TOXICOGENOMICS
insights into the present and future

Adverse drug responses are an important post-marketing public health issue, occurring many times in subsets of treatment populations. Promising new approaches to predicting physiological responses to drugs are focused on 'genomic responses' or toxicogenomics[1]. This article provides a current perspective on toxicogenomics technologies that are aimed at: 1) providing new tools and systems for more rapid, accurate and complete toxicity assessments in advance of human exposure; 2) enhancing the thoroughness and accuracy of toxicity assessments achievable with currently available test systems, and 3) predictive assessments of individualised risk for developing adverse drug reactions.

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Advances in drug development and characterisation technologies hold the promise of better design, execution and interpretation of efficacy and safety testing of drug candidates in advance of human exposure and market launch. Toxicogenomics is a term that has been coined to describe one of these technology areas which, in the broadest sense, can be defined as the application of genomics-based and related technologies for the assessment of the toxicological impact(s) of new chemical entities (NCEs) or established pharmaceutical compounds on biological systems[2]. Pharmacogenomics, in contrast, is typically used to describe genomics-based technologies or approaches to optimise the therapeutic efficacy of drugs through the identification and/or characterisation of genetic differences in target patient populations. However, it is not difficult to identify situations in which the definitions of these terms substantially overlap. For example, polymorphisms in the multidrug resistance gene or MDR1 can impact the bioavailability of drugs, impacting aspects that affect both drug efficacy and potential drug toxicity[3] and recently it was discovered that genetic variations in the drug metabolism gene CYP3A5 may explain drug metabolism, and thus efficacy and toxicity, differences in many individuals[4].

Contemporary drug-based medical interventions have fostered a tremendous impact on both human lifespan and quality of life. However, despite the advances made and the technology represented by today's pharmaceutical-based medicines, there is enormous room for improvement in the preclinical testing and development and the clinical development and application of pharmaceutical-based therapies for human diseases. Current development and testing processes often yield therapeutics that are sub-optimally effective in many individuals and toxic to varying degrees in patient subpopulations. The impact of drug toxicity on human health is highlighted by the estimate that adverse drug reactions rank as between the fourth and sixth leading cause of mortality in the United States[5]. As such, adverse drug responses are a major human health concern from both morbidity/mortality and health economic perspectives. The application of new technologies and tools for better safety and efficacy assessments of new drugs are urgently needed.

Traditional/current methods for the assessment of potential drug toxicities in humans remain heavily dependent upon whole animal models and, within these models, upon conventional clinical chemistry and histopathology endpoints for assessments of tissue damage. These test system and endpoint choices are driven predominantly by

