Identification and Elucidation of the Biology of Adverse Events: The Challenges of Safety Assessment and Translational Medicine

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Abstract

There has been an explosion of technology-enabled scientific insight into the basic biology of the causes of adverse events. This has been driven, in part, by the development of the various "omics" tools (e.g., genomics, proteomics, and metabolomics) and associated bioinformatics platforms. Meanwhile, for decades, changes in preclinical testing protocols and guidelines have been limited. Preclinical safety testing currently relies heavily on the use of outdated animal models. Application of systems biology methods to evaluation of toxicities in oncology treatments can accelerate the introduction of safe, effective drugs. Systems biology adds insights regarding the causes and mechanisms of adverse effects, provides important and actionable information to help understand the risks and benefits to humans, focuses testing on methods that add value to the safety testing process, and leads to modifications of chemical entities to reduce liabilities during development. Leveraging emerging technologies, such as genomics and proteomics, may make preclinical safety testing more efficient and accurate and lead to better safety decisions. The development of a U.S. Food and Drug Administration guidance document on the use of systems biology in clinical testing would greatly benefit the development of drugs for oncology by communicating the potential application of specific methodologies, providing a framework for qualification and application of systems biology outcomes, and providing insight into the challenges and limitations of systems biology in the regulatory decision-making process. *Clin Cancer Res; 17(21); 6641–5. ©2011 AACR.*

Introductory Note

At the 2010 Conference on Clinical Cancer Research, co-convened by Friends of Cancer Research and the Engelberg Center for Health Care Reform at the Brookings Institution, participants explored 4 pressing new challenges in the field. Articles summarizing the panel's recommendations on each of these topics are featured in this issue of *Clinical Cancer Research* (1–4).

Gaps in Current Testing and Safety Assessment Paradigms

The toxicity of new oncology drugs is a leading cause of pharmaceutical attrition and a major impediment to

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efficient and successful drug development. Safety, or lack thereof, is also a major factor in regulatory decisions involving drug approval, labeling, risk evaluation, and mitigation and even withdrawal from the marketplace. The current battery of preclinical safety studies required to support the clinical development of new drugs and marketing approval is mapped out in International Conference on Harmonisation (ICH) guidelines that include ICH M3, E14, and S1 to S9 (5). In addition, various other documents from regulatory agencies provide recommendations regarding specific toxicities or adverse events, such as hepatotoxicity (6). However, these testing methods and risk assessments have not kept pace with the rapid evolution of technology, biomedical research, and knowledge generation. For example, the studies required to meet international regulatory guidelines for drug development and approval rely almost exclusively on *in vivo* animal testing protocols and endpoint assessments that have changed little in decades. These current *in vivo* methods as they are being used do not fully predict complex, serious, and low-incidence effects in humans, and in many cases are not amenable to generating knowledge that leads to mechanistic insight into the causes or biology of adverse events (7). Importantly, without relevant knowledge about the pathophysiology of potential adverse events, we are unable to predict or understand the

occurrence of low-incidence or idiosyncratic events in humans, a task that is perhaps the most challenging one we face in drug development. Although the long history of *in vivo* animal studies has served the scientific and regulatory community well, there is a timely and compelling need to incorporate changes into the earlier components of drug discovery and development that can lead to more focused animal studies. It is clear that no single new method or testing paradigm will replace entirely the need for *in vivo* testing, but adopting new science and technology on a case-by-case or fit-for-purpose basis from an array of emerging methods in the safety scientist's toolbox has the potential to improve research and development productivity, enable the ongoing efforts to understand and mitigate adverse events, and most importantly, facilitate and expedite the access of new therapies for patients.

