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PANEL 2

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

Ken Turteltaub, Battelle Memorial Institute
John Leighton, Food and Drug Administration
Myrtle Davis, National Cancer Institute
Leigh Ann Burns-Naas, Pfizer Inc
Adam Clark, FasterCures

The material contained in this pre-meeting brief is taken in large part from the annotated references and is not intended to reflect the opinions of the panelists or their respective organizations.

Summary and Proposal

Application of Systems Biology methods can accelerate the introduction of safe, effective drugs. Systems Biology adds insights to 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 modifies chemical entitities to reduce liabilitities during development.[1,2] The FDA should issue a guidance document to encourage and accelerate the adoption of systems biology in the development of drugs for oncology.

Background

Safety is a leading cause of pharmaceutical attrition and a major impediment to efficient and successful drug development. Safety is also a major factor in regulatory decisions involving drug approval, labeling, risk evaluation and mitigation and even withdrawal from the marketplace. Current testing methods and risk assessment have not kept pace with the rapid evolution of technology, biomedical research and knowledge generation. For example, the battery of studies required to meet regulatory guidelines for development and approval of pharmaceuticals rely almost exclusively on *in vivo* animal testing protocols and endpoint assessments that have changed little in decades. However, 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 (AE).[3] It is clear no single new method or testing paradigm will replace entirely the need for *in vivo* testing, but adopting new science and

[1] Raschi E, Vasina V, Ursino MG, Boriani G, Martoni A, De Ponti F. Anticancer drugs and cardiotoxicity: Insights and perspectives in the era of targeted therapy. Pharmacol Ther. 2010 Feb;125(2):196-218.
[2] Pujol A, Mosca R, Farrés J, Aloy P. Unveiling the role of network and Systems Biology in drug discovery. Trends Pharmacol Sci. 2010 Mar;31(3):115-23.
[3] Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B, Heller A. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol. 2000 Aug;32(1):56-67.

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 R & D productivity, enable the ongoing efforts to understand and mitigate adverse events and most importantly, facilitate and expedite the access of new therapies to patients.

Driven by rapidly emerging technologies, there has been a nascent transformation of the safety sciences, from empirical, subjective and observation based disciplines to scientifically grounded, objective and data driven sciences. This evolution has spawned new methods and experimental tools, capable of defining the biological basis of adverse events at the cellular, molecular and biochemical level. These tools create the capability to elucidate complex, highly networked and pleiotropic pathways of toxicities and enable the identification of specific biomarkers of impending undesirable events. These factors provide 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 made 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/benefit. Rather than safety decisions being made on some predetermined threshold, safety multiple or scientific judgment, today's toxicologist can contribute to a systematic and objective decision making process based on specifically relevant data.

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 Systems Biology and new testing paradigms will not replace the need for confirmatory and screening animal studies to fully protect human health. However, as described in a recent study published by the National Research Council, "Toxicity testing is approaching a scientific pivot point and is poised to take advantage of the revolutions in biology and biotechnology. Advances in toxicogenomics, bioinformatics, Systems Biology, epigenetics, and computational toxicology could transform toxicity testing from a system based on whole animal testing to one founded primarily on *in vitro* methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin." [4,5,6,7]

Gaps in Current Testing and Safety Assessment Paradigms

The current battery of preclinical safety studies that is required to support the clinical development of new drugs and marketing approval is mapped out in a library of ICH guidelines that include ICH M3, E14 and S1 to S9. In addition, there are various documents from regulatory agencies that provide recommendations regarding specific toxicities or adverse events such as hepatoxicity and vasculitis. In the preclinical area, the mainstay of activity that attempts to create a foundation of knowledge that demonstrates the safety and limitations of a new drug candidate still relies primarily on animal studies using rigorously defined screening protocols that have not been redefined in decades because of the herculedean efforts that are required to to make changes. Consequently, there is a major knowledge gap in our

[4] Toxicity Testing in the 21st Century. A Vision and a Strategy. The National Academies Press, Washington D.C. 2007.
[5] Andersen ME, Al-Zoughool M, Croteau M, Westphal M, Krewski D. The future of toxicity testing. J Toxicol Environ Health B Crit Rev. 2010 Feb;13(2-4):163-96.
[6] Krewski D., et al. Toxicity Testing in the 21st Century. A vision and a strategy. J Toxicol Environ Health B Crit Rev. 2010 Feb;13(2-4):51-138.
[7] Holsapple MP, Afshari CA, Lehman-McKeeman LD. Forum Series: the "Vision" for Toxicity Testing in the 21st Century: promises and conundrums. Toxicol Sci. 2009 Feb;107(2):307-8. Epub 2008 Dec 9.

understanding of the biology of adverse events (AE's) that markedly restricts the ability of regulators to adopt specific methods to mitigate, manage or avoid exposure of patients to unwarranted drug safety risk. By necessity arising from the etiologic uncertainties of potential drug safety issues, regulators must make decisions to broadly protect public health, rather than consider a specific decision affecting patients at risk while enabling access to those that might receive maximum benefit.

