of phenotypes (depending on the center) and which are available for further study.

The current large-scale mutagenesis centers are designed around specific phenotypic areas and are intended to systematically identify mutants of potential interest. From the Jackson Laboratory, Bar Habrbor (ME, USA; http://nmf.jax.org/about.html): 'models will be identified using an extensive phenotype-driven approach that targets a broad range of recessive neurological phenotypes and characterizes them sufficiently to attract further detailed study.' It is not within the mandate of the NIH-funded mutagenesis centers to clone out the mutated genes. For the price of a positional cloning project (about one graduate-student-year in many labs), a researcher can obtain a mutant mouse, the cloned gene carrying the mutation and an intriguing initial phenotype, such as high platelet levels, low HDL cholesterol, decreased exploratory behavior, or reluctance to run the wheel.

But what about the pharmaceutical industry? Although transgenic mice are a workhorse of target validation, applications of random mutagenesis to obtain mouse models of disease have lagged behind. This is likely for several reasons: (1) they require large mouse colonies, (2) accurate, high-throughput phenotypic screens for subtle effects are challenging to implement and interpret, (3) there is a large investment of time and money between initial mutagenesis and final gene cloning and proof of mechanism, and (4) there is no certainty that the mutated gene will be fall into a 'druggable family'.

A plethora of mutant mice from the academic centers begins to address the first three points and provides a potential entry point to take advantage of ENU mutagenesis without the up-front investment.

Another limitation, from the pharmaceutical perspective, of many current mutagenesis programs is that they begin with wild-type mice and seek aberrant phenotypes. As Russ *et al.* point out, it is also possible to begin with a disease model, to mutagenize and screen for a normal phenotype (suppressor screen). Although this approach limits the range of phenotypes that can be sought in a project, the resulting mutated genes might be more likely to resemble targets for therapeutic intervention.

The strength of the mutagenesis centers is in reproducible large-scale methods for initial phenotypic characterization. The mice that they produce are great first steps in unraveling complex phenotypes of interest to the pharmaceutical industry.

Reference

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Advancing technologies for accelerated drug development

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The subject of the *1st International Drug Discovery and Development Summit* (December 2–5, 2002, Honolulu, HI, USA) *– From Lead to Drug in Five Years –* is definitely a worthwhile goal. Characteristic of events organized by The Institute for Scientific Exchange, the meeting had an honest air, with provocative presentations and lively discussions. Presentations focused on technologies with the potential to accelerate the drug development process, namely: how to predict drug properties early so that the right candidates can be chosen; how to accelerate the necessary bioassays and clinical trials; and, most important of all, those technologies that need to be developed and validated. The meeting was attended by approximately 100–110 international delegates, mostly from the pharmaceutical industry.

Lead selection using virtual screening

Keynote speaker Peter Smith (Millennium Pharmaceuticals, http://www.mlnm.com) began the conference by emphasizing the importance of cross-discipline communications during drug development. He supports the gene-to-patient concept to accelerate the development of safe and efficacious drugs, using promising new technologies such as pharmacogenetics, pharmacogenomics, metabolomics, as well as *in silico* approaches. Alan Wilson (Pharmacia, http://www.pharmacia.com) spoke more on *in silico* systems for absorption, distribution, metabolism

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and elimination (ADME), and toxicity prediction, giving examples of 'good prediction' of Ames mutagenicity results using a compilation of multiple predictive softwares. He pointed out, however, that current *in silico* approaches are not able to predict events that involve modification of chemical structures and activities by drug-metabolizing pathways. Continuing the discussion on *in silico* approaches, Dale Johnson (Eos Biotechnology, http://www.eosbiotech.com) emphasized the need for high-quality data before developing a predictive *in silico* system. He argued that the performance of existing predictive systems has suffered owing to the inclusion of inappropriate data.

