rapidly than past empiric approaches [4]. Regardless of the way biomarkers are identified, outside of clinical trials, there had previously been no mechanism to qualify them for regulatory use. That changed in 2006 when the FDA proposed a biomarker qualification process [5], and this has been used successfully in 2008 by the FDA and the European Medicines Agency (EMA) to qualify urinary protein biomarkers for use in rats [103]; efforts are underway to qualify these biomarkers for use in humans. Translation of biomarkers from animals to humans and *vice versa* is an important key in moving medicine forward. However, this offers some unique challenges. For example, in preclinical-testing species, biomarkers are usually evaluated in concert with histological changes; yet in the clinical realm, biopsies are

future
medicine part of fsg

Box 1. Definitions of terms used.**Genomics**

- A measurable DNA and/or RNA characteristic that is an indicator of normal biologic processes, pathogenic processes and/or response to therapeutic or other interventions [102].

Metabolomics

- Comprehensive measurement of metabolite levels in biofluids or tissue samples to determine the effects of different stimuli that result in altered tissue morphology.

Biomarkers

- Prodromal/predictive: change early and prior to overt tissue injury.
- Diagnostic: altered at the time of disease onset.
- Prognostic: predict long-term outcome of a patient.

rarely performed. Thus, one may be left with comparing the value of new biomarkers with older ones (e.g., serum creatinine, blood urea nitrogen and alanine aminotransferase) that are known to be lacking. Difficult to attain short-term measurements of success in affecting drug development and disease management, extremely high standards for biomarker acceptance and reimbursement issues have slowed the move to personalized medicine.

One encouraging sign is the establishment of consortia devoted to qualifying biomarkers in the context of their use [6,7]. The Critical Path Institute has multiple consortia efforts [104]. The most well known at the time of this writing may be the Predictive Safety Testing Consortium. This group is comprised of multiple consortia, each involved in an important area of drug development, including nephrotoxicity and hepatotoxicity. It was the nephrotoxicity group that played a pivotal role in the FDA and EMEA approval of renal biomarkers for preclinical use, as described earlier. Another consortium, overseen by the Critical Path Institute, is looking to qualify the EGF receptor as a diagnostic biomarker of cancer as a pilot program to 'standardize the development path and performance requirements of diagnostic tests for cancer' [104]. Additional information on this group and other consortia within the Critical Path Institute can be found at their website. The Health and Environmental Sciences Institute branch of the International Life Sciences Institute has several efforts underway to identify biomarkers of tissue injury, with the Committee on Development and Application of Biomarkers of Toxicity being one prominent example [105]. In May of 2008, they submitted a Voluntary Exploratory Data Submission package to the FDA and EMEA for the evaluation of additional renal biomarkers. These and other consortia are critical to the regulatory qualification of new biomarkers and in lobbying for their use and reimbursement.

The type of biomarkers required can vary from the preclinical testing realm to clinical medicine. For example, in the clinic it would be informative to know if an individual that once responded adversely to a specific drug will adapt to the drug or experience adverse events if rechallenged. Experiments addressing the response of nonclinical species to drug rechallenge are not normally performed during preclinical testing, so biomarkers are not identified. The types of studies and supporting information can be dramatically different if the biomarkers being sought are prodromal and/or predictive (i.e., change early and prior to overt tissue injury), diagnostic (i.e., are altered at the time of disease onset and/or tissue injury) or prognostic (i.e., predict the long-term outcome of a patient). The most difficult type of biomarker to qualify is probably one that is a surrogate for a clinical end point [6,8]. As discussed later, examples of biomarkers are beginning to emerge, although most have not been officially qualified for regulatory use at the time of writing this article.

Genomic biomarkers**■ Usefulness in preclinical & clinical arenas**

FIGURE 2 reflects the concept behind the use of a genomics approach to identify biomarkers altered by external factors, such as response to a drug or dietary change. The use of a genomics approach to identify biomarkers related to toxicity responses in preclinical species, usually referred to as toxicogenomics, has been the subject of study for over a decade. Most investigators have employed this technology to identify the mechanism behind a particular drug's toxicity, usually with the goal of generating a screening assay to avoid such toxicity in subsequent pipeline compounds. Some have used this approach to determine whether the toxicity observed in rats with certain compounds appears to be a good harbinger of adverse events in humans. By contrast, investigators have explored the use of a toxicogenomics approach, applied in rats or primary rat hepatocytes, to identify drugs that cause idiosyncratic effects in humans [9–12]. These approaches have employed mechanistic analyses of unique genes, as well as computational modeling approaches.

The use of genomics-based biomarkers in tissue samples are appropriate for *in vivo* studies in animals and in human cancer, for example colorectal carcinoma [13], as such material is normally available for study. However, in many human disease situations and adverse events, there is no removal of tissue for therapeutic or

diagnostic purposes and, thus, there is a need to find biomarkers in body fluids. One approach is to use the large discovery net that a genomics approach offers and, from these data, identify expression changes in genes that encode secreted and cell-surface proteins. This genomics method can be used to examine selected genes in multiple tissue types, in order to determine the likely proteins with sufficient tissue specificity [14]. Another approach is the use of gene-expression perturbations in circulating white blood cells. One investigation reported that 80% of genes in the human genome are expressed by human peripheral blood cells and approximately the same percentage was shared with nine different tissue types studied [15]. Transcriptional profiling of human blood cells has been reported to identify biomarkers of [16–26]:

- Neurological conditions (e.g., stroke, post-traumatic stress disorder and multiple sclerosis);
- Inflammatory diseases of the intestine;
- Cardiac transplant rejection;
- Individuals exposed to high levels of acetaminophen.