There are a large number of emerging technologies that are enabling new and more focused approaches to unraveling the biology of disease, drug treatment and adverse effects, especially in the areas of Systems Biology, biomarkers, imaging and information technologies. There have been modifications to the conduct of clinical trials as seen with screening IND's, micro-dosing 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 US and the Innovative Medicines Initiative in Europe, for example. On a smaller scale, much has been learned about the utility and limitations of data derived from new science and enabling technologies from the FDA's voluntary exploratory data submission program 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. FDA has also sponsored a number of scientific based meetings to solicit broad input into the challenges of creating and utilizing drug safety knowledge in the mining of AE databases and predicting AE's such as the Office of Clinical Pharmacology and Biotherapeutics meeting in January 2011. These laudable efforts will be accelerated by creating 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 have made significant advances in the last few years, but as these therapies control these diseases, the onerous effects of the drug treatment begin to emerge. Current topics in the sequellae of cancer therapy could provide the momentum and focus to urgently apply these concepts in a similar fashion that was reflected in FDA's laudable approach to facilitating the evaluation of, expedited access to, and approval of drugs for HIV therapy.

Relevant Emerging Technologies

There exists new and highly robust platforms that can potentially generate millions of data points around a biological event, and new statistical and informatics tools have emerged that transform these mountains of data and phenomena into knowledge and meaningful context of both desirable and undesirable drug effects. We believe these tools have the potential to transform early safety testing into much more reliable and predictive methods that rely less on animals and much more on human tissue or cell based assays. These activities can lead to enhanced safety decision making based on understanding the biology of an AE rather than as a default, relying on the perception of risk from an observation utilizing an outdated or irrelevant

testing protocol or screening animal study.[8] These improvements are capable of generating a much needed stream of de-risked drug candidates that endure the rigors of clinical trials and informed regulatory scrutiny. Implementating knowledge-based risk mitigation strategies and focused post marketing pharmacovigilance has the potential to significantly impact the utilization and delivery of new medicines to patients. A number of expert panels in both the US and Europe have called for this change and what is now needed is an embracing of this activity by policy makers and a plan for implementation articulated by by all stakeholders, including regulators. An overview of the many common technologies that have offered some utility to drug safety research is provided in the attached appendices.

Systems Biology

Up to this point in time, the pharmaceutical industry has relied primarily on reductionist approaches to drug discovery, preclinical drug development and most certainly in drug safety methods and assessments. For example, by seeking a single drug for a single target, a single assay for predicting human response and single etiologies for toxicity, these approaches have not been amenable to exploring the dynamic complexity of drug-induced disease or toxicity. Moreover, there is a paralyzing insistence on "validation" that defies a unifying definition for any new approaches and a dogged adherence to *in vivo* models that themselves have not been validated. There is a compelling legacy therefore, to seek alternative approaches that incorporate the ability to explore and elucidate complex and dynamic biogical phenomena.

