Quantitative and Systems Pharmacology in the Post-genomic Era: New Approaches to Discovering Drugs and Understanding Therapeutic Mechanisms

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Abstract

Document: This white paper arises from two workshops held at the NIH to review the state of the art in systems biology and pharmacology. Workshop participants included leaders in the two fields working in academia, industry and government (including the FDA). They asked whether a merger of systems biology and pharmacology via the emerging discipline of *Quantitative and Systems Pharmacology* (QSP) could advance the discovery, development and clinical use of therapeutic drugs. Preparing the White Paper involved a year of consultation with a diverse community of academic, industrial and government scientists but the views expressed herein are solely those of the authors.

Definitions: QSP is defined as an approach to translational medicine that combines computational and experimental methods to elucidate, validate and apply new pharmacological concepts to the development and use of small molecule and biologic drugs. QSP will provide an integrated "systems-level" approach to determining mechanisms of action of new and existing drugs in preclinical and animal models and in patients. QSP will create the knowledge needed to change complex cellular networks in a specified way with mono or combination therapy, alter the pathophysiology of disease so as to maximize therapeutic benefit and minimize toxicity and implement a "precision medicine" approach to improving the health of individual patients.

Findings: The QSP working group has identified an urgent need to reinvigorate academic pharmacology as a core discipline of translational medicine. It is our unanimous conclusion is that this should be accomplished by integrating concepts, methods and investigators from computational biology, systems biology and biological engineering. Differing views of QSP in academe and industry need to be unified under a single umbrella. Genomics will play an important role in this effort but is expected to be insufficient in and of itself as a means to revitalize pharmacology and revolutionize practical drug discovery.

Expected outcomes: The primary results of a successful QSP effort are expected to include: (i) advances in fundamental understanding of therapeutic and toxic drug activities in individuals with diverse genotypes; (ii) new approaches and tools to link pre-clinical and clinical studies of drugs and disease (iii) increased probability that newly discovered drugs will prove therapeutically beneficial in patients (iv) identification of new uses for existing drugs, particularly generic drugs, as a means to improve human health and reduce the cost of medical care and (iv) a multi-faceted training program that will educate graduate students, postdoctoral fellows and clinician-scientists to become leaders in academic and industrial pharmacology and translational biomedicine.

Recommendations: We recommend the establishment of NIH-supported interdisciplinary research and training programs focused initially on eight specific research challenges. These programs involve activities at a variety of scales from individual research teams to multi-investigator (and multi-institution) laboratories and centers. Both the research and training goals of QSP, particularly those involving clinical trials, will benefit greatly from collaboration among academic and industrial scientists and between clinicians and basic scientists involved in multi-year pre-competitive private-public partnerships.

Executive Summary

Background:

This white paper arises from two workshops held at the NIH in 2008 and late 2010 with participation from academia, industry and government (http://meetings.nigms.nih.gov/?ID=8316). The goals of these workshops were (i) to review the state of the art in systems biology and in pharmacology and (ii) to determine whether a merger of the two in the new discipline of *Quantitative and Systems Pharmacology* (QSP) might significantly advance the discovery, development and clinical use of therapeutic drugs. Whereas the first QSP workshop focused on approaches and methods, the second workshop focused on impact, significance and recommendations.

The meetings revealed that there exists an urgent need to reinvigorate academic pharmacology as a core discipline of translational medicine. The need for new approaches to drug development is clear: although the rate of progress in basic biomedical research is high, it remains difficult to translate preclinical discoveries into meaningful medical progress. Pharmaceutical companies face growing difficulties bringing new drugs to market, and very little academic research is focused on the question of how to improve the efficiency and predictability of this process. The reinvigoration of pharmacology can best be accomplished, in our opinion, by introducing concepts, methods and investigators from computational biology, systems biology and biological engineering, thereby allowing modern pharmacologists to apply systems-level ideas to practical problems in drug development. QSP has deep roots in classical pharmacology and physiology but adds a molecule and systems-level approach that allows drug responses to be studied in the context of increasing knowledge of the complex and subtle interconnectedness of signaling, transcriptional and metabolic networks, as well as the variation in individual patients arising from differences in genetics and environment.

Innovation:

QSP will require innovative science and new organizational structures: simply scaling up existing ideas and methods will not work. A growing understanding of cellular and tissue-level networks suggests that the therapeutic and toxic effects of drugs can best be understood at a systems level. Biochemical networks targeted by drugs are qualitatively similar but quantitatively different in different tissues, genetic backgrounds, development stages and disease states, and the operation of these networks is profoundly impacted by patient lifestyle and history. The effect of a drug on a network — positive or negative — can therefore only be fully understood in terms of multi-factorial and quantitative differences. QSP is predicated on the thesis that quantitative experiments and mathematical analysis of biological networks in health and disease will lead to a better understanding of the multiplicity of factors influencing drug effects, and that this understanding will reveal new ways to intervene therapeutically in disease pathophysiology, while minimizing toxicity. Thus, QSP is innovative in breaking decisively with a "one-gene, one-receptor, one-mechanism" approach in favor of a network-centric view that relies on mathematical models to achieve the necessary integration of data and hypotheses. It also embraces the concept that quantitative measurements made throughout the drug discovery process, often with new and emerging technologies (a bedside to bench paradigm), are essential for informing and testing models.

Significance:

We anticipate that QSP will have a significant impact on multiple stages of the drug discovery pipeline and will complement prevailing approaches involving genomic medicine. QSP draws on existing ideas and established concepts from traditional pharmacology, physiology and target-based drug discovery and will therefore serve as a link between pharmacology/physiology and new systems-level and "omics" approaches.

To evaluate the full impact of QSP, one must remember that the mechanisms of action of most drugs are not fully understood and the origins of patient-to-patient variability in therapeutic and adverse responses are often obscure. For both new and existing drugs, the commercial, regulatory and legal environment in which drugs are approved and sold discourages research into these topics. Once new drugs reach the stage of clinical development there is a reluctance to pursue basic research, often for fear of triggering regulatory review. And drugs that fail at late stages of clinical trials are rarely investigated further to determine the reasons for their failure. Moreover, since industry scientists are focused on the discovery and approval of new drugs, and academics have limited capacity to study drugs in patients in the absence of industry involvement, even less research is done on the mechanisms of action of approved and off-patent drugs. These factors tend to create a scientific vacuum around the pharmacology of both standard-of-care and investigational therapeutics, hindering their effective use and presenting a wide range of potential opportunities for innovative research.

Approach:

The distinguishing feature of QSP is its interdisciplinary approach to an inherently multi-scale problem. QSP will create understanding of disease mechanisms and therapeutic effects that span biochemistry and structural studies, cell and animal-based experiments and clinical studies in human patients. Mathematical modeling and sophisticated computation will be critical in spanning multiple spatial and temporal scales. Models must be grounded in thorough and careful experimentation performed at many biological scales. To achieve this, QSP must promote institutional structures that support integrative, systems-based computational and experimental approaches to pharmacology and reward interdisciplinary research in addition to traditional field-specific knowledge.

Anticipated Outcomes:

The primary results of a successful QSP effort are expected to include: (i) advances in the fundamental understanding of how drugs act, specifically with regard to the beneficial and toxic effects of drugs on healthy and diseased cells with diverse genotypes; (ii) new approaches and tools for aligning pre-clinical and clinical studies and translating discoveries made in cells to tissues and then to patients; (ii) new pharmaco-dynamic biomarkers that assay directly the effects of drugs in tissues and patients (iv) an increase in the probability that newly discovered drugs will prove therapeutically beneficial in individual patients, together with a reduction in the risk of serious adverse events, thereby increasing the rate of success in clinical trials (particularly with respect to efficacy in Phase II); and (v) a multi-faceted training program for educating graduate students, postdoctoral fellows and clinician-scientists to become a new generation of leaders in academic and industrial pharmacology and translational sciences.

Recommendations:

To make QSP a reality we recommend interdisciplinary research programs focused initially on eight specific research challenges, multi-faceted training activities and private-public partnerships aimed at engaging investigators from industry, regulatory agencies and academics in the biological, mathematical, engineering and medical communities. Because industry has an acute need for trainees with strong skills in quantitative reasoning, network biology, and animal and human pharmacology, industry should be engaged in education as well as research. The emphasis in academia should be on creating and testing new ideas that form the intellectual foundation for QSP: the classical approach to pharmacology was developed over fifty years ago and requires overhaul. The eight research areas we recommend emphasizing are:

- Characterizing quantitatively and precisely the biochemistry of drug targets, the networks in which they are embedded and the effects of small molecule and biologic drugs
- Investigating the origins of variability in drug response at the single-cell, organ and patient level that arise from differences at the level of the proteome, genome and environment

- Exploiting diverse clinical and omic data to create pharmacodynamic biomarkers that inform integrated, multi-scale models of drug response determinants in distinct patient populations
- Developing better animal and tissue models for pre-clinical pharmacology with the aim of better target validation and fewer Phase II failures
- Reconnecting tissue physiology with chemistry to facilitate pharmacological experimentation and phenotypic screening on complex systems (cells and model organisms)
- Developing and supporting information exchanges for QSP, particularly in the area of clinical data and electronic medical records
- Developing new multi-scale computational models of pharmacological mechanism that span the divide between cell-level biochemical models and organism-level PK/PD models
- Developing approaches to "failure analysis" as a means to understand why drugs fail in clinical trials and how such failure might be avoided in the future

DEFINITIONS OF SYSTEMS PHARMACOLOGY

- Quantitative and systems pharmacology is currently defined differently in academia and industry based on a fundamental disconnect between cell-level and organism-level models.
- We propose a common definition that spans pre-clinical and clinical studies and includes both experimental and computational approaches.

Background

Quantitative and systems pharmacology (QSP) is a newly emerging discipline and its definition is therefore in flux (see for example the July 2010 issue of the journal *Clinical Pharmacology and Therapeutics*). An important insight from the NIH-sponsored workshops is that academic and industry scientists mean rather different things by "systems pharmacology" but that merging the two is likely to be highly productive (Figure 1). Academics have generally defined systems pharmacology as an extension (or even rediscovery) of classical pharmacology and an area of application of systems biology: "*systems pharmacology involves the application of systems biology approaches, combining large-scale experimental studies with model-based computational analyses, to study drug activities, targets, and effects*" [1]. The discipline is often defined with reference to engineering and pharmacological principles as "*the quantitative analysis of the dynamic interactions between drug(s) and a biological system… [that] aims to understand the behavior of the system as a whole, as opposed to the behavior of its individual constituents*" [2]. In contrast, the term "systems pharmacology" in industry is largely associated with pharmacodynamic (PD) and pharmacokinetic (PK) modeling [3], in which complex physiological processes involved in the distribution and action of drugs are coarse-grained and modeled as a series of interconnected "black boxes" or "compartments". While not mechanistic at a molecular level, PK/PD engages directly with drug activities and disease processes at the level of human patients. PK/PD models are used widely in clinical trials and even in financial prediction and are regarded as key drivers of drug development in industry[4]. We see these differences as a positive for the field: each group has much to learn from the other and both aspire use post-genomic experimental methods and multiscale modeling methods to address many of the problems that are primary concerns of classical pharmacology [5]. The vision of QSP that emerged from the NIH workshops and articulated in this white paper encompasses both previous definitions.

Proposed working definition

Quantitative and Systems Pharmacology (QSP) is an emerging discipline focused on identifying and validating drug targets, understanding existing therapeutics and discovering new ones. The goal of QSP is to understand, in a precise, predictive manner, how drugs modulate cellular networks in space and time and how they impact human pathophysiology. QSP aims to develop formal mathematical and computational models that incorporate data at several temporal and spatial scales; these models will focus on interactions among multiple elements (biomolecules, cells, tissues etc.) as a means to understand and predict therapeutic and toxic effects of drugs. Creation of multi-scale models that ultimately span knowledge of molecules, cells, tissues and patients will be particularly critical for pre-clinical and clinical research teams evaluating target selection and testing therapeutic proof of concept. QSP draws on several existing disciplines, including classic pharmacology, chemical biology, biochemistry and structural biology, molecular genetics and genomics, pathology, applied mathematics, and medicine, and has an intrinsic and extensive experimental component that incorporates approaches from tissue and organ physiology, pharmacology and cell biology as well as bioinformatics and "-omics" approaches. QSP will accelerate drug discovery and development by helping to identify and validate targets (and druggable) networks, uncover drug-response biomarkers, design better drugs and drug combinations, select appropriate doses and dosage regimens and identify those patients most likely to respond to new therapeutic agents and combinations. It will therefore become a core discipline of translational medicine.

HISTORICAL AND CONCEPTUAL CONSIDERATIONS

- Systems pharmacology has deep connections, conceptual and historical, to physiology and classical pharmacology, as well as to newer systems biology and "–omic" approaches.
- Pharmacology is an inherently multi-scale discipline that seeks to integrate knowledge gained through molecular studies in simple biological settings (purified proteins, cells) to efficacy in multi-organ systems and animal models and ultimately efficacy and toxicity in man.
- Systems biology in academia has four branches, all of which are relevant to pharmacology: (i) systematic measurement and "omic" analysis involving genes, proteins, metabolites etc.; (ii) elucidation of broad principles of biological design and the role of noise in signaling networks; (iii) precise mathematical modeling of biological networks as a means to test specific hypotheses and understand disease; and (iv) synthetic biology.
- Systems biology, like classical pharmacology, is inherently quantitative; however systems biology is more "horizontally integrated" and explicitly focuses on networks and multifactorial control over biological processes rather than on drugs and targets in isolation.
- Systems pharmacology adds "vertical integration" to the existing discipline of systems biology and a strong commitment to studying drugs in humans. Achieving vertical integration implies the need for multiscale approaches and the ability to integrate data and concepts from the level of molecules to the levels of cells, tissues and organisms.
- QSP will draw new kinds of scientists into the business of discovering and studying drugs – bioengineers, computer scientists, physicists and mathematicians – many of whom have traditionally eschewed the "applied" world of drug development.

The emerging discipline of systems pharmacology has deep conceptual and historical connections to traditional disciplines such as physiology and classical pharmacology. Physiology in particular is fundamentally a system-level discipline, informed by concepts such as "*homeostasis, set-points, regulation, feedback control and redundancy to explain and model the interactions between cells, organs, systems and organisms*" [6]. Pharmacology addresses how drugs alter this physiology, ideally to restore normal function. Pharmacology has always been a quantitative science, concerned with time and dose-response relationships that accompany interaction of drugs with multifaceted, multi-scale physiology [7].

Because the disciplines of pharmacology and systems biology share an interest in precise, mathematical relationships between perturbations (such as drug dose and exposure, but also genotypic and environmental variation) and physiological consequences (most often drug action, broadly defined, and measured by biomarkers and disease metrics) the idea of merging the two approaches is conceptually compelling. Systems approaches to pharmacology will benefit from mechanistic insight built on decades of molecular and structural studies, genome-wide data on the constituents of biological networks, multiplex "-omic" technologies that can rapidly interrogate the levels and states of many biomolecules (genes, proteins, lipids, metabolite, etc.), a plethora of modern imaging methods that provide information at scales ranging from the single molecule to the whole patient, and profound advances in computer science. The successful development of QSP will make it possible to interrogate normal and disease states using molecular and pathway markers, and to understand pharmacological effects with a breadth and analytical sophistication that would have astounded (and undoubtedly delighted) classical pharmacologists such as James Black.

