Article details

Hasselt J.G.C. van & Iyengar R. (2019), Systems Pharmacology: Defining the Interactions of Drug Combinations, *Annual Review of Pharmacology and Toxicology* 59: 21-40.
Doi: 10.1146/annurev-pharmtox-010818-021511

ANNUAL REVIEWS

Annual Review of Pharmacology and Toxicology

Systems Pharmacology: Defining the Interactions of Drug Combinations

J.G. Coen van Hasselt[1,2] and Ravi Iyengar[1]

[1]Department of Pharmacological Sciences, Systems Biology Center, Mount Sinai Institute for Systems Biomedicine, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA; email: ravi.iyengar@mssm.edu

[2]Division of Systems Biomedicine and Pharmacology, Leiden Academic Center for Drug Research, Leiden University, 2333 Leiden, Netherlands; email: coen.vanhasselt@lacdr.leidenuniv.nl

Annu. Rev. Pharmacol. Toxicol. 2019. 59:21–40

First published as a Review in Advance on September 27, 2018

The *Annual Review of Pharmacology and Toxicology* is online at pharmtox.annualreviews.org

https://doi.org/10.1146/annurev-pharmtox-010818-021511

Keywords

systems pharmacology, combination therapy, pharmacodynamics, systems biology, modeling, QSP, quantitative systems pharmacology

Abstract

The majority of diseases are associated with alterations in multiple molecular pathways and complex interactions at the cellular and organ levels. Single-target monotherapies therefore have intrinsic limitations with respect to their maximum therapeutic benefits. The potential of combination drug therapies has received interest for the treatment of many diseases and is well established in some areas, such as oncology. Combination drug treatments may allow us to identify synergistic drug effects, reduce adverse drug reactions, and address variability in disease characteristics between patients. Identification of combination therapies remains challenging. We discuss current state-of-the-art systems pharmacology approaches to enable rational identification of combination therapies. These approaches, which include characterization of mechanisms of disease and drug action at a systems level, can enable understanding of drug interactions at the molecular, cellular, physiological, and organismal levels. Such multiscale understanding can enable precision medicine by promoting the rational development of combination therapy at the level of individual patients for many diseases.

1. INTRODUCTION

Complex diseases such as cancer, diabetes, infectious diseases, and cardiovascular diseases are associated with multiple alterations in molecular pathways and complex interactions at the cellular and organ levels (1). The Cancer Genome Atlas (TCGA) studies, which provided detailed molecular characterization at the level of individual patients for many cancers (2–5), have shown that similar disease phenotypes can have differing underlying molecular networks (6, 7). This recognition of multiple molecular definitions for disease phenotypes gave rise to the idea of precision medicine, wherein diseases and treatments are guided by these molecular disease definitions in individual patients (8, 9).

When considering drug treatment strategies in complex diseases, single-target monotherapy approaches have fundamental limitations in terms of the optimal treatment effects that may be attainable, as disease phenotypes are rarely driven by single molecular entities; these limitations have led to an increasing interest in the development of combinatorial drug therapies (10–13). In oncology, the use of combination regimens is well established, and was so even long before the introduction of genomic technologies and insights into the molecular complexity of cancer (14). Yet for many other complex diseases, drug development is still primarily focused on monotherapeutic regimens, even though the molecular diversity of these diseases is often equally complex to that of cancer.

The discovery, translation, and clinical development of optimal combination drug regimens remains a major challenge in drug development (15, 16). Identification of drug combinations has been driven to a large extent by high-throughput phenotypic screens (17, 18). Although such assays are valuable, they have important limitations, including phenotypic readouts that oversimplify the complexity of disease phenotypes, in addition to practical limitations related to scalability in terms of the number of testable multidrug combinations or cell lines.

A holistic approach that considers both molecular interactions and multiscale physiological and pathophysiological mechanisms is relevant to the identification and evaluation of the effects of combinatorial drug regimens. Quantitative systems pharmacology (QSP), which combines systems biology analyses with the quantitative reasoning used in pharmacokinetic–pharmacodynamic (PK-PD) modeling, provides such a holistic approach (19–21). Systems pharmacology analyses focus on the quantitative relationships and interactions between drugs and biological systems, considering the behavior of the system as a whole rather than its individual constituents (22). Thus, systems pharmacology approaches can enable meaningful integration of the individual biological and pharmacological entities that are relevant for a pathophysiology of interest. Such integration can help us understand and predict systems-level effects of drug combination regimens.

Key challenges that could be addressed using systems pharmacology approaches include the consideration of variability of response between patients and, within patients (e.g., tumor heterogeneity), variability in terms of efficacy of drug therapy development or resistance to therapy and mitigation of drug-induced adverse events. This can be done by establishing multiscale relationships between drug action at the receptor and at the signal transduction level that describe the propagation of drug effects through cellular regulatory networks that give rise to the organ and organismal response to drug therapies.

New experimental technologies such as single-cell RNA-Seq (23), induced pluripotent stem cell–derived differentiated cells, organoids, and microfluidic microphysiological systems (MPSs) for drug testing (24–26) are of significant relevance to support systems pharmacology–guided combination therapy development. New computational methods that utilize machine learning and artificial intelligence approaches (27) are likely to have significant impact on systems pharmacology approaches in the coming years.

In this review, we discuss current and future approaches in systems pharmacology that can be used to enable rational identification of combination therapies, including repositioning of existing drugs (28) and development of personalized combination therapies.

2. RATIONALE FOR USING DRUG COMBINATIONS

Drug combination regimens may confer therapeutic benefits through different mechanisms. Drugs can interfere with disease-associated signal transduction pathways, either in serial, through different proteins in a single pathway, or in parallel, through proteins associated with different pathways (29), which can lead to synergistic drug effects (**Figure 1*a***). Such synergy arises from cooperation between different pathways in producing physiological responses; this cooperation is often a complex feature in many cellular regulatory systems. Drug combinations that target multiple pathways can also delay the development of therapy resistance (11). Synergistic drug combinations may also allow for the reduction of dose levels of individual drugs used in the combination and thus reduce the risk of exposure-driven drug toxicities and drug resistance due to desensitization at the level of drug targets.

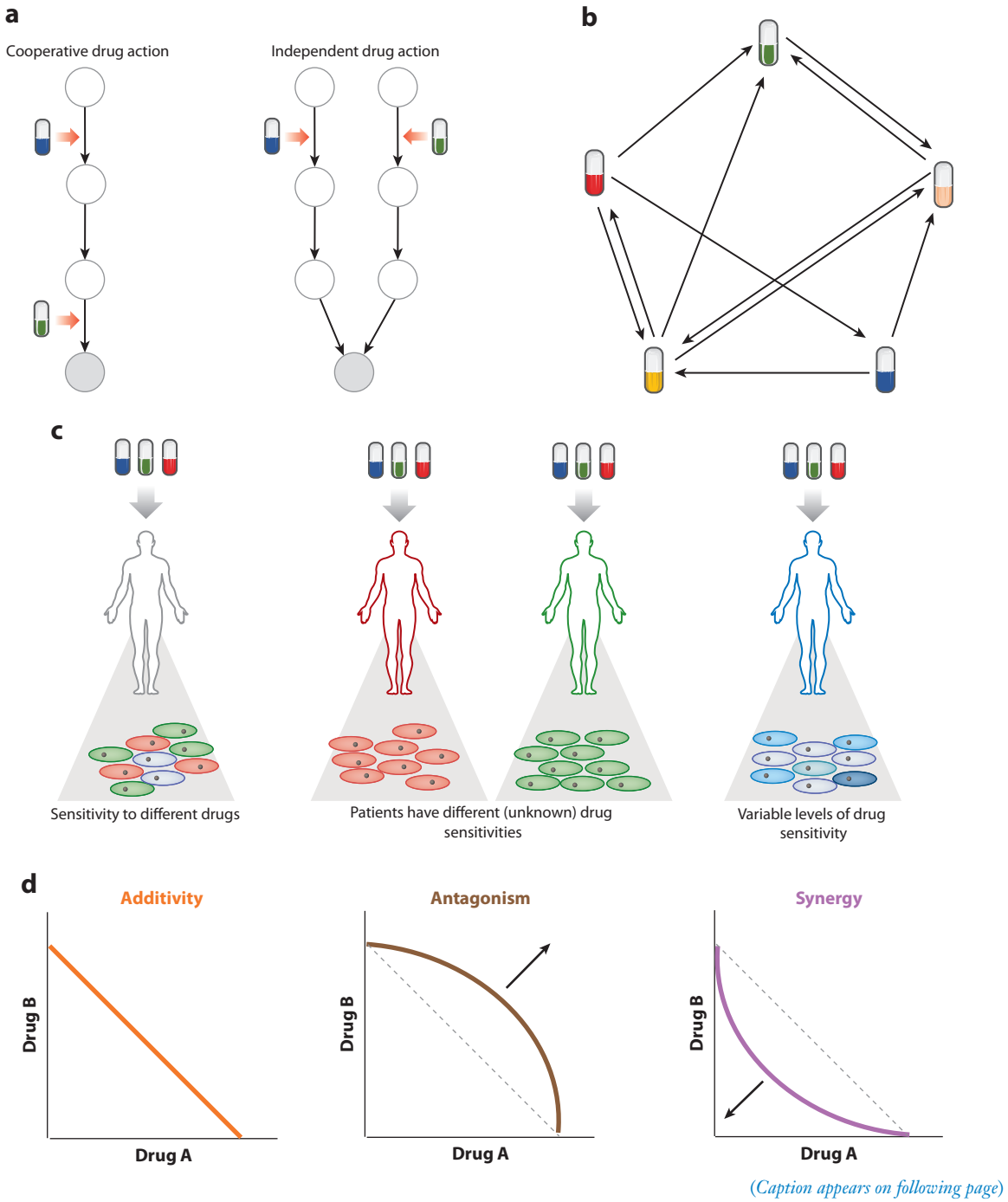
Heterogeneity in molecular characteristics of the disease within patients and variability between patients in molecular disease characteristics represent other rationales for the use of combinatorial therapies. Within-patient disease heterogeneity, e.g., tumor clonal variability, may be associated with variability in drug sensitivities across individual cells, and thus, combinatorial regimens may enable more efficient eradication of cells with variable drug sensitivities (30). When considering within-patient heterogeneity, adaptations or evolution of diseased cell populations can make selection of drug regimens even more complicated (31).

