Application of Quantitative Biomeasures in Early Drug Discovery

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Abstract Mathematical models of drug action are essential in contemporary drug discovery and development. Applications include exposure-response modeling (pharmacokinetics-pharmacodynamics or PK-PD); quantitative understanding of biological target and pathway; and systems approaches that integrate characteristics of the biological system with associated drug exposure. Encompassing empirical, mechanistic, or semi-mechanistic approaches, these mathematical models are informed by experimental data quantifying not only drug exposure (pharmacokinetics) and associated biological response (biomarkers), but also system-specific parameters intermediate between drug exposure and response. These system-specific endpoints, or biomeasures, include target-specific measurements such as density, turnover, shedding, and internalization rate. Quantifying these pharmacokinetic and pharmacodynamic endpoints—which include small molecule, biological, and cellular measures—requires a diverse repertoire of analytical instrumentation and approaches. The discipline partnership between quantitative bioanalytics and systems modeling provides an invaluable tool to improve the success of pharmaceutical research and development. The authors will provide a perspective on the interface between laboratory science and mathematical modeling to improve assessment of exposure-response relationships, and ultimately successful drug development.

Keywords Biomeasure · Biomarker · Target · Exposure-Response · PK-PD · Systems pharmacology · Systems models · Quantitative

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Introduction: Quantitative Methods in Translational Research

Quantitative understanding of biological targets and pathways is ever more important in modern drug discovery and development, where the tolerance for risk is low and R&D productivity unsustainable [1]. Recently, it has been demonstrated how understanding drug exposure at the desired site of action, associated target engagement, and subsequent disease modulation (the *Three Pillars of Survival*) greatly contributes to de-risking drug discovery and development programs, particularly where direct measurement and quantification of exposure-response endpoints is obtained [2]. Program success appears related to the rigorous assessment of well-defined endpoints resulting from specific biological queries. These seemingly simple queries include: Will the drug get to where it is supposed to go (*is there exposure at the biological target*)? Does anything happen to the intended target when the drug is present (*is there target binding*)? Once that happens, is the target modulated as would be expected (*is there pharmacological activity*)? Though it is recognized that direct measurement of exposure, binding, and pharmacology are not sufficient to guarantee robust inference of drug action per se, application of quantitative bioanalysis of well-defined endpoints in support of this goal is necessary.

The drug discovery and development paradigm has made great strides, evolving from a largely empirical discipline to one increasingly driven by predictive approaches and science. Given the complexity of modern drug programs, and especially the pathways they are intended to modulate, use of mathematical models to interpret data, predict outcomes, and design experiments is becoming paramount. Pharmacokinetics-pharmacodynamics (PK-PD), or exposure-response modeling, both in the translational [3] and the clinical setting [4], are exemplary tools that have reshaped drug discovery into a predictive science. Mathematical PK-PD models have been extensively used in drug development to interpret available data, test mechanistic hypotheses or, at best, design experiments prospectively [5, 6]; a natural evolution of PK-PD is the emerging science of mechanistic, or systems, modeling [7].

Systems approaches, as applied in drug discovery and development, integrate aspects of a biological system with understanding of the drug's exposure (i.e., concentration), the ultimate goal being to link preclinical and clinical environments. When this is successful, compartmental models of the drug concentration profile associated with a certain dosing scheme can be integrated with subsequent target modulation or changes in relevant biomarkers, in a causal cascade that allows the researcher or clinician to propose a mechanism of action [8]. Requiring postulation of a causal relationship, models can be crafted and tested against existing data sets or, once validated, used prospectively, such as when data sets are limited or sparse [9]. The detail with which such causal relationships can be represented, or "modeled," is a function of the data informing the models, and will therefore dictate the required characteristics of biomeasure and biomarker data (e.g., assessments of

