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European Journal of Pharmaceutical Sciences 109 (2017) S15–S21

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Integrating clinical metabolomics-based biomarker discovery and clinical pharmacology to enable precision medicine

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ARTICLE INFO

Keywords:
Metabolomics
Biomarkers
Precision medicine
Pharmacokinetic-pharmacodynamic modeling
Pharmacology

ABSTRACT

Novel developments in biomarkers discovery are essential in modern health care, notably in treatment individualization and precision medicine. Clinical metabolomics, which aims to identify small molecule metabolites present in patient-derived samples, has attracted much attention to support discovery of novel biomarkers. However, the step from discriminatory features of disease states towards biomarkers that can truly individualize treatments is challenging. Biomarkers used for treatment individualization can either be dynamic or static prognostic biomarkers. Dynamic biomarkers are relevant for describing the clinical response, including dynamical disease progression and associated treatment response. Static (prognostic) biomarkers do not describe but rather predict a clinical response, and typically reflect aspects of the physiological state of a patient related to drug treatment response or disease progression dynamics. Pharmacokinetic-pharmacodynamic (PK-PD) modeling represents an established approach for drug treatment individualization based on drug exposure or treatment response biomarkers, as well as for the description of disease progression dynamics. Here, we discuss how novel treatment individualization biomarkers can be identified using a clinical metabolomics-based approach, and how concepts inspired from the field of PK-PD modeling can be integrated in this process in order to increase the clinical relevance of identified biomarkers and precision medicine.

1. Introduction

Biomarkers have been defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"(Biomarkers Definitions Working Group 2001). Biomarkers form the cornerstone of treatment individualization and precision medicine. Here, treatment individualization biomarkers should not only inform the choice of the best drug for each patient, but, equally important, the optimal individualized choice of dose regimen. In this context, we emphasize the important distinction between dynamic and static biomarkers, illustrated in Fig. 1. Dynamic biomarkers describe disease progression and associated treatment response (Fig. 1), and are widely used in patient care and drug development. Examples are prostate specific antigen dynamics for prostate cancer treatment response (van Hasselt et al. 2015), or neutrophil count dynamics to monitor drug-induced hematological toxicity (van Hasselt et al. 2013). Static biomarkers are prognostic and aim to predict a clinical response or a dynamical biomarker thereof (Fig. 1). Examples include gene expression signatures to predict clinical benefit of anti-cancer drugs (Cardoso et al. 2016) or the prediction of risk of cardiac function declines after trastuzumab treatment (van Hasselt et al. 2011).

Mathematical pharmacokinetic-pharmacodynamic (PK-PD) modeling has developed into an established quantitative approach to support both drug development and clinical treatment individualization (Knibbe and Danhof 2011; Van Hasselt et al., 2014a; Van Hasselt et al., 2014b). PK-PD modeling uses biomarkers for drug exposure and their relation with drug effects and disease progression, as well as their explicit consideration of the dynamics and inter-individual variation (Danhof et al. 2005). The field of PK-PD has now co-evolved together with the increasing biochemical characterization of disease and drug response to the emerging area of quantitative systems pharmacology (QSP) modeling (Danhof 2016; Danhof et al. 2008; van der Graaf and Benson 2011; van Hasselt and van der Graaf 2015).

Abbreviations: CNS, central nervous system; MS, mass spectrometry; PD, pharmacodynamics; PK, pharmacokinetics; QSP, quantitative systems pharmacology

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http://dx.doi.org/10.1016/j.ejps.2017.05.018
Received 9 May 2017; Accepted 10 May 2017
Available online 11 May 2017