In the case of patient-to-patient variability in drug sensitivities, combination regimens may increase the possibility of at least one drug being efficacious and leading to a favorable treatment response (32) (**Figure 1*c***). Similarly, such an approach has been used for empirical combination regimens for bacterial infections, where the antimicrobial susceptibility of the pathogen is often unknown (33). Ultimately, however, the development of personalized combination therapies for complex diseases needs to be guided by molecular characterization of the disease state to ultimately reach the goal of optimal treatment outcomes in every patient (34).

Combinatorial drug interactions may also occur at the pharmacokinetic (PK) level, where drugs can affect the absorption, metabolism, or elimination of another drug, for instance, through induction or inhibition of drug-metabolizing enzymes. This may lead to undesired changes in drug concentrations, resulting in toxicities or reduced efficacy. Alternatively, such interactions may also be therapeutically exploited, for instance, to boost drug exposure (35, 36).

For diseases in which the immune system plays a role, interest in combinatorial therapies that target the immune system has increased. For instance, the field of immuno-oncology has led to major advances in the treatment of malignancies, and strong interest exists in strategies to develop combination therapies that contain drugs to stimulate the immune system (37). However, the complexity of the immune system challenges rational identification of such regimens. In this case, multiscale systems pharmacological approaches where the responses of the immune system are considered in the context of the diseased tissue response could be used to develop rational combination therapies.

Prevention and reversal of resistance to drug treatment are two important applications of antimicrobial therapies. To this end, interest is increasing in the development of combinatorial therapies to combat the challenge of antimicrobial resistance. The occurrence of collateral sensitivity (**Figure 1*b***), where resistance to one antibiotic leads to sensitivity to another antibiotic, has been



(Caption appears on following page)

Figure 1 (*Figure appears on preceding page*)

Several rationales exist for the effects of combination therapies. (*a*) Drugs may act in combination either in a serial fashion (*left*) or in a parallel fashion (*right*) on a signaling pathway component (*white circles*) that is associated with a downstream effect (*gray circle*). (*b*) The occurrence of collateral sensitivity, here depicted as a network of relationships between drug resistance (*arrow origin*) and associated drug sensitivity, can be exploited to design combinatorial therapies to restore drug sensitivity. (*c*) Heterogeneity in cellular drug sensitivity within patients (*left*) and between patients (*middle*) forms an important rationale for combinatorial regimens. Relative variability in drug sensitivity across cell types (*right*) can be addressed using resensitizing combination regimens. (*d*) Additive, synergistic, and antagonistic combinatory drug effects may be quantified by isobologram analysis. Collateral sensitivity network depicting relationships between drug resistance (*arrow origin*) and associated drug sensitivity.

suggested to enable a new paradigm for antibiotic combinatorial dosing to address antimicrobial resistance (38–41).

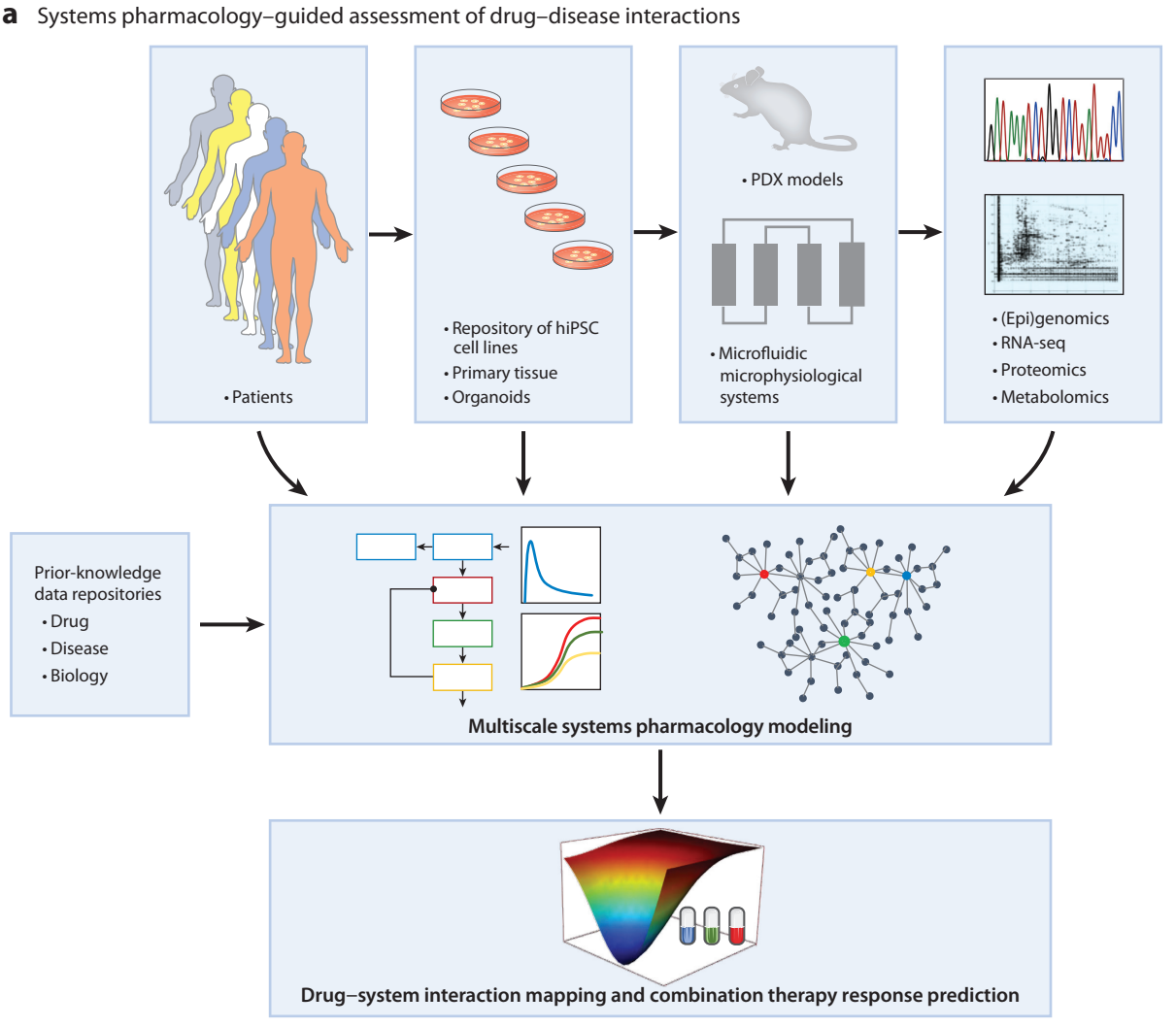
The design of drug combination regimens goes beyond the selection of particular therapeutic agents to be used together. PK and pharmacodynamic (PD) characteristics (42) can be very different for different drugs, and clinical dose regimens have to take such differences into account. Moreover, sequential combinatory drug regimens may have significant benefits, as well, as was shown for anticancer agents (43).

The quantification of combinatorial drug effects remains a debated field, where the Loewe additivity principle–based interaction index and the Bliss independence criterion remain the most important metrics (44, 45). The Loewe additivity principle (46) assumes that additivity of a combinatorial drug treatment is cooperative, as when it is mediated through a single mechanism. Isobologram analysis can be used to define the concentration of two drugs required for the maintenance of a constant level of output activity (**Figure 1*d***). The Bliss independence criterion (47) assumes independence of drug effects and does not consider nonlinearities in dose–response curves. Antagonism or synergy is determined through observed deviations from the predicted additive response. These metrics are, however, primarily relevant to quantifying the end effects in an empirical fashion. For prediction of dosing regimens, quantitative systems-level approaches that consider molecular and physiological complexity are needed.

