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Predicting Drug Safety: Next Generation Solutions

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One of the major stumbling blocks in successful of drug development continues to be the less than optimal prediction of potential human drug toxicity both during the early discovery phase as well into late stage clinical trials. This continues to be a major area of discussion in the fields of drug metabolism and toxicology particularly as it pertains to interspecies extrapolations from non-clinical animal studies. Even though significant advancements have been made in establishing a clearer definition of benefit vs. risk for most medications, adverse drug reactions (ADRs) continue to be a major cause of morbidity and mortality worldwide [1]. It is now time to invest in and develop an innovative, comprehensive system for predicting human drug toxicity and/or safety throughout the drug discovery and development process. The system must include a substantial level of data transparency which would allow us to gain from a wealth of experience from academic, industry, and governmental sources. The consequences of not developing such a system have been evident by the cost of drug safety issues to the health care system and the increasing costs of developing new drugs which in great part reflect the difficulty in establishing and proving adequate risk/benefit ratios in the expected patient populations. New 'omics technologies coupled with bioinformatics-systems biology approaches including genome wide association studies (GWAS) and biological pathway analysis are adding new insights into possible mechanisms, identification of susceptible individuals, and points of potential intervention [2,3].

Creating a fully functional system is, however, far from simple. The factors involved in human ADRs are complex and include the drug itself, the patient with or without unique susceptibilities and concomitant medications, prescribing and medication errors, compliance issues particularly in the elderly, and complications with multiple drugs in complex regimens given to sick individuals. Some of the key factors noted above never come into play before a drug is approved and many of the mainstays of predicting risk/benefit – such as non-clinical safety evaluations - have not given us a true picture of who and why certain individuals are more at risk for developing ADRs than others. At the discovery stage, we tend to concentrate solely on the drug, specifically how the chemistry and particularly chemical motifs of potential lead compounds predict unwanted effects including metabolic conversion into reactive metabolites. Many of these predictions come from both commercial and open source algorithms where the applicability of the training sets used to construct the models may only work with a very limited set of analogues in a structurally-related series. In some cases the chemistry involved in building the models remains undisclosed and therefore the predictive algorithms exist as "black box" tools. QSAR modeling is frequently used in drug toxicity prediction, with acknowledgement that this type of prediction does have both promises and pitfalls [4]. It has become increasingly recognized that QSAR models should be structured to provide: a defined endpoint; an unambiguous algorithm; a defined domain of applicability; appropriate measures of goodness-of-fit, robustness, and measures of predictability; and a mechanistic correlation. Ideally, the choice of chemical descriptors, would take into account the mechanism of action and most desirably the rate limiting step in the endpoint and/or the biological process being modeled [4].

The new system we envision must include chemical motif predictions and metabolic estimates from expert systems, incorporate information on potential patient populations and potential susceptible individuals, and must continually incorporate knowledge from new technologies and innovations. Filtering out the potential "bad actors" either through motif-driven *in silico* analyses or through targeted screening for specific organ toxicities, both *in vitro* and *in vivo*, has proven to be effective, particularly in the prediction of cardiotoxicity and mechanism-based hepatotoxicity [5-7]. However, a major area of innovation is needed to be able to identify where data (or predictive) gaps exist and to identify the key tool(s) that could and should be used to fill the information gap. These key tools will change over time as new technologies are introduced and validated. As an example, most of us in this field participated in large scale toxicogenomics experiments with the idea of creating predictive technologies linked to globally constructed databases. In the end we discovered that the real value was in elucidating mechanisms of action of chemicals rather than predicting toxicity. In addition, most of the early toxicogenomics studies concentrated on attempting to predict animal toxicity rather than directly dealing with human effects. Newer efforts focused on next generation sequencing in the study of disease as well as predicting human drug responses both positive and negative are emerging. We anticipate an extensive body of knowledge to develop, which will enable a predictive process that takes the theoretical drug directly to the bedside and back during the design phase [2,3,8-11].