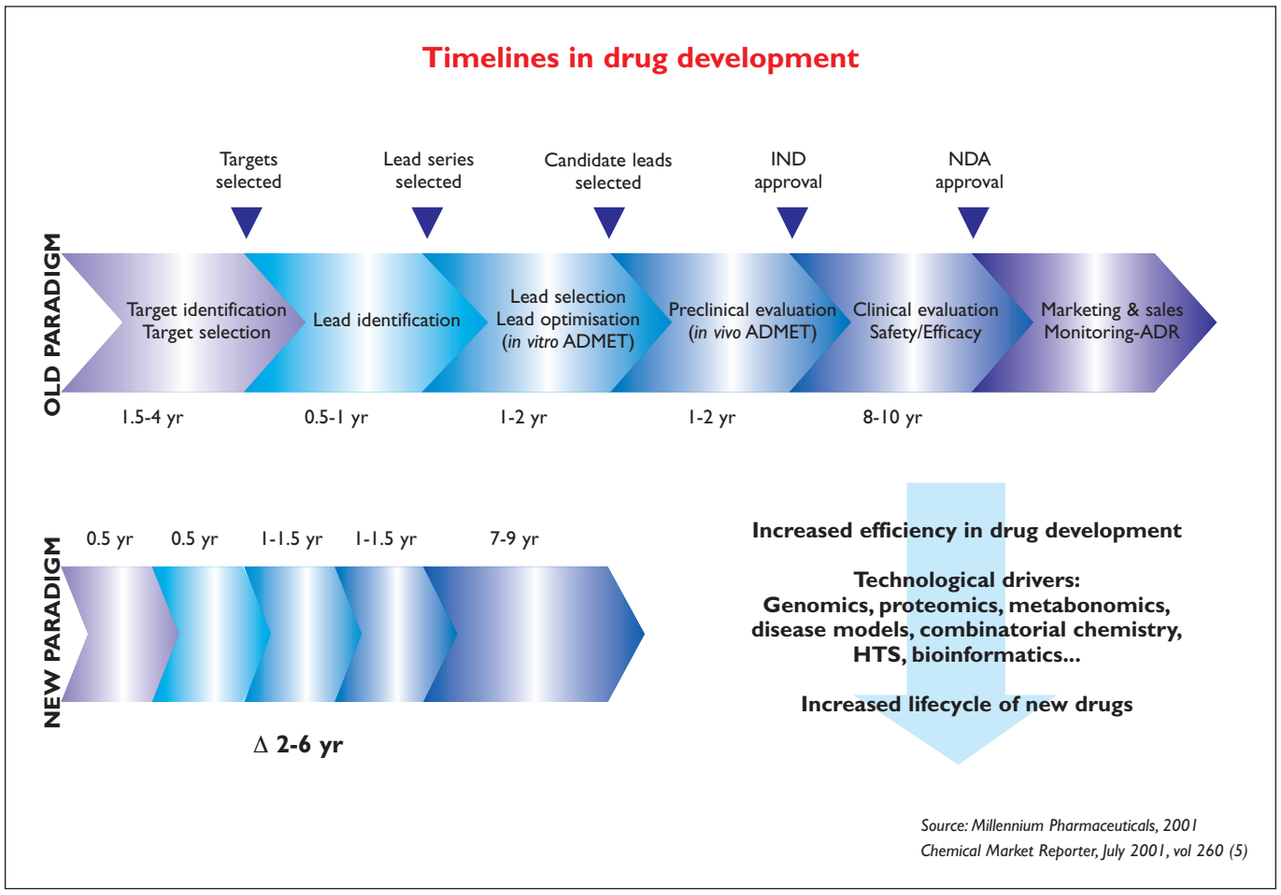

Figure 1

industry experience, regulatory requirements and historic precedence. The limitations associated with animal models include characterised and uncharacterised discrepancies between animal model systems and humans regarding adsorption, distribution, metabolism and excretion of drugs and drug metabolites. In addition, most of the animal models used in drug development and safety testing today utilise individual animals that are genetically similar or practically identical and are subjected to homogeneous environments. In contrast, drugs that are developed and safety tested with animal systems are subsequently tested in genetically heterogeneous human populations and those that make it to the market are subsequently prescribed by clinicians for similarly heterogeneous human patient populations.

What exactly are some of the limitations associated with current methods of preclinical drug toxicity testing? One of the most comprehensive studies to date was conducted by Olson, et al in which they looked at human and animal safety data that had been compiled by 12 different pharmaceutical companies for 150 compounds spanning over 200 different human toxicities. This analysis revealed an overall concordance between animal (both rodent and non-rodent species) and human toxicities of 71%. When different types of toxicities were examined, the highest concordance values between animal test model results and human toxicities were observed for hematological (91%), gastrointestinal (85%) and cardiovascular (80%) toxicity events and lowest for cutaneous toxicity (<40%) and liver toxicity (<60%). It was concluded that animal models are of considerable value for predictive toxicology, but that there is much room for improvement with and beyond these models, especially in the areas of liver toxicity and cutaneous/hypersensitivity reactions[6]. The consequences of development of a drug despite its propensity to cause a particular human toxicity include the potential threat to the health of human study participants and major increases in development costs associated with aborted clinical development studies. Upwards of

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1 Although the term 'toxicogenomics' has been generally applied to gene expression-based methods for toxicity assessments, for the purposes of clarity, we have used it throughout this article to refer to all of the '-omic' technologies discussed.

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Continued on page 21

75% of drug research and development costs have been attributed to compound failures, a third of which include toxicity problems[7].

New methods for preclinical and clinical drug safety assessment have and are being rapidly developed as a result of 1) completion of the Human Genome Project; 2) substantial progress toward the completion of genome projects for a number of model animals that have been central to drug safety testing; 3) advances in throughput capacity for gene and protein expression measurement technologies; 4) the development of improved *in vitro* models for drug metabolism and toxicity assessments and; 5) new tools for assessing metabolic changes in response to drug treatments. Progress on all of these fronts is anticipated to greatly shorten drug development timelines (**Figure 1**), but challenges in regard to data analysis and interpretation (ie what does it all mean?) will have to be overcome before a true impact on drug development time is realised.