3. EXPERIMENTAL PLATFORMS TO EVALUATE DRUG COMBINATIONS

Combination therapy identification has been primarily driven by (large-scale) phenotypic screens in in vitro cell line–based assay systems (17, 18, 48, 49); these screens have limitations in terms of physiological relevance, i.e., translatability to humans from immortalized cell lines, and when considering complex diseases that involve more than one cell type. Furthermore, such screens can quickly become infeasible when expanding the therapy to more than two drug combinations or when evaluating larger panels of cell lines to recapitulate variability in drug response. Systems pharmacology approaches may enable more efficient identification of combinatorial regimens, and experimental platforms to screen and validate drug combination regimens in physiologically meaningful yet efficient ways are thus crucially important. An example of a systems pharmacology–enabled work flow is shown in **Figure 2**.

The use of human induced pluripotent stem cell (hiPSC)-derived differentiated cell lines in drug discovery and development has been quickly becoming more common (50–53). hiPSC-derived cell line panels, which can be subjected to selective mutagenesis, may allow us to recapitulate pharmacogenomic determinants of drug response present in the patient population, thus allowing conduct of in vitro clinical trials (54) through utilization of patient-derived cell lines. Genetic engineering approaches such as CRISPR-Cas9 can be used to create cell lines with particular drug response genotypes (55) or to reconstruct relevant disease-related molecular networks in cell lines



(Caption appears on following page)

Figure 2 (*Figure appears on preceding page*)

Systems pharmacology approaches for combination therapy development. (*a*) Systems pharmacology–guided assessment of drug–disease interactions is enabled by a combination of patient-derived data and cell lines and state-of-the-art translational models and molecular profiling technologies, which enable characterization of multiscale drug–disease systems pharmacology models. (*b*) Patient-specific (liquid) biopsies analyzed with molecular profiling technologies can be used as inputs to established systems pharmacology platforms to predict optimal individualized combination regimens. Abbreviations: hiPSC, human induced pluripotent stem cell; PDX, patient-derived xenograft.

that can then be potentially useful in identifying efficacious drug combinations. For instance, a multiplexed CRISPR-Cas9 approach has been described utilizing barcoded combinatorial gene perturbations in human cell lines that could be translated into novel drug combination strategies (56).

Organoid 3D cell culture models of human cells are increasingly recognized as ex vivo models that are potentially more relevant to study disease and drug effects as these models better represent cellular heterogeneity and differentiation (25, 57). hiPSC-derived cell lines, as well as primary human cells, have been used to develop organoid models. A noteworthy development has been the creation of a living organoid biobank of colorectal cancer patients, enabling screening of drug treatment response (58). The majority of organoids have been developed for identification of cancer therapy (57). However, for many other tissues, organoid models have been generated using hiPSC-derived cell lines, including for the kidney (59) and the lung (60) and for human tissue–derived pancreatic organoids with endocrine differentiation potential (61).

Complex diseases typically involve multiple physiologically interconnected tissues. MPSs represent a rapidly developing field that combines multiple organ-on-a-chip cell or organoid cultures on a single microfluidic platform (62). Several complex multi-organ MPS models have been described, including an MPS that uses human cells to couple intestine, liver, kidney proximal tubules, the blood–brain barrier, and skeletal muscle (24). MPSs are of significant interest for drug discovery (63) and could potentially allow the study combinatorial drug treatments with more physiological relevance than single-cell-type or tissue cultures and with the possibility for medium-throughput scalability. MPSs naturally integrate with multiscale systems pharmacology models (64, 65) and have been explored for screening combinatorial therapies in patient-derived tissue (66).

Patient-derived xenograft (PDX) models consisting of engrafted tumor tissue from patients are of increasing interest for screening of drug combination regimens in the field of oncology. PDX models take into account the tumor microenvironment and allow assessment of genomic variability between tumors as seen in the patient population. Large biobank PDX repositories have been established (67, 68) that can enable preclinical phase II trials of drug (combination) regimens (67–70). Although some concerns have been raised regarding PDX models (71), they currently remain among the best systems to test drugs to predict responses in humans. One feature that makes PDX models valuable is that they preserve intratumor heterogeneity (68).

4. SYSTEMS-LEVEL MEASUREMENT OF COMBINATORY DRUG INTERACTIONS

Molecular profiling methods such as RNA-Seq (72), proteomics (73), and metabolomics (74–76) are of great relevance to mapping systems-level molecular interactions in response to (combinatorial) drug treatments. These technologies can be used for the development of novel combinations of drugs to study mechanisms through which such combinatorial regimens act and to study how

variability across cell lines or patient samples may explain differences in combinatorial drug treatment response.

Integrative analyses of multiple molecular profiling data sets can add insight into combinatorial drug response. For instance, combining (phospho-)proteomics and transcriptomics has allowed for systems-level analysis of drug combinations and synergy in imatinib-resistant chronic myeloid leukemia cells (77). Other examples include the use of whole genome sequencing of patient-derived melanoma circulating tumor cells to evaluate the potential for personalized combination therapies for melanoma (78) and the combination of genomic and transcriptomic profiles in cell line panels to predict two-drug combination therapies (79).

During drug development of novel combinatorial regimens, chemogenomic (80) approaches can be used to elucidate the role of a particular genomic background and the resulting molecular systems-level responses to individual and combinatorial regimens. For instance, chemogenomic approaches have been used to identify novel antibiotic combination therapies (81). Similarly, CRISPR-Cas9 screens are relevant to the systematic mapping of drug combinations associated with particular genetic profiles to identify combinatorial therapies to prevent anticancer drug resistance (82).

Cellular molecular heterogeneity is now increasingly recognized as a major factor that can explain the failure of drug therapy, particularly in cancer. Combinatorial therapies have been suggested as a crucial approach to addressing such intratumor heterogeneity and associated treatment response (30, 31, 83). Emerging single-cell analytical tools, such as single-cell transcriptomics, are thus of great interest for their potential to support development of combinatorial therapies (84–86). For instance, single-cell phosphoproteomic profiling allowed the characterization of signaling dynamics and the selection and validation of combination therapies for a glioblastoma in vivo model (86).

The Connectivity Map (87) offers insights into similarities and differences in drug perturbation molecular response profiles, as it contains transcriptomic signatures of a variety of drugs in human cell lines. The Library of Integrated Network-based Cellular Signatures (LINCS) program of the National Institutes of Health (NIH) could be viewed as a follow-up to this effort and contains multi-omics drug perturbation response profiles in healthy and disease-associated human cell lines, including hiPSC-derived cell line panels (88). Tools such as L1000FWD further enable exploration of such databases in an efficient manner (89). An overview of some resources to support systems pharmacology–based modeling efforts is provided in **Table 1**.

5. ANALYSIS OF MOLECULAR DRUG–DISEASE NETWORK INTERACTIONS

High-throughput omics data, which can be generated to profile the molecular response to single or combination drug treatments in experimental models and patients, require a formalized framework to transform these data into biological and pharmacological knowledge at a systems level. Such analyses can be supported by existing large-scale databases that contain information about molecular interaction networks (90), transcriptomic drug perturbation profiling (87), chemical binding affinity and bioactivity profiles (91), and existing approved or investigational drug combination regimens (92). Analysis of omics data in conjunction with such databases of prior knowledge can enable development of molecular networks to map and quantify interactions of drug combinations (93–100).

Different mathematical formalisms may be used for this purpose depending on the available level of molecular and kinetic information, including undirected molecular interaction networks (e.g., protein–protein interaction networks), directional logic models, and full kinetic models that

Table 1 Overview of relevant data repositories to support systems pharmacology combination therapy discovery

Repository	Description	URL	Reference
DCDB	Database of approved and investigational drug combinations	**http://www.cls.zju.edu.cn/dcdb/**	92
NIH LINCS	Repository containing large-scale cellular drug perturbation data	**http://lincsportal.ccs.miami.edu/**	135
STITCH	Chemical–protein interaction network database	**http://stitch.embl.de/**	136
DrugBank	Drug characteristics database including drug targets and pharmacology	**http://drugbank.ca/**	NA
ChEMBL	Database of bioactive molecules and bioactivity data	**https://www.ebi.ac.uk/chembl/**	91
ConsensusPathDB	Pathway database with integration of multiple pathway databases available	**http://cpdb.molgen.mpg.de**	90

Abbreviations: DCDB, Drug Combination Database; LINCS, Library for Integrated Network-based Cellular Signatures; NA, not applicable; NIH, National Institutes of Health.

may describe, for instance, signal transduction kinetics. Such models, which capture both network configurations and signaling dynamics, can be used to develop enhanced pharmacodynamics models that can quantitatively account for genomic and epigenomic alterations that lead to drug sensitivity or drug resistance (101).

