

to regulatory submission due to pharmacokinetics (PK) and bioavailability was reduced from about 40% in 1991 to less than 10% in 2000, and below 1% in phases II and III by 2007–2011.^{6,8–10} Clearly, the concerted efforts in the 1990's to assess ADME/DMPK (absorption, distribution, metabolism, excretion/drug metabolism and pharmacokinetics) much earlier as part of the lead qualification process, coupled with new technologies making that possible (e.g., combinatorial chemistry and high throughput screening), markedly reduced PK/bioavailability as a significant cause of clinical trial failure.¹¹ More recently, the use of biomarkers for clinical trial patient selection has demonstrably improved success rates: drug development programs that used such biomarkers had 25.9% likelihood of approval from phase I to approval while those that didn't had only 8.4% approval rates,¹² with benefits found at every phase transition. As patient selection biomarkers are typically related to drug mechanism-of-action, these data suggest that improving understanding of mechanisms can be highly beneficial to clinical success, if utilized in trial decision making.

These reasons of pharmaceutical success and failure provide guidance to where MPS technology might best be applied to impact translation of preclinical results to clinical success. Given the current state of MPS technology—showing promise but not fully demonstrated—our aims in this Perspective are to examine how MPS technologies could improve preclinical-to-clinical translation, and to show how utilizing a quantitative systems pharmacology (QSP) framework would be highly beneficial in those efforts.

2. How MPS technologies may improve preclinical drug discovery efforts

MPS technologies provide new *in vitro* approaches, early in the drug discovery and development process, to understand disease mechanisms and progression and assess drug–biology interactions in a more physiologically-relevant manner. The advantages compared to traditional cell cultures and animal testing are numerous. The increased tissue complexity in disease MPSs provides a more realistic assessment platform than immortalized cell lines and two-dimensional monocultures common to *in vitro* assays, and may suffice as alternatives for animal models in some cases. The use of human cell sources, including primary cells and iPSCs, also eliminates the issue of cross-species specificity that often confounds results from animal models. MPSs or multi-MPS platforms provide new testbeds in early development for evaluating potential drug–drug interactions as well as dosing and dose–response relationships of drug combinations for both efficacy and toxicity purposes. Assessment of responses may be in the form of measuring chemical entities *via* sampling of media or tissue or in some MPSs *via* rapid, noninvasive measurements such as electrical and mechanical outputs (e.g., neural action potentials and muscular contractile activity¹³). MPS

models of disease have potential for discovery of response biomarkers. Further, tissue or cell sourcing from multiple donors could provide insights into patient variability and enable patient stratification regarding who will benefit most from a therapy. Sourcing from human iPSCs provides additional advantages for disease modeling, such as monogenetic disorders for studies of disease mechanism and drug responses, as well as generating the equivalent of a patient population harboring the same mutation. Examples of MPSs focused on disease biology include efforts to represent cancer metastasis in liver,^{14,15} thrombosis in lung,¹⁶ chronic obstructive pulmonary disease in lung,¹⁷ Barth syndrome in heart,¹⁸ diabetes in an islet–liver pair,¹⁹ non-alcoholic fatty liver disease (NAFLD)²⁰ and systemic inflammation in interconnected gut and liver MPSs.²¹ Complex diseases that involve multiple organs, such as diabetes and metabolic syndrome, can now be modeled by platforms comprising multiple interconnected, interacting MPSs. Within an MPS or multi-MPS platforms, sourcing all cells from a single patient or differentiated from a single iPSC source is desirable for precision medicine applications. Although some therapeutic areas have substantially better drug approval rates than others, all have great room for improvement and so none are particularly more or less valuable targets for MPS development.^{6,8–10,12}

The characteristics of MPSs that hold promise for efficacy studies may also improve early *in vitro* assessment of adverse effects. For example, immune-competent MPSs would enable studies of the effect of immune cells on drug–biology interactions. Inflammation is hypothesized to be a major cause of idiosyncratic drug toxicity in humans^{22,23} and appropriately designed immune-competent MPSs could enable much earlier assessment than presently available. Additionally, numerous MPSs are being designed to operate for weeks or months, which could improve the *in vitro* assessment of chronic toxicities. Evaluation of toxic response markers over time could provide guidance for clinical patient monitoring. This promise notwithstanding, MPSs tend to be more operationally complex and resource intensive to use than typical *in vitro* toxicity screens, so the most realistic applications may be in assessment of toxicities related to multi-cell or -organ interactions that are difficult to detect with simpler but higher throughput assays.