Studies in rats have illustrated that analysis of blood gene-expression changes can discriminate subtoxic and toxic exposure levels of acetaminophen in rats and humans, and that this approach is superior to classical measurements including clinical chemistry and histopathology [21]. A recent study reported on the use of circulating miRNA analysis to detect acetaminophen-induced hepatotoxicity in mice [27]. Another study explored the use of cell-free mRNA in the peripheral blood as potential biomarkers. These investigators report that elevated levels of liver-specific mRNAs were discovered in rat plasma following treatment with either acetaminophen or galactosamine, both hepatotoxicants [28]. Thus, a genomics approach can be used to find genomic biomarkers, or begin to identify potentially valuable protein-based biomarkers, in body fluids.

■ Examples of biomarkers

There are many examples of genes(s) identified as potential biomarkers of disease and injury, although few have been fully qualified for regulatory use. For a list of validated genomic biomarkers present on drug labels, the reader is directed to a FDA website [106]. For the purposes of this article, several validated biomarkers will be discussed, as well as those still in the experimental phase.

Breast cancer

Herceptin is used to treat patients with breast cancer who have elevated levels of the HER2 receptor. The presence of this receptor is examined on sections of breast tissue by measuring either the amount of mRNA or protein. Unfortunately, in most instances, the measurement of one gene and/or protein does not have sufficient precision. Thus, the analysis of multiple genes is required to provide the required accuracy. Such multivariate assays have been produced that utilize algorithmic models to produce a score instead of a change in one

Figure 1. Systems biology approach to biomarker discovery. Data collected using the genomics and metabolomics platforms is mined using bioinformatics/computational methods to create a list of biomarkers that, once qualified, can be used in future animal studies and/or to stratify a patient population.

Figure 2. Environmental factors can alter phenotypic status. For both genomics and metabolomics approaches, body fluids and/or tissue samples are collected. For monitoring gene-expression alterations, samples are collected before and after a challenge (e.g., drug exposure) or from normal and diseased patients. Metabolomics can be applied to tissue and body fluids, with the latter being particularly amenable to sequential study enabling a real-time measurement. Comparison of the data provides potential biomarkers specific to the challenge, as well as the ability to integrate the data into networks that describes the changes on a molecular level.

analyte. One such example is MammaPrint®, a prognostic test that examines the expression of 70 specific genes to provide insight into the breast tumor's potential to metastasize. Both the test for HER2 and MammaPrint evaluate the tissue itself, which is appropriate when tissue is routinely removed from the patient. However, in most cases, tissues are not easily obtained and, thus, there is a focus on finding biomarkers in body fluids.

Heart disease

AlloMap®, an FDA-approved multivariate assay, monitors the expression of 11 test genes on the peripheral blood mononuclear cells of patients who have received a heart transplant. The test is used to identify patients at low risk of acute transplant rejection [22,23,29].

Gastrointestinal disease

In the area of inflammatory bowel disease, accurate diagnosis remains a clinical challenge. A recent study employed a molecular modeling

approach to identify genes whose expression levels can classify various types of inflammatory bowel disease. Using statistical modeling approaches, investigators identified genes in circulating blood cells that distinguish Crohn's disease from ulcerative colitis [20], while others have identified biomarkers within intestinal samples that differentiate irritable bowel syndrome, Crohn's disease and ulcerative colitis [30]. The former approach, of course, would provide a less invasive assay if these biomarkers can be qualified for use.

Neurological diseases

New blood biomarkers to identify diseases of the brain, where access to tissue is particularly problematic, would be a major medical advancement. Examination of gene-expression alterations in circulating blood cells has found potential biomarkers for diseases, including post-traumatic stress disorder, stroke and migraine [18,19,24,25]. A recent investigation describes the identification of genes in

peripheral blood cells that can distinguish strokes due to cardioembolism and atherosclerosis. Since the treatments differ for each disease, the correct identification of the underlying event is critical.

Liver

Effort is being made in the area of improving preclinical assessment of toxicity. As previously discussed, some investigators are identifying panels of genes in the rat liver or primary rat hepatocytes that may be useful in studying new drug compounds [9–12]. For use in screening compounds, such biomarkers do not have to be subjected to regulatory qualification so their accuracy remains somewhat unclear. However, the Predictive Safety Testing Consortium's Carcinogenicity Working Group is pursuing a set of genomic biomarkers to assist in the identification of drugs that cause nongenotoxic carcinogenicity and have published some early findings [31].

Kidney

As briefly described earlier, a set of urinary protein biomarkers have been qualified for use in rats. One of these proteins, Kim-1 was discovered through a genomics effort and illustrates the power of using this approach [32].

However, most of the biomarkers described here have not been fully qualified for use at the time of this writing. Only when they undergo the rigorous process, will the extent of their accuracy and usefulness become known.

■ Challenges

Technical

There used to be a concern regarding inter-laboratory assessment of gene expression, particularly when different microarray platforms were used. The Microarray Quality-Control Consortium (MAQC), a large group comprised of individuals from the government, pharmaceutical field and other groups, studied such variability and found that biological meaning can be deduced between laboratories and platforms [33–36]. Differences in analysis methods used to identify biomarkers, experimental design and subsequent statistical validation approaches have resulted in differing lists of putative biomarkers, and this has led to criticism of its applicability. The second phase of MAQC is studying how computational modeling approaches and statistical validation of biomarkers can affect the resultant gene selection. Such analysis requires individuals

experienced with biological modeling and statistical assessment. It is imperative that such individuals be involved from the beginning of the process, as their advice can influence the study design and success of the project.

Metabolomics biomarkers

■ Usefulness in preclinical & clinical arenas

Metabolomics [37] or metabonomics [38] involves the comprehensive measurement of metabolite levels in whole organisms at multiple time points to determine the effects of different stimuli that might include, but are not limited, to toxic insults, changes in diet or altered health status due to disease progression, including tumor growth. The observed metabolic profile provides phenotypic information that is determined by the genotype but is also influenced by other environmental factors, including diet, exercise, health-to-disease status, gut microflora content and age (FIGURE 2). Some of these factors have been shown to alter responses to FDA-regulated products. For instance, grapefruit juice has been shown to interfere with a number of drugs that are metabolized by cytochrome P450 enzymes. A metabolomics study generally uses relatively noninvasive biofluids, such as urine and plasma, but is also amenable to a wide range of fluids [39] and tissue biopsy samples [40,41]. Furthermore, little sample preparation is required, which can lead to rapid disease diagnosis and allow for closer monitoring of drug therapy in a clinical setting once the appropriate biomarkers have been defined.