Classical pharmacology: a brief Overview

In its classical period—roughly from the acceptance of the receptor hypothesis in the 1930s—to the advent of reductionist, molecular biology-focused programs in the mid-1980s (see below), pharmacology was a systems-based science. It had two primary pillars—the study and quantification of drug behavior in the body comprising pharmacokinetics (PK: "what the body does to the drug") and pharmacodynamics (PD: "what the drug does to the body") and the "receptor hypothesis", the idea that drug action is mediated through binding to specific target molecules (which are usually proteins). That this was only a hypothesis seems as peculiar today as the "atomic hypothesis" developed at the turn of the 20th century, but in its time the receptor hypothesis was controversial. Also surprising to the modern eye is the way receptors were defined and studied using drugs. Whereas today we begin with

specific proteins and seek drugs that bind to or alter them, classical pharmacology began with collections of structurally related organic molecules that, based on their similarities and differences with respect to assayable activities on tissues and organisms, were used to define receptors and then the pathways downstream of these receptors in whole tissues or whole organisms[8].

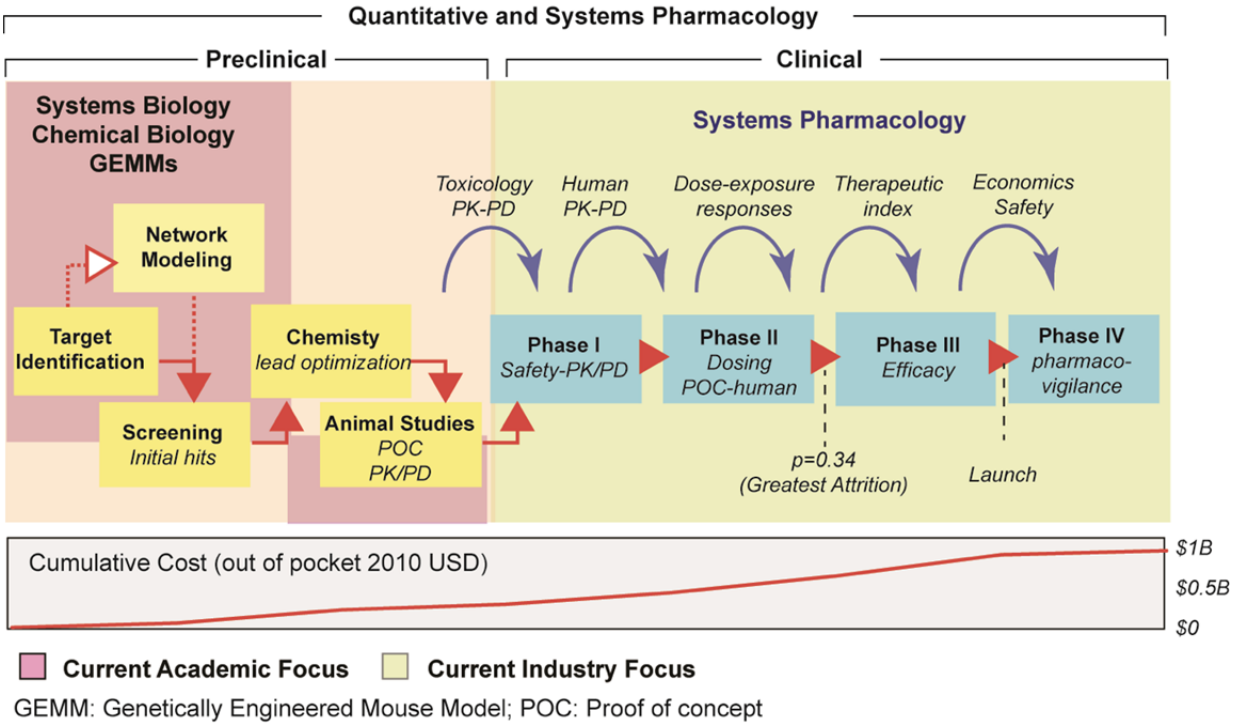

Figure 1. Traditional stages in drug development and discovery showing potential impact of QSP. Systems pharmacology is already established as an activity in the pharmaceutical industry with a focus on pharmacokinetic/pharmacodynamic (PK/PD) modeling, predicting dose-exposure responses and evaluating market potential (blue arrows). In contrast, the academic disciplines of systems biology, chemical biology and mouse modeling of human disease focus on pre-clinical studies and particularly on understanding the activities of potential drug targets (and other biomolecules) in the context of cellular networks, on designing chemicals to probe these networks and on creating mouse models that recapitulate key aspects of human disease. These areas of academic research are poorly coupled to systems pharmacology as it is practiced in industry and bridging this gap represents a key opportunity for QSP. Aspects of this figure were derived from references [10] and [9].

Thus, Ahlquist first divided the adrenergic receptors into α and β sub-classes in 1948 based on differing dose-responses to norepinephrine, epinephrine and isoproterenol in the uterus, nictitating membrane and gut [11]. Twenty years later, Lands assayed fatty acid mobilization by bronchodilation and vasodepression in whole organisms using the same chemical compounds ("ligands") and further divided the β-adrenergic family into β1 and β2 receptors based on the specificities of effects [12]. The distinction between α and β and between β1 and β2 adrenergic receptors was further strengthened by the appearance of the first β-blockers (e.g. propranolol) and subtype selective agonists (salbutamol for β2 and atenolol for β1 receptors), which were to become life-changing drugs. Similarly, subdivision of the histamine receptor H1 and H2 classes followed from the discovery of histaminergic responses in the isolated guinea pig ileum (involving response to histamine but not to the agonist mepyramine and antagonized by burimamide) [13]. In the same vein, Gaddum in the 1950s based differentiated receptors responding to serotonin into two sub-types on effects either on contraction of smooth muscle or depolarization of cholinergic nerves; the receptors involved were subsequently classified as 5-HT1, 5-HT2 and 5-HT3 families based on antagonism by drugs such as bemestron and tropisetron. In none of these studies were receptors isolated or identified in a molecular sense and the underlying logic was the precise opposite of current "targeted" methods for finding

drugs. However, it is noteworthy that the classical approach created many successful drugs. One factor in this success may be that the molecules identified using classical approaches were intrinsically able to penetrate cells and tissues and were assayed against the integrated circuitry of a whole tissue or organism; in many cases the tool compounds used to elucidate receptors were only a few steps away from the molecules that would become transforming drugs. We anticipate that QSP will enable a reinvention (largely through the application of modern, multiplex measurement approaches) of a drug discovery paradigm based on monitoring drugs in complex biological systems, a process that has historically been largely empirical and in some cases even serendipitous.

Historically, pharmacokinetics has been a major challenge in classical drug discovery: many compounds failed because they had unfavorable biological half-lives or distribution in humans [14]. However, isolation and characterization of Phase I and II drug metabolizing enzymes coupled to better medicinal chemistry and improved measurement methods, tissue culture models of human barrier epithelia and sustained efforts to develop mathematical models of pharmacokinetics had a major impact. As a consequence, these days few new drugs fail because of poor PK properties (Figure 2) [14]. Today, the pressing research need is no longer in the area of classical medicinal chemistry, but rather improving efficacy, which means identifying new and better targets (or combinations of targets). This requires better prediction, which in turn requires better measurement and computational modeling. The historical success of measurement and modeling in the context of PK is significant with respect to arguments in favor of QSP: both involve combining measurement and mathematical modeling in the study of drug action.

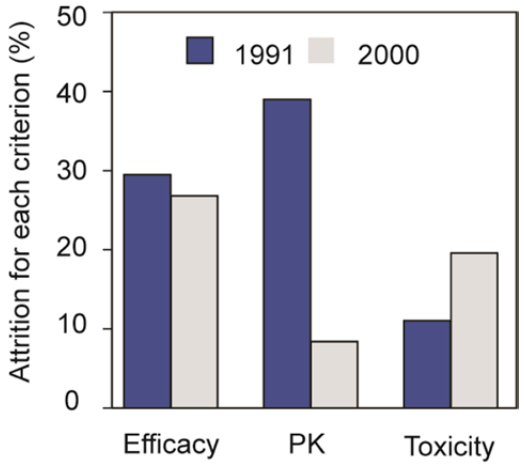

Figure 2. Historical success of pharmacokinetics in reducing attrition in drug discovery. Some common causes of attrition in drug discovery and development are shown over a ten year period. The greatest success in reducing attrition has been achieved with a PK approach that combines modeling and measurement to address bioavailability. Other sources of attrition have not been addressed successfully (e.g. efficacy) and some have gotten worse (e.g. toxicity). If anything, overall success rates decreased from 1991 to 2000. Numbers do not add to 100% because several causes of attrition are not accounted for in the graph. A complete picture is provided by reference 14 from which this figure was adapted.

Targeted drug discovery

Notwithstanding its successes, the target-by-inference approach was deeply frustrating to its practitioners because it limited research and drug development to areas where tool compounds were available. When molecular methods emerged to characterize and isolate receptors, beginning with radio-ligand displacement methods in the 1970s and followed by cloning and receptor transfection in the mid-1980s, pharmacologists abandoned their classical tool set in favor of molecular tools. A contemporary reductionist, or "targeted," program in pharmacology begins by identifying targets hypothesized to have a direct connection to human disease, often based on molecular studies in cells and animal models (including genetically engineered mouse models or GEMMs). Even with the advent of highly multi-factorial genomic approaches and large-scale analysis of patient samples, the favored means for finding new drugs has involved reducing a disease to the simplest possible set of defined targets against which new drugs can be sought. It is evident that such reductionist approaches have been highly successful and have yielded rapid progress in understanding protein mechanism and identifying candidate drug targets for many diseases. The rapid development of highly effective drugs that *in combination* inhibit at least five different targets in the HIV life cycle has turned AIDS from a lethal infection into a manageable, chronic disease. Also highly significant are drugs such as HMG-CoA reductase inhibitors for hypercholesterolemia (statins; e.g Atorvastatin) and inhibitors of oncogenes such as Bcr-Abl (imatinib - Gleevec® - for the treatment of chronic myelogenous leukemia). Design of

protein drugs and therapeutic antibodies is particularly dependent on a targeted approach and important successes include trastuzumab (Herceptin® for breast cancer) and infliximab (Remicade® for rheumatoid arthritis). Targeted drug discovery is more "rational" than exposing complex tissues to compounds, it makes extensive use of advances in genetic engineering, it is more amenable to optimization, and it has yielded therapeutic drugs more likely to survive regulatory review.

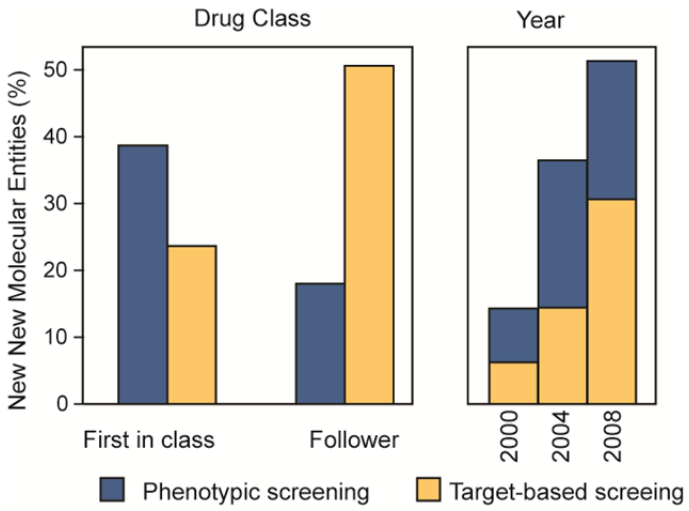

Figure 3. Impact of phenotypic and target-based drug discovery for small molecules 1999-2008. Phenotypic approaches relied on assays that measure a specific physiological phenomenon, with little understanding of molecular mechanisms of action. Left panel compares first in class new medical entities (NMEs) with follower drugs and right panel the proportions of first in class NMEs discovered using each approach. Numbers do not add to 100% because biologic drugs and natural products are not accounted for. The data underrepresent the impact of targeted approaches since follower drugs are ~3-fold more numerous than first-in-class drugs and biologics generally involve targeted approaches. A complete picture is provided by Swinney and Anthony (2011) from which this figure was adapted (Reference 8).

As a natural outcome of the dominance of targeted drug discovery approach, pharmacologists have tended to study the behavior of the drug at two extremes: (i) idealized conditions such as the purified protein (or a tagged protein in a cell or animal) during the preclinical phase of development, or (ii) drug distribution and effects on the whole organisms (including humans, both healthy and diseased) during the clinical phase of development. This dichotomy largely explains the differing perspectives of academics and industry-based scientists with respect to systems pharmacology: academics involved in network-based biology have focused on modeling as a means to revamp the preclinical research paradigm, whereas industry has focused on using modeling to advance PK/PD (Figure 1).

Challenges to the current approach to discovery

Although highly reductionist approaches have increased the rate at which new targets can be identified and pursued, it is not as clear that this has led to a corresponding increase in the rate of delivering effective clinical candidates [10] (Figure 3). Indeed, a recent review highlights the value of phenotypic screens and their successes relative to target-focused methods [8]. This appears to be particularly true of complex diseases, including most cancers, inflammatory diseases, metabolic diseases and diabetes, and most neurological and psychiatric diseases. Similarly, a targeted approach has had very little impact on the development of antibacterials even though it has revolutionized antivirals. In the case of antibiotics discovered in the traditional manner, such as penicillins for example, it is not clear that a focused target-based approach would have worked: the efficacy of penicillins arises from their ability to block multiple distinct transpeptidases. Other successful antibiotics also attack complex protein machines, not single targets. The same can be said of many CNS active drugs, such as the anti-psychotics, which appear to owe their efficacy to an ability to act on multiple targets (usually neurotransmitter receptors or pumps) and for which the target-oriented approach has proven notoriously unreliable. It can therefore be argued that, by shifting to highly target-driven approaches, pharmacologists surrendered a valuable systems-level perspective in which consideration of complex homeostatic networks was implicit.

"High-content screening" (HCS) has come to the fore in the past decade as a response to the limitations of the target based approach and to exploit technology advances. In HCS drugs are screened for activity in living cells and in organisms such as worms, flies and fish [15, 16]. HCS

attempts to recapture the benefits of the "target by inference" approach from the 1940s-1960s in a modern context, by screening for a beneficial effect of a drug on a whole pathway or organism and then seeking out the molecular interaction responsible for the effect. This attractive approach suffers three main limitations, (i) it is not possible to model many diseases accurately in simpler organisms that are amenable to high throughput screening; (ii) target identification is usually needed subsequent to screening for HCS hits to have any realistic chance of translation, and identifying targets of small molecules remains a major challenge to chemical biologists; and (iii) HCS has not yet applied approaches that quantify drug action at a network level using computational and omic approaches. We envision a highly effective integration of HCS with QSP, since they are similar in spirit in their goals of measuring and modeling drug action at the network level, including pathways within cells or between cells in the same or different tissues. The tools and approaches developed by QSP are likely to facilitate target identification in HCS. At a practical level, we anticipate a strong interaction between new QSP programs and existing chemical biology programs in academe and between QSP and medicinal chemistry in industry.