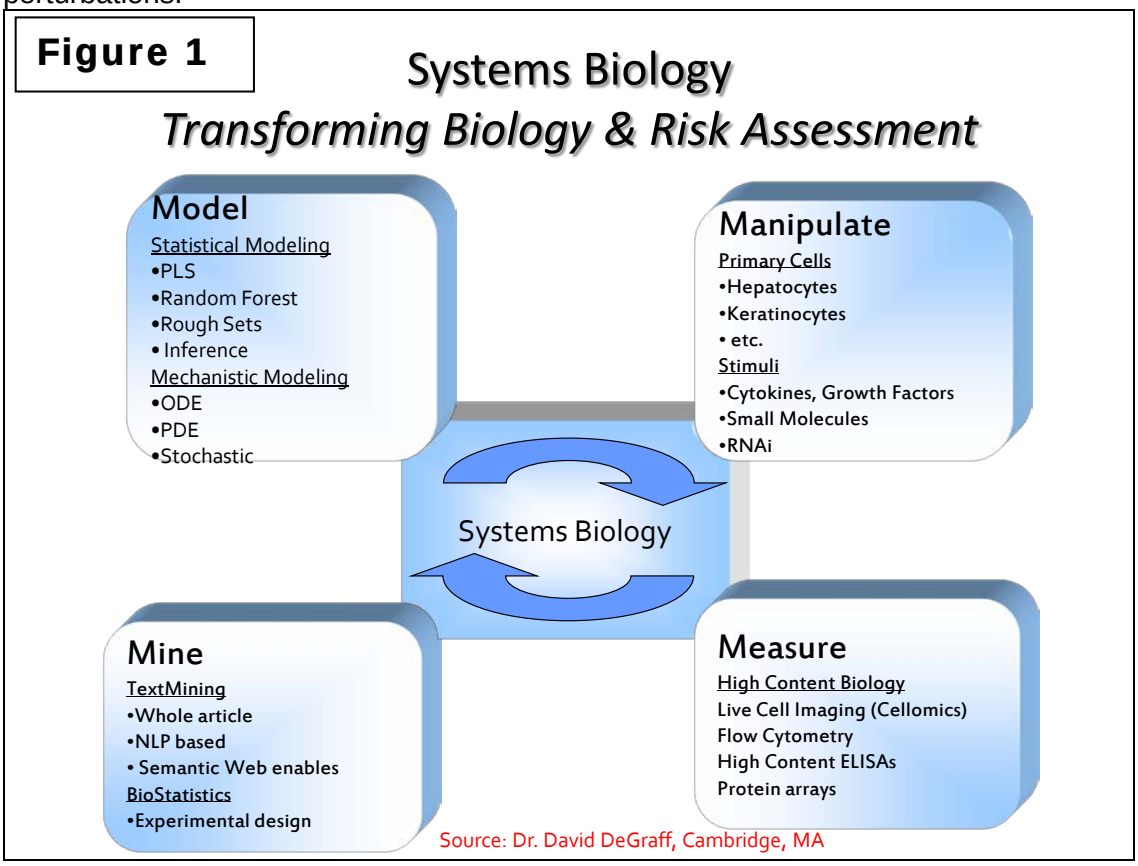
Systems Biology has been defined as the iterative and integrative study of biological systems as they respond to perturbations.[9] Systems toxicology comprises the integration of molecular endpoints and conventional toxicity endpoints into a Systems Biology approach. In a sense, contemporary Systems Biology is a renaissance of physiology, a traditional integrative discipline. As defined by some, there are four basic pillars of activity that define Systems Biology (Figure 1). Biological 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 biological systems, due not only to the large number of their constituents, but also to the intricate web of interactions between these constituents, have proven difficult to understand with reductionist approaches. Research has to be conducted at a more global, systems-level in order to gain understanding of the overall behavior of the biological 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.[10] [11]

Driven by Systems Biology approaches, significant progress has been made in the elucidation and characterization of "cellular response" networks, i.e., the interconnected pathways

[8] Gibb S. Toxicity Testing in the 21st Century. A vision and a strategy. Reprod Toxicol. 2008 Jan;25(1):136-8. Epub 2007 Nov 1.
[9] Auffray C, Imbeaud S, Roux-Rouquié M, Hood L. From functional genomics to Systems Biology: concepts and practices. C R Biol. 2003 Oct-Nov;326(10-11):879-92
[10] Butcher EC, Berg EL, Kunkel EJ. Systems Biology in drug discovery. Nat Biotechnol. 2004 Oct;22(10):1253-9.
[11] Plavec I, Sirenko O, Privat S, Wang Y, Dajee M, Melrose J, Nakao B, Hytopoulos E, Berg EL, Butcher EC. Method for analyzing signaling networks in complex cellular systems. Proc Natl Acad Sci U S A. 2004 Feb 3;101(5):1223-8. Epub 2004 Jan 26.

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] These methods are enhancing our knowledge of cellular response networks and allowing safety scientists to elucidate and model the biology and pathophysiology of adverse events elicited by drugs and chemicals. One example of the value of mapping the interconnected cellular signaling pathways is depicted in Figure 2. The myriad of all the 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 impact 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 plethora of activities. Mining complex and disparate databases is essential to generate non-intuitive insights and testable hypotheses of the causes and sequelae of undesirable perturbations.