To date, metabolomics studies have been focused upon the preclinical arena, especially with regard to toxicity, safety and efficacy of a particular compound [42–53]. In the preclinical setting, factors other than genetics that influence the phenotype can largely be controlled to minimize interanimal variation, unlike in a clinical setting, where there will be a great deal of heterogeneity among individuals. There was an effort put forth by Jeremy Nicholson of Imperial College (London, UK), who, along with five major pharmaceutical companies, put together the Consortium for Metabonomic Toxicology to generate a metabonomic database based upon proton (^1H) nuclear magnetic resonance (NMR) spectra that were used to develop an expert system for the prediction of target-organ toxicity in preclinical drug development [54–56]. Metabolomics studies have also been used in a preclinical setting to study animal

models of disease, such as Alzheimer's disease [57], lung disease [58], diabetes [59], hepatic steatosis [60] and Huntington's disease [61,62]. In both preclinical drug toxicity and disease studies, metabolomics has the potential to identify novel biomarkers that can then be translated into a clinical setting.

One of the earliest applications of metabolomics in a clinical setting was in the diagnosis of inborn errors of metabolism in children at birth [63]. However, recently, there have also been numerous examples of the use of metabolomics in other clinical applications. Urine samples have been analyzed using metabolomics methods to detect renal cell carcinoma and discover biomarkers for it [64]. Metabolic perturbations, as a result of human colorectal cancer, have also been investigated [40]. Spratlin *et al.* recently reviewed the clinical application of metabolomics analyses in the field of oncology [65]. In addition to the use of metabolomics studies for cancer research, clinical applications include monitoring patients after renal transplant [66], predicting exercise-induced ischemia in individuals with suspected heart disease [67], the diagnosis of meningitis and ventriculitis [68], detecting coronary heart disease [69] and investigating subarachnoid hemorrhage [70]. Metabolomics, as a technology, is still in its infancy and the potential applications are numerous. Metabolic flux analysis will also aid in the understanding of mechanisms of disease through the evaluation of ^{13}C -labeled substrates through specific metabolic pathways [71]. Metabolomics promises to have a great impact in both the preclinical and clinical arenas as noted from the breadth of research already being carried out in many diverse fields.

■ Examples of biomarkers

Metabolomics is a technique that has the potential to discover useful new biomarkers that are more predictive and selective in cases where biomarkers, such as serum creatinine or blood urea nitrogen, already exist. Furthermore, metabolomics technologies also show promise in the identification of new biomarkers where there are no current standard measures. Most metabolomics studies are currently focused on the discovery of biomarkers, and little work has been carried out in order to qualify and validate these biomarkers. In their discussion of metabolomic biomarker discovery, Koulman and coworkers highlight some recent examples in a range of projects that included animal and human studies [72].

Liver

Using a liquid chromatography/mass spectrometry (LC/MS)-based approach, some investigators have identified biomarkers in serum of deteriorating liver function associated with chronic hepatitis B [73]. The study identified five potential biomarkers, of which four were lysophosphatidyl cholines. The other potential marker was identified as glycochenodeoxycholic acid, which has been previously linked to liver function [74]. A LC/MS-based metabolomics approach was also employed in serum to investigate biomarkers of intestinal fistulas. In total, nine potential biomarkers were identified. The altered metabolites indicated hepatic injury and abnormal metabolism of two key amino acids [75]. An example of a study carried out in preclinical testing species identified ophthalmic acid as a potential biomarker of hepatotoxicity. Mouse serum and liver extracts were examined using capillary electrophoresis/MS following acetaminophen administration. Serum ophthalmic acid, an oxidative stress biomarker, was found to be a sensitive indicator of hepatic glutathione depletion in rodents [76].

Kidney

A study was initiated to identify biomarkers of acute kidney injury in children who had undergone cardiac surgery with cardiopulmonary bypass. Urine samples collected prior to and after surgery were analyzed using an LC/MS-based method. Homovanillic acid sulfate was identified as a potential novel, sensitive and predictive biomarker of acute kidney injury that was elevated at 12 h after surgery, prior to the rise in serum creatinine (48–72 h after surgery) [77]. Wishart reviewed the use of metabolomics-based analyses to monitor kidney transplants [66,78]. Previous studies have described the utility of metabolomics-based approaches to assess immunosuppressive drug toxicity, as well as in the evaluation of organ function and to localize organ damage. It was concluded that there are a number of easily measured metabolites in serum and urine that can be used to assess kidney function, kidney injury and immunosuppressive drug toxicity.

Prostate cancer

In a recent report in the journal *Nature*, Sreekumar and coworkers identified sarcosine as a potential biomarker in prostate cancer [41]. Sarcosine was increased in individuals with localized tumors versus the benign group and further increased in the metastatic versus localized group. In separate experiments, enzymes that convert

glycine to sarcosine and *vice versa* were knocked down and demonstrated opposing effects on the invasiveness of the cancer cells. Furthermore, sarcosine was also shown to impart an invasive phenotype when added to benign prostate epithelial cells. This study indicates that sarcosine may be a key biomarker of prostate cancer progression.

General toxicity

Hypercreatinuria has been identified as a potential general marker of toxicity [50,79–81]. It has been related to hepatotoxin-induced liver damage, specifically by compounds that induce hepatocellular necrosis [79,80]. Toxicants that caused fatty liver and cholestatic damage did not demonstrate increases in creatine following dosing. The increase in creatine may be directly related to a decrease in glutathione as it is utilized to detoxify reactive metabolites or reactive oxygen species.