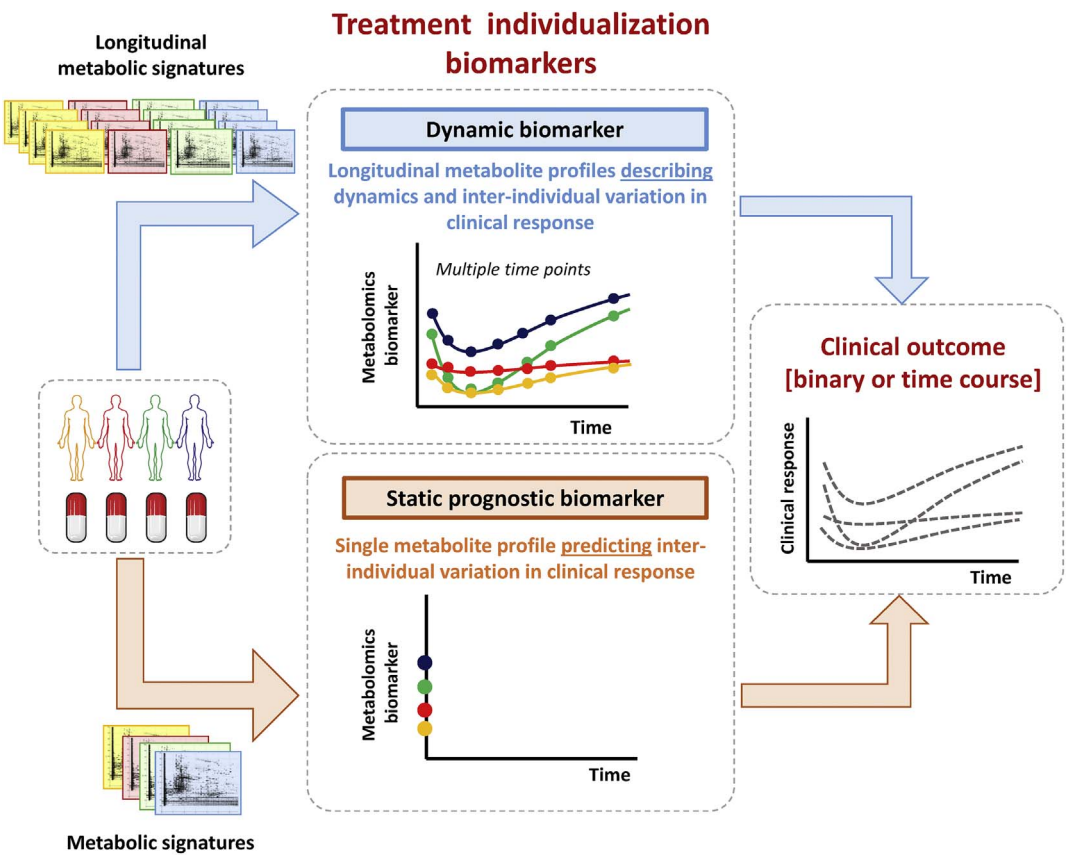

Fig. 1. Biomarkers discovery for drug treatment individualization. This schematic representation illustrates the role of metabolomics in combination with pharmacological PK-PD and QSP modeling in biomarker discovery. The importance of prognostic and dynamic biomarkers, which can be used to respectively predict and describe the inter-individual variation in disease progression and treatment response, is highlighted. PD, pharmacodynamics; PK, pharmacokinetics; QSP, quantitative systems pharmacology.

Current clinical practice relies on a limited selection of diagnostic molecular biomarkers to predict or monitor the response to a treatment in individual patients. The majority of these biomarkers have been identified decades ago, even though for many indications adequate biomarkers are missing. For instance, clinical biomarkers for pain are still lacking (Goulooze et al. 2016), upon which a recent IMI initiative focusing on pain biomarkers has been proposed (Innovative Medicines Initative 2016).

Innovative strategies are needed to identify novel biomarkers. In this context, metabolomics represents an attractive molecular profiling technology (Beger et al., 2016; Koen et al. 2016; Wishart 2016). The metabolome comprises molecular intermediates and end-products resulting from different cellular and physiological processes, encompassing a large diversity of small molecules that are closely related to (patho)physiological conditions and treatment response phenotypes (Fiehn 2002; Patti et al. 2012; Ramautar et al. 2013).