Relevant concepts in systems biology

Systems biology is a relatively new discipline although one with deep historical roots in the physiological models of Hodgkin and Huxley [17], Purcell's studies of signaling in bacteria [18, 19], Guyton's models of cardiac output and multi-organ regulation of electrolyte balance in the plasma [20], and Noble's electrophysiological analysis of the human heart [21]. Interest in this discipline has increased dramatically for several reasons: (i) the perception that biology is entering a "post-genomic" phase in which most of the molecular constituents of humans have already been identified, shifting the emphasis to a deeper characterization of interactions among biomolecules involved in physiological responses and the emergence of specific responses from those interactions; (ii) widespread interest from engineers, mathematicians, computer scientists and physicists in the problems of biology; and (iii) the increasing power of modern computation. The distinguishing feature of systems biology is its combination of computational models and quantitative experimental data as a means to generate formal representations of biological process and discover network-level ("emergent") properties not evident from the study of individual components [22, 23].

In engineering and physical sciences, it is widely recognized that formal models are more rigorous, extendable and predictive than verbal descriptions and simple drawings ("word models") [24]. Some experimental biologists find systems biology hard to accept, arguing that mathematical models are too theoretical and too dependent on assumptions to be useful (although drawing a picture of a biochemical pathway does not involve any fewer assumptions), or that quantitative analysis can only add trivial details to an existing conceptual framework. It is not the task of this report to address arguments about the current impact or future potential of systems biology, but it is worth noting a few obvious examples of success. Understanding how three proteins control circadian rhythm in cyanobacteria has proven difficult using "word models" but possible using a combination of kinetic modeling and experiments [25]. At the opposite pole of biological complexity, Tanaka and Augustine have shown how a positive feedback loop of signaling molecules, predicted nearly a decade before [22], can control long-term depression in Purkinje neurons in rat brains [26]. Systems biology approaches have been valuable in elucidating master regulators of both physiological [27] and pathological [28] processes in brain tumors based on the reconstruction of context-specific gene regulatory networks from large scale molecular profiles data. A combination of systems and genomic methods has identified relationships between heritable genetic variants and specific obesity and diabetes phenotypes [29]. Moreover, it is worth noting that the idea of iterating between models and data, with new experiments suggesting model modification, and model prediction guiding experimental design, has been a mainstay of physical sciences and engineering for decades and is not new to systems biology or pharmacology.

Contemporary systems biology: four complementary approaches

Systems biology is advancing in four distinct but complementary directions, all of which are relevant to pharmacology. The first involves large-scale measurement and network inference. This approach aims to discover interactions among hundreds or even thousands of genes and proteins using systematic, high-throughput measurements (e.g. mRNA profiling, two-hybrid screening, mass spectrometry-based proteomics and metabolomics). The resulting data, which typically derive from high-throughput genomic, proteomic or other –omic approaches are assembled into complex networks whose properties are studied using graph-based methods derived from computer science. Networks of this type have been used to characterize drug targets in a systematic manner [30-32] and are increasingly important in developing disease classifiers based on sequence or transcription data (sometimes called "systems medicine" [28, 33]). The second direction involves attempts to elucidate the principles of biological design or function based on analogies with engineering or physics. Properties elucidated for one biological network may be generalized into concepts such as "feed forward control", "robustness", "adaptation", etc. A notable success of these efforts has been the recognition that noise plays an important role in limiting the accuracy of biochemical circuits and in creating cell-to-cell variability; conversely, the ability of some regulatory motifs (positive feedback for example) to increase precision in the face of this variability has attracted interest in it as a design feature [34]. The third thrust in systems biology involves combining mathematical modeling of regulatory and signaling pathways with multiplex and single-cell experimental data as a means to understand the precise biochemistry, dynamics and functions of the networks that control normal cellular physiology and cause disease. This approach is a natural complement to molecular, structural and cellular biology [35, 36]. At the moment, this type of analysis is often limited to pathways of 20-100 components, but the size of networks that can be analyzed is expected to increase rapidly in the future. Because systems pharmacology is necessarily multi-scale, all three of these systems biology approaches are expected to be important in the future development of the field. The fourth approach, which may have a large impact in the long term, is "synthetic biology". The synthetic strand in systems biology aims to create fundamentally new biological devices based on discoveries from other areas of systems biology and new approaches to genetic engineering. Synthetic biology adds the fields of biochemical engineering and industrial process optimization to systems biology and also adds problems outside the purview of conventional biomedicine, such as bioenergy and bioremediation.

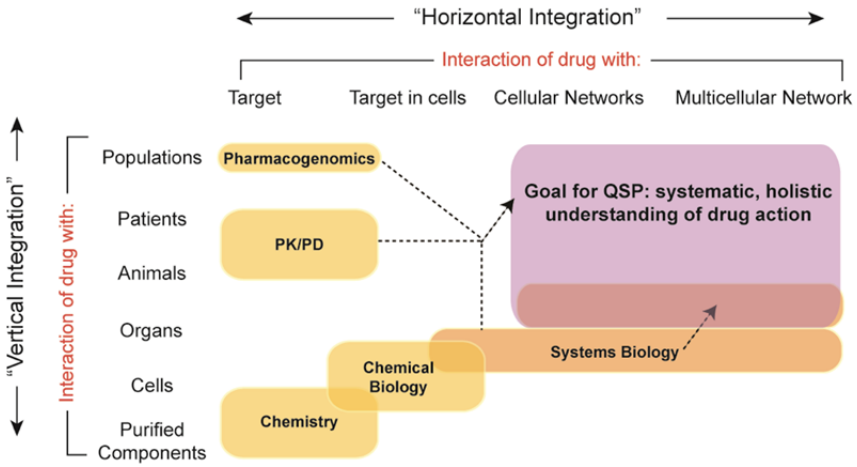

Figure 4. Horizontal and vertical integration in systems biology and pharmacology. One representation of horizontal and vertical integration emphasizing changes in physiological complexity, which tends to parallel changes in time scales (from seconds and minutes to years and lifespans). The goal for QSP is to bring network-level understanding of drugs to the complex physiology of patient responses. The arrows denote trend lines.

Achieving horizontal and vertical integration through multiplex measurement and modeling

The 2008 white paper on quantitative and systems pharmacology (summarized in Appendix 1) carefully considered the complementary strengths of "horizontal" (Appendix 2) and "vertical" (Appendix 3) integration in pharmacology (Figure 4). Many practical and conceptual challenges remain in achieving effective horizontal and vertical integration of biological knowledge, and the difficulties are magnified by the tendency of practitioners to focus on a single type of data (proteomics or genomics, for example) and of funding agencies and academic organizations to value specialists over integrators. Cultural

barriers are also evident: success with integrated pharmacology requires spanning pre-clinical and clinical studies and engaging both academic and industrial scientists.

Horizontal integration refers to studying multiple receptors, signaling networks, metabolic pathways or cell types at the same time. This is significant because biomolecules do not act in isolation but are embedded in complex multi-component networks subject to strong homeostatic control. Thus, even if a target is perturbed by a drug with great selectivity, the biochemical and physiological consequences of such a perturbation can be complex, involving changes in the states or activities of numerous biomolecules. Moreover, real drugs almost always bind to and affect multiple targets [37, 38] and pharmaceutical intervention therefore must be modeled as imposing a multi-factorial perturbation on a complex multi-component network. These networks vary considerably from one cell type to the next with respect to connectivity and input-output behaviors. Multiplex (-omic) measurements are needed to assay these sorts of networks, and a variety of modeling methods are required to understand the data; these include detailed multi-compartment kinetic models and coarse-grained logic-based or "influence" models.

Vertical integration involves linking information together at multiple spatial and temporal scales and at different levels of biological complexity. This includes data from studies on molecules, cells, tissues, organs, organisms (patients) and populations. Vertical integration is particularly critical in pharmacology because drug discovery begins at a very detailed level, typically with *in vitro* characterization of relationships between compound structures, target binding and effects of related drugs (so-called structure-activity relationships involving atomic-level data). It then proceeds to analyze compounds on pathways in cells, on phenotypes in animals and ultimately efficacy and toxicity in patients. The greatest barrier to successful drug discovery is navigating these massive changes in scale and complexity, and ensuring that hypotheses validated in one setting (e.g. cells and animals) are also valid in humans. Failure to correctly track and revalidate key hypotheses is one explanation for frequent (and expensive) failures in Phase II or III for "lack of efficacy". Failure to consider the behavior of networks in different cell types and organs, which may be quantitatively or qualitatively different from the behavior in target tissues, is presumably the main source of failure due to "on target" toxicity (e.g. the expensive post-marketing failure of rofecoxib - Vioxx®). Computational analysis of "vertical" data is generally known as multi-scale modeling and is widely recognized to be extremely challenging, with many failures and few successes [5]. Successes have had enormous impact, however, as illustrated by application of multi-scale models of the human heart to patient care [21].

IMPACT: A VISION FOR QSP AS A CENTRAL PILLAR OF TRANSLATIONAL MEDICINE

- The first goal of QSP is to reinvigorate the classical discipline of pharmacology and to develop new concepts that can be applied to specific problems in drug development including:
    - identifying novel drug targets and pharmacodynamic response markers;
    - validating targets and markers during successive stages of drug development;
    - predicting which drugs will work together (as combination therapies) to achieve defined pharmacological endpoints;
    - determining the full mechanisms of action of existing drugs and whether they might be effective in diseases for which they were not originally tested; and
    - optimally exploiting a growing set of generic drugs as a means to improve the quality and reduce the cost of health care.
- The second goal is to complement genomic medicine as a means to predict which patients will respond to and benefit from a given therapy and to understand, in quantitative terms, the reasons for variability in efficacy.
- The third goal aims to improve our ability to perform key steps in drug discovery and mitigate the current crises in industrial drug development.
- A final goal will involve developing systematic approaches to understanding adverse drug reactions and identify subsets of patients who are at increased risk for acute and delayed toxicity.

Reinvigorating classical pharmacology

A major goal of systems pharmacology is to reinvigorate classical pharmacology, conceptually and practically, using an outlook that substitutes the classic "one-gene, one-receptor, one-mechanism" hypothesis [39] with detailed knowledge of the dynamic, homeostatic networks that control human physiology. This knowledge, instantiated in formal computational models, will be used to rationalize and then predict the effects of drug action on specific network nodes and overall physiology. This is expected to involve new approaches to traditional problems that include: (i) understanding the mechanisms of action of existing drugs on diverse disease states; (ii) increasing the effectiveness of drug development by creating better approaches to target validation, thereby reducing the frequency of phase II failures of efficacy; (iii) developing approaches for identifying effective combination therapies and poly-pharmacology and for predicting and analyzing the properties of such combinations; and (iv) predicting and evaluating the potential action of new drugs and unrecognized actions of old drugs in patients based on knowledge of their individual genotypes. "Action" in this context includes both the desired therapeutic and non-desired or toxic effects (both target-related and often off-target toxicities). "Understanding" entails a quantitative, pathway-level description of relevant normal and disease physiologies and how they differ. "Predicting" involves the development of quantitative computational models capable of extrapolating how new drugs might perform with greater precision and effect than current, largely informal, approaches.

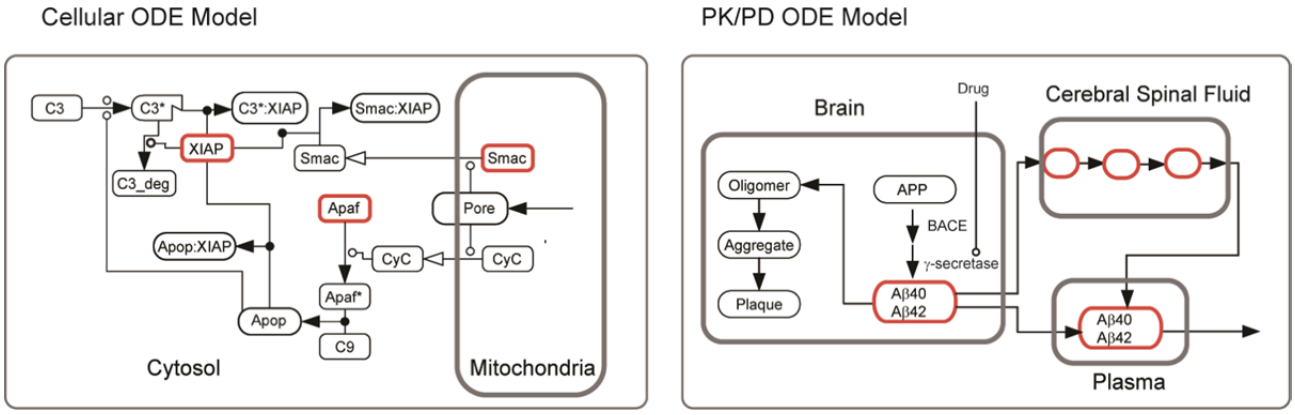

Figure 5. Comparing cell-based and PK/PD models. The left panel shows a portion of a differential equation-based model (a compartmental ODE network) depicting proteins critical in the final steps of receptor-mediated apoptosis. Individual species in the model (ellipses) correspond to proteins with and without various posttranslational modifications. Compartments refer to distinct, membrane-bounded subcellular organelles. The graph is drawn in Systems Biology Graphical Notation [40] and is a component of a more complex model of the cell death network that responds to the investigational therapeutic TRAIL [41]. Right panel shows a simplified depiction of a compartmental ODE model depicting the deposition of amyloid protein (which causes dementia) in the brain and its partitioning into the blood and cerebral spinal fluid (Courtesy Julie Stone, Merck Research Laboratories). Gamma secretase is the potential drug target under investigation. In this case compartments refer to distinct organs or regions of organs. In both the cellular and PK/PD models the underlying mathematics are very similar.

A closely related aspect of QSP will be developing network models spanning multiple tissues that can predict "on-target toxicity". On-target toxicity is an adverse event caused by binding of a drug to its intended target but with an unanticipated and unwanted outcome. By way of example, a pathological process such as insulin resistance influences multiple organs including muscle, adipose tissue, liver, pancreas, heart and the brain, and alters the levels and activities of multiple genes in each organ. Drug discovery efforts that have focused exclusively on one gene or one aspect of insulin resistance have done poorly. For example, the FDA has withdrawn marketing approval for multiple members of the glitazone family, a once-exciting class of drugs that binds to and stimulates PPARs (peroxisome proliferator-activated receptor γ in particular). The ligands for PPARs include free fatty acids and eicosanoids and glitazones seek to alter transcriptional responses to these metabolites, thus increasing sensitivity to insulin; however they also increase the incidence of heart attacks and strokes in a subset

of patients. Toxicity in these patients is on-target and mechanism-based: it involves inhibition of PPARs, rather than non-specific binding to other proteins. Glitazone's problems therefore have their origins in a failure to consider the multiplicity of signaling pathways affected by the drugs in different tissues. A more subtle and difficult problem with glitazone stemmed from a failure to consider the slow onset of adverse cardiovascular effects. We regard glitazones to be exemplars of drugs that have suffered from "systems-level" failures and we anticipate that QSP will help to prevent such failures by providing the tools to identify adverse outcomes at a network level.