Background on ADRs

Previous analyses on the extent and consequences of ADRs, some now outdated and others derived from small sample sets, nevertheless present a staggering picture. Lazarou et al. [12] estimated that there were over 2 million serious ADRs each year in the US and that ADRs ranked fourth in cause of death in hospitalized patients and those in nursing homes. At the time this placed ADRs ahead of deaths from pulmonary disease, diabetes, AIDS, pneumonia, and accidental deaths. Passarelli et al. [13] came to similar conclusions in studies on elderly hospitalized patients in Brazil showing almost 50% developed ADRs while in the hospital. The authors discovered that this was a major factor in complicating the course of diseases being treated and in many cases created requirements for additional therapeutics to be given to these patients. Laroche et al. [14] in another study showed that the

major factor in ADRs in elderly hospitalized patients in France was the inappropriate use of drugs. There were larger numbers of drugs taken by individual patients developing ADRs, and a higher number of patients that developed ADRs were given inappropriate medications both prior to admission and during hospitalization. Franceschi et al. [15] reported similar findings in Italy, where they showed that over 5% of hospitalizations were ADR related and potentially avoidable. Several studies have shown that ADRs in children are also a significant public health issue. In a meta-analysis of seventeen studies in hospitalized children in Italy, Impicciatore et al. [16] showed the overall ADR incidence – and cause for hospitalization - in the children studied to be almost 10% with approximately 1/8th of these considered to be severe. Regardless of the analysis or reporting system cited, there is widespread agreement that ADRs continue to be an enormous public health problem. Therefore, establishing risk factors for individuals or groups of individuals has taken on new importance. Pharmacogenomics has emerged as the primary focus of determining the influence of genetic variation on drug response, both from an efficacy and safety standpoint. Pharmacogenomic information is now contained in about ten percent of labels for FDA approved drugs. These genomic biomarkers are classified for the following general uses: clinical response and differentiation; risk identification; dose selection guidance; susceptibility, resistance, and differential disease diagnosis; and polymorphic drug targets.

ADRs Resulting in Drug Withdrawals from the Market

MacDonald and Robertson [17] surveyed drugs withdrawn from the US, European, and Asian markets due to ADRs over a period of 1998-2008, and categorized the type of ADR by organ system with the number of drugs in each category. These categories with number of drugs include: Cardiac and/or cardiovascular - 17, liver - 15, psychiatric/addiction - 4, gastrointestinal - 1, muscle - 1, and other - 11 including renal, accelerated carcinogenicity or death, mutagenesis, severe drug-drug interaction with alcohol, hypersensitivity, and hypertension. They point out that hepatotoxicity from the drugs listed resulted from a wide-range of modes-of-action including mitochondrial toxicity, cholestasis, reactive metabolite-mediated cytotoxicity, and unknown idiosyncratic events.

Man et al. [18] looked at ~150 drugs removed from the market since 1960. The top safety reasons were hepatotoxicity (27.9%), cardiovascular toxicity (17.4%), hematologic toxicity (10.4%), cutireaction (7.0%) carcinogenicity (6.3%), neurotoxicity (6.3%), nephrotoxicity (5.6%), allergy (3.5%), and drug abuse (3.5%). Zhang et al. [19] analyzed 10 of these drugs, which included 8 that were included in the MacDonald and Robertson paper, to derive associations between ADRs and potentially related genetic factors and polymorphisms. The implication was that prospective screening could identify specific individuals with a higher level of risk from these specific drugs.

While these specific examples are enlightening, they do have the drawback that they describe drug-ADR linkages retrospectively and also only lead to a conclusion of which organ-specific screening approaches may have been useful. For instance, we now understand the value of cardiotoxicity and hepatotoxicity screening early on and can eliminate compounds that fall into certain classes for which the mode-of-action falls within the screening paradigm. However, what is needed is a comprehensive approach, combining all known information on chemical-related toxicities and predictive models that include a relevant applicability domain such as ADRs occurring after repeated dosing. In addition, comprehensive systems biology approaches such as biological pathway mapping of ADRs both from the chemical and biology aspects are emerging and offering increased visibility to these issues.