One example of such analyses includes the interaction networks informed by the gene expression Connectivity Map data that were used to rank optimal drug combination therapies (102). Another example is the development of protein interaction networks to characterize TRAIL-induced apoptosis, and to identify potential targets for combination therapy (103). Molecular interaction network analysis has also been used to identify combinatorial regimens that may reduce the risk for cardiovascular adverse drug reactions (104). Coupling of hiPSC-derived cell lines and pharmacogenomic profiling could potentially allow the development of individualized combinatorial therapies that reduce the risk for adverse drug reactions (54).

Logic-based network models use Boolean relationships to define stimulatory or inhibiting relationships between nodes; they are increasingly being used in QSP as middle-out network models that do not require full kinetic parametrization but are much more informative than an undirected network (105–107). Several variants of logic modeling have been used, including those that utilize fuzzy logic and those that use Hill equations (105, 106, 108). Logic models have been shown to be relevant to the prediction of drug combination effects. For instance, a fuzzy logic ensemble model of intracellular signaling in hepatocellular carcinoma was used to predict the effects of key transcription factors associated with treatment response (109). Another example is the development of Boolean modeling of ErbB signaling pathways to investigate combinatorial drug targets for treatment of trastuzumab-resistant breast cancer (110).

Kinetic models describing molecular interaction networks such as for signal transduction allow us to consider multiscale models to predict combinatory drug effects. Parameterization of kinetic models can be challenging. Recently, it has been shown that transcriptomic profiling data derived from glioblastoma patient-derived tumor samples can be used to inform kinetic systems pharmacology models to conduct virtual drug combination trials (111). Another example described the prediction of combination therapies for B-cell lymphoma using a kinetic model of the B-cell receptor signaling network (112).

6. PHARMACOKINETIC–PHARMACODYNAMIC MODELING

To characterize and predict clinical-level effects of combinatory treatments at the organ and organismal levels, scaling from molecular-level network models to higher-level physiological response markers is needed. To this end, PK and PD mathematical models (42, 113, 114) are relevant. PK models describe the absorption, distribution, elimination, and metabolism of drugs in the body, where systems pharmacology–based, physiologically based PK (PBPK) models can predict local tissue target site concentrations based on system-specific tissue parameters and drug-specific physicochemical parameters (115). Drug combinatory effects may already occur at the PK level, for instance, through inhibition or induction of drug metabolizing enzymes. PBPK modeling can be used to quantify and predict such drug–drug interactions (116). Moreover, PBPK modeling approaches may help to translate among experimental models that do not account for the effects of drug metabolites or the occurrence of drug protein binding.

PK-PD models typically described biomarker-based physiological responses at the time scale of clinical treatment regimens. They allow one to effectively quantify and model interindividual variability and to account for comorbidities that may be present. PK-PD modeling is used extensively to support dose optimization in translational and clinical drug development (21, 117) and has been specifically used to investigate combinatory effects of drugs, for instance, to predict synergistic activity of gemcitabine and trabectedin in pancreatic cancer cells (118), to model tumor growth and anticancer effects of combination therapy in animal models (119), and for interspecies scaling of combination therapies for vasoconstriction (120).

7. MULTISCALE SYSTEMS PHARMACOLOGY MODELS TO PREDICT COMBINATORIAL TREATMENT REGIMENS

Systems pharmacology models that aim to predict optimal (pre-)clinical combination dose regimens should ideally consist of multiscale models that include PBPK models for drug target site concentrations, target binding kinetic models, kinetic or network-based downstream signal transduction models, and PD models that integrate molecular-level events with higher time scales and treatment response biomarkers. Such integration of network-based models for signal transduction and higher-level response have been described for the epidermal growth factor receptor pathway (101). Another example explicitly integrated PK-PD modeling with a model of the vascular endothelial growth factor signaling pathway to explore combinatory treatment regimens (121). Approaches to potentially associating logic-based models with PK-PD models have also been described (122). Agent-based models linked to network and PK-PD models are also of interest to the computational study of the impact of cell-to-cell heterogeneity in response to combinatorial treatments (123). A schematic overview of how the different technologies and modeling formalisms can be integrated to support combination therapy development is provided in **Figure 3**.

One current challenge in which multiscale systems pharmacology modeling approaches could be relevant concerns the identification of combinatorial regimens that target the immune system, such as in the field of immuno-oncology (37, 124, 125). Such models need to take into consideration the complex PK of typical large molecule immune therapeutics, as well as both inter- and intracellular immune signaling mechanisms.

8. BRIDGING SYSTEMS PHARMACOLOGY AND MACHINE LEARNING

Developments in the field of machine learning and artificial intelligence–based learning approaches are occurring at a brisk pace (126). As larger electronic health record data sets become

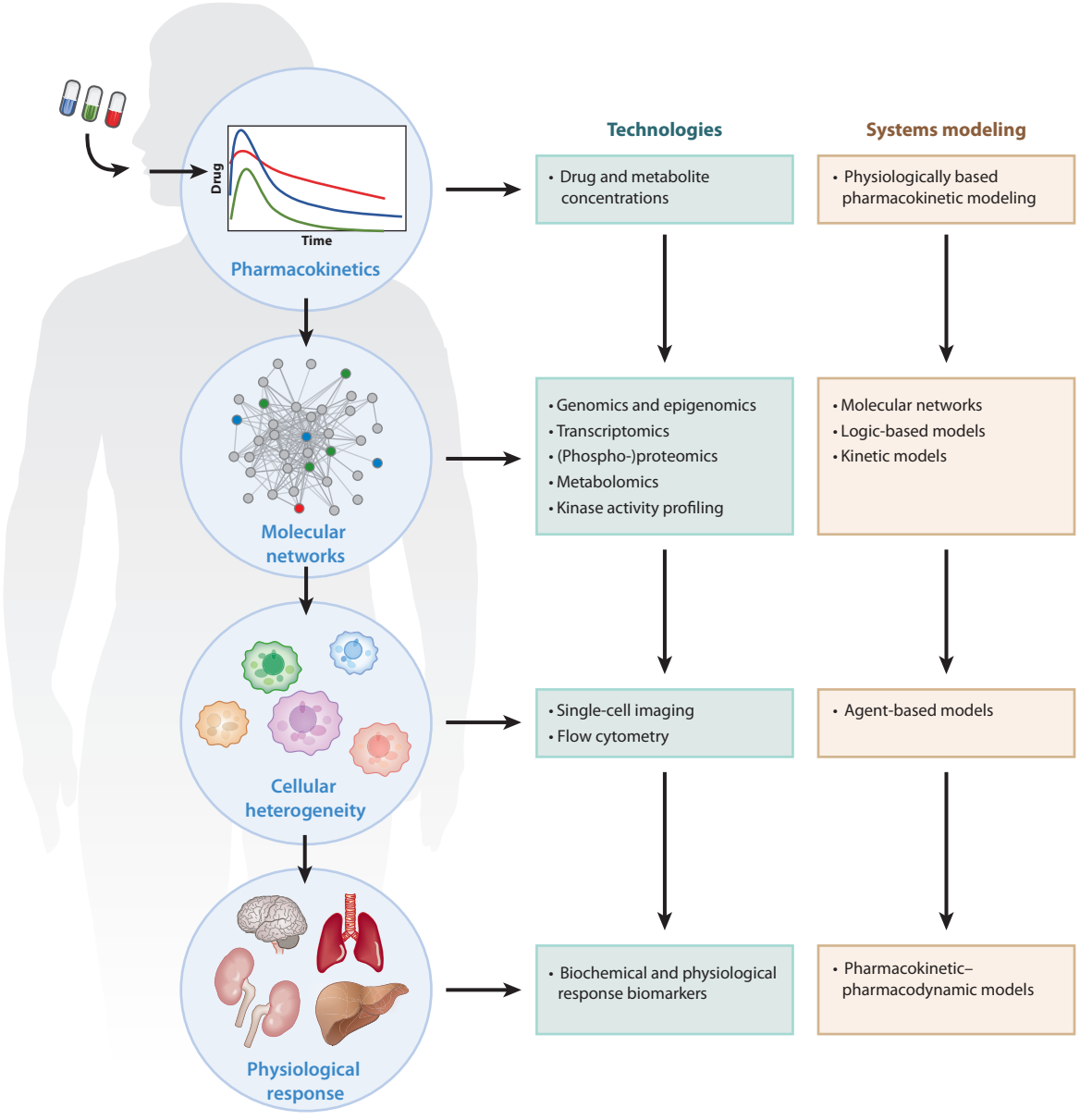


Figure 3

Drug efficacy of combination therapies is mediated by the interaction of several physiological and pharmacological processes that occur across multiple scales of time and space, including drug (target-site) pharmacokinetics, signal transduction, the effects of cellular heterogeneity, and ultimate organ (system)-specific physiological response. Quantitative systems pharmacology approaches allow us to integrate prior knowledge and patient-specific data across these scales to infer optimized combination regimens.

available, further integration of the genomic and epigenomic information of individual patients with clinical data can help us understand the genetic contributions to many complex diseases. Additionally, efforts to combine large-scale multi-omics data sets with kinetic systems pharmacology models could benefit from the use of machine learning algorithms (127). Already, machine learning–based approaches have already proven to be relevant to the prediction of optimal combination therapies (27, 128) and allow integration of phenotypic, therapeutic, chemical, and genomic properties to predict drug–drug interactions (129). Also, the integration of signaling network models with machine learning approaches has recently been reported (130). Further development of machine learning approaches may also enable us to fully utilize existing and rapidly expanding large-scale databases for experimental and clinical drug combination studies. For such data sets, it remains crucial to ensure high data quality standards, particularly for the field of systems pharmacology (131). Recent initiatives such as the NIH LINCS project have ensured that such standards are being met (88).