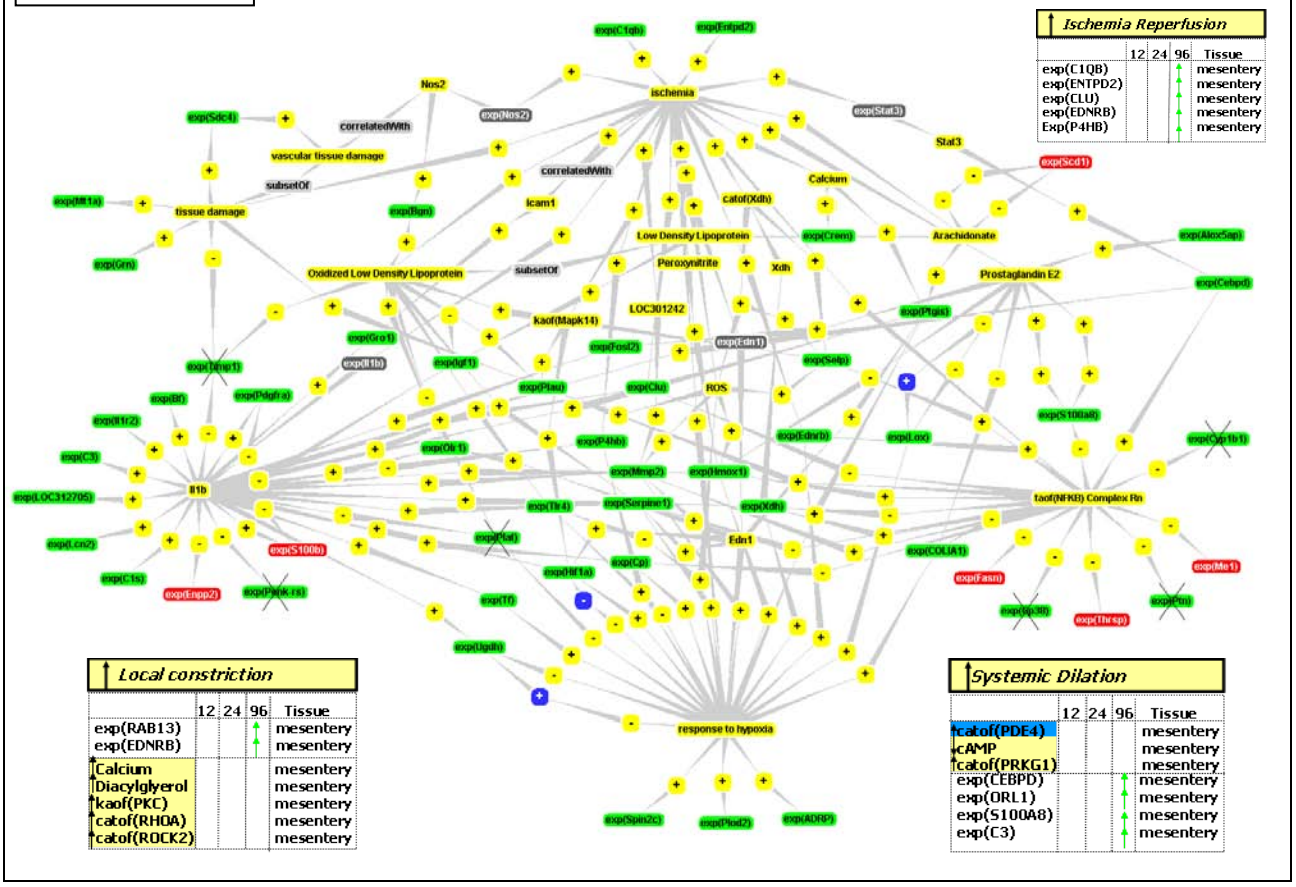
Figure 1



Some of the key platforms that enable the practice of Systems Biology are listed in the appendices and include:

- Proteomics
- Metabolomics/metabonomics/metabogenomics
- Genomics
- Bioinformatics
- Imaging

Figure 2 Representative Pathophysiology Model



Case 1

Case Study 1: Integrated approach to organ injury

DIVI (Drug Induced Vascular Injury)

This case demonstrates how a Systems Biology approach can elucidate the pathophysiology of complex and dynamic biological 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.[12] Moreover, this dataset was shared with the FDA under their voluntary genomics submission program and rigorously evaluated by their scientists.