The current impact of metabolomics biomarkers in daily clinical practice is still limited, which is probably associated with the specific challenges encountered in clinical metabolomics, namely, study design, bioanalysis and data analysis, that still need to be overcome (Kohler et al. 2016). Guidelines addressing some of these challenges have been already proposed and discussed elsewhere (Beger et al., 2016; Dunn et al. 2011; Dunn et al. 2012; Kohler et al. 2016; Sumner et al. 2007; Want et al. 2010). However, limited attention has been given to the challenges associated with discovery of treatment individualization biomarkers that would include explicit consideration of the disease progression dynamics and drug-exposure response relationships. Integrating established PK-PD and QSP concepts as well as techniques to support the discovery of clinical metabolomics-based static prognostic and dynamical disease treatment individualization biomarkers can represent a powerful and relevant approach to bridge the gap between current metabolomics practices and development of effective treatment individualization biomarkers, as emphasized in Fig. 2. This review provides practical considerations for development of metabolomics-based treatment individualization biomarkers to enable precision medicine. We focus specifically on the characterization of dynamic biomarkers that describe disease progression and treatment response, and static prognostic biomarkers that predict a clinical response (Fig. 1).

2. Clinical Study Design and Bioanalytical Metabolomics Strategies

This section discusses specific considerations for controlling and reducing variation through appropriate study design and adequate bioanalytical experiments to obtain high-quality metabolomics data as well as optimize conditions for identification of the most relevant treatment individualization biomarkers.

2.1. Prospective and retrospective studies

Metabolomics biomarker discovery studies are ideally based on prospectively designed studies with a clear defined clinical response outcome, although there is also significant value in the analysis of archived samples from well-designed and previously conducted studies. Retrospective studies either consist of short-term clinical studies that may have been already designed to investigate a disease or treatment of interest, or epidemiological studies. Epidemiological studies that collect patient-derived samples (e.g., blood, urine, etc.) together with other health data are relevant for the identification of disease progression biomarkers (Hofman et al. 2015; Marmot and Brunner 2005; Pardo et al. 2005; Salomaa 2016).