9. CASE STUDY: COMBINATION THERAPY DISCOVERY THROUGH TRANSCRIPTOMIC PROFILING, NETWORK ANALYSIS IN IN VITRO CELL LINES, AND PATIENT-DERIVED XENOGRAFT MODELS

Recent studies in our laboratory (132) have demonstrated how systems pharmacology approaches can be used to identify drug combinations using bulk transcriptomic data. Network modeling of these data allow us to extract signatures of expression patterns that could be used to predict responsiveness to drug therapy. We computationally analyzed TCGA data (133) for lung adenocarcinoma patients and identified a subset in which xanthine dehydrogenase expression correlated with decreased survival. We tested whether xanthine dehydrogenase inhibits proliferation in a panel of human non–small cell lung cancer (NSCLC) cell lines and identified sensitive and resistant cell lines. Bulk gene expression profiles of these cell lines were used to identify six-gene signatures for allopurinol-sensitive and -resistant cell lines. Network development and analyses identified JAK2 as an additional target in allopurinol-resistant lines. Treatment of resistant cell lines with allopurinol and the JAK2 inhibitor CEP-33779 resulted in cell death. We then utilized the six-gene signatures to predict five additional allopurinol-sensitive NSCLC lines and four allopurinol-resistant lines susceptible to combination therapy. We found that drug treatment of all cell lines yielded the responses that were predicted by the genomic signatures. We used these signatures to search a repository of PDX NSCLC tumors to identify tumors that would be predicted to be sensitive to monotherapy or combination therapy. Patient-derived tumors predicted to be allopurinol sensitive or susceptible to combination therapy showed the predicted drug response. Although this study does not show that we can predict responsiveness in the clinic, it provides a systems-based approach to predicting both drug resistance and sensitivity to combination therapy.

10. CASE STUDY: COMBINATORY THERAPY DISCOVERY FOR NERVE REGENERATION

Tissue organization adds an additional level of complexity to consider in setting up combination therapy. In an example from our laboratory (134), we used a systems pharmacology approach to develop a four-drug combination to functionally regenerate axons after nerve crushes. We reasoned that some drugs could be protective and promote regeneration by acting at the cell body, while other drugs might be more efficacious if applied at the site where the nerves were injured. This application of drugs at different locations could increase neuronal regenerative

capacity by regulating multiple subcellular processes at the cell body, while drugs near the injury site would help grow long axons in inhibitory environments. Dynamical computational models of neurite outgrowth showed that the transcriptional effects of drugs applied at the cell body served as a base, such that a combination of drugs that work locally near the site of the injured axons could produce extensive synergistic growth. We used the optic nerve crush in rats to test the drug combinations. We intravitreally injected two drugs, cannabinoid receptor-1 agonist HU-210 and IL-6 (interleukin 6 receptor agonist) to stimulate retinal ganglion cells whose axons had been crushed, and applied two drugs in gel foam, Taxol to stabilize microtubules and activated protein C to potentially clear the injury site debris field. Morphology experiments using the injured optic nerve showed that the four-drug combination promoted robust axonal regeneration from the retina to the optic chiasm and on to the visual cortex. The four-drug treatment restored pattern electroretinograms, and approximately 25% of the animals had detectable visual evoked potentials in the brain. These studies show that combination therapy that takes into account morphological complexity can promote functional axonal regeneration after nerve injury.

11. CONCLUSIONS

Systems pharmacology–based approaches offer a unique tool set to characterize and predict the multiscale interactions of drug combinations and the resulting effects, as they allow multiscale integration of molecular-level profiling data with data from the organ level and clinical treatment response. A key potential of systems pharmacology–based approaches is to combine information regarding genomic determinants of individuals with multiscale data on drug action to predict the action of specific drug combinations in individuals. These types of predictions are likely to be very useful in precision medicine. Systems pharmacology approaches can support the development of combinatorial regimens beyond the initial phases of drug discovery, providing valuable insights for both preclinical animal studies and clinical drug development. Encouraging results have been described in recent years with respect to predictions of combinatorial regimens guided by systems pharmacology approaches. However, further studies and development of new approaches are needed to optimize and catalog strategies to allow us to develop more straightforward applications for combination therapy in drug development and to enable personalized medicine in the clinic for many complex diseases. The majority of current developments have been in the field of oncology, but in many cases, these approaches may be adapted for other diseases as larger molecular and tissue- and organ-level, as well as clinical, data sets become available.

SUMMARY POINTS

1. Systems pharmacology approaches that integrate consideration of drug action at the molecular, cellular, organ and organismal levels can enable discovery of new combinatorial drug treatments to improve treatment of diseases.
2. Enabling technologies, such as single-cell transcriptomics, hiPSC-derived cell line libraries, and organ-on-a-chip MPSs, represent key platforms to enable systems pharmacology–guided combination therapy identification.
3. Multiscale systems pharmacology modeling approaches, including network analysis, dynamical modeling of signal transduction, agent-based modeling, and PBPK and PK-PD modeling, offer tool sets to enable data integration across experimental models and to allow patients to support discovery and development of novel combination regimens.

4. Integration of systems pharmacology modeling approaches with machine learning and artificial intelligence–based approaches may further enhance our ability to select combination regimens.
5. Combinatorial therapies that rationally address disease variability across patients and within-patient cellular disease heterogeneity, as well as the development of host-directed combinatorial therapies that utilize the immune system, offer unique strategies to improve outcomes in patients.

DISCLOSURE STATEMENT

R.I. has a collaboration with researchers at GlaxoSmithKline and is an invited speaker at a Merck symposium. The authors are not aware of any other affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Research in systems pharmacology in the Iyengar laboratory is supported by National Institutes of Health grant GM54508 and the Systems Biology Center Grant GM071558. J.G.C.v.H. is supported by a Marie Sklodowska Curie Postdoctoral Research Fellowship from the European Commission.

LITERATURE CITED

1. Loscalzo J, Kohane I, Barabasi A-L. 2007. Human disease classification in the postgenomic era: a complex systems approach to human pathobiology. *Mol. Syst. Biol.* 3:124
2. McLendon R, Friedman A, Bigner D, Van Meir EG, Brat DJ, et al. 2008. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 455(7216):1061–68
3. Bell D, Berchuck A, Birrer M, Chien J, Cramer DW, et al. 2011. Integrated genomic analyses of ovarian carcinoma. *Nature* 474(7353):609–15
4. Radovich M, Pickering CR, Felau I, Ha G, Zhang H, et al. 2018. The integrated genomic landscape of thymic epithelial tumors. *Cancer Cell* 33(2):244–58.e10
5. Burk RD, Chen Z, Saller C, Tarvin K, Carvalho AL, et al. 2017. Integrated genomic and molecular characterization of cervical cancer. *Nature* 543(7645):378–84
6. Patel VN, Gokulrangan G, Chowdhury SA, Chen Y, Sloan AE, et al. 2013. Network signatures of survival in glioblastoma multiforme. *PLOS Comput. Biol.* 9(9):e1003237
7. Wu G, Feng X, Stein L. 2010. A human functional protein interaction network and its application to cancer data analysis. *Genome Biol.* 11(5):R53
8. Meyer JM, Ginsburg GS. 2002. The path to personalized medicine. *Curr. Opin. Chem. Biol.* 6:434–38
9. Whirl-Carrillo M, McDonagh EM, Hebert JM, Gong L, Sangkuhl K, et al. 2012. Pharmacogenomics knowledge for personalized medicine. *Clin. Pharmacol. Ther.* 92:414–17
10. Iyengar R. 2013. Complex diseases require complex therapies. *EMBO Rep.* 14(12):1039–42
11. Fitzgerald JB, Schoeberl B, Nielsen UB, Sorger PK. 2006. Systems biology and combination therapy in the quest for clinical efficacy. *Nat. Chem. Biol.* 2:458–66
12. Keith CT, Borisy AA, Stockwell BR. 2005. Multicomponent therapeutics for networked systems. *Nat. Rev. Drug Discov.* 4(1):71–78
13. Al-Lazikani B, Banerji U, Workman P. 2012. Combinatorial drug therapy for cancer in the post-genomic era. *Nat. Biotechnol.* 30(7):679–92
14. DeVita VT, Young RC, Canellos GP. 1975. Combination versus single agent chemotherapy: a review of the basis for selection of drug treatment of cancer. *Cancer* 35:98–110