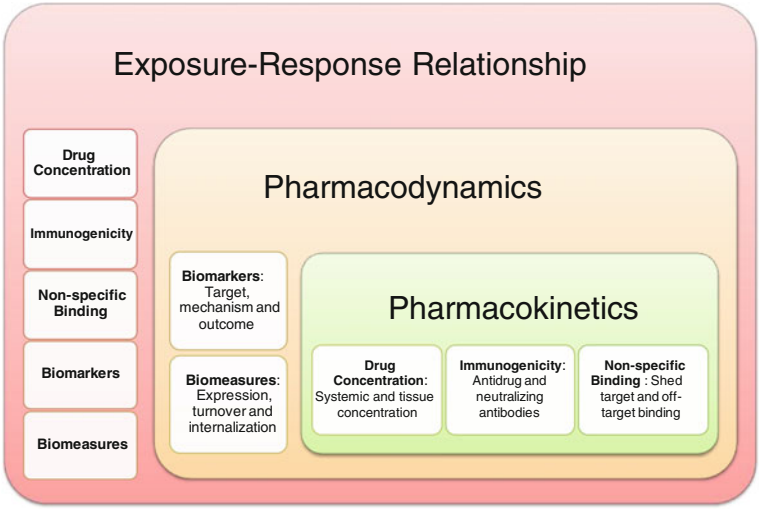

Fig. 1 Accumulated knowledge of in vivo and in vitro exposure-response relationships for investigative compounds is the result of nested interactions between understanding of the agent's pharmacokinetics (exposure) and pharmacodynamics (response). While this applies to small and large molecules and in general holds across modalities, some features of exposure-response (e.g., immunogenicity) are specific to biotherapeutics

selectivity, sensitivity, number of variables, etc.) for the model to produce valuable predictions. Clearly, with increasing detail in the system's mathematical representation, equally detailed experimental information is necessary to drive model building and incorporate the appropriate level of mechanistic detail. As such, models can be empirical [10], mechanistic or semi-mechanistic [11], or include richly detailed system-level factors [12]. We will describe how laboratory science and mathematical modeling interface, and how one influences the other to address the challenges pharmaceutical research and development is facing [1], specifically the granular understanding of the in vitro and in vivo drivers of exposure-response (Fig. 1).

We begin with a brief review of how PK-PD and system-level models have been used in drug discovery and development, after which we will move to a description of how these concepts can be integrated with quantitative bioanalysis to improve our understanding of how key targets and pathways can be modulated through pharmacological intervention.

Value and Application of PK-PD and Systems Models in Drug Discovery

As mentioned, mathematical models of drug action have a distinguished history and continue to evolve [13]. Most often these models address PK-PD relationships,

linking drug exposure, whether systemic or at the site of action, with target modulation, most often inferred or measured directly through associated biomarkers [14]. The best possible circumstance is that biomarkers are specific to a given drug target or mechanism, lending confidence in selective target engagement. Because biomarker endpoints vary greatly depending on target, biology, and site of action, the bioanalytical techniques used to measure and characterize them are equally varied and include, but are not limited to, instrument-, antibody-, and cell-based assays [15]. For example, in oncology programs to discover kinase inhibitors, phosphoproteins are routinely measured as proximal substrates where inhibition of the target can be monitored. These time courses can then be integrated into PK-PD models that encompass drug exposure, target modulation and antitumor effect [16]. These models are increasingly popular and can be used to characterize maximal effect and half-maximal effect exposure, quantitatively describing a drug's pharmacodynamic properties against its intended target. Again we recognize that PK-PD models, despite all their value, are still best classified as empirical or semi-mechanistic models. True system-level models, where signaling or metabolic pathways are explicitly represented in their constituent parts, remain comparatively rare [8]. Their emergence is arguably the next frontier for applied modeling and simulation in pharmaceutical research. The emerging discipline of systems pharmacology offers promise for drug discovery especially when it is symbiotically linked to available laboratory methods that provide unprecedented quantification of in vitro and in vivo biology [17]. Systems pharmacology is a unique evolution of systems biology in the following ways [12]:

1. Systems pharmacology recognizes the importance of time-dependent data and time series, thus using differential as opposed to algebraic or steady-state equations;
2. Systems pharmacology uses drugs and pharmacologic agents to probe the system and investigate how it responds, thus focusing on dynamic changes, as opposed to homeostatic behavior;
3. Systems pharmacology uses tools defining pathway analysis, i.e., PK-PD and systems biology, to ultimately predict the in vivo behavior of intact systems and their response to a variety of perturbations.