There have been numerous other examples of metabolomics studies to identify biomarkers for a broad spectrum of conditions or diseases, including breast, ovarian, pancreatic and cervical cancers [82,83], asthma [84], Type 2 diabetes mellitus [85] and arthritis [86]. As is the case with many genomics biomarkers, there has been little work done to date to qualify proposed metabolites in the process described by Goodsaïd and coworkers [5,87]. In order to advance the field of metabolomics, more research is required to qualify proposed biomarkers in order for them to gain universal acceptance.

■ Technology challenges

Initially, the most commonly used platform for metabolic profiling studies of drug toxicity and disease has been ^1H NMR. However, with advances in analytical technologies, there has been a growing use of LC/MS and, to some degree, gas chromatography/MS for metabolomics analyses. Additional platforms that have been utilized for metabolomics studies include chromatographic methods (gas chromatography, HPLC and capillary electrophoresis), Fourier transform infrared spectroscopy, Raman spectroscopy and electrochemical array detection. However, NMR and MS methods have the capacity to provide detailed metabolite identification and have been the most commonly applied to metabolomics studies. Each platform has advantages and disadvantages, two articles in particular discuss this in detail [88,89]. While there will be some overlap in metabolites across platforms, there will also be many differences in the biomarkers measured. Therefore, there is no single platform that fits all metabolomics

studies. Rather, a combination of techniques is necessary to generate the information to completely understand the perturbations in a system due to a toxic insult or altered health status.

Regardless of the platform used, one of the major challenges lies in the identification of the peaks of interest. Early metabolomics studies employed chemometrics approaches to identify spectral features that distinguished the different classes being analyzed [90]. In these cases, the actual metabolites were not identified or quantified. However, in order to advance metabolomic biomarkers to a broader audience, it is imperative that the metabolite be identified and validated. Many studies now take a quantitative metabolomics or targeted profiling approach, in which an attempt is made to identify and quantify as many metabolites in the spectra as possible [91]. Identification is complicated by the fact that there are few public databases available [66]. It has been reported that an NMR spectrum may contain from 2000 to 3000 peaks, of which 10% are known and an MS spectrum may contain up to 15,000 peaks, of which 5% are known [92]. While there is little variation in intra-laboratory NMR spectra, there are a number of factors that will affect the intra-laboratory mass spectra, such as column, flow rate, voltage and gradient conditions, making it difficult to generate a single database of metabolites for MS. The generation of appropriate databases is a major challenge for metabolomics studies. Another technology challenge arises owing to the wealth of data produced, especially in MS analyses. The breadth of data require a strong bioinformatics component in order to distill the data into knowledge that can be applied in both preclinical and clinical arenas. The lack of standardization of metabolomics experiments also presents a technology challenge. The goal of standardization is to optimize the amount and quality of the biological information generated in metabolomics experiments by defining the use of chemometrics and quantitative approaches, defining the best practice procedures and defining the minimum reporting requirements, such as that for microarray analysis [93]. While the Metabolomics Standards Initiative was formed in 2005 and, in 2007, this group published reporting standards for the Metabolomics Society and publication in the journal *Metabolomics* [94], there still remains no set of standards for metabolomics analyses that have been accepted by other journals and the medical community at large [95]. In order to advance the field, these technology challenges will need to be addressed and uniform practices adopted.

Issues hindering biomarker use

■ Biomarker adoption

As noted in the report from the Health and Human Services Secretary's Advisory Committee on Genetics, Health and Society, some pharmaceutical companies have been hesitant to embrace the value of biomarkers as it is contrary to their blockbuster business model in that the target population will be smaller and they fear loss of revenue. However, this may not always be true. For example, a marketed drug may find an expanded targeted population if used with specific biomarkers [107]. When real-life examples are available, this may encourage companies to utilize this approach.

■ Reimbursement

Another problem linked to adoption is the cost of biomarker development, whether insurance companies and the Centers for Medicare and Medicaid Services (CMS) will pay for it and the level of reimbursement. Herceptin is an expensive drug and the use of the assay for the *HER2* gene enables stratification of patients who will benefit from the drug, thus enabling its adoption. In another situation, researchers directly studied the cost–benefit ratio of AlloMap, and found that it is less expensive and poses much less risk to the patient than the endomyocardial biopsy currently used as the gold standard [96]. However, in a recent setback to personalized medicine, CMS announced that it will not pay for a genetic test used to guide warfarin dosing as it has not been shown to improve ‘health outcomes in Medicare beneficiaries’. Since private insurance companies tend to follow the guidance of CMS, this has a ripple effect in the industry [107]. Tests that are developed as part of a drug–kit combination and demonstrate usefulness during clinical trials make the costs of using the assay more likely to be reimbursed. However, outside of that paradigm, new biomarkers face difficult hurdles in proving their usefulness to the satisfaction of

third-party payers. Insurance companies and CMS tend to have more rigorous standards than the evidence required for FDA approval of biomarker assays [97]. Even if a test is covered, unfortunately, the reimbursement levels are not optimal and may not be linked to true value, thus leading to a lower adoption rate for the test. Such issues have been brought to the attention of the Secretary of Health and Human Services by the Secretary's Advisory Committee on Genetics, Health and Society [107].

Future perspective

The discovery of new biomarkers will be made at a pace previously unseen as the discovery platforms become less expensive and more accurate. Next-generation sequencing has clear advantages over some aspects of microarray-based transcriptomics, and may overtake the use of such platforms, particularly for certain applications [98]. Proteomics has demonstrated great promise but is relatively low throughput when used as a global discovery tool. Both metabolomics and proteomics are in need of quality standards to assess inter-laboratory accuracy and efforts are underway to examine these issues.

