

## REVIEW

# Pharmacometabolomics in Early-Phase Clinical Development

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## INTRODUCTION

Pharmacometabolomics is an emerging field that uses the body's complement of metabolites to identify individuals likely to experience treatment or adverse effects. Nevertheless, review of clinicaltrials.gov reveals that <1% of trials used metabolomic principles and only 1.5% of 469 metabolomic studies were of new molecular entities. We review the history, current usage, and potential for future use of pharmacometabolomics in early-phase drug development, and conclude with recommendations for applications in clinical trials.

## PHARMACOMETABOLOMICS – A NOVEL TRANSLATIONAL TOOL

Metabolomics represent the downstream end-products of cellular reactions, the “foot soldiers” of the genomic-transcriptomic-proteomic-metabolomic process, and the components that are most closely associated with the phenotype.<sup>1</sup> Indeed, the organism's metabolic composition, the “metabotype,” is a phenotype in its own right, a convergence of genetic, environmental, and pathophysiological effects.<sup>2</sup> One of the fields that evolved from metabolomics is “pharmacometabolomics,” initially termed “pharmacometabonomics” and defined as “the prediction of the outcome (for example, efficacy or toxicity) of a drug or xenobiotic intervention in an individual based on a mathematical model of preintervention metabolite signatures”.<sup>3,4</sup> Pharmacometabolomics complements genomic, transcriptomic, proteomic, and epigenomic “systems biology” approaches to drug development and contributes to a comprehensive and holistic understanding of drug effects by taking into account both intrinsic and extrinsic contributions to interindividual variation in drug response.<sup>5,6</sup> Most importantly, there is the potential to understand and manage nonresponders and partial responders to conventional treatments, phenotypes that are likely the product of our incomplete understanding of pathophysiology and incorrect nosology, and grouping of diseases. For example, conditions such as coronary artery disease and schizophrenia are likely syndromes composed of many entities, with distinct etiologies and management requirements, as suggested by the wide variation in response to treatment and high percentage of nonresponders, partial

responders, and remitters, and those suffering from adverse drug reactions.<sup>7</sup> In that capacity, pharmacometabolomics hold the promise not only of delivering personalized drug treatment, but also improving the efficiency of drug development. This review describes the role and utilization of pharmacometabolomics as a tool in early-phase clinical development (i.e., the first human testing of new drugs), and its potential to facilitate translational effectiveness in drug development. We include assessment of utilization of pharmacometabolomics in clinical drug development using an analysis of clinicaltrials.gov records, discuss the related challenges and opportunities, and conclude with recommendations for future development of the field.

## HISTORY

The concept of metabolomics, as manifested in the use of bodily products to infer the state of health of the individual, dates back to antiquity. Examples include references in ancient Chinese and Ayurvedic medical literature to insects attracted to patients with sweet-tasting urine as markers of diabetes, and the use, albeit erroneous, of “black bile” and “phlegm” as markers of mood and alertness, respectively.<sup>5</sup> However, because of the complexity of interactions between the multitude of metabolites and respective physiological and pathological states, and consequent dependence on sophisticated bioinformatics and powerful analytical and computational tools, the field has made significant progress only in last few decades (**Table 1**).<sup>1,4,5,8,10,14–28</sup>

The term “metabonomics” (later converging with the parallel coined “metabolomics”) was coined in 1999 as “the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification.”<sup>8</sup> It captures a dynamic process of metabolic changes over time in response to internal and external influences. One such influence is pharmacotherapy, and the term “pharmacometabolomics” was introduced in 2006 to describe the use of the metabolome to study drug effects, first applied to an animal model of liver damage associated with paracetamol metabolism.<sup>4</sup> The analysis revealed that a certain metabolic profile was associated with increased liver damage after paracetamol treatment. Later studies provided further insight into the application of

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**Table 1** Metabolomic studies in clinicaltrials.gov