Because it is well differentiated from other modeling approaches not only in terms of complexity, but also in terms of intended impact and use, systems pharmacology has generated increasing interest from both academia and industry [18]. This requires parallel advancements in quantitative bioanalytical platforms and methodologies, necessary to inform these more complex mathematical models. Convergence of these independent disciplines, quantitative bioanalytics and systems models, has the potential to create a differentiated toolkit for pharmaceutical research—a truly quantitative approach to the understanding of biological targets and their pathways—building on the inherent reciprocity of these sciences. Specifically, this can inform a *virtuous circle*, i.e., how establishment of systems pharmacology models can motivate bioanalysis, and vice versa.

Data Requirements to Inform Quantitative and Systems Pharmacology Models

In principle, a well-characterized mathematical model or prediction should describe and reconcile appropriately quantified biological variables. Data requirements vary depending on the model's intended purpose, for example, whether a mathematical model is used to make pharmacokinetic predictions, which can be validated against readily obtainable data [19], or pharmacodynamic scaling [20], where efficacy predictions can be made using nonclinical models, then compared to clinical observations. In the clinic, given patient availability and logistical constraints, measurements are often limited to drug concentrations. There is, however, the potential for quantitative bioanalysis to provide essential mechanistic parameters for systems pharmacology models, whose predictions can then be iteratively tested and updated when new experimental evidence comes to light. The concept of "*biomeasure*" is an example of successful synergy between bioanalysis and modeling and simulation. Biomeasures can be defined as drug-independent characteristics of the biological system, such as receptor density or target turnover, necessary to successfully implement mechanistic, predictive models [21].

What Are Biomeasures? Their Context and Application

A Brief Introduction to Biomeasures

Biomeasures are a relatively new concept finding favor in applied drug discovery [13]. Biomeasures are system-specific parameters that are intermediate between drug exposure (measured by concentrations) and its response (quantified through biomarkers). Examples of biomeasures in a drug discovery program may include target density, target turnover, target shedding, and rate of internalization. Biomeasures are necessary to fully characterize how drug molecules trigger the mechanistic cascade that ultimately leads to effect. Depending on how much detail is needed to make informed predictions on the system, the number of biomeasures required to inform a model can be large or small, and the choice of what biomeasures to monitor depends on the priorities of a particular program. It is important to note this is not a one-size-fits-all approach. Differences in target properties, e.g., turnover, can motivate different approaches in how quantitative bioanalysis is performed and prioritized, especially in large molecule development [22].

Key Biomeasures of Interest in Drug Development

Depending on how the drug interacts with the target and how complex the pathway of interest is, a varying amount of detail is necessary to elucidate the therapy's

mechanism of action [23]. This is the role played by emerging tools such as proteomics [24] and techniques to measure the immune response [25] that may, for example, be required to obtain a mechanistic understanding of immunogenicity [26].

PK-PD methods have a long and illustrious history in facilitating the understanding of reversible inhibitors' mechanisms of action [27]. An example of where target properties become crucial even for small molecule drugs is in irreversible inhibitors, valuable in treating various diseases [28]. The development of irreversible inhibitors is relatively recent [29] and in this therapeutic class target turnover plays a major role in drug target engagement and pharmacology, as shown in [30], thus indicating turnover as a key biomeasure to assess in a comprehensive model.