In vitro screening for drug toxicity

The application of *in vitro* systems to accelerate drug development was addressed by the next set of presentations. Albert Li (PHASE-1 Molecular Toxicology, http://www.phase1tox.com) highlighted the inadequacies of present toxicology testing approaches. He presented a multiple-parameter hypothesis for idiosyncratic drug toxicity, with the key parameters being exposure level, chemical properties, environmental effects and genetic effects. Data were presented that support the use of gene expression in the prediction of drug toxicity in rats. Toxicogenomics data using human hepatocytes illustrated the activation and suppression of toxicologically relevant genes by the toxic drug troglitazone, and the lack of such activities by the less-toxic drugs rosiglitazone and pioglitazone. Li emphasized the importance of human-based experimental models (e.g. human hepatocytes) with appropriate metabolizing enzyme activities for the prediction of human drug toxicity.

Norman Sussman (Amphioxus Cell Technologies, http://www.amphioxus.com) presented data from the C3A cell-line (derived from HepG2 cells), where multiple toxicity endpoints (ATP content, P450 induction, and enzyme release) were used to rank chemical toxicity. Another multiple-endpoint assay for the screening of chemicals with toxic potential was described by Peter O'Brien (University of Toronto, http://www.utoronto.ca), who emphasized the importance of the pro-oxidants in drug toxicity, especially for nonsteroidal anti-inflammatory drugs (NSAIDs). Vangala Subrahmanyam of Johnson & Johnson (http://www.jnj.com) discussed the application of *in vitro* assays – especially rat and human hepatocytes – in the evaluation of drug metabolism, drug–drug interactions and drug toxicity. The importance of *in vitro* systems was also highlighted by Peter Bullock (Purdue Pharma, http://www.pharma.com) who exemplified the use of expressed P450s, liver microsomes, and hepatocytes in the evaluation of drug metabolism properties. The first day of the meeting was concluded by Yuichi Sugiyama (University of Tokyo, http://www.u-tokyo.ac.jp) who stressed the importance of transporter inhibition in drug-induced hepatoxicity and drug–drug interactions. He presented data from a 'double transfectant' cell line – cells transfected with influx and efflux transporters – in the screening of transporter inhibitors.

Solubility and bioavailability

The second day began with a stimulating presentation by Chris Lipinski (Pfizer, http://www.pfizer.com) on the importance of chemical space on drug properties. He explained the rule-of-five and the application of this rule in the prediction of solubility and intestinal permeability. Solubility should be a key parameter in the selection of drug candidates, which can be facilitated using *in silico* approaches. In addition, he voiced his dismay in the lack of melting-point data for chemicals used for pharmacological screening. Ikuo Horii (Pfizer) discussed the use of high-throughput toxicity screening assays in drug discovery to enable the early elimination of compounds with high toxic potential. He emphasized the need to incorporate all facets of toxicity testing, from the use of different cells and culture systems (e.g. spheroids) to the application of all 'omics' platforms, to predict drug toxicity early and accurately in drug discovery and development. The importance of drug evaluation at the discovery–development interface was highlighted by Marcus Brewster (Johnson & Johnson). Such an evaluation provides a continuum of knowledge rather than the currently practiced 'over-the-wall' process, which results in a duplication of efforts during drug development for information already gained during drug discovery. The technical focus of his presentation was on the prediction of intestinal absorption, where he emphasized that formulations that can enhance solubility often also enhance intestinal absorption (e.g. cyclodextran for itraconazole).

Animal models: accelerating discovery

Selecting an appropriate animal species for the prediction of human drug properties is paramount in preclinical studies. Rakesh Dixit (Merck, http://www.merck.com) emphasized that the key properties in such a selection should be drug metabolism and pharmacological similarities with humans. Although drug metabolism as a key parameter was emphasized by other speakers, Dixit was the first to emphasize the importance of pharmacological similarities. An example of a successful prediction of *in vivo* pharmacokinetic properties was presented by Jiunn Lin (Merck), using data from *in vitro* drug metabolism with the drug indinavir. The predicted hepatic clearance of indinavir was 5.5–36.0 ml min^{-1} kg^{-1}, compared with the observed clearance

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of 4.2–17.0 ml min^{-1} kg^{-1}. Such a successful prediction was achieved by the inclusion of an *in vitro–in vivo* ratio, using *in vitro* and *in vivo* data from rats. Lin stated that although *in vitro* approaches could successfully predict hepatic clearance, they might not successfully predict half-life, owing to the current inability to predict volume distribution in humans.