Table 1: Types of models used in systems biology, PK/PD and QSP (Courtesy of Pfizer Inc.)

Heuristic model	Model with no specific assignment of biochemical or physiologic representation and mechanism and typically developed by trial and error; often provides intuition
Semi-mechanistic model	Model that that aims to represent some mechanistic aspects of a physiologic processes or measured endpoints with some heuristic features. Often used when data are available only for some model features; can be multi-scale.
Mechanistic model	A model that explicitly represents biomolecules and their mechanisms of interaction at a physico-chemical level. Often confined to cellular networks of relatively restricted scope but has ability to represent pharmacological mechanism of action in detail.
Network model	A graphical model that describes a set of interacting components in an extended network at a relatively low level of detail and is typically inferred from high-throughput omic data (interactomes are one example).
Multiscale systems pharmacology model	A model that links phenomena at two or more spatial and temporal scales such as drug-target interaction, signaling networks in cells, physiological processes operating at the organ and tissue level, and animal or clinical data.

Building, validating and exploiting network models of drug action

An important function of network modeling in QSP is to understand drugs in the context of cells, tissues, and multi-organ systems with a consideration both for the structural aspects of target-drug interaction and the biological functions of the target. Key concerns will include tackling diseases involving multiple interacting genes (and epigenetic modifications) and potential drug targets; designing and exploiting poly-pharmacy [42] (including combination therapies, agents with multiple pharmacophores and "dirty" — i.e. multifunctional — drugs) and optimizing the design of experiments to discover and validate drug targets. Proprietary models have already been developed to test clinical hypotheses and model the outcomes of clinical trials. A significant benefit of a vigorous academic effort will be to move such models out of "black boxes" and into the public domain, thereby allowing them to be validated and further developed [31, 32].

Systems biologists are currently developing and evaluating multiple modeling methods that aim to be integrative while spanning rather different levels of biochemical detail and biological complexity (Table 1). At one extreme, detailed stochastic simulation and differential equation models have helped to elucidate the origins of noise in biological processes [43], the spatiotemporal dynamics of complex signaling pathways [44] and the origins of cell-to-cell variability in cellular responses to drugs [45]. Logical and regression models have been used to dissect more complex pathways and to compare drug responses in normal and diseased cells [46, 47]. At a very different level of detail, sophisticated data mining and bioinformatics approaches are being used to investigate diversity in patient responses to drugs [48] and PK/PD models remain a mainstay of practical drug discovery. QSP will likely incorporate many of these approaches but will be distinguished by its focus on drugs and drug targets and on the creation of multi-scale models that are "actionable" and can be used to guide practical aspects of drug development (Figure 6).

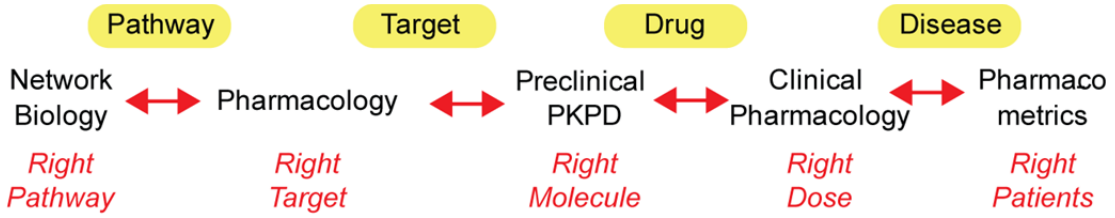

Figure 6. Areas in which QSP models will impact drug discovery. An alternative view of the impact of combined modeling and measurement approaches on key steps in drug discovery which range from identifying the right target to treating the right patients. (Courtesy of Piet Van Der Graaf, Pfizer Inc.)

Multi-scale temporal and spatial modeling to span different levels of biological complexity

Multi-scale models are needed to develop an integrated picture of the therapeutic and toxic effects of drugs over multiple spatial and temporal scales, particularly when short-term therapeutic benefit must be balanced with long-term risk of serious adverse events. Of particular importance will be methods for linking mechanistic models at the levels of proteins and cells to PK/PD models at the levels of organisms and patients. This will require advances in our ability to scale from animals to humans while correctly accounting for physiological and genetic differences. In this regard, it will be important to encourage studies that carefully investigate differences between humans and rodents and do not paper over distinctions to sell a "perfect" animal model (e.g. [49]). Also critical will be implementing an effective "bedside-to-bench" transfer in which data obtained during clinical trials is informed by, and informs, mechanistic understanding of the therapeutic hypothesis and pharmacological strategy. At the moment, it is common for the research teams responsible for target selection and validation (key inputs to the therapeutic hypothesis) to be disbanded before the first human data become available; this exacerbates the difficulty in performing effective Phase II trials, the point in development at which most contemporary drugs fail (Figure 7). In all of these endeavors data management and integration are expected to be significant challenges (Appendix 4).

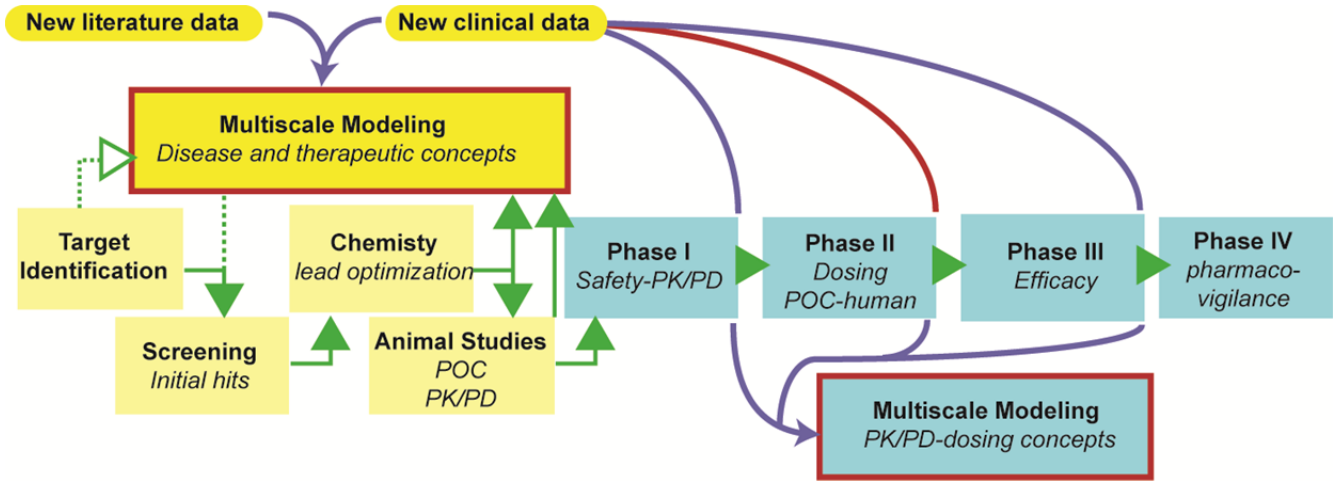

Figure 7. Importance of bedside to bench studies. The universally accepted progression in drug development from target identification to Phase IV studies usually results in dispersal of the teams involved in target selection and validation by the time proof of concept (POC) studies are conducted during Phase Ib-II. As a consequence, the ability to perform sophisticated analysis of patient-derived data is typically lost, and this makes Phase II highly vulnerable to errors made many years earlier during the pre-clinical phase. In a QSP-driven discovery process the underlying disease and therapeutic concepts are continually revisited and refined via a multi-scale model that incorporates all available preclinical, literature and patient-derived data (yellow and red box). This model would be the counterpart of models that are currently assembled to develop PK/PD and dosing regimens (blue and red box).

Patient-specific variation in drug responses and resistance mechanisms

One way in which systems pharmacology will differ from traditional pharmacology is that it will address variability in drug responses between tissues and cells in a single patient as well as between patients. Variability in response arises from differences in human genotype, genetic variation in pathogens that cause infectious disease, and the impact of current or past environments (Figure 8). Variability has proven particularly problematic in complex diseases such as cancer, type 2 diabetes, arthritis, chronic pain and psychiatric disease. At a mechanistic level, variability likely arises from a complex interplay among stochastic biochemical processes (that is, random fluctuations arising from the probabilistic interaction of individual molecules), epigenetic switching, genetic instability (particularly in cancer) and normal genetic variation. It directly impacts the effectiveness of therapy even in responsive individuals. Variation has been studied empirically, based on phenotypic measures [50, 51], but must be understood mechanistically for personalized medicine ("precision medicine") to truly succeed. Noteworthy successes have already emerged from the field of pharmacogenomics, where variation in drug response has been mapped to polymorphism in drug metabolism genes, leading to clinical useful predictions [52].

New or modified theoretical, experimental and computational tools are required to measure non-genetic variation [53] in a biological context. Good methods exist for isolated cells but a focused program is needed to develop approaches that can be used in organisms and ultimately in the clinic. In many cases, modifications of existing proteomic, FACS and high-throughput sequencing techniques may be applicable. For example, intravital optical imaging makes it possible to monitor the spatio-temporal dynamics of complex physiological processes in living tissue. Developing the probes, hardware and software to apply intravital imaging to pharmacology will be an important direction for systems pharmacology. Translation into the clinic should involve co-development of non-invasive imaging methods based on technologies such as MRI and PET, so that data obtained by studying the behavior of cells in model organisms can be extrapolated to humans[54, 55].

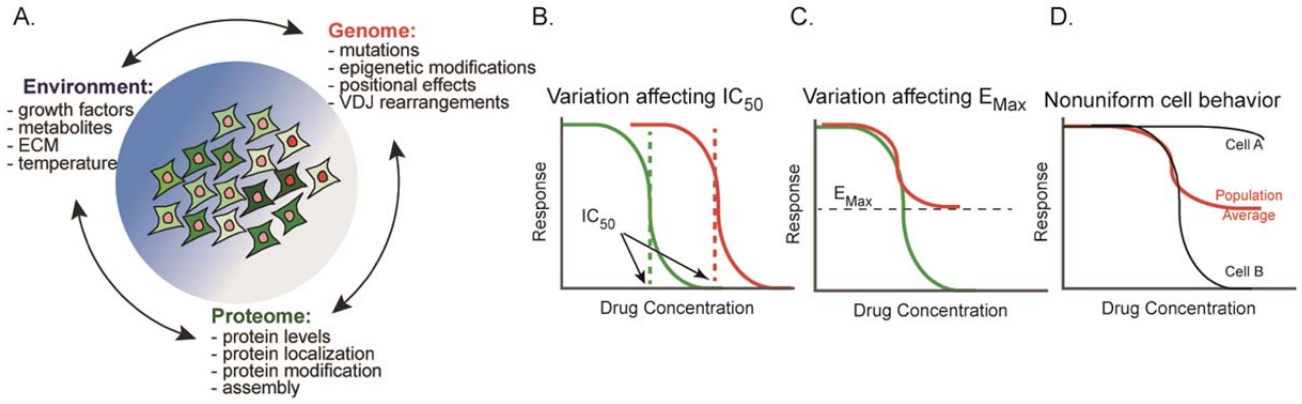

Figure 8. Origins and impact of variability in responses of cells to drugs. (A) Interaction among factors that determine average cellular phenotype and variance around the average. Combinations of environmental (blue), genomic (red), and proteomic (green) variation can cause heterogeneity in the physiological states of cells in an initially homogenous population and this can give rise phenotypic heterogeneity. (B) A standard view of drug response in which two population-level measures of IC_{50} vary, with green depicting a more sensitive and red a more resistant population. (C) Often two cell populations differ in E_{max}, the maximal response that can be achieved at even saturating drug doses. (D) One cause of suboptimal E_{max} is that not all cells in a population respond identically (black lines) and the average response at a population level is therefore midway between two distinctly different outcomes. Detecting this type of nonuniform behavior requires single-cell techniques [45]. Panel A is adapted from reference [56]; Panels C-D were adapted from reference [57].

New clinical trial designs as a means to test disease and therapeutic hypotheses

A key goal of systems pharmacology is to improve our ability to test therapeutic hypotheses at each stage of drug discovery and development, from target identification to phased clinical trials, and to use such drugs effectively in diverse patient populations [58, 59]. It may ultimately be possible to simulate first use of a drug in humans *in silico* based on studies in animals: some progress is already evident [60], and research is underway to develop simulation approaches for translation from preclinical to clinical settings [61]. However, achieving this routinely, and with confidence, requires more effective two-way flow of information from clinical trials back to the laboratory, and from the laboratory to the clinic (Figure 7). Optimizing the design and execution of clinical trials based on simulation and modeling is also expected to be an important, continuing area of research. The growing availability of electronic medical records (EMRs) can facilitate this process but a significant investment is required to develop computational and analytical tools for mining EMR data. This will encompass the validation of new types of trials, including adaptive trials, optimizing the collection and analysis of clinical data during Phase I/II, and predicting drug-drug interactions, particularly those that involve combinations of new agents with standard-of-care therapies.

Failure analysis as a means to improve drug discovery

The complexity of drug development is such that, even with the best science, a significant fraction of all drugs are likely to fail in clinical trials. In engineering, detailed study of failure is an essential part of systems engineering, and "failure analysis" is a key discipline. An important effort in systems pharmacology will be to integrate failure analysis into every stage of drug development from preclinical development to post-marketing surveillance. The goal is to understand precisely where a clinical or therapeutic hypothesis failed in the past, to predict future failures, and to develop procedures for minimizing the impact of failure [10]. We anticipate that this will be a key area for development of private-public partnerships. Among other things, failure analysis will require restructuring of current concepts of proprietary information and agreement on common approaches to data-sharing.

Synthetic biology and the development of new "smart drugs".

Current drugs are mostly activators or inhibitors of one target, or of several poorly defined targets. Synthetic biologists aspire to build fundamentally smarter molecules and therapeutic devices. An example would be a protein or nucleic acid molecule that makes some non-trivial "measurement" - for example the co-expression of two proteins on the surface of a cancer cell - before making a "decision" such as delivering a toxic drug or insult. Using current developments in chemical biology, protein engineering and nucleic acid "origami", quite sophisticated molecules are possible; but progress with smart drugs will hinge on better understanding of disease networks that must be modulated [62]. Progress in gene targeting in humans will powerfully complement synthetic biology approaches, starting with the most serious diseases, where the risk to pioneer patients is justified. There will also be therapeutic opportunities for engineered microorganisms, most obviously in the gut and vagina, which are naturally colonized by bacteria. Using current technology it may be easier to engineer a microorganism to make decisions based on measurement than to engineer a single molecule to perform a similar calculation. Engineered human cells, suitable for long term transplantation, will hopefully come out of stem cell initiatives. The current focus in stem cell research is replacement parts, but a longer-term goal might be to develop improved cells, e.g. cells that can deliver therapeutic molecules when needed.

QSP as a complement to genomic medicine

New biochemical, pathway-oriented and physiological approaches to QSP are necessary to complement a major ongoing investment in genomics as a means to understand drug-target interactions and pathophysiology. Much of the emphasis in human genetics, molecular biology, and translational medicine over the past few decades has been on identifying disease genes and determining their molecular functions. Pharmacology and complementary approaches to studying disease have received less emphasis, particularly in academic programs based on reductionist molecular approaches (both computational and experimental). Genomics approaches provide a wealth of data and many clues to

disease mechanisms and drug targets, but genomics is, in and of itself, insufficient as a means to develop and study drugs: the operation of biological networks is strongly affected not only by changes in coding sequence or gene expression level but also by transient responses to external signals at the level of protein activity, posttranslational modification, stochastic processes, etc. It seems highly unlikely that these latter effects can be understood at a mechanistic level solely on the basis of genomic data.