Driven by rapidly emerging technologies, a nascent transformation of the safety sciences has taken place from empirical, subjective, and observation-based disciplines to scientifically grounded, objective, and data-driven sciences. This evolution has spawned new methods and experimental tools that are capable of defining the biologic basis of adverse events at the cellular, molecular, and biochemical level. These tools include platforms such as genomics, proteomics, metabolomics, and bioinformatics. Together, they enable the practice of systems biology. Systems biology creates the capability to elucidate complex, highly networked, and pleiotropic pathways of toxicities and to identify specific biomarkers of impending undesirable events (8, 9). This provides the opportunity for the contemporary toxicologist to take a more active and visible role in safety-related decisions. Historically, due to the gap in our knowledge of most toxicities, many safety decisions were based solely on the perceived risk of a toxicity, and often disregarding the potential benefit of a drug. Elucidating the biology of an adverse event allows the supplanting of the perception of risk with specific data that form the underpinning of a robust decision on risk and benefit (10). With this new knowledge, toxicologists can contribute to a systematic and objective decision-making process that identifies patients at risk for serious adverse events and at the same time enables access to individuals that might receive maximum benefit.

Emerging technologies, particularly in the areas of systems biology, biomarkers, and imaging, have begun to be incorporated into clinical development programs. Modifications to the conduct of clinical trials include screening of Investigational New Drug (IND) applications, microdosing protocols, adaptive clinical trials, translational medicine, and risk management planning or risk mitigation strategies. Regulatory agencies have extended explicit overtures and shown a readiness to embrace change through the Critical Path Initiative in the United States and the Innovative Medicines Initiative in Europe, for example. On a smaller scale, a great deal has been learned about the utility and limitations of data derived from new science and enabling technologies from the U.S. Food and Drug Administration's (FDA) voluntary exploratory data submission program (11) and active participation in a number of scientifically driven public–private consortia. The recent announcement of NIH and FDA grants directed at improving drug development and regulatory sciences is a timely testimony to the importance of these topics. The FDA has also sponsored a number of scientific meetings to solicit broad input into the challenges of creating and using drug safety knowledge in the mining of adverse events databases and prediction of adverse events. These laudable efforts create the opportunity to articulate a coordinated framework for policy change that can be understood and engaged by the pharmaceutical industry and broadly communicated to patients and the public. The treatment of cancers has undergone significant advances in the past few years, but as patients are living longer with their diseases, the onerous effects of drug treatment begin to emerge. Current topics in the sequelae of cancer therapy could provide the momentum and focus to urgently apply new technologies to preclinical toxicology.

Systems Biology

Systems biology is an attractive complementary approach to preclinical testing. It has been defined as the iterative and integrative study of biologic systems as they respond to perturbations (12). Systems toxicology comprises the integration of molecular endpoints and conventional toxicity endpoints into a systems biology approach. In a sense, contemporary systems biology is a renaissance of physiology, a traditional integrative discipline. Biologic research has enjoyed decades of success in dissecting the structures and functions of individual molecular and cellular components comprising an organism. However, the inherent complexity of biologic systems, due not only to the large number of their constituents but also to the intricate web of interactions among these constituents, has proved to be difficult to understand with reductionist approaches. Research must be conducted at a more global, systems level if we are to gain understanding of the overall behavior of the biologic networks that maintain normal physiology and the perturbations in these networks that lead to toxicity and disease. Environmental stressors, including physical and chemical agents, exert adverse effects by initially impinging on specific molecular or cellular targets. The ensuing responses triggered from the initial interactions and subsequently propagated along the normal molecular, cellular, or systemic networks, will ultimately affect the health of the intact organism. The application of computational systems biology in risk assessment focuses on developing quantitative simulation models of the dose-response relationships for network perturbations by chemical stressors and drugs (13, 14).

Driven by systems biology approaches, significant progress has been made in the elucidation and characterization of cellular response networks—the interconnected pathways composed of complex biochemical interactions of

genes, proteins, and small molecules that maintain normal cellular function, control communication between cells, and allow cells to adapt to perturbations in their environment (4). The myriad potential sites of interaction and impact that any given perturbation might have on a cell or organ function and the resulting complexity of gaining insight into how these can affect the entire system can be envisioned (2). This complexity can only be overcome and be of utility through the systematic and integrated approach to manipulation, modeling, and measuring the wide spectrum of activities. Mining of complex and disparate databases is essential to generate nonintuitive insights and testable hypotheses of the causes and sequelae of undesirable perturbations. Two case studies that exemplify the potential of systems biology are discussed below.