There is currently no sensitive and reliable biomarker for monitoring of the vascular lesions induced by PDE4 inhibitors in preclinical models.[13] Moreover, the pathogenesis of these lesions in animals is still unclear. The value of an integrated and systematic approach to the study of the biology of complex pathopysiologic processes that is enabled by Systems Biology approaches is depicted in Figure 3. Using modern “omics” technologies, knowledge generation

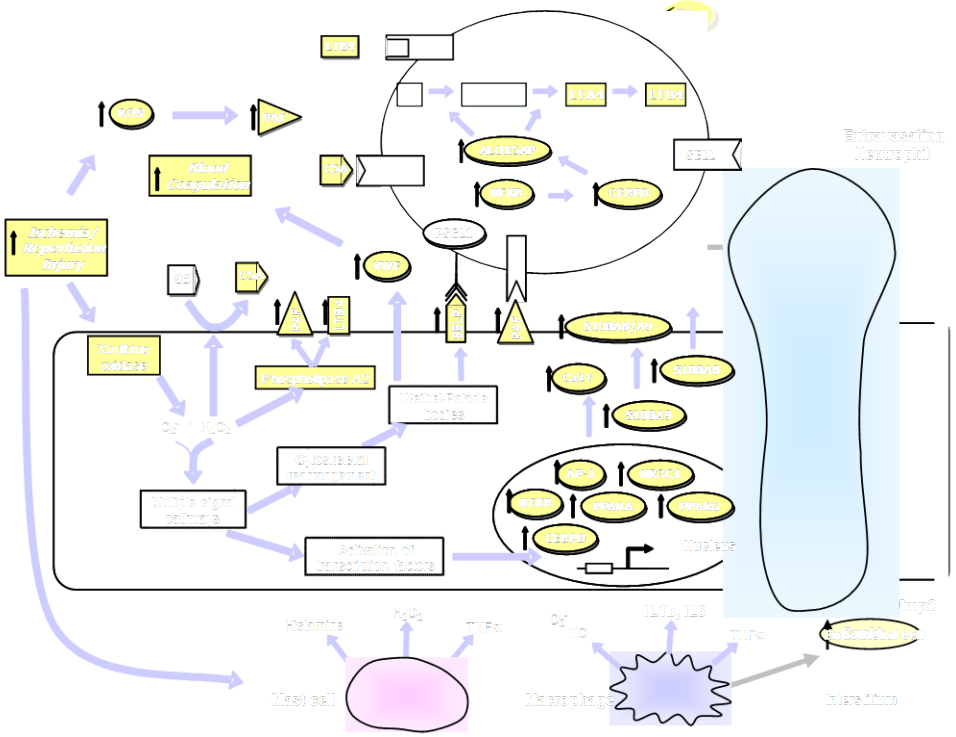
[12] Marrer E, Dieterle F. Impact of biomarker development on drug safety assessment. Toxicol Appl Pharmacol 2010 March 1;243(2):167-79.
[13] Kerns W, et al. Drug-induced vascular injury--a quest for biomarkers. Toxicol Appl Pharmacol. 2005 Feb 15;203(1):62-87.

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 also rigorously reviewed and analyzed by the FDA who reached essentially the same conclusions of pathophysiology of drug-induced ischemia and subsequent reperfusion after analyzing data that had been submitted under their voluntary exploratory data submission program.

Phosphodiesterase (PDE) 4 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.[14] 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 drug-induced vascular injury biomarkers to confirm the candidate drug safety for administration to humans.[15] In order to investigate the early molecular

Figure 3

Pharmacodynamic Induced Vascular Injury and Inflammation: A Schematic



[14] Zhang J, Snyder RD, Herman EH, Knapton A, Honchel R, Miller T, Espandiari P, Goodsaid FM, Rosenblum IY, Hanig JP, Sistare FD, Weaver JL. Histopathology of vascular injury in Sprague-Dawley rats treated with phosphodiesterase IV inhibitor SCH 351591 or SCH 534385. Toxicol Pathol. 2008;36(6):827-39.

[15] Zhang J, Defelice AF, Hanig JP, Colatsky T. Biomarkers of Endothelial Cell Activation Serve as Potential Surrogate Markers for Drug-induced Vascular Injury. Toxicol Pathol. 2010 Aug 17.