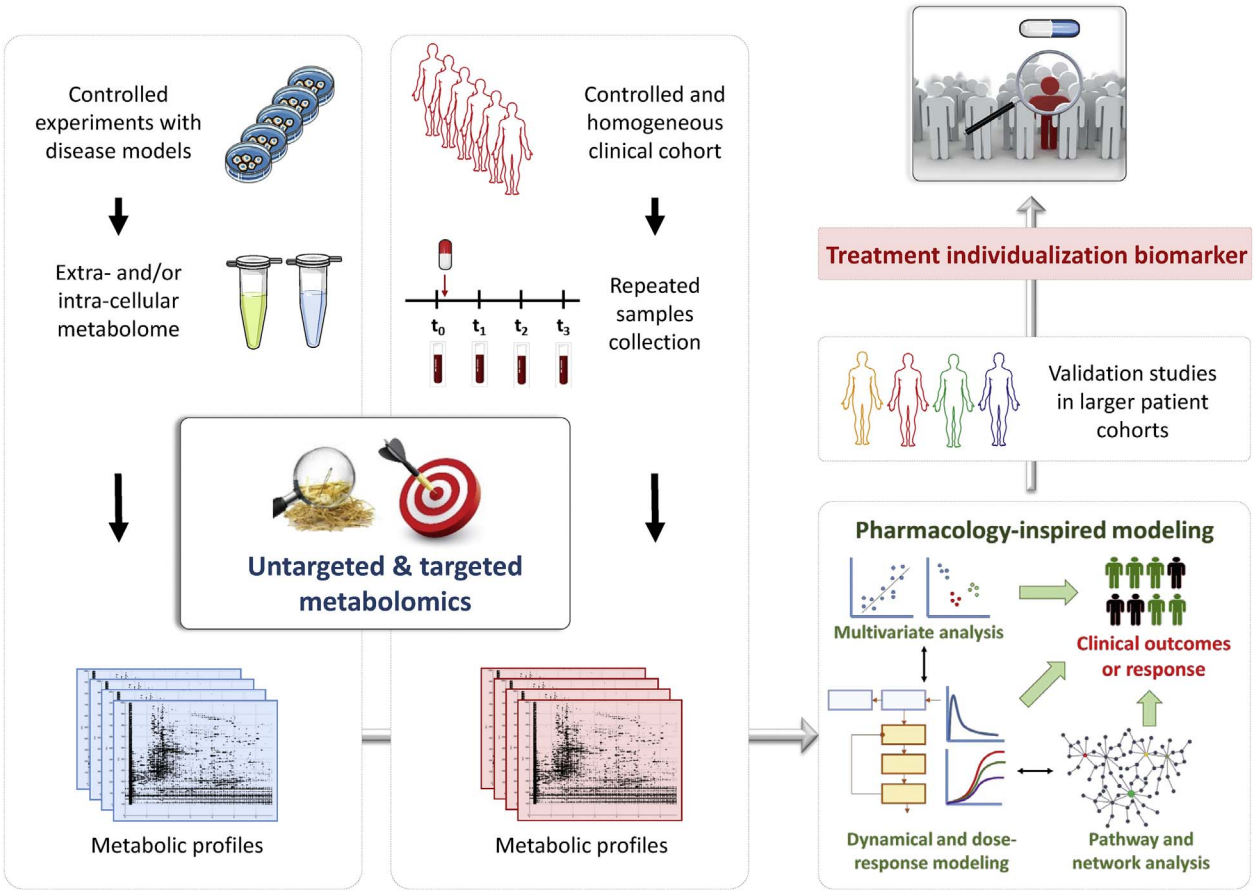

Fig. 2. Illustration of the workflow for developing metabolomics-based treatment individualization biomarkers.

2.2. Dichotomic and time course biomarkers

The majority of clinical metabolomics studies intended for biomarker discovery are case-control studies aiming to identify differences between the metabolite signatures of groups of patients (e.g., healthy vs. diseased). However, such categorizations are often inappropriate oversimplifications, especially for treatment individualization biomarkers, because disease and treatment response are inherently dynamic processes. Moreover, metabolic profiles are associated with intra-individual variability, resulting in convolution of intra- and inter-individual variation in metabolic signatures. Therefore, collection of repeated samples is of great importance for both static prognostic and dynamical biomarkers to separate intra- from inter-individual variation. Additionally, discretization of clinical outcome markers should be prevented, since longitudinal and continuous clinical biomarkers contain more information (e.g., disease severity scores). Fortunately, some of these considerations are being increasingly recognized (Nagele et al. 2016; Poldrack et al. 2015; Sengupta et al. 2016).

2.3. Patient-specific factors introducing variability

Multiple patient-specific factors may influence the metabolome, and thus need to be controlled in the design phase to minimize variation. Patient-specific factors that may affect inter-individual variation in response and metabolome include demographics (e.g., gender, age, body weight), comorbidities, and concomitant medications (Beisken et al. 2015; Dunn et al. 2012; Emwas et al. 2015). Patient-specific characteristics may vary not only between but also within patients over short periods of time; and include for instance circadian rhythms (Giskeodegard et al. 2015) or the menstrual cycle (Wallace et al. 2010). Diet and changes in bodyweight may also influence the metabolome (Scalbert et al. 2014). To this end, appropriate selection criteria for patients should be used and adequately recorded.

2.4. Characterizing disease progression and treatment response

Treatment individualization biomarkers are ultimately developed to provide insight into the inter-individual variability of disease progression dynamics and drug treatment response. Characterization of the dynamics and exposure-response relationships of novel metabolomic biomarkers is therefore essential. In order to assess disease progression, epidemiological biobanking cohort studies are of relevance, combined with appropriate focus on specific disease stages or stratification across baseline disease states. For exposure-response relationships, information on drug exposure in individual patients is essential, i.e., plasma drug concentrations or by individual patient dosing histories (amount, timing). Secondly, sufficiently spread or prospective stratification of drug exposure and/or dose regimens is needed to allow characterization of the exposure-response relationship.