As previously noted, the progress of biomarker discovery and qualification will be stymied if appropriate reimbursement of successful assays is not forthcoming. Since knowledge of this issue has reached the highest levels of the US government, hopefully, action will be taken.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Biomarkers of tissue injury can serve useful purposes in the identification of new drug targets for disease prevention and/or progression.
- Such biomarkers can also serve as indicators for disease management and assessment of adverse drug effects.
- To identify biomarkers that will survive qualification, a multidisciplinary team must be convened so that the appropriate study design is developed and correct steps used to statistically evaluate the accuracy of selected biomarkers.
- Tissue biomarkers may be appropriate in certain circumstances (e.g., cancer diagnosis in humans and preclinical screening of compounds), while body fluid-based biomarkers may be more applicable in other situations (e.g., clinical differentiation of inflammatory bowel disease).
- Consortia will play a major role in qualifying biomarkers.
- Public and private payers must identify appropriate means to reimburse successful assays to ensure innovation and the promise of personalized medicine.

Bibliography

Papers of special note have been highlighted as:

■ of interest

■ of considerable interest

- 1 Spear BB, Heath-Chiozzi M, Huff J: Clinical application of pharmacogenetics. *Trends Mol. Med.* 7, 201–204 (2001).
- **Ground-breaking article exploring the efficacy rate of marketed compounds.**
- 2 Woodcock J: The prospects for “personalized medicine” in drug development and drug therapy. *Clin. Pharmacol. Ther.* 81, 164–169 (2007).
- 3 US Department of Health and Human Services. *Personalized Health Care: Pioneers, Partnerships, Progress*. US Department of Health and Human Services, DC, USA (2008).
- 4 Laterza OF, Modur VR, Ladenson JH: Biomarkers of tissue injury. *Biomarkers Med.* 2, 81–92 (2008).
- 5 Goodsaid FM, Frueh FW, Mattes W: Strategic paths for biomarker qualification. *Toxicology* 245, 219–223 (2008).
- 6 Mattes WB, Walker EG: Translational toxicology and the work of the predictive safety testing consortium. *Clin. Pharmacol. Ther.* 85, 327–330 (2009).
- 7 Mattes WB: Public consortium efforts in toxicogenomics. In: *Essential Concepts in Toxicogenomics*. Mendrick DL, Mattes WB (Eds). Humana, NJ, USA, 221–238 (2008).
- 8 Biomarkers Working Group: Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin. Pharmacol. Ther.* 69, 89–95 (2001).
- 9 Leone AM, Kao LM, McMillian MK *et al.*: Evaluation of felbamate and other antiepileptic drug toxicity potential based on hepatic protein covalent binding and gene expression. *Chem. Res. Toxicol.* 20, 600–608 (2007).
- 10 Hultin-Rosenberg L, Jagannathan S, Nilsson KC *et al.*: Predictive models of hepatotoxicity using gene expression data from primary rat hepatocytes. *Xenobiotica* 36, 1122–1139 (2006).
- 11 Buck WR, Waring JF, Blomme EA: Use of traditional end points and gene dysregulation to understand mechanisms of toxicity: toxicogenomics in mechanistic toxicology. In: *Essential Concepts in Toxicogenomics*. Mendrick DL, Mattes WB (Eds). Humana, NJ, USA, 23–44 (2008).
- 12 Martin R, Rose D, Yu K, Barros S: Toxicogenomics strategies for predicting drug toxicity. *Pharmacogenomics* 7, 1003–1016 (2006).
- 13 Hunt JL: Molecular pathology in anatomic pathology practice: a review of basic principles. *Arch. Pathol. Lab. Med.* 132, 248–260 (2008).
- 14 Mendrick DL, Daniels KK: From the bench to the clinic and back again: translational biomarker discovery using *in silico* mining of pharmacogenomic data. *Biomarkers Med.* 1, 319–333 (2007).
- 15 Liew CC, Ma J, Tang HC, Zheng R, Dempsey AA: The peripheral blood transcriptome dynamically reflects system wide biology: a potential diagnostic tool. *J. Lab. Clin. Med.* 147, 126–132 (2006).