A Review of Established and Emerging Tools Informing PK-PD and System Models

The predictive quality of exposure-response models relies heavily on the quality of data used to inform the model. Data quality itself—accuracy, precision, and specificity, for instance—is a function of analytical methodology or platform, access to reliable reference standards and controls, and understanding of the biological system itself, among other variables. A modeler can obtain data from a number of sources. Often data on target expression and internalization may be published in the literature, and text mining [31] may provide biomeasure data sufficiently robust to initially inform a model in early development or for biomeasure endpoints that are particularly difficult or impractical to obtain. Target expression and related biomeasure data may also be available from biological studies that characterize protein expressing using Western blot or immunohistochemistry (IHC). However, in order to provide reliable data that comparably informs both "halves" of the PK-PD continuum, so to speak, bioanalytical approaches that independently quantify exposure, response, and target assessment, applying more rigorous analytical tools, may be favored or required. This is particularly relevant in systems where the predictive strength of the model necessitates higher precision, accuracy, or specificity. "Stress points" in the model, obtained via one of the many flavors of sensitivity analysis [32], can highlight the need to quantify a specific flux or control parameter most accurately, or, conversely, can suggest that accurate quantification is not crucial for model predictions. Such approaches can provide a quantitative basis to set laboratory objectives for a new discovery program (potency, target engagement mechanism, extent of target modulation desired, etc.).

Assessing Drug Exposure—PK

In order to make quality drug exposure measurements, well-characterized standards and biological matrix free of endogenous analyte is critical. Established bioanalytical platforms such as mass spectrometry and antibody-based approaches such as enzyme-linked immunosorbent assays (ELISA), arguably the workhorse methodologies of the contemporary bioanalytical pharmacokinetic laboratory, are well suited for these bioanalytical applications, and acceptance criteria for assay performance have been established and applied for pharmacokinetic, bioavailability, and bioequivalence assessment for a number of years [33]. Additional analytical capabilities inherent in mass spectrometry and antibody-based analytics include multiplexing [34, 35], automation, including data interchange [36], and common platform expertise allowing assay transfer between laboratories. These characteristics of established PK bioanalysis are worth noting, as they have arguably become the benchmark used to assess the quality of non-PK bioanalytical methods.

Assessing Drug Effect and Target Engagement—PD, Biomarkers and Biomeasures

In comparison to therapeutic drug assessment, biomarkers and biomeasures are, by definition, endogenous endpoints and are often not fully characterized, particularly since they are often macromolecules that exist in multiple isoforms or chemically modified states. This adds complexity and ambiguity to the analysis. To address the inherent ambiguity in biomarker data obtained by various assays, Lee et al., have recommended a system to categorize biomarker assay data based on the type of assay employed [37]. This nomenclature defines biomarker assays as (a) definitive quantitative, (b) relative quantitative, (c) quasi-quantitative, and (d) qualitative, reflecting variability in access to, or purity of, definitive reference standards and specifics of experimental design. This scaled approach characterizing the quantitative rigor of endogenous analytes provides a convenient framework to recognize the quantitative limits of the analytical methodology, mitigating the risk of over-interpretation of model projections or over-interpretation of model estimates. This is of equal relevance when investigating a molecule's safety and efficacy [38].

Emerging and Innovative Tools

Innovative bioanalytical tools are evolving with unique capabilities in addressing biomeasure endpoints, including target expression, turnover, and internalization. Two of these approaches, imaging flow cytometry and mass cytometry, build on the flow cytometry platform, which, like mass spectrometry and antibody-based

methods, is a cornerstone of the contemporary pharmaceutical laboratory, as shown by published applications addressing mechanism-based assessment of target engagement and safety [39]. Imaging flow cytometry builds on the capabilities of flow cytometry, adding spatially separated imaging and digital microscopy [40] that provides unique capabilities in assessing biomarkers and biomeasures [41]. Mass cytometry couples fluorescent-based flow cytometry with inductively coupled mass spectrometry to quantify epitope-specific antibodies custom labeled with rare earth isotopes, providing unparalleled multiplexing capabilities in assessing surface antigen expression [42, 43]. These and other tools promise to provide differentiated improvements, provided interpretative models continue their symbiotic relationship with laboratory sciences, and vice versa.

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