Advancing practical problems in drug discovery and development

The pharmaceutical industry is facing unprecedented challenges in discovering new drugs and moving them into the clinic: the cost of developing drugs is rising rapidly, even though the number of new drug approvals is constant or even declining [63]. Ninety percent of investigational drugs—i.e., those approved for trial—fail before being approved for use in patients, driving up the total cost of development (currently estimated at $1.8 billion per newly approved drug [10]). The success rate is better for antibody drugs [64], which is one reason industry is actively pursuing them. However antibodies are costly to manufacture and are limited to extracellular targets. Costs arising from a high rate of failure are passed on to patients, third party payers, and the government in terms of higher costs for those drugs that do succeed. In this context it is noteworthy that of all the factors that impact the overall cost of drug development (e.g. cost of discovery, cost of studies at each phase of clinical development, long discovery/development timelines, etc.), the most important is the probability of success in Phase II clinical development (Figure 1; [10]). Phase II is generally when a proof-of-concept study is conducted to evaluate the efficacy/safety of new molecules in patients and the high impact of Phase II success on the overall cost of drug development derives in large part from high failure rate at this stage, especially for novel mechanisms of drug action (success rates of 20-30% are typical; Figure 1). Moreover, Phase II studies are particularly relevant to systems pharmacology because it is at this stage that correct selection of targets, drugs, dosing regimens and therapeutic hypotheses is tested rigorously. In contrast, failure in Phase III (which occurs about 30-50% of the time [10]) often involves poor differentiation of a new drug relative to standard of care.

As discussed above, drug discovery currently focuses on the identification and optimization of drugs that bind to a specific target (or to a family of closely related targets), even though it has become abundantly clear that most drugs affect not only the intended target but also myriad other processes at the cellular, organ and organismal level. Increasing ability to identify "off-target" effects has had both advantageous and problematic consequences. In some cases, the recognition of unexpected effects in patients coupled with the identification of new drug targets has led directly to the development of new therapeutic agents (one aspect of drug repositioning or repurposing; e.g. sildenafil (Viagra®) in the treatment of pulmonary arterial hypertension [65]). Nonetheless, it is remarkable that the mechanism of action (i.e. how target engagement leads to patient responses) remains obscure for most therapeutic drugs. Contemporary clinical pharmacology and pharmacometrics primarily search for statistical correlations between variability in subject response and drug exposure [66] which, although clinically valuable, often lacks a known mechanistic basis [35]. Contemporary pharmacogenetics focuses primarily on variation in proteins that mediate drug absorption and metabolism, an approach that has succeeded in cases where these factors dominate clinical variation. When variation lies instead in response pathways at the level of proteins, as is often the case, this approach has had only modest success.

It is sometimes asserted that the job of developing and testing therapies should be left to industry. In our view this simply is not working: the problems are so difficult that long-term commitment to the development of new approaches is necessary. The problem is compounded by the fact that the pharmaceutical industry is reducing its commitment to basic research. In this white paper we argue that fundamentally new concepts and approaches are required to better translate knowledge of biochemical mechanisms into new therapies, to predict the effects of perturbing a target on the behavior of cellular networks, to determine the effect of perturbing cellular behavior on the behavior of a tissue, and to anticipate the consequences for whole-organism physiology. These are ideal areas for academic investigation. Moreover, through collaboration with industry (see below) it should be possible

to accelerate the rate at which new ideas can be integrated into the drug discovery pipeline in a manner that supports practical decision making.

Also important will be more extensive and effective study of standard of care and generic drugs. These are areas in which industry cannot invest major resources, but the potential economic and medical benefits to society are enormous [67]. Standard of care drugs are, by their very definition, in widespread use, but their precise mechanisms of action are often not fully understood and it is not obvious how to repurpose them as either mono or combination therapies for new indications. The very fact that many patients, particularly the elderly, are prescribed multiple drugs in combinations that have never been rigorously tested poses a conceptual and practical challenge for QSP. We see intensive study of existing drugs as one area in which QSP could both advance fundamental biomedical science and have a dramatic near-term impact on healthcare.

We anticipate that concepts from QSP developed in industry and academia will have an impact on multiple stages in the drug discovery/development pipeline, from preclinical studies of networks to improved prediction of therapeutic and toxic responses in patients (Table 1). This does not mean, of course, that QSP subsumes the totality of drug discovery, but rather that it adds a quantitative and integrative perspective to activities that are currently qualitative or isolated. Pursuit of QSP will require a greatly increased investment in computational, statistical and mathematical analysis during preclinical research and more consideration of mechanistic and network perspectives in development of translational PK/PD models. One occasionally encounters the prediction that drug development will become a largely *in silico* exercise. We do not see any prospect of this happening in the foreseeable future, but it is nevertheless the case that relatively modest investments in sophisticated mathematical approaches are likely to make QSP extremely valuable to a drug discovery process that is currently largely driven by empirical observation and qualitative reasoning.

Systematic approaches to toxicology and better prediction of drug safety

Many drugs fail for overt safety reasons, and many more fail because safety concerns limit dose to a level that is not efficacious. Conceptually, it is useful to distinguish three causes of toxicity: on-target and on-pathway (toxicity arising from the same pharmacological effects as efficacy); on target and off pathway (toxicity arising from a second function of the target) and off-target (toxicity arising from binding to molecules other than the target; e.g.[68]). In principle, the difficulty of predicting toxicity increases along this series but in practice, predicting all of these forms of toxicity is complicated by poor quantitative understanding of human physiology.

As part of the development of QSP, drug safety prediction and evaluation must evolve from a qualitative to a quantitative science[69]. Historically, statements about drug efficacy were qualitative, consisting of a clinical impression, perhaps supported by some physiological measurement. We now require statements of efficacy to be based on rigorous statistical analysis bounded by confidence intervals as compared to either placebo or active controls. This will require metrics that can be summed or integrated across different aspects of toxicity (predicted or observed) to allow determination of a point estimate of risk bounded by confidence intervals (as compared to a placebo or active control comparator). QSP should therefore seek to develop quantitative metrics on both sides of the risk/benefit equation so that rigorous statements can be made about clinical benefit and clinical utility of a drug.

Existing efforts to predict specific organ toxicity based on the chemical structure of a drug have met with some success, particularly in the case of hepatotoxicity and cardiac toxicity (e.g. electrocardiographic QT prolongation). In some ways, this work is derivative of efforts to link the characteristics of chemical structures to therapeutic effects. Much less success has been reported with more general predictions of toxicity based on chemical structure, and this certainly presents a much more complex problem. The difficulty probably arises because the universe of possible therapeutic targets for a given chemical structure is much smaller than the known universe of targets that confer off-target toxicity. Open-source databases that link drug structures to the known universe of toxic

targets (similar to, but ultimately more exhaustive than, similar databases in industry) would have great potential in enabling prediction of new toxicity. Development of such a translational bioinformatics program is underway at the FDA in collaboration with pharmaceutical companies, cheminformatics and bioinformatics groups in academia and NIH (see the NCTR Centers of Excellence at http://www.fda.gov). The activities required to create a generally useful predictive approach to drug safety include (i) creating a standardized ontology for the universe of "molecular toxic targets" (ii) using bioinformatics tools to network these terms to organ specific drug toxicities, and (iii) developing multiple scale networks that link molecular targets and organ toxicities to clinical toxicity and adverse events [70].

QSP must seek to develop additional links between cheminformatics resources and vertically or horizontally integrated network models. This will advance the goal of achieving a robust and flexible approach able to accommodate the diversity of candidate drug-target pairs submitted to the FDA for regulatory review. An immediate challenge is developing the capacity to make rigorous statements about both sensitivity and specificity for a predicted risk: when data-mining exercises are conducted to explore possible clinical risk from a drug, a number of possible risks are identified but this is of little value unless both sensitivity (likelihood of correctly predicting a true risk) and specificity (likelihood of not predicting if it is not a true risk) are known. Fortunately, calculation of such ROC (receiver operating characteristic) curves has become common in systems biology.

1. Analyze networks to identify optimal points of intervention	Model network behavior and use a combination of experimentation and computation to predict how drugs affect network behavior [47]. Incorporate knowledge on genetic variation.
2. Use models to improve selection of primary and backup targets	Use modeling of multi-variant effects to identify how desired outcome could best be achieved by modulation of one or more targets with drugs; consider combination approaches..
3. Model outcomes and variance	Measure and model functional relationships between dose and response. Identify determinants of variation in response across populations of cells in isolated organ cultures and across patient cohorts. Develop models that account for differences in disease mechanism between man and animals.
4. Predict on-target and off target safety	Identify and measure "on-target" interactions and develop network-level understanding of mechanism-based toxicity and of networks affected by drug but not relevant to efficacy.
5. Model absorption, distribution, metabolism, excretion, target engagement	Develop multi-scale models that incorporate molecular- and cellular knowledge with PK-PD. Provide insight into how trials should be designed.
6. Sustain target validation throughout drug development	Develop models that can sustain validation of the target and therapeutic hypothesis throughout preclinical and clinical development.
7. Model therapeutic re-purposing	Model potential alternative diseases and/or drug combinations based on known mechanisms.

Table 2. Potential contributions of QSP to drug development. Potential contributions of QSP-inspired computational modeling that span pre-clinical (1-4) and clinical (5-7) phases of the drug development pipeline. Not listed are the medicinal chemistry steps involved in making a compound that is stable, bioavailable, safe, capable of being manufactured on a large scale, and formulated for delivery.

CHALLENGES AND CONTROVERSIES

- Fundamentally new ideas are needed. We cannot rely on existing approaches and paradigms to deal with the challenges of scaling from molecular interactions to organismal physiology.
- It will be difficult but necessary to develop means to complement existing and emerging genomic data with biochemical, cell and organ level mechanistic data and information on lifestyle and environment as a means to construct predictive multi-scale pharmacological models.
- Expertise in translational therapeutics and clinical pharmacology must be sustained in the face of declining numbers of trainees. Pharmacological problems must be made more attractive to the next generation of trainees.
- Cultural barriers to developing integrated rather than highly specific knowledge must be overcome; as in other areas of translational medicine barriers to combining preclinical and clinical, and academic and industrial research, must be addressed.
- Access to data sets on drug effects in patients is needed to develop new approaches to failure analysis.

Need for fundamentally new science in drug development

While it is commonplace in genomics to investigate large scale gene regulatory networks in normal physiology and disease (particularly cancer), much less model and pathway level research has been devoted to understanding networks from other perspectives and of incorporating data on mechanisms of drug action, particularly at the level of protein targets, and the way drug-target interactions impact pathophysiology. Systems biology has generally lacked the chemical and medical sophistication necessary to explore structure-activity relationships at this scale and chemoinformatic methods are not in widespread use in the academic community (even though proprietary systems are common in industry). Further, systems biology has not pervasively attempted to reconcile physiological and pathophysiological function at the clinical level, or attempted to apply systems thinking to understand the beneficial and harmful effects of human drug exposure. Finally, systems biologists generally are unfamiliar with the key concerns of industrial and clinical pharmacologists, which inevitably focus on pharmacotherapy in the clinical setting. Pharmacology, on the other hand, has always related drug action to clinical phenotypes, but clinical relationships are often correlative and phenomenological rather than causal and quantitative; in many cases, the molecular and cellular information that is available is insufficiently integrated with clinical testing. An exception to this observation is pharmacogenomics, which has emerged as a vital and growing field.

Integration with genomics

In our opinion, QSP represents an ideal complement to genomics as a means to study disease and characterize patient responses to drugs, serving both to help interpret mutational data and to provide insight into those aspects of drug mechanism that cannot be deduced from sequence alone. Achieving success will involve combining genetic data with data on temporal dynamics of proteins, small molecules and organelles, as well as detailed information on how regulatory networks behave in different tissues and individuals. Spatial data derived from microscopy of cells and imaging of tissues and organisms (e.g. with CT and MRI) will also be critical.

Success in QSP will hinge on success in elucidating the impact of specific therapies in individual patients based on knowledge of genotypes [71]. With the advent of next generation sequencing and the impending likelihood of large scale sequencing of individual "exomes," genomics has become the primary approach to finding disease genes and identifying patients who might benefit from specific therapeutic drugs. For example, by screening small cell lung cancer patients for EGFR mutations it is possible to identify the subset who will benefit from treatment with geftinib (Iressa®) [72] and by screening simultaneously for Kras mutations it is possible to identify those who are drug resistant or for whom treatment is contraindicated [73]. Unfortunately, knowing which genes are mutated in disease does not necessarily provide a clear path to an effective drug. In many situations even highly potent inhibitors of disease genes are not useful as drugs. This arises for several reasons. First, drug targets often carry out essential cellular functions in normal tissues, and cannot be blocked completely without causing excessive toxicity. Second, many disease processes involve dysregulation of multiple pathways,

and blocking any single pathway involved in disease does not necessarily produce the desired beneficial effect. Third, many drug targets have different sensitivity to inhibition in different tissues so that the concentration of drug sufficient to cause toxicity in one tissue may not be sufficient for efficacy in a tissue where the effect is therapeutic (moreover, these differences can rarely be predicted based simply on the levels of the target in various tissues). Fourth, biological pathways can adapt to challenges, including those imposed by drugs, by adjusting the levels of pathway components. This is one reason for insensitivity to drugs at a cellular level and it is also likely to contribute to the acquisition of actual resistance. Fifth, it is not always possible to target the product of the disease-related gene; not all proteins are "druggable", and drugs that restore function to a damaged or absent protein can be especially hard to develop.

Investigating therapeutic mechanism at different levels of detail and complexity

A topic of debate among academic and industrial investigators discussing contemporary pharmacology is whether the emphasis should be on studies performed with purified components (e.g. structure-guided drug design), tissue or organ culture, animal models of disease or human patients. In our opinion this represents a false set of choices since all are required, at the very least for regulatory and ethical reasons. In contrast to genomic analyses of disease processes, there is no realistic prospect that drugs can be tested in animals without prior analysis in cells or that humans can be exposed to new drugs without extensive testing in animals. We must, therefore, learn how to better combine data from all of these levels of analysis: detailed mechanistic studies are much easier *in vitro* and in culture, but only studies in man, where the drug and the target are both exposed to the relevant biological milieu, can establish whether a drug is efficacious and sufficiently nontoxic for use as a therapeutic agent. Once again, the key requirement for QSP is horizontal and vertical integration. We suspect that many opportunities exist for improving drug studies in cells and animals by improving our understanding of how cell regulation is affected by the paracrine, endocrine, and contact signals that characterize the behavior of cells in their pathophysiologic milieu. It appears that while the endpoint of drug action on cells (e.g. lethality for anti-cancer drugs) may not necessarily be recapitulated in patients, the targets and effects on networks are often highly conserved.