2.5. Sampling sites

Body fluids such as blood or urine are commonly used for identification of biomarkers. Blood-derived samples are usually preferred because of a relatively lower daily variation in the blood metabolome compared to the urine and the on-demand availability in patients. Blood-based metabolomics primarily provides information on the extracellular metabolome, or the intracellular metabolome when relevant (e.g., peripheral blood mononuclear cells). Depending on the disease type, blood may sometimes be insufficient to reflect the disease progression. For instance, in some central nervous system conditions, other sample types such as cerebrospinal fluid may be needed to gather

complementary information (Mehta and Adler 2016).

2.6. Sample collection, preparation and storage

Variability in metabolic profiles can be affected by sample preparation and storage conditions. Rapid inhibition of enzymatic activity (quenching) is particularly important for processing plasma or serum samples because of the high concentration of still active enzymes after collection (Kohler et al. 2016). Samples stored at − 80 °C generally present rather stable metabolome for at least 6 months, although some metabolite classes may be sensitive to chemical and enzymatic reactions during storage (Kanani et al. 2008; Vigor et al. 2014). Multiple freeze-thaw cycles can lead to significant changes in the metabolome, stressing the relevance of direct post-collection aliquoting (Kohler et al. 2016).

2.7. Sample size

The required sample size for treatment individualization biomarker studies depends on several factors including the metabolic baseline concentrations and expected changes thereof, sampling design, and patient heterogeneity. Many of these factors may be uncertain for metabolomics studies, and it is often unclear which particular metabolite candidates will be of interest. Therefore, conducting metabolomics studies following a two-stage design is recommended for obtaining initial estimates of variability (Lenth 2001; Hyotylainen and Oresic 2016; Lenth 2007). Power analyses of prognostic or dynamic metabolic biomarker studies are complex if accounting for repeated measurements and multivariate metabolite profiles. Hierarchical simulation analyses offer a practical approach to evaluate different study designs. Additionally, the increasing public availability of metabolomics cohort studies in healthy and diseased patient populations is of great relevance to support efficient design methodologies. Historically, clinical metabolomics studies have led to successful discriminatory identification of metabolite profiles including approximately 30–50 subjects per group, based on case control or time course studies, although these numbers are dependent on design details and should be increased if various factors in the study design are expected to influence the metabolic variation.

2.8. Sampling times

For dynamic biomarker studies, the selection of sampling time points within individuals is dependent on key aspects such as: i) the expected time course of disease progression and the time of therapeutic interventions to affect this progression; ii) whether the objective is to study, for instance, early stage response to therapeutics or rather the response during a long-term treatment scenario; and iii) whether metabolomic sampling is combined with repeated PK sampling to characterize also the drug concentration time course. Finally, even though prognostic biomarkers are static, inclusion of multiple samples remains essential to discriminate between intra- and inter-individual variations in the blood metabolome.

2.9. Bioanalytical strategies for metabolomics

Nuclear magnetic resonance spectroscopy and mass spectrometry (MS)-based techniques, especially in combination with chromatography, are the major analytical platforms for clinical metabolomics. Unique advantages and limitations of such state-of-the art techniques have been already discussed elsewhere (Kohler et al. 2016; Wishart 2016). Both techniques can be used for untargeted or targeted approaches.

Untargeted metabolomics strategies cover a large part of the molecular metabolite space and allow for potential identification of compounds that may not have been previously identified. However, untargeted strategies do not directly identify molecular structures but rather “features” typically defined by a retention time, mass-to-charge ratio, and signal intensity. Moreover, untargeted strategies usually only result in relative or semi-quantitative data.