mechanisms underlying vascular injury, time-course studies were performed by treating rats for 2–24 h with high doses of a candidate PDE4 inhibitor. Transcriptomics analyses in mesenteric tissue were performed using oligonucleotide microarray and real-time RT-PCR technologies and compared to histopathological observations. In addition, protein measurements were performed in serum samples to identify soluble biomarkers of vascular injury. The results indicate that molecular alterations preceded the histological 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 pharmacological 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. TIMP-1 appeared to be an early and sensitive predictive biomarker of the inflammatory and the tissue remodeling components of PDE4 inhibitor-induced vascular injury.[16] 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 an acute to a chronic disease, cardiotoxicity of anticancer treatments has become an increasingly important clinical problem faced by cardiologists. Left ventricular systolic dysfunction and heart failure generate the most concern, but clinical features and prognosis vary considerably depending on the causative agent. Anthracycline related cardiomyopathy differs fundamentally from effects associated with newer targeted agents, such as trastuzumab. Other forms of cardiovascular disease that occur as a result of cancer treatment include hypertension, thromboembolic disease, pericardial disease, arrhythmia, and myocardial ischemia. The approach to cardiovascular disease in patients with cancer is often different from that in the general population, not only because of distinct underlying mechanisms and clinical features of their heart disease, but also because of the potential ongoing need for additional cancer treatment as well as the altered duration of anticipated survival. In an effort to maximize both quality of life and survival, cardiologists and oncologists should collaborate with the aim of balancing the risks of cardiotoxicity with the benefits of oncologic therapy.[17]

The prototypical example of cardiotoxicity from anticancer treatment is anthracycline related cardiomyopathy. Early observations demonstrated that left ventricular (lv) systolic dysfunction was related to cumulative anthracycline dose, damage was permanent on the cellular level, and could lead to refractory heart failure and cardiac death. Novel drugs, such as the monoclonal antibody trastuzumab, have been introduced that also cause cardiomyopathy, but with clinical features that are fundamentally different from anthra cyclinerelated disease, and with a more favorable prognosis. Trastuzumab related cardiac damage is now known to be less destructive to the myocyte than the damage caused by anthracyclines, and is usually transient, mimicking the stunning or hibernation phenomenon seen with myocardial ischemia.[7] The anthracyclines, and other drugs that predominantly cause irreversible cell destruction, have been designated as type i agents, and those without cell destructionas a dominant characteristic are classified as

[16] Daguès N, Pawlowski V, Guigon G, Ledieu D, Sobry C, Hanton G, Freslon JL, Chevalier S. Altered gene expression in rat mesenteric tissue following in vivo exposure to a phosphodiesterase 4 inhibitor. Toxicol Appl Pharmacol. 2007 Jan 1;218(1):52-63.
[17] Ewer MS, Ewer SM; Medscape. Cardiotoxicity of anticancer treatments: what the cardiologist needs to know. Nat Rev Cardiol. 2010 Aug 24.

type ii agents.[7] Type ii agents do not exhibit toxicity that is related to cumulative dose, and the rever sibility of their effects has been demonstrated in several multicenter adjuvantclinical trials.[6,12,13,18,19] These characteristics have allowed type ii agents to be administered for years without the sequelae that are a cardinal feature of the toxicity associated with type i agents, and to be reintroduced after cardiac recovery with acceptable risk.[3] Although reversibility associated with type ii agents has been called into question,[11] these concerns have arisen from the effects of combined therapy rather than from trials that have used type ii agents in the absence of an anthracycline.[1,11] These considerations do not alter the premise that agents associated with cell death are fundamentally different from those that do not cause cell death. Nevertheless, it is widely agreed that ongoing monitoring of patients for potential longterm cardiotoxicity is prudent. Some of the anticancer agents associated with impaired cardiac function have been enumerated.[16]

Hypothetical agent to be discussed by panel and audience:

- Kinase inhibitor for oncology, second generation (e.g., back-up)
- Prototype has cardiovascular liabilities in the clinic (hypertension, contractility…decreased LVEF, no specific evidence of QT though other kinase inhibitors do)
- A lead series for the BU has a significantly better kinase selectivity profile, but still hits about 10 in potential Ceff range (unknown significance of the hits). Noteably - does <u>not</u> hit Abl.
- Preclinical Data on the BU shows **hypo**tension but a profound decrease in contractility that would be considered clinically-relevant and occur well-below the free plasma concentration producing tumor growth inhibition (TGI). Hepatotoxicity and changes in WBC that may be associated with bone marrow suppression are also evident, though at free plasma concentrations at or very slightly above those producing TGI in mice.
- Mitchondrial toxicity testing does not show a significant effect of the BU kinase inhibitor (the lead looks similar).
- Expression profiling from heart tissue suggests that some genes associated with ion channel biology, calcium homeostasis, and autophagy may be altered. Further work is needed to confirm expression changes and evaluation in additional tissues.

Systems Biology approach to understanding the mechanisms of drug-induced cardiotoxicity associated with use of a broad array of oncology agents would be useful in the following areas.[1,16]

- Identification of individuals at high risk for DICT

[18] Louden C, Brott D, Katein A et al. Biomarkers and mechanisms of drug-induced vascular injury in non-rodents. Toxicol Pathol 2006;34(1):19-26.