While failures due directly to PK and bioavailability have been greatly reduced, drug safety and efficacy are directly related to dose, exposure, biotransformation to active metabolites, and uptake at sites of action, so improved early evaluation of PK and drug disposition can still impact preclinical-to-clinical translation. For example, for drug metabolism modes that utilize ubiquitously expressed enzymes instead of CYP or UGT (which are adequately modeled in liver-derived cultures),^{24,25} a grouping of MPSs representing liver, kidney, gut, lung, heart, fat, *etc.*, could be used to assess drug disappearance in many tissues.²⁶ Further, MPSs utilizing primary human cell sources may enable more relevant assessment than cell lines, for example, BBB or kidney MPSs^{27,28} utilizing



primary or iPSC-derived cells compared to existing *in vitro* models using MDCK-MDR1 or HK-2 cell lines, respectively.

An essential requirement for pharmacologic applications of MPSs is the quantitative evaluation of drug behavior and tissue responses with markers and endpoints as relevant to the clinic as possible, and subsequent *in vitro-in vivo* translation of these findings to human. The utility of computational models to design MPS technologies and analyze MPS data is well-demonstrated,^{26,30–34} however, there is still a scientific gap on how to correlate, extrapolate or otherwise translate quantitative MPS data to human clinical outcomes. In the following sections, we describe how quantitative systems pharmacology (QSP) could and should be utilized to fill that gap.

3. Quantitative systems pharmacology (QSP) is coming of age

Despite MPSs better representing human physiology, some level of post-experimental analysis is needed because MPSs are still *in vitro* systems that lack true systemic blood flow, waste removal, hormonal and immune systems, a microbiome, and many other defining *in vivo* contextual systems and cues. While some measures from MPSs may scale easily from *in vitro* to *in vivo*, for example based on relative cell numbers, many have no direct analog *in vivo* and will require more sophisticated analysis to determine meaning for the *in vivo* context. We believe QSP is a powerful, integrative framework for such interpretation and translation efforts (Fig. 1).

QSP can be defined 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”.³⁵ As such, QSP thinking goes beyond traditional pharmacokinetic/pharmacodynamics (PKPD) modeling to integrate more mechanistic information from a broader range of sources, such as mechanistic *in vitro* data (e.g., from MPSs), target characteristics, drug properties, *in vivo* data, human physiology, genetic information, human pathology and prior clinical data.³⁶

The transition from standard PK models to physiologically-based PK (PBPK) models is a major example of moving towards “systems thinking,” by placing preclinical data into a more complete human context. Use of PBPK models in pharmaceutical development is increasing rapidly.^{37,38} Integrating PBPK modeling with mechanistic models of relevant biology and PD will enable the interrogation of mechanisms of action and response to drugs for diverse dosing regimens, while accounting for interspecies, population, and disease state differences.

Several examples demonstrate how QSP modeling can incorporate numerous data sources into a quantitative mechanistic framework to test hypotheses, explore the relative importance of uncertainties, and extend understanding to multiple patient populations. While these examples do not utilize data from MPSs, the possibility to do so is clear.



Fig. 1 Quantitative systems pharmacology models of the biology and pharmacology for specific applications can be used to translate *in vitro* results from MPSs into the *in vivo* human context. QSP models are initially developed based on an array of biological and (patho) physiological data as well as information about target and drug characteristics (upper right). Then, quantitative information derived from MPS experimentation provides values or ranges of specific parameters to the models (top). MPS results might relate specifically to drug activity or might provide biology-specific parameters with which to define virtual patient biology for simulations of drug activity derived from other sources. Simulation and analysis using MPS inputs are then possible to predict aspects of drug efficacy, toxicity, DMPK, etc., for virtual patients (individuals, populations, subpopulations) accounting for knowledge gained from MPSs (bottom).

In one of the most far reaching, integrative QSP efforts within the pharmaceutical industry to date, Kirouac *et al.* developed a mechanism-based model of the MAPK signaling network in a BRAFV600-mutant colorectal cancer to investigate the potential benefit of combining three oncology drugs with targets on the same biochemical pathway.³⁹ The QSP model integrates information from multiple sources on multiple scales (cellular biochemistry, genetics, *in vitro* cell growth, *in vivo* tumor kinetics in patients and xenograft models, and clinical tumor responses). With it, they identified mechanisms of differential activity of MEK and ERK inhibitors as well as differential effects of supra-physiological MEK activity on resistance to MEK, ERK, and RAF inhibitors. They simulated combination dose-response matrices for the three drugs to inform combination studies for multiple cancer indications, and identified alternate dosing regimens that might have better tolerability. They report they are using the results to strategize indications and drug combination regimens for clinical trials.