Toxicogenomic technologies

Many technology platforms exist for toxicogenomics and related investigations, ranging from cheminformatics-based structure-toxicity assessments to gene expression and metabolism byproduct and intermediate measurements. For the purposes of this review, we have categorised and described some of the biological-based technology areas according to the type of biomolecular characterisation addressed. This categorical list is intended to provide a highlight of some of the technology areas that have and will continue to contribute to our ability to characterise and understand drug effects on *in vitro*, animal and ultimately human systems.

Gene Expression

Technologies to measure or compare gene expression levels are increasingly being applied to *in vivo* and *in vitro* drug toxicology and safety assessment[8,9,10]. Expression technologies can be used to provide information about gene transcript levels in individual samples or to compare the levels of gene transcripts in 'control' samples to 'treated' samples. Expression measurement can be divided into the two general categories of either 'open' or 'closed' systems. Open systems are theoretically capable of identifying any gene transcript that is expressed at different levels between two samples. Examples of open expression analysis systems include some established technologies such as serial analysis of gene expression or SAGE[11] and differential display[12] and recently developed techniques such as massively parallel signature sequencing or MPSS[13]. Closed systems are comprised of specific sets of genes that have been previously identified and characterised. To date, many of the reported studies of gene expression-based changes associated with drug toxicity have relied upon microarray-based gene expression assays. See Clarke et al for a detailed review of the technologies behind microarray-based gene expression measurement[14].

Genetic polymorphisms

A large amount of effort has been applied and attention paid to technologies aimed at the identification of small variations in genes, otherwise known as single nucleotide polymorphisms or SNPs. Because SNPs occur very frequently within the human genome (one SNP in every 100-300 base pairs of DNA), they offer considerable utility as markers for finding or 'mapping' genes involved in complex genetic diseases and responses to environmental factors. SNPs and other genetic differences have been directly linked to variations in drug metabolism efficacy[4], with important implications regarding individual human differences in drug response. The identification of individuals who are poor metabolisers, based upon examination of key drug metabolism genes, is also useful for the identification of persons who may be predisposed to suffering adverse effects associated with a drug treatment and thus possibly at increased risk for drug toxicity. The wide inter-individual variation identified in drug metabolism contradicts the current 'one-dose-fits-all' philosophy that is currently applied to prescription medicines. This is further emphasised by a recent study by Wilson et al suggesting that genetic variation-based differences in drug response are common within different racial and ethnic groups. These authors conclude that too much genetic diversity exists within drug metabolism genes within these type of 'groups' to use general descriptors such as race to categorise human subjects in regard to drug metabolism and drug response[15].

A number of drugs have been removed from the market in recent months and years due to rare adverse drug reactions or 'idiosyncratic' toxicities (**Table 1**). The identification of SNP-based differences in affected subpopulations in advance of drug exposures has the potential to provide the necessary insight for improved dosing recommendations, and avoidance recommendations for individuals who are likely to develop an adverse drug reaction due to genetic factors.

Continued from page 20

Table 1
Recent examples of marketed drugs that have been withdrawn due to to subpopulation (idiosyncratic) toxicity

MANUFACTURER	PRODUCT/DRUG	DATE OF MARKET WITHDRAWAL
Bayer	Baycol (cerivastatin)	08/01
Glaxo Wellcome	Raxar Tablets (greoafloxacin)	10/00
Janssen Pharmaceutica	Propulsid (cisapride)	06/00
Glaxo Wellcome	Lotrex (alosetron hydrochloride)	11/99
Janssen Pharmaceutica	Hismanal (astemizole)	06/99
Wyeth-Ayerst Laboratories	Duract (bromfenac)	06/98
Roche	Posicor (mibefradil)	06/98
Warner-Lambert (Parke-Davis)	Rezulin (troglitazone)	12/97 (UK) 05/00 (USA)
Aventis	Seldane D (terfenadine)	12/97
Interneuron Pharmaceuticals	Redux (dexfenfluramine)	09/97
Robins	Pondimin (fenfluramine)	09/97

Proteomics: beyond gene transcripts

Protein analyses offer distinct advantages over gene expression techniques due to the role that proteins play as final functional (enzymatic) mediators of gene expression, compared to the intermediate role represented by gene transcripts. Technological approaches to protein characterisation and quantitation are generally much more complex than gene expression measurement technologies due to additional aspects of proteins, including secondary structures and post-translations modifications. Because of their complexity, proteomics technologies lag behind in their application to drug development, including toxicology studies.