- 16 Burczynski ME, Dorner AJ: Transcriptional profiling of peripheral blood cells in clinical pharmacogenomic studies. *Pharmacogenomics* 7, 187–202 (2006).
- **Excellent article on the use of transcriptomics to identify biomarkers in circulating blood cells.**
- 17 Sharp FR, Xu H, Lit L *et al.*: Genomic profiles of stroke in blood. *Stroke* 38, 691–693 (2007).
- 18 Sharp FR, Xu H, Lit L *et al.*: The future of genomic profiling of neurological diseases using blood. *Arch. Neurol.* 63, 1529–1536 (2006).
- 19 Segman RH, Shefi N, Goltser-Dubner T, Friedman N, Kaminski N, Shalev AY: Peripheral blood mononuclear cell gene expression profiles identify emergent post-traumatic stress disorder among trauma survivors. *Mol. Psychiatry* 10, 500–513 (2005).
- 20 Burczynski ME, Peterson RL, Twine NC *et al.*: Molecular classification of Crohn's disease and ulcerative colitis patients using transcriptional profiles in peripheral blood mononuclear cells. *J. Mol. Diagn.* 8, 51–61 (2006).
- 21 Bushel PR, Heinloth AN, Li J *et al.*: Blood gene expression signatures predict exposure levels. *Proc. Natl Acad. Sci. USA* 104, 18211–18216 (2007).
- **Interesting article demonstrating the potential of translational, genomic biomarkers.**
- 22 Deng MC, Eisen HJ, Mehra MR *et al.*: Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. *Am. J. Transplant.* 6, 150–160 (2006).
- 23 Fang KC: Clinical utilities of peripheral blood gene expression profiling in the management of cardiac transplant patients. *J. Immunotoxicol.* 4, 209–217 (2007).
- 24 Tang Y, Xu H, Du X *et al.*: Gene expression in blood changes rapidly in neutrophils and monocytes after ischemic stroke in humans: a microarray study. *J. Cereb. Blood Flow Metab.* 26, 1089–1102 (2006).
- 25 Moore DF, Li H, Jeffries N *et al.*: Using peripheral blood mononuclear cells to determine a gene expression profile of acute ischemic stroke: a pilot investigation. *Circulation* 111, 212–221 (2005).
- 26 Xu H, Tang Y, Liu DZ *et al.*: Gene expression in peripheral blood differs after cardioembolic compared with large-vessel atherosclerotic stroke: biomarkers for the etiology of ischemic stroke. *J. Cereb. Blood Flow Metab.* 28, 1320–1328 (2008).
- 27 Wang K, Zhang S, Marzolf B *et al.*: Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc. Natl Acad. Sci. USA* 106, 4402–4407 (2009).
- 28 Miyamoto M, Yanai M, Ookubo S, Awasaki N, Takami K, Imai R: Detection of cell-free, liver-specific mRNAs in peripheral blood from rats with hepatotoxicity: a potential toxicological biomarker for safety evaluation. *Toxicol. Sci.* 106, 538–545 (2008).
- 29 Heidecker B, Hare JM: The use of transcriptomic biomarkers for personalized medicine. *Heart Fail. Rev.* 12, 1–11 (2007).
- 30 von Stein P, Lofberg R, Kuznetsov NV *et al.*: Multigene analysis can discriminate between ulcerative colitis, Crohn's disease, and irritable bowel syndrome. *Gastroenterology* 134, 1869–1881 (2008).
- 31 Fielden MR, Nie A, McMillian M *et al.*: Interlaboratory evaluation of genomic signatures for predicting carcinogenicity in the rat. *Toxicol. Sci.* 103, 28–34 (2008).
- 32 Ichimura T, Bonventre JV, Bailly V *et al.*: Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. *J. Biol. Chem.* 273, 4135–4142 (1998).
- 33 Shi L, Reid LH, Jones WD *et al.*: The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. *Nat. Biotechnol.* 24, 1151–1161 (2006).
- 34 Guo L, Lobenhofer EK, Wang C *et al.*: Rat toxicogenomic study reveals analytical consistency across microarray platforms. *Nat. Biotechnol.* 24, 1162–1169 (2006).
- 35 Patterson TA, Lobenhofer EK, Fulmer-Smentek SB *et al.*: Performance comparison of one-color and two-color platforms within the MicroArray Quality Control (MAQC) project. *Nat. Biotechnol.* 24, 1140–1150 (2006).