[19] Korkmaz S, Maupoil V, Sobry C, Brunet C, Chevalier S, Freslon JL. An increased regional blood flow precedes mesenteric inflammation in rats treated by a phosphodiesterase 4 inhibitor. Toxicol Sci 2009 January;107(1):298-305.

- Enlightened assessment of benefit risk to enable a) reduction of DICT and b) elevation of the chemotherapeutic dose to optimize efficacy
- Determine the optimal choice of chemotherapy agents
- Identification of cardioprotective agents or dosing regimens/schedules
- Develop preventive strategies through identification of pre-dosing risk biomarkers such as Trop I that guide reduction or elimination of DICT
- Identify therapies for DICT once it shows onset leading to early or optimal treatments that minize the oncolytic effects of the cancer therapy
- Identify rationale strategies for prevention of DICT and early interventions

A Pathway Forward

The current state of safety sciences and the related emerging technologies represent an unprecedented and timely opportunity to profoundly impact 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. Over-coming current challenges of safety assessment through new technologies improves the efficiency of of drug R &D and the probability of success, adding value to patient communities in terms of improved access to promising new therapies. It will be poised to have a profound impact on the pharmaceutical and chemical industries business model and stem the safety driven downward spiral of the industry.

Using Systems Biology for characterizing the inherent risks of pharmaceuticals can markedly improve drug development and post marketing processes in close collaboration with the FDA and other regulatory agencies. The science and methods will be centered in Systems Biology and enabling the observations and outcomes of laboratory investigations to be readily validated using *in vivo* models and rapidly assessed in humans. The evolving concept of Systems Biology however is not widely known or adopted in drug discovery or development.

Goals for Next Steps

1) Systems Biology approaches offer major improvement opportunities for identifying safety issues in drug development
2) The FDA should issue a guidance document that would encourage and accelerate the adoption of Systems Biology in the development of drugs for oncology

Appendices

Proteomics

The proteome is the entire set of proteins expressed by a genome, cell, tissue or organism. The proteome is the entire complement of proteins, including the modifications made to a particular set of proteins, produced by an organism or system, which will vary with time and distinct requirements, or stresses, that a cell or organism undergoes. More specifically then, it is the set of expressed proteins in a given type of cells or an organism at a given time under defined conditions. Proteomics, the study of the proteome, has historically been practiced through the separation of proteins by two dimensional gel electrophoresis, but there have been considerable advances in separtation methods in the last few years.

While the genome is clearly important and defines the potential for an organism, we believe proteomics may provide a much better understanding of how an organism is functioning dynamically than genomics. First, the level of transcription of a gene gives only a rough estimate of its level of expression into a protein. An mRNA produced in abundance may be degraded rapidly or translated inefficiently, resulting in a small amount of protein. Second, many proteins experience post-translational modifications that profoundly affect their activities; for example some proteins are not active until they become phosphorylated. Methods such as phosphoproteomics and glycoproteomics are used to study post-translational modifications. Third, many transcripts give rise to more than one protein, through alternative splicing or alternative post-translational modifications. Fourth, many proteins form complexes with other proteins or RNA molecules, and only function in the presence of these other molecules. Finally, protein degradation rate plays an important role in protein content. Understanding the proteome, the structure and function of each protein and the complexities of protein-protein interactions will be critical for developing the most effective diagnostic techniques and disease treatments in the future.

Biomarkers

A biomarker can be defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. A BM is best when it is directly involved mechanistically in phenomena of interest, but can be of value if a strong quantitative and robust correlation exists. Biomarkers can range from a simple small or large molecule to complex patterns of molecular markers or fingerprints, often called a signature, of a biological condition or a response to a specific perturbation.

Metabonomics

Metabonomics is defined as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification". The word origin is from the Greek *meta* meaning change and *nomos* meaning a rule set or set of laws. This approach was pioneered by Jeremy Nicholson at Imperial College London and has been used in toxicology, disease diagnosis and a number of other fields. Historically, the metabonomics approach was one of the first methods to apply the scope of Systems Biology to studies of metabolism.

Metabonomics is potententially useful approach because disease, drugs or toxins either result from or cause perturbations of the concentrations and fluxes of endogenous metabolites

involved in key biochemical pathways. For example, the response of cells to toxic or other stressors generally results in an adjustment of their intra- and/or extracellular environment in order to maintain constancy of their internal environment (homeostasis). This metabolic adjustment is expressed as a fingerprint of biochemical perturbations which is characteristic of the nature or site of a toxic insult or disease process. Urine, in particular, often shows changes in metabolite profile in response to toxic or disease-induced stress because the attempt to maintain homeostasis in the face of a toxic challenge results in changes to the composition of biofluids, particularly excreted fluids like urine. Hence, even when cellular homeostasis is maintained, subtle responses to toxicity .or disease are often expressed in altered biofluid composition.