An example of the success of a QSP framework for toxicity applications is DILIsym®, a mathematical model for



predicting drug induced liver injury (DILI) from mechanistic *in vitro* toxicity assays such as those for bile acid transporter inhibition, mitochondrial oxygen consumption rate and oxidative stress.⁴⁰ DILIsym® comprises multiple interacting sub-models (e.g., reactive metabolite generation, immune responses, bile acid homeostasis) to simulate the effect of a drug on the liver as a whole across multiple mechanisms of toxicity. Important insights into risks and mechanisms of DILI have been gained through its use regarding the toxicity and relative DILI risk of multiple drugs, as well as insights into species differences.^{41–43} DILIsym® is a particularly good example of integrating experimental and modeling paradigms, in that the toxicity assays provide quantitative metrics and the model utilizes these as inputs to its mechanistic algorithm to translate *in vitro* measurements into risk of hepatotoxicity *in vivo*.

Another example is a study used to extend the intravenous application of Tamiflu to infants as young as 2 weeks, a population at increased risk of complications and death from influenza.^{44,45} Complementing the existing data on oral treatment in children under 2 years and toxicology data in newborn animals, an age-dependent PBPK model of the prodrug and its metabolite in both monkey and human was utilized to demonstrate that intravenous infusions of Tamiflu would produce plasma profiles similar to oral doses and thereby not have higher safety risks. This new analysis provided the basis for the US Food and Drug Administration to forego requiring an additional toxicological study and to approve Tamiflu treatment of influenza in infants as young as 2 weeks.⁴⁴

As these examples illustrate, QSP models that account for human biology and (patho)physiology as well as drug characteristics can provide actionable insights at multiple stages of drug development. Other reviews and publications provide additional examples.^{35,40,46–50}

4. Maximizing MPS value through QSP for *in vitro*–*in vivo* translation

While integration of QSP models with MPS-based research is still nascent,⁵¹ early applications for MPS design and data analysis have already demonstrated its value. The seminal work on model-guided MPS design and data analysis was carried out by Shuler *et al.*,^{30–32,52–54} who utilized PBPK models to design multi-MPS platforms based on scaling blood residence times in organs, and to analyze mechanistic data on drug toxicity studies with multi-MPS platforms. More recently, mechanistic models of drug kinetics in single liver and liver-gut MPS platforms have been shown to be more useful than traditional PK models for estimating intrinsic ADME parameters, probe mechanisms of LPS-induced inflammation, and evaluate inter-MPS communication on PK processes.^{26,33} Multiple studies have demonstrated the critical nature of quantitative modeling for pharmacological applications of multi-MPS platforms, which are even more complex than single MPSs.^{29,54} To address the difficult question

of appropriate scaling in MPS design^{51,55–57} and move scaling from allometry to function-based, Maass *et al.*³⁴ devised a new, functional approach. Their mechanistic model-based, multi-functional scaling method identifies physical design and operational parameters for single and multi-MPS platforms based on initial specification of required biological function. A proof-of-concept study focused on drug exposure demonstrated significant differences in MPS design based on this functional scaling approach compared to allometric scaling approaches.

With the benefits of model-based MPS design and data analysis demonstrated, it is time to extend MPS-QSP integration to the translation of MPS-derived data (Fig. 1). QSP models are helpful whenever multiple functions operate simultaneously and/or are nonlinearly related, which is the case in most of human (patho)physiology and is necessarily repeated in MPSs that strive to recapitulate that. The examples in section 3 illustrate the power of integrative, mechanistic models to translate *in vitro* experimental data to humans, in the context of known human physiology, drug and target properties, and other available knowledge. A seminal application of QSP for *in vitro*–*in vivo* translation of MPS data was the study of Tsamandouras *et al.*²⁵ assessing population variability in hepatic drug metabolism using the liver MPS. In this, a population PBPK model was developed for lidocaine to extrapolate *in vitro* MPS data to the observed PK variability *in vivo*.

To understand how QSP models could be used to interpret MPS-generated data, a few thought experiments are useful. In the DILI study in section 3, for example, MPSs that embody biological functions or context important for evaluation of specific toxicity mechanisms, and better than existing *in vitro* assays, could provide inputs to DILIsym® or similar QSP models for hepatotoxicity predictions. In the study by Kirouac of the MAPK pathway for cancer therapy,³⁹ a possible use would be MPSs representing tumor-organ systems to provide alternate (human) data to xenograft models on individual drug dose-responses as inputs for the model investigations of drug combinations. Conversely, such MPSs could provide human-based *in vitro* testbeds for validating the dose-response matrices of drug combinations that were predicted prior to or instead of further animal studies.