- 36 Canales RD, Luo Y, Willey JC *et al.*: Evaluation of DNA microarray results with quantitative gene expression platforms. *Nat. Biotechnol.* 24, 1115–1122 (2006).
- 37 Fiehn O: Metabolomics: the link between genotypes and phenotypes. *Plant Mol. Biol.* 48, 155–171 (2002).
- 38 Nicholson JK, Lindon JC, Holmes E: 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data. *Xenobiotica* 29, 1181–1189 (1999).
- 39 Lindon JC, Nicholson JK, Everett JR: NMR spectroscopy of biofluids. In: *Annual Review of NMR Spectroscopy*. Webb GA (Ed.). Academic Press, SD, USA, 2–88 (1999).
- 40 Chan EC, Koh PK, Mal M *et al.*: Metabolic profiling of human colorectal cancer using high-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy and gas chromatography mass spectrometry (GC/MS). *J. Proteome Res.* 8, 352–361 (2009).
- 41 Sreekumar A, Poisson LM, Rajendiran TM *et al.*: Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. *Nature* 457, 910–914 (2009).
- 42 Craig A, Sidaway J, Holmes E *et al.*: Systems toxicology: integrated genomic, proteomic and metabolomic analysis of methapyrilene induced hepatotoxicity in the rat. *J. Proteome Res.* 5, 1586–1601 (2006).
- 43 Coen M, Hong YS, Clayton TA *et al.*: The mechanism of galactosamine toxicity revisited; a metabolomic study. *J. Proteome Res.* 6, 2711–2719 (2007).
- 44 Robertson DG, Datta K, Wells D *et al.*: Metabonomic evaluation of metabolic dysregulation in rats induced by PF 376304, a novel inhibitor of phosphoinositide 3-kinase. *Chem. Res. Toxicol.* 20, 1871–1877 (2007).
- 45 Sun J, Schnackenberg LK, Holland RD *et al.*: Metabonomics evaluation of urine from rats given acute and chronic doses of acetaminophen using NMR and UPLC/MS. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 871, 328–340 (2008).
- 46 Chan W, Cai Z: Aristolochic acid induced changes in the metabolic profile of rat urine. *J. Pharm. Biomed. Anal.* 46, 757–762 (2008).
- 47 Espandiari P, Zhang J, Schnackenberg LK *et al.*: Age-related differences in susceptibility to toxic effects of valproic acid in rats. *J. Appl. Toxicol.* 28, 628–637 (2008).
- 48 Wei L, Liao P, Wu H *et al.*: Toxicological effects of cinnabar in rats by NMR-based metabolic profiling of urine and serum. *Toxicol. Appl. Pharmacol.* 227, 417–429 (2008).
- 49 Park JC, Hong YS, Kim YJ *et al.*: A metabolomic study on the biochemical effects of doxorubicin in rats using ¹H-NMR spectroscopy. *J. Toxicol. Environ. Health Pt A* 72, 374–384 (2009).
- 50 Schnackenberg LK, Chen M, Sun J *et al.*: Evaluations of the trans-sulfuration pathway in multiple liver toxicity studies. *Toxicol. Appl. Pharmacol.* 235, 25–32 (2009).
- 51 Wei L, Liao P, Wu H *et al.*: Metabolic profiling studies on the toxicological effects of realgar in rats by ¹H NMR spectroscopy. *Toxicol. Appl. Pharmacol.* 234, 314–325 (2009).
- 52 Boudonck KJ, Mitchell MW, Nemet L *et al.*: Discovery of metabolomics biomarkers for early detection of nephrotoxicity. *Toxicol. Pathol.* 37, 280–292 (2009).
- 53 Portilla D, Li S, Nagothu KK *et al.*: Metabolomic study of cisplatin-induced nephrotoxicity. *Kidney Int.* 69, 2194–2204 (2006).
- 54 Lindon JC, Nicholson JK, Holmes E *et al.*: Contemporary issues in toxicology and its evaluation by the COMET project. *Toxicol. Appl. Pharmacol.* 187, 137–146 (2003).
- 55 Lindon JC, Keun HC, Ebbels TM, Pearce JM, Holmes E, Nicholson JK: The Consortium for Metabonomic Toxicology (COMET): aims, activities and achievements. *Pharmacogenomics* 6, 691–699 (2005).
- 56 Ebbels TM, Keun HC, Beckonert OP *et al.*: Prediction and classification of drug toxicity using probabilistic modeling of temporal metabolic data: the consortium on metabolomic toxicology screening approach. *J. Proteome Res.* 6, 4407–4422 (2007).
- 57 Barba I, Fernandez-Montesinos R, Garcia-Dorado D, Pozo D: Alzheimer's disease beyond the genomic era: nuclear magnetic resonance (NMR) spectroscopy-based metabolomics. *J. Cell Mol. Med.* 12, 1477–1485 (2008).
- 58 Serkova NJ, Van RZ, Tobias M, Pitzer JE, Wilkinson JE, Stringer KA: Utility of magnetic resonance imaging and nuclear magnetic resonance-based metabolomics for quantification of inflammatory lung injury. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 295, L152–L161 (2008).
- 59 Altmaier E, Ramsay SL, Graber A, Mewes HW, Weinberger KM, Suhre K: Bioinformatics analysis of targeted metabolomics – uncovering old and new tales of diabetic mice under medication. *Endocrinology* 149, 3478–3489 (2008).
- 60 van Ginneken V, Verhey E, Poelmann R *et al.*: Metabolomics (liver and blood profiling) in a mouse model in response to fasting: a study of hepatic steatosis. *Biochim. Biophys. Acta* 1771, 1263–1270 (2007).
- 61 Holmes E, Tsang TM, Tabrizi SJ: The application of NMR-based metabolomics in neurological disorders. *NeuroRx* 3, 358–372 (2006).
- 62 Tsang TM, Woodman B, McLoughlin GA *et al.*: Metabolic characterization of the R6/2 transgenic mouse model of Huntington's disease by high-resolution MAS ¹H NMR spectroscopy. *J. Proteome Res.* 5, 483–492 (2006).
- 63 Moolenaar SH, Engelke UF, Wevers RA: Proton nuclear magnetic resonance spectroscopy of body fluids in the field of inborn errors of metabolism. *Ann. Clin. Biochem.* 40, 16–24 (2003).
- 64 Kim K, Aronov P, Zakharkin SO *et al.*: Urine metabolomics analysis for kidney cancer detection and biomarker discovery. *Mol. Cell. Proteomics* 8, 558–570 (2009).
- 65 Spratlin JL, Serkova NJ, Eckhardt SG: Clinical applications of metabolomics in oncology: a review. *Clin. Cancer Res.* 15, 431–440 (2009).
- 66 Wishart DS: Metabolomics: a complementary tool in renal transplantation. *Contrib. Nephrol.* 160, 76–87 (2008).
- 67 Barba I, de LG, Martin E *et al.*: Nuclear magnetic resonance-based metabolomics predicts exercise-induced ischemia in patients with suspected coronary artery disease. *Magn. Reson. Med.* 60, 27–32 (2008).
- 68 Coen M, O'Sullivan M, Bubb WA, Kuchel PW, Sorrell T: Proton nuclear magnetic resonance-based metabolomics for rapid diagnosis of meningitis and ventriculitis. *Clin. Infect. Dis.* 41, 1582–1590 (2005).
- 69 Brindle JT, Antti H, Holmes E *et al.*: Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using ¹H-NMR-based metabolomics. *Nat. Med.* 8, 1439–1444 (2002).
- 70 Dunne VG, Bhattachayya S, Besser M, Rae C, Griffin JL: Metabolites from cerebrospinal fluid in aneurysmal subarachnoid haemorrhage correlate with vasospasm and clinical outcome: a pattern-recognition ¹H NMR study. *NMR Biomed.* 18, 24–33 (2005).
- 71 Maguire G, Lee P, Manheim D, Boros L: SiDMap: a metabolomics approach to assess the effects of drug candidates on the dynamic properties of biochemical pathways. *Expert Opin. Drug Discov.* 1, 351–359 (2006).