The third day of the meeting focused on the use of transgenic animals in toxicity testing. A presentation by Carl Alden (Millennium Pharmaceuticals) offered the challenging view that current rodent two-year bioassays do not predict human carcinogenicity. He proposed rather that transgenic carcinogenicity assays using TgrasH2- and p53-knockout mice should be used to enhance the efficiency of drug development. Tetsuya Kamataki (Hokkaido University, http://www.hokudai.ac.jp) gave a lively presentation, entertaining the audience with photographs of his 60th birthday party, in addition to presenting interesting data. A transgenic mouse – transfected to express human fetal Cyp3A7 – was presented as a promising experimental system to test for human fetal toxicity; currently there is no acceptable assay for human fetal drug toxicity.

The role of disease conditions on drug toxicity – a parameter ignored in most preclinical and clinical drug evaluation – was described by Urs Boelsterli (HepaTox Consulting, http://www.hepatox.com). Data were presented supporting the case that adverse drug reactions are often more prevalent in the diseased rather than the healthy human population. He proposed that animal models of mitochondrial diseases be used as toxicological models. Roland Wolf (University of Dundee, http://www.dundee.ac.uk) described mice and rats models that contain reporter genes for CYP1A induction within hair follicles, thereby enabling induction to be evaluated without sacrifice of the animal. He also presented data on 'P450 knock mice' – mice with no P450 activities through a knocking-out of the reductase.

The subsequent session focused on analytical chemistry. Timothy Olah (Bristol-Myers Squibb, http://www.bms.com) described an automated routine-sample analysis process, providing data on the automation of sample preparation, *in vitro* drug metabolism, P450 inhibition assays, and Caco-2 transport assay. An exciting presentation was given by Colin Garner (CBAMS, http://www.cbams.co.uk) who described the use of accelerated mass spectrometry (AMS), which enables accurate quantification of radioactivity-based [^{14}C]:[^{12}C] ratios. The sensitivity of AMS (1000-fold more sensitive than liquid-scintillation counting), enables human *in vivo* pharmacokinetic studies to be performed during the early phases of drug discovery.

Accelerating clinical studies

The final day of the conference did not lack audience or exciting presentations. Frank Sistare, speaking as a private citizen, although he is an employee of the US FDA (http://www.fda.gov), discussed the lack of clinical biomarkers for early phases of toxicity, including liver toxicity, kidney toxicity, neurotoxicity and myocardial tissue damage. He presented data supporting the use of various biomarkers: troponin T for cardiac toxicity, various serum proteins for vasculitis, and C-reactive protein for inflammation. He also presented a biomarker-confirmation protocol for the validation of biomarkers. The application of safety biomarkers was addressed by Rakesh Dixit, who presented data supporting the use of glutathione-*s*-transferase as biomarker for renal toxicity.

The integration of *in vitro* data into physiologically based pharmacokinetics (PBPK) models is key to the successful prediction of preclinical and clinical pharmacokinetics and toxicokinetics, as highlighted by Ryosei Kawai (Novartis, http://www.novartis.com). Kawai also emphasized the importance of bridging studies; that is, using clinical data from one country to accelerate clinical trials in another country.

Concluding remarks

The Drug Discovery and Development Summit was an excellent meeting. A good cross section of the pharmaceutical industry and technology providers was represented, with excellent speakers and critical and interesting discussions. The technologies were presented objectively and from a scientific perspective (as opposed to 'commercial' presentations, which are prevalent in some conferences). Discussions were lively, honest and critical, reflecting the expertise and confidence of the participants.

The Summit emphasizes the importance of an intelligent, coordinated approach to drug development. Choosing the wrong drug candidate remains the major stumbling block for drug development, and there are technologies, such as *in vitro* systems, *in silico* approaches and genomic approaches, that, when used appropriately, can minimize the probability of committing this fatal mistake. The use of transgenic animals can facilitate preclinical studies. Clinical studies are still hampered by the lack of appropriate biomarkers for organ toxicity and the 'classical' markers might not be definitive enough to allow an accurate safety assessment. The speakers and the attendees seemed to agree that drug development can be accelerated – and needs to be accelerated. Acceleration of drug development requires continual technology development and intelligent application of these technologies.