Cultural barriers to systems pharmacology

A recurrent theme in our review of pharmacology in the 21st century is the need for fundamentally new perspectives on problems associated with linking data, models and ideas at different scales. This will require the participation of investigators with different skillsets, but will also benefit from the insight of individuals who can bring concepts and information from several relevant fields (integrators rather than specialists). This poses a challenge, since the current culture of grant review and academic biomedicine has evolved to reward individuals who are embedded in the culture of a single field, with the unintended consequence that interdisciplinary innovation is made harder. Efforts to promote systems biology are promising in this regard. For example, the National Centers of Systems Biology (established by NIGMS; www.systemscenters.org) have (in our view) been extremely successful in promoting interdisciplinary research. These centers required interdisciplinary collaboration, but crucially, allowed individual centers to initiate new projects without the need for (usually conservative) external peer review (except every five years). A similar set of centers would help to accelerate the establishment of systems pharmacology, but with the additional challenge of promoting translational research. In establishing these centers, it will be important to encourage both interdisciplinary collaboration and interdisciplinary research. Reforming peer review to give new ideas and interdisciplinary projects a better chance in the broader NIH-funded ecosystem is also an important topic, but for another forum.

<u>Linking Academic and Industrial Activities</u>

In industry, pharmacological modeling and simulation have gained the greatest acceptance during early and late clinical development, specifically with respect to studying the uptake and distribution of drugs in cells and organisms, determining which drugs engage their targets (PD), designing phased clinical trials, and informing go/no-go business decisions (see above). There has been relatively little penetration of modeling and simulation into preclinical activities such as target identification, lead generation, etc., precisely the areas that most engage academic systems investigators [74-77].

Conversely, academic systems biologists have little exposure to concepts such as PK/PD, and there is little evidence that even "physiological" or network PK/PD has incorporated contemporary "-omic" approaches and systems biology thinking. This provides a clear opportunity for creating strong industrial-academic collaborations and consortia [78], the latter modeled on the collaborations that have proven effective in genomics research [79, 80]. Developing a formal approach to drug "failure analysis" will also require the cooperation of industry (which has much of the necessary data) and academics (who have the time and expertise to analyze the data). Ideally as much data would be released into the public domain as possible, increasing the possibility of unanticipated breakthroughs.

EDUCATION AND TRAINING IN QSP

- We require more trainees in the area of QSP and better preparation of pharmacologists in general; industry currently has openings for many individuals with such training.
- Doctoral training programs in systems pharmacology are required as are systems pharmacology tracks in existing pharmacology, systems biology, bioengineering, pharmaceutical science and cell and molecular biology and potentially other Ph.D. programs (e.g. chemical biology, genetics and bioinformatics).
- Innovative and interdisciplinary approaches to training are essential so that investigators can be engaged at all career stages.
- New teaching materials are required for QSP, and many of these will be non-conventional in the sense that they are electronic and model or database-oriented.
- Active involvement of academic, industrial and regulatory scientists is necessary.

One of the major impediments to widespread adoption of QSP by the scientific community is a shortage of appropriately trained pharmaceutical scientists, pharmacologists, and bioengineers and of clinicians who have sufficient breadth and depth in their knowledge of quantitative approaches to drug discovery and development. Current curricula in pharmacology include coursework in biochemistry, cellular biology and mammalian physiology, drug action and PK/PD modeling, as well as biostatistics. However, if physiology and pathophysiology are to be considered at the systems level, pharmacologists must be trained to think quantitatively about multiplex -omic methods, quantitative molecular models, and the connections of -omics and models to physiology. Furthermore, trainees need a fundamental understanding of PK/PD as well as chemistry and structure-activity relationships. How should we educate students to be well prepared for interdisciplinary research in systems pharmacology without requiring them to take years of foundational courses to develop a knowledge base in the field?

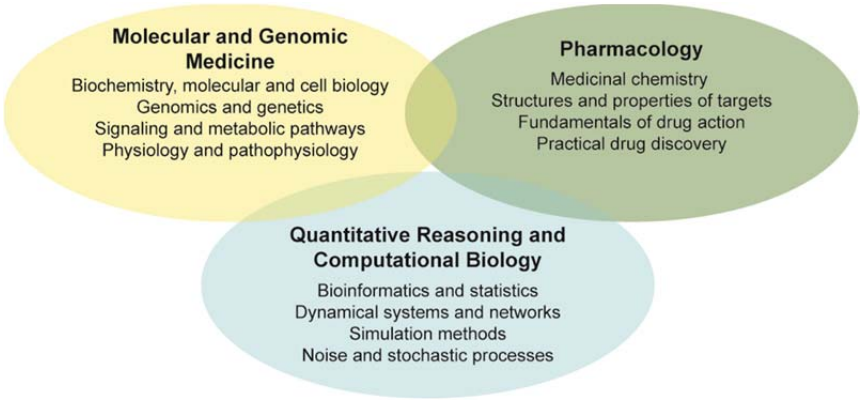

Figure 9. Graduate Training in QSP. Concepts and application areas for a training program in QSP emphasizing the importance of combining molecular medicine and pharmacology with quantitative approaches emerging form systems biology. Further details about a potential QSP curriculum are described in reference [77].

An important step in establishing new training programs in QSP, whether at the graduate or postdoctoral levels, is to define the skill-sets that appropriately trained systems pharmacologists should

have. What do we expect trainees to be proficient and competent in doing once they have completed their training program? It is not as easy to answer this question as one might think: we already face the problem that trainees are spending too much time in graduate school and there is considerable danger in simply adding further to the list of required knowledge. It will be necessary to deemphasize some topics in order to make room for others.

Core Competencies: Students who are proficient in QSP will become practitioners of next generation pharmacology and will need the following core competencies (Figure 9):

- Essentials of biochemistry, cell and molecular biology
- Essentials of genetics and genomics, including bioinformatics and modern statistical approaches to the genome
- Essentials of chemistry, protein structure and molecular visualization software
- Essentials of human physiology and anatomy relevant to drug action and pharmacokinetics
- Mathematical (at least ODEs) and programming (at least MATLAB or equivalent) skills

In addition they should receive in-depth training in some of the following areas, ideally more than one, though we see a need for both specialists and integrators.

- Modeling mammalian physiology at the cellular level and systems levels linking models to empirical experiments
- Approaches to elucidating disease processes and studying pathophysiology
- Drug action in physiology and drug metabolism at molecular, cellular and organismal levels including principles and applications of PK/PD modeling and simulation at the level of tissues and organisms.
- Clinical studies and trials as analyzed using statistical and mathematical methods.
- Use of biomarkers, gene signatures and statistical metrics to determine disease progression and pharmacological responses.

Prerequisites for Trainees: Students entering graduate or postdoctoral training programs in QSP are expected to come from diverse backgrounds including biochemistry, cell biology, physiology, biochemical or biomedical engineering, statistics, as well as the pharmaceutical and clinical sciences, (including MDs and PharmDs). At a minimum, students should have a background in chemistry, biochemistry, physiology, cell biology, calculus/differential equations, and statistics at the level of a college sophomore. In some cases it will be necessary to provide basic training in biology (including physiology) and chemistry and in other cases basic training will be required in quantitative and mathematical methods. Systems biology graduate programs have become familiar with the complexities of training students from varied backgrounds to address biological questions using quantitative and mathematical techniques, although this remains a somewhat challenging issue. Student background could be used to pass some students out of courses in required competencies to allow more time for others. For example, undergraduates with degrees in subjects emphasizing cell and molecular biology might spend more time training in math and modeling in their first year, and undergrads from engineering or computational programs more time in biochemistry, cell biology and genetics.

Coursework for Training Graduates in Quantitative Pharmacology

A complete program of study for systems pharmacology might include:

- Human physiology and pathophysiology with case studies discussed at the molecular, cellular, organ and whole organism scales.
- Systems Biology I/II: Technology and measurements in biology and physiology and central pathways and mechanisms in normal physiology and pathophysiology
- Statistics and Probability at the Graduate level with applications to life sciences
- Modeling and Simulation in Biological Systems – modeling of networks, simulation of dynamic systems, statistical and probabilistic modeling
- Variation in drug responses at the levels of cells, model organisms and patients

- Basic Computer Programming (PERL/Python, JAVA and SQL)
- Drug Action, Delivery, Transport, Metabolism, PK/PD Modeling and mechanisms of resistance
- Experimental approaches to biological systems
- Experimental/ Clinical Pharmacology and Toxicology
- Pharmacogenomics

Elective Courses: In addition to the Core coursework described above, students should have the option of taking elective courses in areas ranging from advanced drug metabolism and pharmacogenomics to mathematical methods and advanced computer science (algorithms, data mining, machine learning etc.). Depending on the program, course content could be delivered through traditional 10-15-week-long courses, or via intensive "mini" courses. The short-course approach, common in many universities and independent laboratories throughout the US and Europe, has proven to be an effective way to deliver a wide range of diverse information.

Training a new generation of quantitative and systems pharmacologists should include a mix of didactic lectures and hands-on practical (laboratory and computer) training. The training will involve multifaceted integration of concepts and principles in structural biology, biomedical sciences, applied mathematics and engineering sciences, and integration among these disciplines [81]. Key concepts for drug discovery and drug action should be interwoven into these areas. A case-based approach where concepts are integrated across the scale of organization will be critical. Furthermore, hands-on modeling experiences are needed. Trainees should gain modeling experiences through lab-based courses, journal clubs and lab research rotations, and most importantly with their doctoral dissertation or fellowship research project(s).

Expectations of Trainees: This new generation of quantitative and systems pharmacologists will be expected to advance fundamental knowledge of mechanisms of drug action including how complex networks respond to drug-induced perturbation in healthy and diseased cells, organs and patients, including an understanding and prediction of target and off-target effects. A Ph.D. thesis might reasonably be expected to address one or more of the following topics:

- Drug discovery based on systems level physiology and pathology
- Quantitative modeling of pathology and drug action
- Precise design of clinical studies from a systems perspective
- Modeling of target and off-target drug effects
- Identification of markers of disease and drug response to stratify patients as responders and non-responders
- Drug choice and dose titration targeted at personalized treatment
- Development of rational approaches to combination therapy
- Use of population-based resources (electronic medical records, adverse event reporting systems) to generate and test drug-response hypotheses
- Variation in drug responses at the levels of cells, tissues and individuals

Industrial internships: Given the need for industry-academic collaboration to make QSP a success, it would be interesting for training programs to experiment with internships as an integrated part of the program. The design of such a component would likely vary with the enthusiasm and availability of local companies, and we feel the goals should be training and experience, not job placement. However, such internships (typically 3-6 months) are common in engineering and could become a distinguishing feature of a QSP program. Done correctly, they would be a major attraction for undergraduate recruitment.

Job Placement and Opportunities: Leaders in the pharmaceutical industry have expressed enthusiasm for systems pharmacology training and have indicated that they have many positions available for scientists with quantitative pharmacological training (see Appendix 5 for an outline of a traditional pharmacometrics curriculum). However, many entry-level PhD scientists interested in the pharmaceutical industry lack a strong foundation in basic pharmacology and in quantitative investigation. This represents an opportunity for government, academia and industry to collaborate in

the development of training programs to advance the discipline and address a shortage of skilled scientists. An acute need for faculty trained in systems pharmacology is also evident. How programs will develop a core of faculty qualified to teach systems pharmacology is a major concern. In addition to hiring talented junior faculty who have completed one of the few training programs in existence, senior faculty with appropriate backgrounds and interest in the field will need to re-tool. Funding mechanisms (e.g., self-training with experienced colleagues via administrative supplements to existing NIH grants, sabbaticals of various lengths, formal training etc.) are needed to help expand the existing cadre of faculty capable of teaching a new generation of systems pharmacologists.

The training environment for systems pharmacology

Systems pharmacology is, by nature, multi-disciplinary, and it will be difficult for some institutions to provide the diversity of training experiences that are needed by aspiring systems pharmacologists. The ideal training environment would be a major research university with a comprehensive health care campus that includes a strong systems biology program or department, teaching hospital, translational research/clinical trials unit and faculty active in bioinformatics, biostatistics, genetics/genomics, classical pharmacology, etc. A strong relationship with the pharmaceutical industry and relevant federal agencies that can provide appropriately interactive training experiences for both graduate students and fellows would be a plus. However, a consortium involving university-based engineering programs and industry would also be a good approach. Publicly available clinical databases and samples from NIH should be developed further so they can be used for training in systems pharmacology. For instance, the ARDS data and samples from the NHLBI Clinical Study [www.ardsnet.org], US NIH and other agency-sponsored studies like the Dallas Heart Study [clinicaltrials.gov/ct2/show/NCT00344903] and the Framingham Heart Study [www.framinghamheartstudy.org] are exemplars of the type of resources needed for training in QSP.

Support for training in systems pharmacology

Over the last two decades, there has been a steady decline in academic programs and training sites focused on the pharmaceutical sciences and capable of preparing the next generation of pharmacologists. It will be crucial for the success and development of QSP to reverse this trend and establish a diverse set of educational programs and training sites. To accomplish this we need to create exciting and attractive research and training programs, recruit junior faculty to these efforts, and convince academic administrators of the value and promise of these programs. Attracting external funding for research is a major concern. Systems pharmacology has not been a major focus of federal funding even though, as we have argued here, it is a core translational science. Strong support for pre- and postdoctoral training programs will ensure a steady supply of appropriately trained systems pharmacologists in industry and academia. Innovative approaches to funding should be considered, including industry-NIH joint/collaborative funding mechanisms for both trainees and faculty. Funding for mini-sabbatical training and re-tooling of faculty also is needed and industry supported postdoctoral fellowships are one mechanism to support the training of quantitative pharmacologists, although these programs are usually much shorter in duration than a typical graduate program, and yield trainees with a more narrow focus of expertise.

ANTICIPATED OUTCOMES

- New approaches to studying the therapeutic and toxic effects of drugs based on a network-level understanding of diverse mechanisms of action and the role that emergent network-level behaviors play in sensitivity, resistance and toxicity
- More effective translation of preclinical discoveries into clinical success, particularly with respect to the efficacy of Phase II clinical trials
- Substantial advances in understanding the mechanisms of action of new and existing drugs in patients having diverse genotypes and environmental factors; consequent improvements in personalized care

- Better understanding of past failures in drug discovery and how to avoid or mitigate these failures in the future
- Approaches to the discovery and development of new drugs, and repurposing of existing drugs, that are more efficient and therefore less expensive in addressing unmet medical needs

The primary results of a successful systems pharmacology effort are expected to be (i) advancing the fundamental knowledge of how drugs act, and how pathways respond to drug-induced perturbation in healthy and diseased cells in the context of tissues and organs within which they exist; (ii) increasing the probability that newly discovered drugs will succeed in clinical trials and prove therapeutically beneficial in patients; and (iii) training a new generation of students, postdocs and physician-scientists to be leaders in academic and industrial pharmacology and translational sciences. Through precise, quantitative and multi-factorial understanding of how drug targets function in the context of pathways, tissues and patients, new and better ways to use existing drugs and guide the development of new drugs will be developed collectively by academic, industry and government investigators.

The foregoing analysis of pharmacology and drug development suggests a pressing need for new ideas and approaches. Among leaders in universities, medical schools, industry and regulatory agencies there is remarkable unanimity of opinion on the outlines of what needs to be done. Nonetheless, the development of QSP (in the sense we use it here) is unlikely to proceed rapidly in either industry or academic departments of pharmacology as currently constituted. We therefore recommend new programs in three areas: research, training and education, and industry-academic collaboration. These new programs will accelerate the development of QSP and innovative thinking about therapeutic drugs in general by engaging diverse investigators in science, engineering and medicine.