Targeted approaches provide absolute concentrations for a set of metabolites with known molecular structures. Targeted platforms cover classes of metabolites with related molecular structures and which have often known associations with (patho)physiological and drug response processes. Examples are acylcarnitines in atherosclerosis, branched-chain amino acids in diabetes, or phosphocholines and acylcarnitines in Alzheimer's disease (Wishart 2016). If the set of metabolites of potential interest have widely different physico-chemical properties, multiple targeted platforms are necessary, thereby lowering throughput and increasing costs.

In clinical metabolomics biomarker discovery, ultimately quantitative data that are chemically and biologically interpretable is of great importance, making targeted metabolomic platforms preferable. However, available knowledge with respect to metabolite classes of interest often remains insufficient. Therefore, two-stage strategies using initial untargeted screening followed by quantitation, or a combination of targeted approaches represent the most adequate solution to such challenge.

2.10. Experimental validation of clinical biomarkers

The use of relevant experimental disease models (i.e., in vitro, in vivo) in conjunction with metabolomics analyses may inform the selection of targeted metabolomics platforms (Fig. 2). Moreover, in parallel to or subsequent to clinical metabolomics biomarker discovery, conduct of such experiments can be of great value to increase the biological and mechanistic understanding of identified clinical metabolomics-based biomarkers, and may support identification of translationally relevant metabolomic biomarkers. Particularly, recent developments in the field of human induced pluripotent stem cell derived cell lines and 3D cell culture in combination with metabolomics may be of significant value for establishing a close link between experimental model systems and clinical biomarker studies.

2.11. Clinical validation of biomarkers

Clinical validation of metabolomics-based biomarkers represents the last but crucial step of biomarker development, aiming to confirm the clinical utility of the biomarkers candidate in treatment individualization. These studies aim to evaluate the clinical relevance of biomarkers candidates under conditions where patient and environmental differences result in increased variability in the metabolome (Lin et al. 2009). Consensus in best-practices for the conduct of such studies in the context of metabolomics are still lacking, although will generally follow validation studies for any other type of biomarker (Mandrekar and Sargent 2009).

3. Multivariate and PK-PD Data Analysis

3.1. Challenges associated with metabolomics treatment individualization biomarkers

The majority of clinical metabolomics studies aiming for treatment individualization have been focusing on the determination of metabolic signatures (i.e., a panel of metabolites) that discriminate between discretized patient groups or predict a therapeutic response. These signatures can be identified using various multivariate statistical regression and classification techniques, e.g., partial least squares regression, random forests. Such metabolite signatures are generally considered to have better predictive performance than single molecule profiles (Beger et al., 2016). Extensive external- or cross-validation procedures during the data analysis process are important to prevent

overfitting and determine metabolic signatures with high significance.

Conventional metabolic signatures do typically not consider patient-specific factors that may introduce variation in the metabolome. Moreover, they often do not take into account the intrinsically dynamic nature of disease progression and treatment exposure-response response relationships. Not correcting or considering these factors also results in a reduced statistical power or bias. Most importantly, these considerations are of critical importance to enable treatment individualization biomarkers that may inform dose regimen optimization.

3.2. Mathematical PK-PD modeling concepts of relevance for metabolomics biomarker discovery

The field of mathematical PK-PD modeling makes use of dynamical compartmental modeling to characterize the dynamics of drug exposure in relation to a biomarker describing the drug effect (Cleton et al. 1999; Danhof et al. 2007; Dayneka et al. 1993) or disease progression (Chan and Holford 2001; Danhof 2015; Schmidt et al. 2010). The resulting dynamical model parameters then describe the shape of time course profiles using a limited set of model parameters. It is worth mentioning that although availability of PK data is the golden standard to quantify exposure, individual drug dosing regimens may also be used as surrogate exposure marker through the use of kinetic-PD modeling approaches (Jacqmin et al. 2007).

Clinical PK-PD models are typically combined with statistical mixed effect models to estimate inter-individual variability. The combination of dynamical modeling, as highlighted in Fig. 3, allows obtaining insight into inter-individual variation for different aspects of biomarker dynamics and drug response, e.g., dose-response slope, maximum drug effect, recovery half-life, resistance development, or rate of disease progression, and their potential association with any prognostic predictor. Combined with the practical considerations described in Section 2, we consider the use of these PK-PD modeling approaches also of major relevance to enable development of meaningful and appropriate metabolomics-based treatment individualization biomarkers.