Case study 1: Drug-induced vascular injury

This case demonstrates how a systems biology approach can elucidate the pathophysiology of complex and dynamic biologic processes, create testable hypotheses related to these phenomena, and identify potential candidate biomarkers that can be assessed and validated as an indicator of the toxicity (15).

No sensitive and reliable biomarker currently exists for monitoring of the vascular lesions induced by chemicals in preclinical models (16). Moreover, the pathogenesis of these lesions in animals is still unclear. Using modern "omics" technologies, knowledge generation and intelligent networking tools, and targeted modeling methods, the pathophysiology of a well-known but enigmatic phenomenon of chemically induced vascular injury has been elucidated. Not only was the application of a systems biology approach essential to the characterization of the signals and pathways of these events, but long-sought-after candidate biomarkers were also identified. This research endeavor generated over one million data points that were shared with the FDA under their voluntary genomics submission program. After a rigorous analysis of the data, FDA scientists reached essentially the same conclusions about the pathophysiology of drug-induced ischemia and subsequent reperfusion.

Phosphodiesterase 4 (PDE4) inhibitors are a class of drugs that can provide novel therapies for asthma and chronic obstructive pulmonary disease. Their development is frequently hampered by the induction of vascular toxicity in rat mesenteric tissue during preclinical studies. Histopathologically, mesenteric vascular injury is characterized by perivascular edema and mixed inflammatory cell infiltration associated with medial necrosis and hemorrhage (17). Whereas these vascular lesions in rats have been well characterized histologically, little is known about their pathogenesis, and in turn, sensitive and specific biomarkers for preclinical and clinical monitoring do not exist. Development of potentially novel life-saving therapies has therefore been hindered due to the lack of biomarkers for drug-induced vascular injury to confirm that a candidate drug is safe for administration to humans (18). To investigate the early molecular mechanisms

underlying vascular injury, time-course studies were performed in which rats were treated for 2 to 24 hours with high doses of a candidate PDE4 inhibitor. Transcriptomics analyses in mesenteric tissue were performed using oligonucleotide microarray and real-time reverse transcriptase PCR technologies, and compared with histopathologic observations. In addition, protein measurements were performed in serum samples to identify soluble biomarkers of vascular injury. The results show that molecular alterations preceded the histologic observations of inflammatory and necrotic lesions in mesenteric arteries. Some gene expression changes suggest that the development of the lesions could follow a primary modulation of the vascular tone in response to the pharmacologic effect of the compound. Activation of genes coding for pro- and antioxidant enzymes, cytokines, adhesion molecules, and tissue inhibitor of metalloproteinase 1 (TIMP-1) indicates that biomechanical stimuli may contribute to vascular oxidant stress, inflammation, and tissue remodeling. This leads to the proposed time-dependent mechanism of toxicity of PDE inhibitors: (i) ischemia reperfusion-like injury initiates the toxic response followed by the induction of oxidative stress; (ii) release of cytokines such as interleukin-6 (IL-6), TNF, and IL-1B activate the innate immune response; and (iii) the release of specific molecular mediators, such as leukotriene B_4, platelet activating factor, C5a, and oxidized low-density lipoprotein, induces an inflammatory response that leads to vascular necrosis. Indeed, TIMP-1 appeared to be an early and sensitive predictive biomarker of the inflammatory and tissue remodeling components of PDE4 inhibitor-induced vascular injury (19). Importantly, some of the candidate biomarkers identified by these studies are now being assessed and potentially validated in animal and human experiments and may lead to the renewed development of a very important class of potential therapeutics.