Identifying Individuals with Increased Risk of Developing ADRs

The ADR issue including potential mechanisms of idiosyncratic toxicities, toxicities identified in animal studies with or without relevance to humans, toxicity caused by metabolism or altered pharmacokinetics, computational approaches for estimating chemical-structure determinants of toxicity, and ethnic variations in drug response has been widely discussed. A key gap that needs to be identified and filled for the future is a more complete understanding of the term risk as it applies to individual drugs and patient populations and the formation of a strategy to move this multi-faceted prediction further into the non-clinical portion of drug discovery and development. Currently we expect new technologies such as genomic screening may help resolve the question of risk from the patient standpoint and this patient-centric information could eventually become a risk/benefit label identifying individuals at increased risk when given certain medications alone or in combination. How widespread this may become is not known, although major efforts are underway to develop wide-spread databases. Two of these include The United States Drug Induced Liver Injury Network (DILIN) (http://dilin.dcri.duke.edu/) which has been established to discover underlying causes of drug-induced liver disease. The endeavor is sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases of the U.S. National Institutes of Health. The overall goal of the program is to discover why some individuals develop hepatotoxicity and others do not. A registry of people experiencing liver injury from one of four drugs since 1994 has been established. A prospective study is on-going and following patients who recently experienced adverse liver reactions to any drug or herbal medicine. In Europe, the European collaboration to establish a case-control DNA collection for studying the genetic basis of adverse drug reactions (EUDRAGENE) (www.eudragene.org) has as its objective to advance the understanding of the basis of adverse drug reactions, which they hope will lead to the development of tests for predicting individual susceptibility to ADRs. The network has 12 participating centres in Europe and Canada and initially is studying six ADRs including myopathy from cholesterol lowering drugs, agranulocytosis from several different drugs, tendonitis and tendon rupture from fluoroquinolone antibiotics, long QT syndrome caused by several classes of drugs, liver injury caused by non-steroidal anti-inflammatory drugs, and neuropsychiatric reactions caused by mefloquine antimalarials.

These and other developments suggest a major effort is now being placed on prospective screening to detect individuals with a higher likelihood of developing ADRs with certain medications. Ingelman-Sundberg [20] reviewed pharmacogenomic biomarkers used for the prediction of ADRs and a continued update on details of specific testing procedures can be found on the USFDA CDER website. (www.fda.gov.cder/genomics/genomic_biomarkers).

Examples that are included or being evaluated in approved drug labels are presented below. These are listed for genes or alleles, some relevant drugs affected, and potential toxicity.

- APOC3 polymorphisms; examples dyslipidemia and lipoatrophy in HIV-infected individuals receiving HAART therapy using d4T and protease inhibitors.

- CYP2C19 variants; examples – Voriconazole, Clopidogrel: altered pharmacokinetics and potential toxicity

- CYP2C9 variants: examples - Celecoxib, Warfarin: altered pharmacokinetics and potential toxicity and VKORC1 for Warfarin; requires lower dose requirements to avoid side effects

- CYP2D6 variants and mutant; examples - tricyclic antidepressants, atomoxetine, fluoxetine; altered pharmacokinetics and potential toxicity

- DPD deficiency; examples - capecitabine, fluorouracil: stomatitis, diarrhea, neutropenia, neurotoxicity

- G6PD deficiency; example - rasburicase; severe hemolysis

- HLA-B*5701 for Abacavir; Hypersensitivity reactions, lactic acidosis and severe hepatomegaly

- HLA-B*1502; example - Caramazepine: serious dematological reactions; certain epilepsy drugs including dilantin, phenytek, and cerebyx can lead to severe skin reactions in Asian patients

- NAT variants; examples - rifampin, isoniazid and pyrazinamide. Altered pharmacokinetics and increased toxicity

- SLCO1B1 variants; - increased risk in statin-induced myopathy

- TPMT variants; example - Azathioprine: increased risk of myelotoxicity

- UGT1A1*28; for Irinotecan: increased risk for neutropenia; Nilotinib: increased risk of hyperbilirubinemia

Systems Biology – Bioinformatics Approaches

The most recent approaches involve a combination of complex information linked to biological networks. Cami et al. [3] developed a mathematical approach to predict adverse events from a training set comprised of 809 drugs and 852 ADRs documented in 2005. Their approach has been termed "predictive pharmacosafety networks" or PPN. This approach as applied in a global sense has great potential merit, even though the described network is limited to the applicability domain of the drugs and events within the training sets of the model. In another systems approach, Wallach et al. [2] designed a computational framework to pair drugs and associated ADRs by using *in silico* protein docking with relevant protein targets and biological pathways to define ADR-pathway associations. This resulted in 32 probable ADR pathways.