3.3. Metabolomics prognostic biomarkers

Prognostic (static) treatment individualization biomarkers typically reflect some aspects of the "physiological state" of a patient that relates to the (expected) drug treatment response or disease progression dynamics. If continuous clinical time course data for treatment response or drug treatment are available, dynamical models can be implemented to capture the variability in different dynamical response characteristics, e.g., maximum drug effect, rate of disease progression. The individual estimates for these dynamical characteristics can then be used to identify patient-specific predictors including metabolic prognostic biomarkers (signatures) that predict a specific characteristic of the clinical response. Standard multivariate regression techniques can be used for this purpose (Valitalo et al. 2016). This approach allows for straightforward integration with dose regimens and derivation of dose adaptation, given the PK-PD framework used.

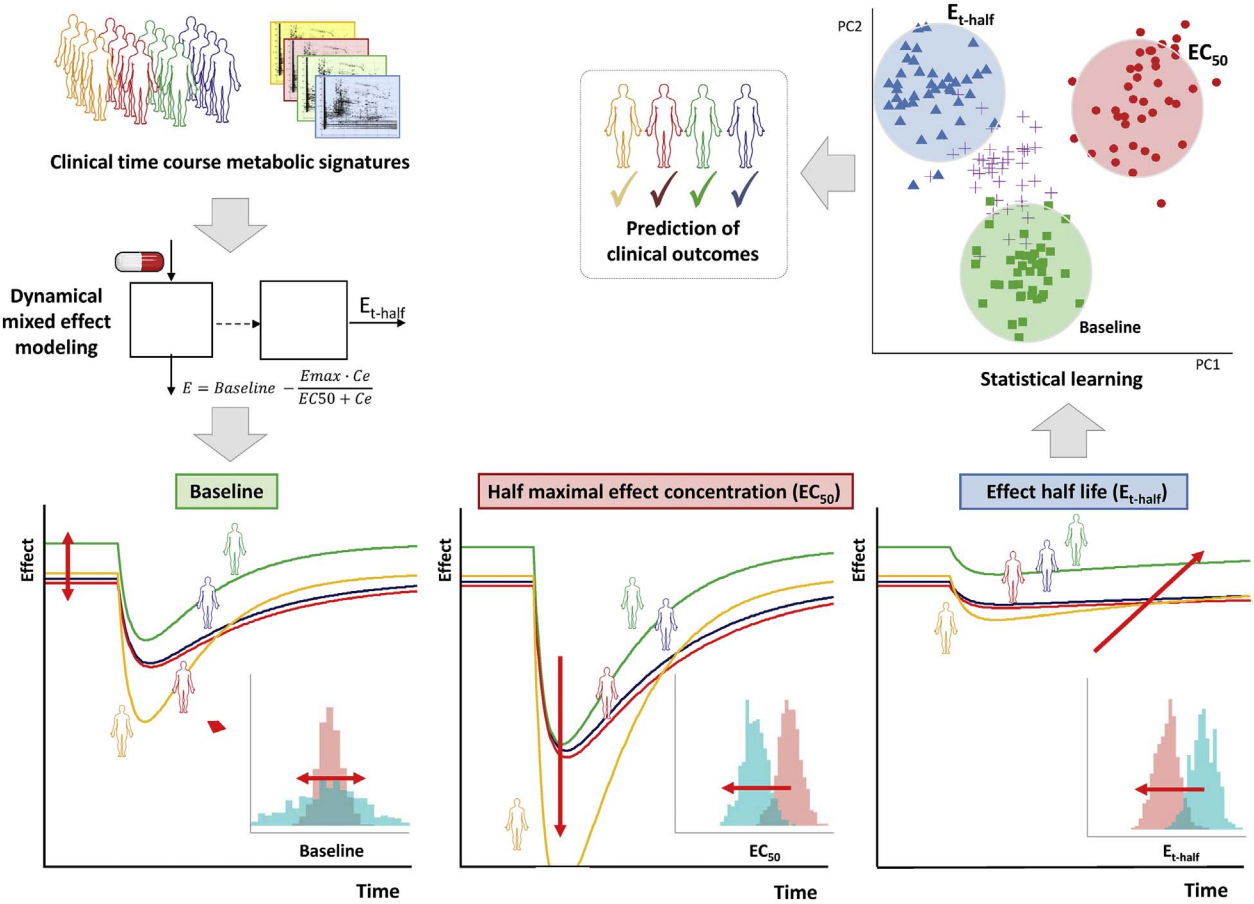

Fig. 3. Combination of metabolomics-based time course data with dynamical modeling. Dynamical mixed effect modeling of can be used to summarize a specific pharmacological effect or an effect on disease progression. The distributions of pharmacological parameters (i.e., half maximum effect concentration, EC_{50}, or effect half-life, $E_{t\text{-}half}$) can be used to identify metabolites associated with pharmacological response. Such distributions can also help in identifying relevant metabolites that can predict variation in the pharmacological response between patients.

3.4. Metabolomics dynamical disease progression and treatment response biomarkers

Time course (or longitudinal) metabolic profiles aiming at identifying dynamical disease progression and treatment response biomarkers can be analyzed using dynamical and mixed effect modeling approaches to summarize longitudinal profiles for each metabolite into a more relevant set of parameters, individually estimated for each patient (Fig. 3). These individual summary parameters can then be evaluated alone or in combination using statistical modeling approaches such as time-to-event- or logistic regression models, to predict available clinical endpoint (e.g., time of death). This approach highlights a major advantage compared to the use of non-parametric analysis approaches described for metabolomics (Nagele et al. 2016). Alternatively, multivariate analysis approaches can be also used first for metabolomics-based datasets of individual patients prior to applying a dynamical characterization (Rasmussen et al. 2010). This has been recently illustrated by van den Brink et al. who demonstrated the relevance of metabolomics combined with PK-PD based selection and analysis of multiplex hormonal data (van den Brink et al. 2016).