Case study 2: Oncology drug-induced cardiovascular toxicity

As multiple types of cancer transition from acute to chronic diseases, the cardiotoxicity of anticancer treatments has emerged as a serious clinical problem (20). Cardiotoxicity can manifest in a variety of ways depending on the type of anticancer treatment being used. For example, anthracyclines generate free radicals, causing permanent myocyte cellular destruction that is related to the cumulative lifetime dose, which limits the usefulness of anthracyclines in oncology (21). In contrast, the monoclonal antibody trastuzumab can mediate transient cardiotoxicity by disrupting cardiomyocyte cellular signaling pathways (22). An understanding of the mechanism of drug-induced cardiotoxicity is crucial in devising methods to treat or prevent this toxicity. Ideally, the use and application of systems biology approaches could provide an opportunity to facilitate or improve (i) selection of the most effective therapies, (ii) identification of specific patients at risk for chemotherapy-induced adverse events, (iii) dose selection and scheduling decisions, and (iv) identification of early

signals of emerging adverse events that may enable prompt clinical responses.

A systems biology approach was used to delineate the signaling pathways involved in imatinib-induced cardiotoxicity. Imatinib is a tyrosine kinase inhibitor that is active against the tyrosine kinase Bcr-Abl in chronic myelogenous leukemia (CML) as well as in the tyrosine kinase C-kit in gastrointestinal stromal tumors (GIST). Unfortunately, imatinib treatment is associated with a 4% incidence of heart failure (23). To identify the mechanism of this toxicity, researchers incubated rat cardiomyocytes with imatinib and determined that imatinib induced the endoplasmic reticulum stress response, leading to cell death (24). The researchers further demonstrated that imatinib mediates cardiomyocyte death through its interaction with Bcr-Abl by transducing into the cardiomyocytes the Abl T315I point mutation which renders the kinase resistant to imatinib (25). Subsequently, a redesigned imatinib was engineered that inhibited only the C-kit tyrosine kinase and no longer had activity against Bcr-Abl (26). Although this redesigned compound was ineffective in treating CML, it retained its activity against GIST and no longer exhibited cardiotoxicity in mouse models. These results demonstrate the potential of systems biology in combination with rational drug design to engineer drugs so that their adverse effects are minimized or eliminated while their desired anticancer effects are preserved.

A Pathway Forward

The current state of safety sciences and the related emerging technologies represent an unprecedented and timely opportunity to make a profound impact on drug development and regulatory decision making. By defining, characterizing, validating and integrating new methods and science into the regulatory decision-making framework, this enterprise will improve public health decision making and enhance the efficiency of bringing new drugs to patients. Overcoming current challenges of safety assessment through new technologies will help us (i) understand the translation (if any) of nonclinical safety signals to the patient population; (ii) aid in the development of safer drugs, beginning at the design phase; and (iii) enhance our understanding of the potential safety impact of a drug on a particular individual by understanding relationships to key personal "omic" signatures. These accomplishments would improve the efficiency of drug research and development and increase the probability of success, adding value to

patient communities by improving access to promising new therapies. Moreover, these changes could also have a profound impact on the business model of the pharmaceutical and chemical industries and help to stem the occurrence of unanticipated adverse effects in late-stage clinical trials or in the postmarketing phase, which can quickly halt the development or availability of novel therapeutics.

The use of systems biology to characterize the inherent risks of pharmaceuticals can markedly improve drug development and postmarketing processes in close collaboration with the FDA and other regulatory agencies. Although resource constraints, computational limitations, and the complexities inherent to human disease may limit the utility of systems biology, data resulting from science and methods centered in systems biology can be readily validated using *in vivo* models and rapidly assessed in humans. The evolving concept of systems biology is starting to be adopted by the pharmaceutical industry and integrated into the safety sciences. A recent example of a substantial move forward in this field is the European Union Framework 6 Project on Predictive Toxicology, which compared conventional toxicologic endpoints for several investigational compounds to transcriptomics, proteomics, and metabolomics profiles (27). These researchers found that the use of systems biology was instrumental in determining mechanisms of nephrotoxicity and hepatotoxicity as well as identifying potential predictive biomarkers. To realize the true potential of systems biology and improve drug safety, a more systematic integration into drug discovery and development is needed. As a direct result of this panel discussion, the FDA has spearheaded an oncology pilot study on the cardiotoxicity of tyrosine kinase inhibitors with the hope that a guidance document can be generated to encourage and accelerate the adoption of systems biology in the development of drugs for oncology.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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