These types of proposals should lead to new bioinformatics methods to create drug-ADR linkages. Large databases that contain well-characterized cases with negative controls are now being interrogated in industry, academia, and regulatory agencies. Tissue and DNA/RNA banks along with detailed clinical annotation are available commercially and through networks including federally-funded projects where results have been deposited in the public domain. Several companies including contract research organizations are also banking samples from large animal toxicology studies with the view that technologies are becoming available to establish the same information in primates and dogs as has been done in humans. It is being discussed that toxicology studies

will have back-up samples available to identify whether animals with specific toxicities have pre-disposing genetic variants that place them at a higher risk. In contract research organizations, this type of service could be a major competitive advantage particularly when clients are smaller biotechnology companies whose entire company success rides on a single drug candidate rather than a portfolio of therapeutic products as seen in larger pharma companies. It is also interesting to suggest these types of technologies could be used to create specific animal models that predict relevant ADRs and risk factors for humans, similar to developing relevant mouse models for certain diseases where the mouse contains the same genetic variants as do humans who are highly pre-disposed to disease development.

Wang et al. [21] reported the results of a study using deep sequencing of human tissue and cell line transcriptomes. The authors indicated that 92-94% of human genes undergo alternative splicing that could lead to multiple mRNA and protein isoforms that may have related, distinct, or even opposing functions. Johnson et al. [8] have recently reported on a process that could prospectively identify causative factors for serious animal and human toxicities from RNA sequencing data coupled with research-based screening tools for tissue-specific splicing phenotypes. These findings, and several related studies, suggest that these rapid phenotypic identifying technologies may be central players in the future of identifying risk factors for ADRs. This would create a major advance in preclinical safety testing.

Next Generation Solutions

As mentioned earlier, the Editor is proposing an innovative, comprehensive system for predicting human drug toxicity and/or safety throughout the drug discovery and development process. The system would include a substantial level of data transparency for which an important part of the transparency is the publication of key articles on drug metabolism and toxicology in open source journals such as the Journal of Drug Metabolism & Toxicology by the OMICS Group. This information source can be linked directly into the system described above as soon as the information appears on-line. It is also hoped that the OMICS Group will start to develop and offer conferences specifically related to this topic to be on the front end of this important revolution.

An important part of this system will be not only a predictive portion based on chemical structure, but also the identification of key assays or studies to run to fill data gaps. Rather than investing in broad screening, a more important process would be to pinpoint the relevant next step to answer a key question that incorporates chemistry, biological effects (both positive and negative), and directly takes into consideration the intended patient population. In the drug development field, we call this "the key study to kill a project". That is, what study should be run that if a compound fails, the project should reconsidered, but if it passes the project's probability of success would increase. In the ideal situation, the first *in vivo* safety study would not be conducted in normal animals but in an animal model that directly predicts for human toxicity suggested from the comprehensive system and based on actual patient information.

I would propose an additional incentive. The FDA should consider granting a similar benefit as now seen with Orphan Drug development to those companies that can identify subsets of patients at risk, which would include pharmacogenomic and/or ethnic variations, and provide a screening test to identify these individuals at the time of

marketing. Rather than shelving potentially important drugs because of rare toxicities, the increased incentive to continue, but prospectively identify patients at risk, could change the dynamics of drug discovery and development. In addition, this could be the impetuous to resurrect potential drugs on the shelf or previously removed from the market because of unresolved toxicities in small subsets of patients. These projects could be handed off to small focused companies that can gain from the new incentives and exclusivity measures with royalties to the original company.

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