- 72 Koulman A, Lane GA, Harrison SJ, Volmer DA: From differentiating metabolites to biomarkers. *Anal. Bioanal. Chem.* 394, 663–670 (2009).
- **Discusses the need to validate metabolic biomarkers and its associated challenges.**
- 73 Yang J, Zhao X, Liu X *et al.*: High performance liquid chromatography-mass spectrometry for metabonomics: potential biomarkers for acute deterioration of liver function in chronic hepatitis B. *J. Proteome Res.* 5, 554–561 (2006).
- 74 Spivey JR, Bronk SF, Gores GJ: Glycochenodeoxycholate-induced lethal hepatocellular injury in rat hepatocytes. Role of ATP depletion and cytosolic free calcium. *J. Clin. Invest.* 92, 17–24 (1993).
- 75 Yin P, Zhao X, Li Q, Wang J, Li J, Xu G: Metabonomics study of intestinal fistulas based on ultraperformance liquid chromatography coupled with Q-TOF mass spectrometry (UPLC/Q-TOF MS). *J. Proteome Res.* 5, 2135–2143 (2006).
- 76 Soga T, Baran R, Suematsu M *et al.*: Differential metabolomics reveals ophthalmic acid as an oxidative stress biomarker indicating hepatic glutathione consumption. *J. Biol. Chem.* 281, 16768–16776 (2006).
- 77 Beger RD, Holland RD, Sun J *et al.*: Metabonomics of acute kidney injury in children after cardiac surgery. *Pediatr. Nephrol.* 23(6), 977–984 (2008).
- 78 Wishart DS: Metabolomics in monitoring kidney transplants. *Curr. Opin. Nephrol. Hypertens.* 15, 637–642 (2006).
- **Interesting article that demonstrates how metabolomics analyses can be applied to monitor patients following kidney transplantation.**
- 79 Clayton TA, Lindon JC, Everett JR *et al.*: An hypothesis for a mechanism underlying hepatotoxin-induced hypercreatinuria. *Arch. Toxicol.* 77, 208–217 (2003).
- 80 Clayton TA, Lindon JC, Everett JR *et al.*: Hepatotoxin-induced hypercreatinemia and hypercreatinuria: their relationship to one another, to liver damage and to weakened nutritional status. *Arch. Toxicol.* 78, 86–96 (2004).
- 81 Schnackenberg LK, Dragan YP, Reily MD, Robertson DG, Beger RD: Evaluation of NMR spectral data of urine in conjunction with measured clinical chemistry and histopathology parameters to assess the effects of liver and kidney toxicants. *Metabolomics* 3, 87–100 (2007).
- 82 Woo HM, Kim KM, Choi MH *et al.*: Mass spectrometry based metabolomic approaches in urinary biomarker study of women's cancers. *Clin. Chim. Acta* 400, 63–69 (2009).
- 83 Beger R, Schnackenberg L, Holland R, Li D, Dragan Y: Metabonomic models of human pancreatic cancer using 1D proton NMR spectra of lipids in plasma. *Metabolomics* 2, 125–134 (2006).
- 84 Saude EJ, Obiefuna IP, Somorjai RL *et al.*: Metabolomic biomarkers in a model of asthma exacerbation: urine nuclear magnetic resonance. *Am. J. Respir. Crit. Care Med.* 179, 25–34 (2009).
- 85 Li X, Xu Z, Lu X *et al.*: Comprehensive two-dimensional gas chromatography/time-of-flight mass spectrometry for metabonomics: biomarker discovery for diabetes mellitus. *Anal. Chim. Acta* 633, 257–262 (2009).
- 86 Weljie AM, Dowlatbadi R, Miller BJ, Vogel HJ, Jirik FR: An inflammatory arthritis-associated metabolite biomarker pattern revealed by ¹H NMR spectroscopy. *J. Proteome Res.* 6, 3456–3464 (2007).
- 87 Goodsaid FM, Frueh FW: Biomarker qualification pilot process at the US Food and Drug Administration. *AAPS J.* 9, E105–E108 (2007).
- 88 Kaddurah-Daouk R, Kristal BS, Weinshilboum RM: Metabolomics: a global biochemical approach to drug response and disease. *Annu. Rev. Pharmacol. Toxicol.* 48, 653–683 (2008).
- **Comprehensive article that provides a good overview of metabolomics, including analytical platforms, informatics tools and potential applications of the technology.**
- 89 Wishart DS: Applications of metabolomics in drug discovery and development. *Drugs R. D.* 9, 307–322 (2008).
- 90 Trygg J, Holmes E, Lundstedt T: Chemometrics in metabonomics. *J. Proteome Res.* 6, 469–479 (2007).
- 91 Wishart DS: Quantitative metabolomics using NMR. *Trends Analyt. Chem.* 27, 228–237 (2008).
- 92 Clarke CJ, Haselden JN: Metabolic profiling as a tool for understanding mechanisms of toxicity. *Toxicol. Pathol.* 36, 140–147 (2008).
- 93 Brazma A, Hingamp P, Quackenbush J *et al.*: Minimum information about a microarray experiment (MIAME) – toward standards for microarray data. *Nat. Genet.* 29, 365–371 (2001).
- 94 Fiehn O, Robertson D, Griffin J *et al.*: The metabolomics standards initiative (MSI). *Metabolomics* 3, 175–178 (2007).
- 95 Sansone SA, Fan T, Goodacre R *et al.*: The metabolomics standards initiative. *Nat. Biotechnol.* 25, 846–848 (2007).
- 96 Evans RW, Williams GE, Baron HM *et al.*: The economic implications of noninvasive molecular testing for cardiac allograft rejection. *Am. J. Transplant.* 5, 1553–1558 (2005).
- 97 Deverka PA: Pharmacogenomics, evidence, and the role of payers. *Public Health Genomics* 12, 149–157 (2009).
- 98 Wang Z, Gerstein M, Snyder M: RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* 10, 57–63 (2009).

■ Websites

- 101 US FDA's Critical Path Report and List. www.fda.gov/oc/initiatives/criticalpath
- 102 European Medicines Agency (EMA): definition of a genomics biomarker. www.emea.europa.eu/pdfs/human/ich/43798606en.pdf
- 103 European Medicines Agency (EMA): rat urinary protein protein biomarkers announcement. www.emea.europa.eu/pdfs/human/biomarkers/25088508en.pdf
- 104 Consortia: Critical Path Institute. www.c-path.org/consortia.cfm
- 105 ILSI: Health and Environmental Science Institute. www.hesiglobal.org/i4a/pages/index.cfm?pageid=3279
- 106 FDA: Table of valid genomic biomarkers in the context of approved drug labels. www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm
- 107 Office of Biotechnology Activities: Report from the Department of Health and Human Services USA. http://oba.od.nih.gov/oba/SACGHS/reports/SACGHS_PGx_report.pdf