Genetic Analysis and Expression Profiling

Genomics

Genomics is defined here as the study of the genomes of organisms and their regulation. The field includes intensive efforts to determine the entire DNA sequence of organisms and fine-scale genetic mapping efforts. The field also includes studies of intragenomic phenomena such as heterosis, epistasis, pleiotropy and other interactions between loci and alleles within the genome. Research of single genes does not fall into the definition of genomics unless the aim of this genetic, pathway, and functional information analysis is to elucidate its effect on, place in, and response to the entire genome's networks.

Transcriptomics

Transcriptomics is the branch of molecular biology that deals with the study of messenger RNA molecules produced in individual cells or tissues. The transcriptome is the set of all RNA molecules, including mRNA, rRNA, tRNA, and non-coding RNA transcripts (miRNA, shRNA, etc.) produced in one or a population of cells. The term can be applied to the total set of transcripts in a given organism, or to the specific subset of transcripts present in a particular cell type. Unlike the genome, which is roughly fixed for a given cell line (excluding mutations), the transcriptome often varies with external environmental conditions. Because it includes all mRNA transcripts in the cell, the transcriptome reflects the genes that are being actively expressed at any given time, with the exception of mRNA degradation phenomena such as transcriptional attenuation. The study of transcriptomics, also referred to as expression profiling, examines the expression level of mRNAs in a given cell population, often using high-throughput techniques based on DNA microarray technology. The use of next-generation sequencing technology to study the transcriptome at the nucleotide level is known as RNA-Seq.

Imaging

Imaging biomarkers, those quantified using imaging modalities including Magnetic Resonance Imaging (MRIand Positron Emission Tomography (PET), are attractive for a variety of reasons: the methods of measurement used are non-invasive, and can provide information that cannot be obtained in other ways including a drug’s pharmacology and side effect profile, interaction of a drug and its target, delivery of a drug to its target, and the drug’s pharmacokinetic profile. In the clinical setting, imaging biomarkers can be used as a screening, diagnostic or prognostic tool as well as for monitoring treatment response and consequently are now widely used in drug discovery development, as well as in clinical care. Imaging can also be used in a clinical context to validate mode of action information developed in animal models.

The development of imaging biomarkers has the potential in many cases, to revolutionize basic research, drug development and treatment by providing non-invasive approaches that are translatable from the laboratory to the clinic and by allowing researchers and clinicians to see in great detail how drugs are behaving. The discovery and development of imaging biomarkers is an exciting and growing area and researchers across the globe are working to develop this vision.

All the technologies described above can and are being used to discover new biomarkers that can be measured via imaging. The imaging technologies available today offer a variety of methods that can be used to quantify information and thus create useful biomarkers. Discovering the biomarker is perhaps the easy step, whilst the clinical follow up studies required to gain a better understanding of the utility of the biomarker are more complex, time consuming and expensive.

Image quantification is improving: Nuclear imaging methods – PET and SPECT – are some of the most important to the field of imaging biomarkers because they have the required sensitivity and are potentially quantitative. The development of new molecular imaging probes is a growing and exciting area. MRI has limitations in terms of sensitivity as opposed to nuclear methods, although the methods are often non-proprietary and more MRI scanners are available in clinical practice. Sensitive contrast agents for MRI need to be very sophisticated. Future improvements in sensitivity, computer aided diagnostics and standardization will improve the potential for imaging biomarkers.

Small animal imaging is a rapidly growing area in the preclinical development of new pharmaceuticals. Instrumentation to allow CT, PET, SPECT, MRI, ultrasound or optical imaging of small animals is available from a large number of suppliers and the largest pharma companies are actively developing their capabilities in this area. Some large pharma companies have also invested in dedicated clinical imaging centers, while others have chosen to outsource to specialist academic centers.

In the clinical setting, MRI represents the most highly utilized technology and includes the diversity of methods available under the MRI banner, such as MRS, DCE-MRI, diffusion weighted MRI, fMRI and arterial spin labeling. The wide availability of MRI machines in hospital settings and imaging centers also makes this an attractive technique for biomarker detection. The use of nuclear imaging methods, such as PET and SPECT, is growing. This is catalyzed by the growing availability of targeted ligands that highlight particular pathways or metabolic events.