RECOMMENDATIONS

Recommendations for research

We recommend the establishment of multi-faceted research programs that combine experimental and computational studies and span biochemical, genetic, animal and clinical approaches. The emphasis must be on generating and testing new ideas because the scale-up of existing ideas will not be sufficient. These new research programs should involve efforts at a variety of scales including research centers that bring cross-cutting expertise to the creation of multi-disciplinary computational, experimental, training and outreach programs. Recent success in the establishment of centers of excellence in systems biology and of biomedical computing (in NIGMS, NCI and the Common Fund) provides a template for similar large-scale efforts in QSP. Smaller-scale projects that involve two to three investigators or even individual labs that have built up the expertise needed to mount interdisciplinary programs also are required. In addition, established programs in pharmacology or systems biology should be encouraged to add elements (and/or collaborators) from other disciplines.

The distinguishing features of all QSP projects must be an explicit attempt to cut across scientific and technological barriers to advance the study of therapeutic drugs at different levels of biological complexity. An integration of computational (or mathematical) and experimental approaches is required. Identifying and evaluating the best such proposals for support will necessarily require appropriately constituting peer review groups comprised of individuals willing to think "outside the box". The following eight research areas have been identified as being of particular importance by the authors of this report:

<u>R1 Quantitative analysis of drug targets, the networks in which they are embedded and the effects of drugs on targets and networks</u>

There is a dearth of information on the absolute abundances, rates of synthesis and degradation, catalytic and binding parameters and intracellular distribution of proteins that are targeted by drugs. Collection of this type of data was common 20-30 years ago but has fallen out of favor in recent years relative to cloning genes and finding new targets. However, the absence of quantitative biochemical data on targets severely impedes the development of accurate quantitative models. We recommend

studies that involve horizontal integration spanning multiple biomolecules in a particular class or pathway (e.g. growth factor receptors, protein kinases, E3 ligases, etc.) or vertical integration that spans spatial and temporal scales (e.g. from cells, to tissues to organisms). Such studies will become particularly valuable in fields such as cancer and inflammation in which many potential targets have been identified but it remains unclear how to design a pharmacological strategy that achieves sufficient therapeutic index. Indeed, a general consensus is emerging that the most pressing need in drug discovery is not to identify new targets but to validate those targets we have and to develop optimal strategies for developing drugs that bind them. To realize the value of the quantitative information that will be collected we also require new and powerful ways to visualize and explore multidimensional protein-level data. Based on recent literature in systems biology, we are confident that quantitative data correctly presented and effectively visualized will inform models of drug action and uncover new pharmacological hypotheses.

R2 Investigating the origins of variability in drug response at the single-cell, organ and patient level based on proteomic, genomic and environmental differences

An important aspect of QSP is that it seeks to understand the origins of the variation in drug response seen under different circumstances. This includes cell-to-cell variation among members of a clonal population that are genetically identical but differ in protein composition. Such variation is likely to be one cause of "fractional killing" by anti-cancer drugs and of suboptimal E_{max} in general (Figure 8). It will also be important to understand variation induced by differences in microenvironment. Finally, given recent progress in genomics and high variability in therapeutic benefit from one patient to the next, an important aspect of QSP will be developing quantitative and predictive understanding of the way genotype impacts drug response. QSP approaches will be informed by genetic data but will include many other types of information as well as a deep understanding of the stochastic nature of biological networks and heterogeneity at the single-cell level. QSP should investigate the impact of variation in time, including circadian rhythms (chronopharmacology) and pulsatile v. continuous drug administration.

At present, substantial resources are being devoted to linking genotype directly to outcome using statistical and data mining approaches (e.g. through the Pharmacogenomics Research Network - PGRN; http://pgrn.org). QSP will complement these efforts by adding spatially and temporally resolved data and developing models that deal with (i) the biochemistry of drug targets, (ii) the gene, protein and metabolic networks in which targets are embedded, (iii) cell-cell interactions and tissue ultrastructure, and (iv) physiology of patient responses. Innovative means to integrate protein, imaging, and metabolic data with genomic data and data on current and past environmental exposure (including the impact of exposure on epigenetic state) will be a feature of the QSP studies we envision. We anticipate that such integration will yield insight into mechanisms of drug sensitivity and resistance that could not be obtained by consideration of genomic data alone.

R3: Exploiting diverse clinical data to understand drug responses in patients

The introduction of electronic medical records and the creation of large-scale omic datasets on individual patients and their treatment histories creates a unique opportunity to improve our understanding of disease and drug responses in diverse patient populations. Similarly, the advent of multiple modes of human imaging and the introduction of bio-banking in most academic medical centers enables studies of human physiology and pathophysiology at scales previously not feasible. We recommend that EMR, biobank and omic-based studies be undertaken with an explicitly quantitative pharmacological foundation. In all of these areas we require new and effective means to model and visualize complex multifactorial data sets. We anticipate that studies based on these approaches, in combination with cell, tissue and animal models will eventually enhance the success rate in moving drug candidates from pre-clinical studies to use in humans.

R4: Improving the use of animal models in testing disease hypotheses and developing new drugs

Animal models play a critical role in testing disease hypotheses and in preclinical drug development. However, there is widespread dissatisfaction with our ability to translate results obtained in animal

models to predictions about clinical success. Nonetheless, animal studies remain a pre-requisite for testing new compounds in humans. We therefore recommend a sustained effort to develop better preclinical pharmacology/drug discovery models based on deeper understanding of rodent physiology coupled with the use of the latest genetically engineered rodent models (ideally in coordination with the NIH GEMS consortium). New animal and tissue models would be informed by, and inform, the research in R1-R3.

R5: Reconnecting tissue physiology with chemistry to facilitate pharmacological experimentation and phenotypic screening on complex systems (cells and model organisms

Classical pharmacology proceeded hand-in-glove with chemical exploration of ligands and their effects on whole organisms or live organs in vitro (indeed, many key receptors were first detected and then identified based on their abilities to bind specific chemical probes). In an era of target-based discovery the connection between physiology and chemistry has largely been dissolved. However, with the advent of genetically engineered model organisms, chemoinformatic and bioinformatic methods to interrogate and classify drug effects, and techniques to screen large libraries of compounds against whole organisms, there is an opportunity to re-forge the association between integrative pharmacology (and human physiology) and chemical biology.

R6: Developing and supporting information exchanges for QSP

Virtually every area of biomedicine is faced with the task of integrating and mining large-scale data sets. This necessarily involves the development of appropriate ontologies and controlled vocabularies. In the case of QSP, we require exchange formats that extend from chemistry to electronic medical records and the subsequent creation of cross-cutting datasets. We recommend a sustained attempt to develop the necessary tools in coordination relevant advisory bodies and agencies (such as the caBIG SAB and the advisory committee on information technology to the Director of NIH).

R7: Developing multi-scale computational models of pharmacological mechanism that span the divide between cell-level biochemical models and organism-level PK/PD models

We require better quantitative models of pharmacological mechanism at all scales, starting with single targets and drugs and scaling to vertically and horizontally integrated multi-scale models. Among the pressing needs are models that can use available structural data to correctly predict the effects of known mutations and drug-target binding events on reaction rates within a physiological network. We also require methods for using molecular data, on receptor biochemistry for example, to build accurate models of paracrine and autocrine signaling among similar and different cell types. We therefore recommend investment in advancing the state of the art in modeling mammalian signaling networks and their perturbation by drugs. Dissemination of these models must also be improved: implementation of Web-based tools for analyzing sequence data (e.g. BLAST and genome browsers) was a breakthrough in bioinformatics and genomics research because it made it possible to perform complex calculations without the need to install and maintain specialized software and data sets. We recommend the development of similarly transparent and effective means to explore QSP models of different types. It remains to be determined which technologies will be most appropriate but web-based modeling environments such as the Virtual Cell (http://vcell.org) represent one avenue, as do rapid advances in cloud-based computing.

R8: Developing approaches to "failure analysis"

The vast majority of drugs that enter clinical development fail, often because of poor target selection, but we have a relatively poor understanding of the precise reasons for failure and how to avoid failure in the future. In engineering, "failure analysis" is an essential and routine part of product development and we recommend the development of failure analysis methods for pharmaceuticals across industry and academia. Such an effort will focus on multiple steps in the discovery pipeline including pre-clinical discovery and clinical trials. For example, for those drugs that fail in Phase II due to insufficient efficacy it might be particularly valuable to re-examine target selection through extended pre-clinical studies. In the case of failures due to toxicity, it would be valuable to determine whether mechanism-based toxicity

might be mitigated by a different therapeutic strategy. In some cases failure analysis might result in drug repurposing and in other cases it would lead to a reconsideration of how targets are validated. Ultimately, it would contribute to the development of means to conduct virtual and adaptive clinical trials.

Recommendations for training and education in QSP

We recommend creation of multi-faceted QSP training programs for predoctoral and postdoctoral students, MD fellows and senior investigators. We also recommend introducing all trainees in biomedical research to at least some contemporary pharmacology. Resources should also be provided to create on-line educational tools similar those available for training in bioinformatics. This will help to engage academic and industrial scientists interested in incorporating QSP concepts into their research.

T1. Defining core QSP competencies for all biomedical trainees

We recommend establishing a core set of competencies in classical and systems pharmacology that would be advantageous for a broad range of pre- and postdoctoral training programs in pharmacology, systems biology, bioengineering, pharmaceutical science and cell and molecular biology and potentially other Ph.D. programs (e.g. chemical biology, genetics and bioinformatics). This set of skills would necessarily be added to already heavy training loads and careful consideration should be given to integration with existing teaching. It seems probable that the development of open-source pedagogical materials for 2-4 lecture hours would be a reasonable initial goal. At the same time, complete tracks in systems pharmacology should be developed for addition to existing doctoral programs; means to incentivize the development of these programs should be considered.

T2. Establishing new pre- and postdoctoral training programs in programs in QSP.

We recommend establishing new training programs in QSP at both the pre- and postdoctoral levels. The possibility that these programs could span institutions (e.g. universities and hospitals) should be considered. Both Ph.D. and M.D. candidates should be included.

T3. Promoting the development of pedagogical resources

Many of the resources needed to teach QSP are poorly documented or are hard to access. Resources and databases that can serve as models, examples, and training sets (e.g. of suitably redacted clinical records and treatment histories or of PK/PD data) should be developed specifically for educational purposes.

T4. Enhancing training in clinical pharmacology

We recommend the development of programs for training MDs and PharmD fellows in QSP within the context of new or existing clinical pharmacology training programs and program for training clinical investigators. Among other advantages this will help address a need for such individuals in industry.

T5. Encouraging industry-academic collaborations

We recommend that industry and academia develop joint training programs that combine the traditional rigor of PhD programs with exposure to practical problems in industry. Specifically, collaborative industry-academic training programs should be developed (at least partly funded by industry) that include an internship experience.

T6. Training established investigators

We recommend the creation of novel pedagogical materials, forums and remote-learning tools for cross-training established investigators in new quantitative and systems pharmacology; such training could take the form of short nano-courses or web-based seminars. Two-week residential courses along the lines of *Cold Spring Harbor* courses should also be considered.

Recommendations for industry-academic collaborations in QSP

We believe that systems pharmacology will require strong academic-industrial links because only industry has the experience and resources needed to optimize drugs for use in humans and to move

drugs through clinical trials. Conversely, only academia is able to pursue new ideas with longer time-lines and without obvious financial payoff. Academia also operates in a less complex regulatory and intellectual property environment. We anticipate that analysis of clinical translation will be an important area in which industry and academia can interact. We therefore recommend the establishment of industry-academic consortia that focus on pre-competitive aspects of QSP and on training and education. Regulatory agencies such as the FDA also should play a role in these consortia. Following past examples, QSP consortia should be included in the portfolio of The Foundation for NIH (http://www.fnih.org/) and their organization should be informed by past experience with the Biomarkers Consortium and I-SPY breast cancer trial.

The purpose of the QSP consortia will be varied but include linking academic research and training to the practical aspects of drug development. This will be particularly important in tackling the problem of target selection, in pharmacokinetics and pharmacodynamics and in determining whether failure analysis can contribute to the effectiveness of preclinical-to-clinical translation. As a first step, proposals for QSP meetings and planning new centers should be considered. The success of the two NIH QSP meetings held thus far at fostering academic-industrial dialogue demonstrates that there is real interest in such interactions. Implementing an internship component of a training program would be another fertile direction and may lead to broader collaborations.

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APPENDICES

Appendix 1: Summary of the 2008 Quantitative and Systems Pharmacology Workshop

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The Quantitative and Systems Pharmacology Workshop, held 25-26 September 2008 at the NIH campus, aimed to provide state-of-the-art-knowledge and perspectives on the interface of systems biology and pharmacology to a highly diverse spectrum of researchers in academia, industry, and government. Addressing the question of where systems biology, modeling, and more quantitative measurements can be applied to pharmacology and drug discovery/action now and in the foreseeable future was the central focus of talks and discussion sessions. Five major aspects of the systems biology/pharmacology interface were explicitly featured: (i) horizontal systems integration; (ii) vertical systems integration; (iii) quantitative biology and pharmacology; (iv) data management; and (v) education and training.

Widespread appreciation arose for the need to create mechanisms for bringing the systems biology and pharmacology communities together more frequently and deeply, because of the clear promise for common benefit. The complexity inherent in understanding and predicting the pathophysiology and drug effects in patients ought to be gainfully attacked by including the methods and concepts being developed by systems biology investigators and, in turn, the envisioned impact of systems biology ought to be pursued vigorously in this important realm of molecular medicine. The arena of systems biology would benefit from greater appreciation of the diverse complexities inherent in the actions of many drugs. Systems and homeostatic perturbations induced by drugs can serve as probes of the validity of systems models.

One dimension of the scientific challenge is the need for more comprehensive 'horizontal integration'. That is, even at the level of cellular pharmacodynamics, understanding drug action requires integration of effects within the myriad interconnecting multi-pathway networks of signaling, metabolism, and gene expression; moreover, operation of these pathways involves molecular communication across cell boundaries into the microenvironment. Hence, quantitative measurement and modeling must emphasize incorporation of multi-pathway information and in tissue environmental context, which will require major advances mainly in experimental technologies including tissue-level imaging with molecular resolution.

The second dimension of scientific challenge naturally follows, the need for more explicit 'vertical integration', in which "bottom-up" models rooted in detailed molecular mechanisms at the cellular level must meet "top-down" models describing organ- and organism-level physiology with the objective of the higher-level observations being interpretable and predictable more explicitly in terms of measurable molecular and cellular properties. This will require advances mainly in computational methodologies via which consequences of multi-pathway molecular mechanistic detail at the lower level can be propagated efficiently as the scope escalates up from cells to tissues to organs to organisms. A high priority goal is to leverage the systems approach to shift the basis for biomarkers from correlative to mechanistic, and likely multi-variate, linking quantitative biology to quantitative pharmacology.