Two-dimensional electrophoresis has been the historical standard for analysis of complex protein mixtures. However, this technology has restrictions of speed, sensitivity and throughput that have limited wide spread application to toxicogenomic applications. Other approaches such as high performance liquid chromatography have been increasingly applied for protein separations, combined with mass spectroscopy for protein identification. A number of variations on these themes have recently been developed, including approaches using protein 'chips' to selectively bind individual protein components for sample analysis. The next few years will yield the development of protein analysis tools that will provide the breadth of analysis and throughput capabilities to move proteomics into the realm of widespread application for biological response characterisations.

Metabonomics: byproducts and intermediates of metabolism

A relatively new approach to characterisation of toxic responses is a nuclear magnetic resonance-based analysis termed 'metabonomics'. It involves the characterisation of biofluids for byproducts and intermediates of drug metabolism and other physiological processes. This technology has the capability to complement and augment gene and protein expression characterisations of physiologic responses to toxic insults and challenges by providing information about the dynamic metabolic status of whole organisms and biological systems[16]. In addition, metabonomic approaches offer the capability of capturing detailed time-course data for toxic responses, with results that can reveal the cumulative effect of multi-organ responses in whole animal models. Because of the focus on metabolism intermediates and byproducts, many of which are small molecules and/or are found in biofluids such as blood, urine and saliva, metabonomic sampling can be performed in repeated fashion. This is in contrast to gene and protein technologies that, due to sample size, the invasive nature of sampling and cost, are often limited to examination of changes or responses in a few organs across a limited number of timepoints following drug or toxicant exposure. In addition, since typical biofluid samples are more readily obtainable from human populations compared to solid tissue samples, metabonomics approaches for drug response characterisation are easily adaptable to human clinical studies.

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Continued on page 22

Continued from page 21

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In vitro test systems: improved human response prediction capabilities

In addition to new and developing technologies for characterising responses at the gene transcript, protein and metabolite level there is also substantial effort being applied toward advances in the development and optimisation of models of human metabolism, leading to better surrogates for human testing. Currently a number of *in vitro* model systems are being used to investigate liver metabolism characteristics of NCEs. These systems include metabolism proteins produced by recombinant DNA methods, primary cultures of human and animal hepatocytes (isolated directly from fresh tissue sources), in addition to microsomal organelles isolated from these cell sources. Such systems have the ability to provide insight about drug-drug interactions and the impact that drug metabolism gene polymorphisms have on drug metabolism efficiency and clearance[17,18]. These and other *in vitro* models for toxicity, metabolism and pharmacokinetic testing have been the centre of recent development efforts[19] along with transgenic animal models, with emphasis placed on the establishment of animals harbouring genetic modifications with greater relevance to human metabolism[20].

Applied toxicogenomics: tomorrow and beyond

The development and application of new and improved toxicogenomic and related technologies for prediction and characterisation of toxic responses will ultimately provide: 1) better resolution and accuracy than current animal models used today; 2) more information from alternatives to animal models such as primary cell strains, cell lines and *in vitro* tissue models, combined with higher throughput than current *in vivo* models; 3) *in vitro* models of human systems offering more 'human relevant' data than current animals models for toxicity and safety, 4) more and better data from human clinical studies, with renewed focus on sampling and endpoints aimed at the definition of mechanism(s) of action and overall physiological impact.

The ultimate goal sought from the combined contributions of all of these technologies is the development of functional and applied physiomics, a biosystem-wide characterisation and modelling of complex biological and biochemical responses to genetic and environmental factors. The availability of physiomics will enable comprehensive characterisations of mechanisms and effects involving all aspects of adsorption, distribution, metabolism and excretion of drugs. In the end, these technologies will contribute to more and better medicines that will be discovered and developed in less time and for less cost. They will also provide large contributions towards the elimination of the 'trial and error' and 'one drug fits all' methods that are an inherent part of drug development and prescription medicine today. DDW

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