3.5. Multicompartimental dynamical systems pharmacology modeling

A mechanistic systems pharmacology extension of the previous section may be envisioned where multi-compartmental dynamical models are derived to describe dynamical associations between multiple metabolomic biomarkers, rather than characterizing each metabolite individually. To this end, as a first step, time course data allows to obtain insight into the underlying network topology of metabolite biomarkers, for instance using Bayesian network analysis (Yazdani et al. 2016). Subsequently, metabolites that have shown relevant clinical associations may be selected, and a reduced dynamical model can be defined and fitted to metabolomic time course data using mixed effect dynamical modeling approaches. Inclusion of relevant biological variation (e.g., circadian rhythms) is also straightforward using this approach (Jacobs et al. 2016; Mochel et al. 2013). In parallel, to support development of such models, a deeper biological understanding may be obtained by using enrichment analysis strategies that identify statistical overrepresentation of metabolites with known biomolecular pathway associations (Chagoyen and Pazos 2011). Finally, the translational value and parametrization of these models can potentially be supported by integrative approaches that combine clinical samples with experimental models.

4. Summary

Clinical metabolomics represents a powerful bioanalytical strategy to identify novel treatment individualization biomarkers for response prediction or dynamical description of both disease progression and treatment response. Integration of clinical metabolomics with pharmacological considerations during may allow for derivation of more clinically and pharmacologically meaningful biomarkers for treatment individualization, eventually enabling personalized medicine. The successful integration of metabolomics with pharmacological concepts and modeling is dependent on explicit consideration of study designs and data analysis techniques that can effectively quantify sources of biological and pharmacological variability.

Acknowledgements

J.G.C.v.H. received funding from the European Union Marie Curie programme (Project ID 661588).

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