15. Pourkavoos N. 2012. Unique risks, benefits, and challenges of developing drug-drug combination products in a pharmaceutical industrial setting. *Comb. Prod. Ther.* 2:2
16. Lopez JS, Banerji U. 2017. Combine and conquer: challenges for targeted therapy combinations in early phase trials. *Nat. Rev. Clin. Oncol.* 14(1):57–66
17. Severyn B, Liehr RA, Wolicki A, Nguyen KH, Hudak EM, et al. 2011. Parsimonious discovery of synergistic drug combinations. *ACS Chem. Biol.* 6(12):1391–98
18. Borisy AA, Elliott PJ, Hurst NW, Lee MS, Lehar J, et al. 2003. Systematic discovery of multicomponent therapeutics. *PNAS* 100(13):7977–82
19. Danhof M. 2016. Systems pharmacology: towards the modeling of network interactions. *Eur. J. Pharm. Sci.* 94:4–14
20. Gadkar K, Kirouac D, Parrott N, Ramanujan S. 2016. Quantitative systems pharmacology: a promising approach for translational pharmacology. *Drug Discov. Today Technol.* 21–22:57–65
21. van Hasselt JGC, van der Graaf PH. 2015. Towards integrative systems pharmacology models in oncology drug development. *Drug Discov. Today Technol.* 15:1–8
22. van der Graaf PH, Benson N. 2011. Systems pharmacology: bridging systems biology and pharmacokinetics-pharmacodynamics (PKPD) in drug discovery and development. *Pharm. Res.* 28:1460–64
23. Islam S, Zeisel A, Joost S, La Manno G, Zajac P, et al. 2014. Quantitative single-cell RNA-seq with unique molecular identifiers. *Nat. Methods* 11(2):163–66
24. Vernetti L, Gough A, Baetz N, Blutt S, Broughman JR, et al. 2017. Functional coupling of human microphysiology systems: intestine, liver, kidney proximal tubule, blood-brain barrier and skeletal muscle. *Sci. Rep.* 7:42296
25. Fatehullah A, Tan SH, Barker N. 2016. Organoids as an in vitro model of human development and disease. 18:246–54
26. Liang P, Lan F, Lee AS, Gong T, Sanchez-Freire V, et al. 2013. Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation* 127(16):1677–91
27. Tsigelny IF. 2018. Artificial intelligence in drug combination therapy. *Brief. Bioinform.* In press
28. Sun W, Sanderson PE, Zheng W. 2016. Drug combination therapy increases successful drug repositioning. *Drug Discov. Today* 21(7):1189–95
29. Yin N, Ma W, Pei J, Ouyang Q, Tang C, Lai L. 2014. Synergistic and antagonistic drug combinations depend on network topology. *PLOS ONE* 9(4):e93960
30. Zhao B, Hemann MT, Lauffenburger DA. 2014. Intratumor heterogeneity alters most effective drugs in designed combinations. *PNAS* 111:10773–78
31. Zhao B, Sedlak JC, Srinivas R, Creixell P, Pritchard JR, et al. 2016. Exploiting temporal collateral sensitivity in tumor clonal evolution. *Cell* 165(1):234–46
32. Palmer AC, Sorger PK. 2017. Combination cancer therapy can confer benefit via patient-to-patient variability without drug additivity or synergy. *Cell* 171(7):1678–82.e13
33. Micek ST, Welch EC, Khan J, Pervez M, Doherty JA, et al. 2010. Empiric combination antibiotic therapy is associated with improved outcome against sepsis due to gram-negative bacteria: a retrospective analysis. *Antimicrob. Agents Chemother.* 54(5):1742–48
34. Jeon M, Kim S, Park S, Lee H, Kang J. 2018. In silico drug combination discovery for personalized cancer therapy. *BMC Syst. Biol.* 12(Suppl. 2):16
35. van Hasselt JGC, van Eijkelenburg NKA, Beijnen JH, Schellens JHM, Huitema ADR. 2014. Design of a drug-drug interaction study of vincristine with azole antifungals in pediatric cancer patients using clinical trial simulation. *Pediatr. Blood Cancer* 61(12):2223–29
36. Oostendorp RL, Huitema A, Rosing H, Jansen RS, Ter Heine R, et al. 2009. Coadministration of ritonavir strongly enhances the apparent oral bioavailability of docetaxel in patients with solid tumors. *Clin. Cancer Res.* 15(12):4228–33
37. Swart M, Verbrugge I, Beltman JB. 2016. Combination approaches with immune-checkpoint blockade in cancer therapy. *Front. Oncol.* 6:233

38. Imamovic L, Ellabaan MMH, Dantas Machado AM, Citterio L, Wulff T, et al. 2018. Drug-driven phenotypic convergence supports rational treatment strategies of chronic infections. *Cell* 172(1–2):121–34.e14
39. Munck C, Gumpert HK, Wallin AIN, Wang HH, Sommer MOA. 2014. Prediction of resistance development against drug combinations by collateral responses to component drugs. *Sci. Transl. Med.* 6(262):262ra156
40. Baym M, Stone LK, Kishony R. 2015. Multidrug evolutionary strategies to reverse antibiotic resistance. *Science* 351(6268):aad3292
41. Kim S, Lieberman TD, Kishony R. 2014. Alternating antibiotic treatments constrain evolutionary paths to multidrug resistance. *PNAS* 111(40):14494–99
42. Danhof M, de Jongh J, De Lange ECM, Della Pasqua O, Ploeger BA, Voskuyl RA. 2007. Mechanism-based pharmacokinetic-pharmacodynamic modeling: biophase distribution, receptor theory, and dynamical systems analysis. *Annu. Rev. Pharmacol. Toxicol.* 47:357–400
43. Lee MJ, Ye AS, Gardino AK, Heijink AM, Sorger PK, et al. 2012. Sequential application of anticancer drugs enhances cell death by rewiring apoptotic signaling networks. *Cell* 149(4):780–94
44. Greco WR, Bravo G, Parsons JC. 1995. The search for synergy: a critical review from a response surface perspective. *Pharmacol. Rev.* 47(2):331–85
45. Foucquier J, Guedj M. 2015. Analysis of drug combinations: current methodological landscape. *Pharmacol. Res. Perspect.* 3:e00149
46. Loewe S. 1928. Die quantitativen Probleme der Pharmakologie. *Ergeb. Physiol.* 27(1):47–187
47. Bliss CI. 1939. The toxicity of poisons supplied jointly. *Ann. Appl. Biol.* 26(3):585–615
48. Tan X, Hu L, Luquette LJ, Gao G, Liu Y, et al. 2012. Systematic identification of synergistic drug pairs targeting HIV. *Nat. Biotechnol.* 30(11):1125–30
49. O'Neil J, Benita Y, Feldman I, Chenard M, Roberts B, et al. 2016. An unbiased oncology compound screen to identify novel combination strategies. *Mol. Cancer Ther.* 15(6):1155–62
50. Zhang J, Li H, Trounson A, Wu JC, Nioi P. 2017. Combining hiPSCs and human genetics: major applications in drug development. *Cell Stem Cell* 21:P161–65
51. Liang P, Lan F, Lee AS, Gong T, Sanchez-Freire V, et al. 2013. Drug screening using a library of human induced pluripotent stem cell-derived cardiomyocytes reveals disease-specific patterns of cardiotoxicity. *Circulation* 127(16):1677–91
52. Engle SJ, Puppala D. 2013. Integrating human pluripotent stem cells into drug development. *Cell Stem Cell* 12(6):699–712
53. Inoue H, Nagata N, Kurokawa H, Yamanaka S, Auffray C, et al. 2014. iPS cells: a game changer for future medicine. *EMBO J.* 33(5):409–17
54. van Hassselt JGC, Iyengar R. 2017. Systems pharmacology-based identification of pharmacogenomic determinants of adverse drug reactions using human iPSC-derived cell lines. *Curr. Opin. Syst. Biol.* 4:9–15
55. Wang Y, Liang P, Lan F, Wu H, Lisowski L, et al. 2014. Genome editing of isogenic human induced pluripotent stem cells recapitulates long QT phenotype for drug testing. *J. Am. Coll. Cardiol.* 64(5):451–59
56. Wong ASL, Choi GCG, Cui CH, Pregernig G, Milani P, et al. 2016. Multiplexed barcoded CRISPR-Cas9 screening enabled by CombiGEM. *PNAS* 113(9):2544–49
57. Mebarki M, Bennaceur A, Bonhomme-Faivre L. 2018. Human-cell-derived organoids as a new ex vivo model for drug assays in oncology. *Drug Discov. Today* 23:857–63
58. Van De Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, et al. 2015. Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 161(4):933–45
59. Takasato M, Er PX, Chiu HS, Maier B, Baillie GJ, et al. 2015. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* 526(7574):564–68
60. Dye BR, Hill DR, Ferguson MAH, Tsai Y-H, Nagy MS, et al. 2015. In vitro generation of human pluripotent stem cell derived lung organoids. *eLife* 4:e05098
61. Loomans CJM, Williams Giuliani N, Balak J, Ringnalda F, van Gurp L, et al. 2018. Expansion of adult human pancreatic tissue yields organoids harboring progenitor cells with endocrine differentiation potential. *Stem Cell Rep.* 10(3):1088–101