Because of practical constraints on proving and achieving the aspired combination of horizontal and vertical integration in human patient studies, one approach proffered is dedicated full-scale tests in animal studies. Although successful accomplishment of an envisioned quantitative systems pharmacology paradigm therein would not have short-term impact in the clinic, it would give confidence in the effective utility of the new paradigm.

A third, complementary challenge arises from the problem of managing—i.e., organizing, storing, accessing, and visualizing—the various kinds of experimental data in concert with the diverse classes of computational models that attempt to capture their salient features. This problem is dissimilar to the more straightforward issue previously experienced by the genomics field, in which data can be

relatively easily managed due to its fairly homogenous structure. The immense degree of heterogeneity of horizontally- and vertically-integrated data for drug actions within complex molecular networks for understanding and predicting organ- and organism-level physiological behavior will require coupled data- and modeling-management approaches that cannot currently be found "on the shelf".

Finally, there was essentially unanimous recognition that a new cohort of scientists and engineers will need to be educated at the systems biology / pharmacology interface, as the current population overlap is vanishingly small. Consensus landed on postdoctoral training as the most effective locus for dedicated educational efforts, because of the need to have a strong research capability foundation first before tackling the daunting inter-disciplinarities involved. One unusual avenue gained significant favor, that of multi-institutional postdoctoral training programs, since few institutions possess the necessary expertise across the many contributing fields.

Appendix 2 Horizontal Integration in Systems Pharmacology
(Adapted from the 2008 QSP Report)

Abstract
In a situation where the overall behavior of the system depends on many different components, horizontal integration synthesizes a composite model from several components having the same spatial and/or temporal granularity. In multi-cellular organisms, many cell and tissue behaviors are the consequences of interactions among local components. Integrated system models are expected to be able to uncover (i) precisely how components (at multiple levels) interact, (ii) the magnitudes of emergent properties (multiscale phenomena not explained by the sum total of local mechanisms), and how this emergent behavior can be modeled, (iii) the extent to which different biological circuits conform to common principles of "design", and (iv) optimal ways to alter cellular and organ phenotypes using pharmacological agents.

Discussion
Systems integration is the act of assembling a composite system—computer models in QSP—from previously autonomous components. Horizontal integration synthesizes a composite from components having the same spatial and/or temporal granularity. An example might be a cell pathway interaction model composed of various networks within and across different cells in various cell systems, but not including tissue or molecular dynamics. A clear statement of current and future uses to which an integrated system will be put is a precondition of systems integration for scientific research (this represents the requirement of fit-to-purpose for models). A use statement typically begins with the current capabilities of the individual components followed by listing the expected capabilities of the integrated system. It seems unlikely that biological component interactions will be exclusively either horizontal or vertical. We must therefore anticipate that issues of horizontal and vertical component integration will overlap and even merge into a single problem of multi-scale integration.

Impact
What are the uses of horizontally integrated system models? Such systems will be used as stand-alone software components to study specific networks, and they will also be used as components in larger horizontally and vertically integrated systems. Such uses depend on the driving biological problems. If the integrated model is intended to represent an organism's pharmacological response, for example, then the duration of the response cycle, the number of cycles considered, and required response granularity each become determining aspects. With that in mind, model and component reuse, flexibility, and adaptability, become important and that feeds back into model and component design. Therefore, it should be relatively easy to reconfigure components to represent different mechanistic hypotheses or different aspects of a key attribute under different experimental conditions. It should also be relatively simple to accommodate additional aspects at the current level of granularity or alter usage and assumptions, without requiring significant component or system reengineering. Components should be constructed so that they can be adapted easily to function as components in different, integrated models.

By way of example, the ability to target signaling and transcriptional pathways that drive diseases such as cancer will be enhanced by a more global understanding of how these pathways interconnect to create, through feedback and cross-talk mechanisms, the full signaling network that integrates all signals into a net outcome or phenotype (e.g. oncogenic transformations). The broader biomedical research community is searching for the underlying rules that govern signaling, while cancer researchers are simultaneously addressing through technology development the need to measure variations between tumor types, between tumors from different patients, and even within tumors through single cell measurements (e.g. integrated microfluidic-based assays). Using cancer models such as Bcr-Abl driven leukemic transformation, we are already collecting high dimensionality phosphoprofiling signaling data focused specifically on subnetworks that involve the cross-talk between a small numbers of signaling modules. Simplification of the problem through subnetwork analysis, allows us to first focus on a more tractable scale, while retaining clinical relevance, with the hope that

we can later expand the network diversity using the technologies and methodologies we are developing. Through iterative rounds of global measurements, perturbation of the signaling proteins involved (e.g. drug inhibition of nodes and mRNA knock down techniques), and measurement of resultant phenotype, we are building experimentally grounded theoretical descriptions of the oncogenic systems, along with establishing the basis of pharmacological interventions.

Challenges

Data integration is expected to be a major challenge in horizontally integrated QSP, as is the development of modeling methods able to describe the precise behaviors of thousands of model species. In principle, horizontally integrated models consist of components having the same granularity, and data used to control, parameterize, and observe them will be semantically grounded at the same level. This should increase the possibility of interoperability among model components. Some progress has been made with exchange formats such as SBML but additional work is clearly required.

Appendix 3: Vertical Integration in Systems Pharmacology
(Adapted from the 2008 QSP report)

Abstract
Vertical integration in pharmacology is an approach that synthesizes a composite system using previously autonomous components that have different spatial and/or temporal granularities; in this case of drug action the relevant scales are: molecular, complex, sub-cellular, cellular, multi-cell, tissue, organ, multi-organ systems, organism, and population levels. Vertical integration can be performed bottom-up, top-down, or middle-out. In each case, the challenge is to establish approaches that make it possible to move up in scale and also back down in scale in a guided manner, so that information gained at one scale can inform our understanding of behavior on another scale. An example might be connecting specific receptor binding properties measured at the cellular level with knowledge of bio-distribution (PK) and ultimate impact on organs and patients. Many existing efforts, which often fall under the rubric of multiscale modeling, are of conceptual and specific relevance to the challenge of vertical integration in systems pharmacology. Like multi-scale models, vertically integrated systems will need to be specified based on clear statements of usage, starting with the capabilities of the components, both in their original context and within the newly formed integrated system.

Discussion
The activities of the NIH Multiscale Modeling PAR which are relevant to a consideration of vertical integration in QSP are the reports of the Interagency Modeling and Analysis Group; www.nibib.nih.gov/research/multiscalemodeling/imag). The prevailing framework for multiscale integration (to the extent it is considered at all by biological scientists) relies on the traditional experimental approach of studying subsystems, which can involve isolating the subsystem from external factors (or attempting to keep them constant) and studying the behavior of the isolated subsystem. Multilevel behavior is then inferred by combining the subsystems. This is largely the approach contemplated and practiced by most computational biologists involved in multiscale modeling initiatives. However, the nature of such an isolated subsystem experimental/conceptual approach may limit one's understanding of the overall biological system, especially when the goal is to understand drug action in disease. An alternate approach to vertical understanding ("integration"), that aims to identify and elucidate the guiding principles of control and communication defining the behavior of an organism across scales is also needed.

Impact
For the pharmacologic and biological sciences, supporting drug discovery and development integration from in vitro to in vivo scales and from pre-clinical to clinical studies is particularly critical. The promise of multiscale modeling is to assess the functional impact of molecular perturbations across changes in scales. This will allow cross-scale biomarker assessment and accelerate target validation for drug discovery research. This in turn should facilitate the entry of targets into the drug development pipeline and thus help focus treatment and reduce development costs. Clinical endpoints include decision support and treatment impact studies. However, vertically integrated models will require continuous revision. New information will require revisions, especially if that new information falsifies the model in some way. To answer new questions, the use statement may need revision. We anticipate needing to deconstruct the vertically integrated system, revise or replace components, and then assemble a revised system to undergo the next round of validation challenge. How can we make it easier to accomplish these tasks? From an algorithm and software development perspective, one needs to avoid having to go through constant, and most definitely, costly revisions. A modular design may be needed, e.g., connecting nodes between pathway modules necessitates translation of code modules that can communicate, which in turn requires shares practices or standards. This is highly non-trivial for a number of reasons, including technical. It could be pushed as a community effort if one could agree on common standards—here is where the NIH could provide leadership. The In Silico Liver (ISL) exhibits elements of both vertical and horizontal integration. It uses an In Silico Hepatocyte [82] model as one of its sub-systems. The two separate aspects (use cases) are: (i) in situ perfusion output fraction

profiles for compounds studied the ISL and (ii) uptake by cultured hepatocytes of compounds for the ISH.

Challenges:

Building re-usable model components for either vertical or horizontal integration requires establishing and using standards, as well as best software engineering practices. "Industrial grade" software engineering talent is needed. The emerging field of "rules-based modeling" is promising in this regard [83-85]. The main challenges confronting multi-scale modeling are: (i) identification of the biological systems (components) with which integration will be done; (ii) quantitative experimental characterization of these components; (iii) development of reproductive and predictive models of each component; (iv) determination of the appropriate degree of model complexity that can be included in the model integration; (v) model validation; and (vi) application of mathematical and computational methods to achieve the integration. In addition, identification of appropriate ways to integrate across different levels of biological organization remains a challenge. As one example, the complexity of how to relate gene expression to protein expression will require understanding of a complex network of interacting parts. Vertical integration, as with all modeling, requires that data used in the modeling be carefully annotated with metadata describing experimental protocols, assays, preparations, etc. This can be called "context". A challenge is developing ontologies, data models and data exchange formats to do this. Moreover, as in horizontal modeling at a single level of integration, it is important that vertically integrated models be tested extensively to determine which data they reproduce, and where they fail. Failures can guide design of experiments that will help fill in the black boxes.

Like any other computational exercise in biomedicine, vertical models must lead to experimentally testable hypotheses, facilitate data integration and eventually enable outcomes to be predicted (as part of clinical translation). The latter is, of course, the truly challenging part, and it relies in large parts on the data integration. More generally, how will integrated models be falsified and/or validated? This will require experimental data on all levels, and models should be trained on clinical data. Construction and evaluation (selection, validation, falsification, and execution) are two fundamental aspects of building any system model. Each comes with many specific methods designed to maximize efficiency and efficacy. Can all necessary methods be assembled into a coherent methodology? Are the families of methods of construction distinct from those of evaluation?

Appendix 4: Data Management in Systems Pharmacology
(Adapted from the 2008 QSP Report)

Abstract
Data management involves three concepts: data formats for exchange; data messaging for communication; and data storage. Special attributes of biomedical data are: heterogeneous data types; data acquisition pipeline; imperfect laboratory information management systems (LIMS); and open and closed databases and tools. QSP places strong requirements on data analysis and management because it involves the union of previously disparate informatics disciplines.

Discussion
Simulation and modeling in QSP require an informatics infrastructure that may be more diverse than those required in other fields. Chemical informatics is critical in describing the structures and activities of small molecules. Bioinformatics focuses on the genes and protein products that are measured in the genome, the transcriptome and the proteome. Physiological modeling is important to connect the molecular scale to organs as an enabling tool for PK/PD. Finally, clinical informatics studies how clinical data can be organized and mined for significant phenotypic trends. At the same time, there are special, new data sources that may be specific to systems pharmacology.

Impact
Ultimately QSP aims to integrate data in specific drug response across many temporal and spatial scales to create cause/effect models that can be analyzed as discrete modeling "units" and used to predict drug responses in patients. Integrated models and the data sets they are built on should be made public, so that the highly complex and innovative data analyses required for model development can be parallelized among many scientists worldwide. Already, genome-enabled data sets as applied to pharmacogenomics and systems biology include large data sets on multiple time scales. After treatment with a drug, multiple changes occur at the molecular level, including protein phosphorylation within seconds, protein localization within minutes, alteration of stored RNAs for translation within minutes, and transcriptional changes within hours. Serum markers and physiological changes can mirror any of these lower level molecular alterations, with added complications of organ-organ effects. Thus it is now possible to 'profile' both low level molecular responses to a drug, and higher level reactions to these responses. What is lacking are means to link these different types of data together and to assemble a multi-scale model of pharmacology.

Challenges
QSP presents challenges in data integration, database design, and public access. Also, the statistical and bioinformatic tools required to evolve multi-scale physiome models of pharmacology are in their infancy. One approach that has emerged in the informatics community is developing workflows where tools and datasets are chained such that the output from one tool feeds into the input of another tool. A major problem we currently face is that we do not store, search or reuse data and models outside of specific areas of genomics. One solution is to add meta-data to existing and new data and models. Many models do not describe specifically the entities that are modeled, limiting their broad utility. This concept has notably been termed "harmonization" as related to work carried out by the Alliance for Cell Signaling. This approach was attempted but achieved only partial success. It is also useful to utilize modeling software that forces authors to heavily annotate their data when submitting papers for publication. Tools are being developed to facilitate the conversion of models and data into sharable formats automatically: e.g. JigCell ability to export models in ODEs and SBML, and tools to convert protein interaction networks and signaling pathways saved in text files into BioPAX format. Scientists are motivated to publish papers and will be willing to dedicate their time and effort to provide meta-data along with their studies. There should also be established data reporting protocols.

More effort also needs to be put into data organization. The design of a global QSP data warehouse that would mine, organize and disseminate data in a cloud computing fashion is one potential major useful undertaking. Many challenges exist that are specific to the integration of data across multiple scales.

We currently have diverse datasets and data types, i.e., data from clinical, pharmacological, and cellular studies. How can we manage and integrate such data to advance translational research? A consensus of the group was that "*there are so many databases... more databases than articles... by the time you learn what is in one database, another appears... it is impossible to be aware of what is available... a lot of efforts are being duplicated because of that.*" Indeed, there are also many modeling tools that do the same thing, being developed in parallel and without knowledge of the work of others. This is also why we need more meta-data and exchange standards: this will improve searching, and speed up the process of understanding the content of databases.

Appendix 5: Coursework for a Curriculum in Pharmacometrics

Formal education programs are needed to prepare pharmacometricians for the workforce. The demand for pharmacometricians currently exceeds the supply in all sectors. Projections based on the limited number of training programs indicate that this is not likely to change in the near future until significant investments are made in the development of training programs through traditional funding mechanisms (NIH-funded T32 predoctoral and postdoctoral programs), as well as creative, collaborative, cross-disciplinary initiatives through industry, academia and regulatory partnerships.

The following coursework should be available in a curriculum in pharmacometrics:

Human Pharmacology Core Coursework

- Principles of Pharmacokinetics and Pharmacodynamics (including Biopharmaceutics, Drug Metabolism and Transport, Pharmacogenomics and practical exercises with WinNonlin)
- Physiologically-Based Pharmacokinetic (PBPK) Modeling (including practical exercises using software such as MatLab)

Pharmacometrics Core Coursework

- Population PK Analysis (including practical exercises with NLME, S-Adapt, NONMEM, Simcyp)
- Bayesian Methods and Approaches
- Clinical Trial Simulation and Experimental Design (including practical exercises with Clinical Trial Simulator/WinP-OPT)

Statistics Core Coursework

- Applied Biostatistics
- Advanced Statistical and Monte Carlo Methods (including practical exercises with SAS and R)

Useful Electives

- Programming (computational methods/application and development; statistical programming)
- Systems Biology
- Drug Development and Regulatory Science
- Decision Analysis
- Advanced Pharmacotherapy