This points out that a key area of MPS-QSP benefit is in the evaluation and understanding of multi-drug situations, related to either efficacy^{39,46} or the complications of drug-drug interactions. An example of the latter is Long *et al.*, in which the effect of tocilizumab, an IL-6 receptor antagonist, on simvastatin PK in rheumatoid arthritis patients⁵⁸ could be recapitulated over two weeks in an immune-competent liver MPS under inflammatory conditions.⁵⁹ Further experimentation to investigate stimulus-response relationships for both drugs and inflammatory levels would benefit from a QSP model encompassing mechanisms of inflammatory modulation of drug metabolism as well as tocilizumab and simvastatin PK to extrapolate the *in vitro* findings to the *in vivo* patient setting.



Numerous other applications in which QSP translation will be needed to maximally utilize MPS-based data can be envisioned. A promising use of MPSs is to understand tissue site of action PD and toxicodynamics as well as evaluate potential efficacy and toxicity biomarkers. Tissue accumulation and therapeutic effects could be quantitatively evaluated in MPSs, and QSP models could extrapolate to *in vivo* organ accumulation and predict PK/PD and toxicokinetic/toxicodynamic relationships at sites of action. Given that patient selection biomarkers result in a more than three-fold increase in likelihood of drug approval,¹² disease MPSs could provide a major new tool for discovery and/or evaluation of disease biomarkers. In concert, QSP models could put such information in human context to understand relationships to disease mechanism and drug mechanism-of-action, possibly helping speed new biomarkers into the clinic. Further, MPS-QSP studies can be used to evaluate the kinetics of biomarkers, which could then inform the timing of biomarker sampling in clinical trials.

MPS technologies have the potential to provide an experimental framework to accurately assess drug responses for patient sub-populations and even individuals, and thus, integrative MPS-QSP approaches should better enable precision and personalized medicine applications to find proper treatment options for individuals. For example, adverse drug effects for populations with renal or hepatic impairment can be experimentally evaluated in appropriate MPSs and then, dose adjustments for these patients can be calculated with QSP models. Moreover, therapeutic windows of combination treatments could be assessed using MPS data-driven QSP models. MPSs from various organ systems can provide testbeds for preclinical evaluation of therapeutic and adverse effects of combination therapies, while QSP models adjust the dosing regimen for the clinic.

A final hurdle for realizing the potential of MPS technologies, QSP models, and their integration for drug development is the acceptance of information derived from them by regulatory bodies.⁶⁰ It is promising that results from computational models, including mechanistic QSP models, are increasingly part of successful regulatory filings.^{46,47,61,62} Modeling contributions in recent years in INDs, NDAs and BLAs include estimating first in human dose,⁶³ predicting drug–drug interactions,^{64,65} extensions of drug PK/PD to special patient populations (e.g., hepatic and renal impairment, and pediatrics^{38,63}), and understanding disease progression as well as dose–exposure–response relationships,^{46,66} among other things. Favorably, a major goal of the US Food and Drug Administration in 2018–2022 is to update its regulatory science and review expertise to facilitate the development and application of “model-informed drug development”.⁶⁷ While use of MPS technologies to directly influence regulatory decisions is not yet demonstrated, it is our expectation that mechanistic systems methods, involving both MPSs and QSP models, will continue to gain acceptance for regulatory decisions.

5. Conclusion

In conclusion, the perspective that MPS technologies hold great promise for impacting preclinical-to-clinical translation in the pharmaceutical arena is supported from multiple directions: an understanding of the unaddressed needs in the drug R&D pipeline; the demonstration that MPSs better recapitulate human physiology than more traditional *in vitro* models; recognition of the value of mechanistic QSP models within biomedical and pharmaceutical R&D for integrating and interpreting data from MPSs alongside other diverse sources; and the support from regulatory agencies to include and accept computational modeling in support of regulatory applications. The MPS field will arguably have its greatest impact going forward by utilizing quantitative model-based approaches, *i.e.*, quantitative systems pharmacology, for MPS design, data analysis and *in vitro*–*in vivo* translation.

Author contributions

CLS and MC conceptualized the research and wrote and edited the manuscript.

Conflicts of interest

None to declare.

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