62. Wikswo JP. 2014. The relevance and potential roles of microphysiological systems in biology and medicine. *Exp. Biol. Med.* 239(9):1061–72
63. Mathur A, Loskill P, Shao K, Huebsch N, Hong SG, et al. 2015. Human iPSC-based cardiac microphysiological system for drug screening applications. *Sci. Rep.* 5:8883
64. Yu J, Cilfone N, Large E, Sarkar U, Wishnok J, et al. 2015. Quantitative systems pharmacology approaches applied to microphysiological systems (MPS): data interpretation and multi-MPS integration. *CPT Pharmacomet. Syst. Pharmacol.* 4(10):585–94
65. Abaci HE, Shuler ML. 2015. Human-on-a-chip design strategies and principles for physiologically based pharmacokinetics/pharmacodynamics modeling. *Integr. Biol.* 7(4):383–91
66. Eduati F, Utharala R, Madhavan D, Neumann UP, Cramer T, et al. 2016. Rapid identification of optimal drug combinations for personalized cancer therapy using microfluidics. bioRxiv 093906. **https://doi.org/10.1101/093906**
67. Gao H, Korn JM, Ferretti S, Monahan JE, Wang Y, et al. 2015. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nat. Med.* 21(11):1318–25
68. Bruna A, Rueda OM, Greenwood W, Batra AS, Callari M, et al. 2016. A biobank of breast cancer explants with preserved intra-tumor heterogeneity to screen anticancer compounds. *Cell* 167(1):260–74.e22
69. Krepler C, Xiao M, Sproesser K, Brafford PA, Shannan B, et al. 2016. Personalized preclinical trials in BRAF inhibitor-resistant patient-derived xenograft models identify second-line combination therapies. *Clin. Cancer Res.* 22(7):1592–602
70. Townsend EC, Murakami MA, Christodoulou A, Christie AL, Köster J, et al. 2016. The public repository of xenografts enables discovery and randomized phase II-like trials in mice. *Cancer Cell* 29(4):574–86
71. Ben-David U, Ha G, Tseng YY, Greenwald NF, Oh C, et al. 2017. Patient-derived xenografts undergo mouse-specific tumor evolution. *Nat. Genet.* 49(11):1567–75
72. Wang Z, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nat. Rev. Genet.* 10:57–63
73. Tedford NC, Hall AB, Graham JR, Murphy CE, Gordon NF, Radding JA. 2009. Quantitative analysis of cell signaling and drug action via mass spectrometry-based systems level phosphoproteomics. *Proteomics* 9:1469–87
74. Wang X, Zhang A, Wang P, Sun H, Wu G, et al. 2013. Metabolomics coupled with proteomics advancing drug discovery toward more agile development of targeted combination therapies. *Mol. Cell Proteom.* 12(5):1226–38
75. Kohler I, Hankemeier T, van der Graaf P, Knibbe C, van Hasselt J. 2017. Integrating clinical metabolomics-based biomarker discovery and clinical pharmacology to enable precision medicine. *Eur. J. Pharm. Sci.* 109S:S15–21
76. Kell DB, Goodacre R. 2014. Metabolomics and systems pharmacology: why and how to model the human metabolic network for drug discovery. *Drug Discov. Today* 19:171–82
77. Winter GE, Rix U, Carlson SM, Gleixner KV, Grebien F, et al. 2012. Systems-pharmacology dissection of a drug synergy in imatinib-resistant CML. *Nat. Chem. Biol.* 8(11):905–12
78. Gulbahce N, Magbanua MJM, Chin R, Agarwal MR, Luo X, et al. 2017. Quantitative whole genome sequencing of circulating tumor cells enables personalized combination therapy of metastatic cancer. *Cancer Res.* 77(16):4530–41
79. Liu Y, Fei T, Zheng X, Brown M, Zhang P, et al. 2016. An integrative pharmacogenomic approach identifies two-drug combination therapies for personalized cancer medicine. *Sci. Rep.* 6:22120
80. Jones LH, Bunnage ME. 2017. Applications of chemogenomic library screening in drug discovery. *Nat. Rev. Drug Discov.* 16(4):285–96
81. Chandrasekaran S, Cokol-Cakmak M, Sahin N, Yilancioglu K, Kazan H, et al. 2016. Chemogenomics and orthology-based design of antibiotic combination therapies. *Mol. Syst. Biol.* 12(5):872
82. Han K, Jeng EE, Hess GT, Morgens DW, Li A, Bassik MC. 2017. Synergistic drug combinations for cancer identified in a CRISPR screen for pairwise genetic interactions. *Nat. Biotechnol.* 35(5):463–74
83. Reinartz R, Wang S, Kebir S, Silver DJ, Wieland A, et al. 2017. Functional subclone profiling for prediction of treatment-induced intratumor population shifts and discovery of rational drug combinations in human glioblastoma. *Clin. Cancer Res.* 23(2):562–74

84. Heath JR, Ribas A, Mischel PS. 2015. Single-cell analysis tools for drug discovery and development. *Nat. Rev. Drug Discov.* 15(3):204–16
85. Xiong Y, Soumillon M, Wu J, Hansen J, Hu B, et al. 2017. A comparison of mRNA sequencing with random primed and 3′-directed libraries. *Sci. Rep.* 7:14626
86. Wei W, Shin YS, Xue M, Matsutani T, Masui K, et al. 2016. Single-cell phosphoproteomics resolves adaptive signaling dynamics and informs targeted combination therapy in glioblastoma. *Cancer Cell* 29(4):563–73
87. Lamb J, Crawford ED, Peck D, Modell JW, Blat IC, et al. 2006. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science* 313(5795):1929–35
88. Keenan AB, Jenkins SL, Jagodnik KM, Koplev S, He E, et al. 2018. The Library of Integrated Network-Based Cellular Signatures NIH program: system-level cataloging of human cells response to perturbations. *Cell Syst.* 6:13–24
89. Wang Z, Lachmann A, Keenan AB, Ma'ayan A, Stegle O. 2018. L1000FWD: fireworks visualization of drug-induced transcriptomic signatures. *Bioinformatics* 34:2150–52
90. Kamburov A, Stelzl U, Lehrach H, Herwig R. 2013. The ConsensusPathDB interaction database: 2013 update. *Nucleic Acids Res.* 41:D793–800
91. Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, et al. 2014. The ChEMBL bioactivity database: an update. *Nucleic Acids Res.* 42:D1083–90
92. Liu Y, Wei Q, Yu G, Gai W, Li Y, Chen X. 2014. DCDB 2.0: a major update of the drug combination database. *Database* 2014:bau124
93. Jia J, Zhu F, Ma X, Cao ZW, Li YX, Chen YZ. 2009. Mechanisms of drug combinations: interaction and network perspectives. *Nat. Rev. Drug Discov.* 8(2):111–28
94. Chen D, Liu X, Yang Y, Yang H, Lu P. 2015. Systematic synergy modeling: understanding drug synergy from a systems biology perspective. *BMC Syst. Biol.* 9:56
95. Hopkins AL. 2008. Network pharmacology: the next paradigm in drug discovery. *Nat. Chem. Biol.* 4(11):682–90
96. Zhao S, Iyengar R. 2012. Systems pharmacology: network analysis to identify multiscale mechanisms of drug action. *Annu. Rev. Pharmacol. Toxicol.* 52:505–21
97. Berger SI, Iyengar R. 2009. Network analyses in systems pharmacology. *Bioinformatics* 25(19):2466–72
98. Boran ADW, Iyengar R. 2010. Systems approaches to polypharmacology and drug discovery. *Curr. Opin. Drug Discov. Dev.* 13(3):297–309
99. Schadt EE, Friend SH, Shaywitz DA. 2009. A network view of disease and compound screening. *Nat. Rev. Drug Discov.* 8(4):286–95
100. Klinger B, Sieber A, Fritsche-Guenther R, Witzel F, Berry L, et al. 2013. Network quantification of EGFR signaling unveils potential for targeted combination therapy. *Mol. Syst. Biol.* 9:673
101. Iyengar R, Zhao S, Chung S-W, Mager DE, Gallo JM. 2012. Merging systems biology with pharmacodynamics. *Sci. Transl. Med.* 4(126):126ps7
102. Huang L, Li F, Sheng J, Xia X, Ma J, et al. 2014. DrugComboRanker: drug combination discovery based on target network analysis. *Bioinformatics* 30(12):i228–36
103. So J, Pasculescu A, Dai AY, Williton K, James A, et al. 2015. Integrative analysis of kinase networks in TRAIL-induced apoptosis provides a source of potential targets for combination therapy. *Sci. Signal.* 8(371):rs3
104. Zhao S, Nishimura T, Chen Y, Azeloglu EU, Gottesman O, et al. 2013. Systems pharmacology of adverse event mitigation by drug combinations. *Sci. Transl. Med.* 5:206ra140
105. Bloomingdale P, Nguyen VA, Niu J, Mager DE. 2018. Boolean network modeling in systems pharmacology. *J. Pharmacokinet. Pharmacodyn.* 45(1):159–80
106. Traynard P, Tobalina L, Eduati F, Calzone L, Saez-Rodriguez J. 2017. Logic modeling in quantitative systems pharmacology. *CPT Pharmacomet. Syst. Pharmacol.* 6(8):499–511
107. Irurzun-Arana I, Pastor JM, Trocóniz IF, Gómez-Mantilla JD. 2017. Advanced Boolean modeling of biological networks applied to systems pharmacology. *Bioinformatics* 33(7):1040–48
108. Shim JV, Chun B, van Hasselt JGC, Birtwistle MR, Saucerman JJ, Sobie EA. 2017. Mechanistic systems modeling to improve understanding and prediction of cardiotoxicity caused by targeted cancer therapeutics. *Front. Physiol.* 8:651

109. Morris MK, Clarke DC, Osimiri LC, Lauffenburger DA. 2016. Systematic analysis of quantitative logic model ensembles predicts drug combination effects on cell signaling networks. *CPT Pharmacomet. Syst. Pharmacol.* 5(10):544–53
110. Sahin Ö, Fröhlich H, Löbke C, Korf U, Burmester S, et al. 2009. Modeling ERBB receptor-regulated G1/S transition to find novel targets for de novo trastuzumab resistance. *BMC Syst. Biol.* 3:1
111. Barrette AM, Bouhaddou M, Birtwistle MR. 2018. Integrating transcriptomic data with mechanistic systems pharmacology models for virtual drug combination trials. *ACS Chem. Neurosci.* 9(1):118–29
112. Du W, Goldstein R, Jiang Y, Aly O, Cerchietti L, et al. 2017. Effective combination therapies for B-cell lymphoma predicted by a virtual disease model. *Cancer Res.* 77(8):1818–30
113. van Hasselt JGC, Gupta A, Hussein Z, Beijnen JH, Schellens JHM, Huitema ADR. 2013. Population pharmacokinetic-pharmacodynamic analysis for eribulin mesilate associated neutropenia. *Br. J. Clin. Pharmacol.* 76(6):2878–81
114. van Hasselt J, Gupta A, Hussein Z, Beijnen J, Schellens J, Huitema A. 2015. Integrated simulation framework for toxicity, dose intensity, disease progression, and cost effectiveness for castration-resistant prostate cancer treatment with eribulin. *CPT Pharmacomet. Syst. Pharmacol.* 4(7):374–85
115. Yamamoto Y, Välitalo PA, Huntjens DR, Proost JH, Vermeulen A, et al. 2017. Predicting drug concentration-time profiles in multiple CNS compartments using a comprehensive physiologically-based pharmacokinetic model. *CPT Pharmacomet. Syst. Pharmacol.* 6(11):765–77
116. Min JS, Bae SK. 2017. Prediction of drug-drug interaction potential using physiologically based pharmacokinetic modeling. *Arch. Pharm. Res.* 40:1356–79
117. van Hasselt JGC, van Eijkelenburg NKA, Beijnen JH, Schellens JHM, Huitema ADR. 2013. Optimizing drug development of anti-cancer drugs in children using modelling and simulation. *Br. J. Clin. Pharmacol.* 76(1):30–47
118. Miao X, Koch G, Straubinger RM, Jusko WJ. 2016. Pharmacodynamic modeling of combined chemotherapeutic effects predicts synergistic activity of gemcitabine and trabectedin in pancreatic cancer cells. *Cancer Chemother. Pharmacol.* 77(1):181–93
119. Koch G, Walz A, Lahu G, Schropp J. 2009. Modeling of tumor growth and anticancer effects of combination therapy. *J. Pharmacokinet. Pharmacodyn.* 36(2):179–97
120. Yin A, Yamada A, Stam WB, van Hasselt JGC, van der Graaf PH. 2018. Quantitative systems pharmacology analysis of drug combination and scaling to humans: the interaction between noradrenaline and vasopressin in vasoconstriction. *Br. J. Pharmacol.* 175(16):3394–406
121. Zhang X-Y, Birtwistle MR, Gallo JM. 2014. A general network pharmacodynamic model-based design pipeline for customized cancer therapy applied to the VEGFR pathway. *CPT Pharmacomet. Syst. Pharmacol.* 3:e92
122. Kirouac DC, Onsum MD. 2013. Using network biology to bridge pharmacokinetics and pharmacodynamics in oncology. *CPT Pharmacomet. Syst. Pharmacol.* 2(9):e71
123. Wang Z, Butner JD, Cristini V, Deisboeck TS. 2015. Integrated PK-PD and agent-based modeling in oncology. *J. Pharmacokinet. Pharmacodyn.* 42:179–89
124. Harris SJ, Brown J, Lopez J, Yap TA. 2016. Immuno-oncology combinations: raising the tail of the survival curve. *Cancer Biol. Med.* 13(2):171–93
125. Iafolla MAJ, Selby H, Warner K, Ohashi PS, Haibe-Kains B, Siu LL. 2018. Rational design and identification of immuno-oncology drug combinations. *Eur. J. Cancer* 95:38–51
126. Silver D, Huang A, Maddison CJ, Guez A, Sifre L, et al. 2016. Mastering the game of Go with deep neural networks and tree search. *Nature* 529(7587):484–89
127. Xie L, Draizen EJ, Bourne PE. 2017. Harnessing big data for systems pharmacology. *Annu. Rev. Pharmacol. Toxicol.* 57:245–62
128. Bansal M, Yang J, Karan C, Menden MP, Costello JC, et al. 2014. A community computational challenge to predict the activity of pairs of compounds. *Nat. Biotechnol.* 32(12):1213–22
129. Cheng F, Zhao Z. 2014. Machine learning-based prediction of drug-drug interactions by integrating drug phenotypic, therapeutic, chemical, and genomic properties. *J. Am. Med. Inform. Assoc.* 21(e2):e278–86
130. Chua HE, Bhowmick SS, Tucker-Kellogg L. 2017. Synergistic target combination prediction from curated signaling networks: machine learning meets systems biology and pharmacology. *Methods* 129:60–80

131. Benson HE, Watterson S, Sharman JL, Mpamhanga CP, Parton A, et al. 2017. Is systems pharmacology ready to impact upon therapy development? A study on the cholesterol biosynthesis pathway. *Br. J. Pharmacol.* 174(23):4362–82
132. Tavassoloy I, Hu Y, Zhao S, Mariottini C, Boran A, et al. 2018. Systems therapeutics analyses identify genomic signatures defining responsiveness to allopurinol and combination therapy for lung cancer. bioRxiv 396697. **https://doi.org/10.1101/396697**
133. Cancer Genome Atlas Res. Netw. 2014. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* 511(7511):543–50
134. Siddiq MM, Zorina Y, Hansen J, Yadaw A, Rabinovich V, et al. 2018. Systems therapeutics for axonal regeneration in the central nervous system. bioRxiv 157099. **https://doi.org/10.1101/157099**
135. Koleti A, Terryn R, Stathias V, Chung C, Cooper DJ, et al. 2018. Data portal for the Library of Integrated Network-based Cellular Signatures (LINCS) program: integrated access to diverse large-scale cellular perturbation response data. *Nucleic Acids Res.* 46(D1):D558–66
136. Kuhn M, Szklarczyk D, Franceschini A, Von Mering C, Jensen LJ, Bork P. 2012. STITCH 3: zooming in on protein–chemical interactions. *Nucleic Acids Res.* 40:D876–80

Annual Review of Pharmacology and Toxicology

Volume 59, 2019

Contents

Indexes

Errata

An online log of corrections to *Annual Review of Pharmacology and Toxicology* articles may be found at http://www.annualreviews.org/errata/pharmtox