Year	Milestones	References
1500–2000 BC	Traditional Chinese and Ayurvedic doctors used ants for the identification of “sweet urine” in patients	van der Greef & Smilde <sup>5</sup>
Late 1940s	“Metabolic profile” terminology proposed. Paper chromatography used (nonquantitative)	Gates & Sweeley <sup>14</sup>
1960s	LC and HPLC, GC, and MS used to characterize physiologic and pathophysiologic states (quantitative)	Ryhage & Stenhagen <sup>16</sup> ; Horning <i>et al.</i> <sup>15</sup>
1970s	Term “quantitative metabolic profiling” was coined	Ward <i>et al.</i> <sup>17</sup> ; Thompson & Markey <sup>18</sup> ; Thompson <i>et al.</i> <sup>19</sup>
1980s	First interfaces for combining LC with MS emerge	Games <i>et al.</i> <sup>20</sup> ; van der Greef <i>et al.</i> <sup>21</sup> ; Bain <i>et al.</i> <sup>22</sup> ; van der Greef <i>et al.</i> <sup>1</sup>
1998	Metabolome was coined by Oliver <i>et al.</i> <sup>23</sup> (see text)	Oliver <i>et al.</i> <sup>23</sup>
1999	Metabonomics was coined by Nicholson <i>et al.</i> <sup>8</sup> (see text).	Nicholson <i>et al.</i> <sup>8</sup>
2002	Metabolomics introduced by Fiehn <sup>24</sup> to the field of plant biology as the study of the link between the genotype and phenotype; the term is essentially equivalent to “metabonomics” but became the preferred one since	Fiehn <sup>24</sup>
2005	Metabolic footprinting introduced by Kell <i>et al.</i> <sup>25</sup> to describe the impact of the metabolome on its biologic environment	Kell <i>et al.</i> <sup>25</sup>
2006	Pharmacometabolomics and metabotype were coined (see text) with the earliest study discussing the principle and applications of pharmacometabolomics in the case of paracetamol liver toxicity	Clayton <i>et al.</i> <sup>4</sup> ; James <sup>26</sup>
2007	The FDA publishes “The critical Path Opportunities” report. Metabolic profiling plays a vital role in improvements to the “critical path” of new drug development	Schnackenberg & Beger <sup>27</sup>
2009	First human pharmacometabolomic study demonstrating that host microbiome and predose urinary metabolite profile may predict drug metabolism	Clayton <i>et al.</i> <sup>10</sup>
2012	IOM Report: provides guidelines for development, evaluation, and translation omics-based test development (including metabolomics) as surrogate biomarkers of treatment development; emphasizes the importance of validation	IOM <sup>28</sup>

The FDA, US Food and Drug Administration; GC, gas chromatography; HPLC, high-performance liquid chromatography; IOM, Institute of Medicine; LC, liquid chromatography; MS, mass spectrometry.

Search of “metabolomics” in clinicaltrials.gov 4 July 2015 yielded 518 trials. After exclusion of absolute bioavailability, the total trials were 469.

the metabolic profile as an early indicator of drug-related metabolism and toxicity in humans.<sup>9,10</sup>

Using a range of complementary platforms for comprehensive chemical analyses, metabolomic approaches enable identification and quantification of physiologic-, pathologic-, and treatment-specific metabolites in cell extracts, tissue, and biological fluids (e.g., serum, plasma, urine, and cerebrospinal fluid). The product is a biochemical fingerprint of the organism at a specific timepoint containing information that may be relevant for diagnostic and therapeutic considerations and may be used to identify causal factors (i.e., biomarkers) most strongly affecting the organism's steady state. The most commonly used analytical platforms are nuclear magnetic resonance spectroscopy, noted for its capability for the comprehensive, simultaneous, “unbiased” quantification of a wide range of compounds, and the highly sensitive mass spectrometry (MS), including liquid chromatography MS (LC-MS), tandem MS (MS-MS), and gas chromatography MS methods, and, more recently, the more powerful ultraperformance liquid chromatography.<sup>8,11–13</sup> The complex multivariate nature of the data obtained with metabolomics requires sophisticated statistical, visualization, chemometric, and bioinformatics methods for analysis and interpretation.<sup>13</sup>

### Modern metabolomics and personalized medicine

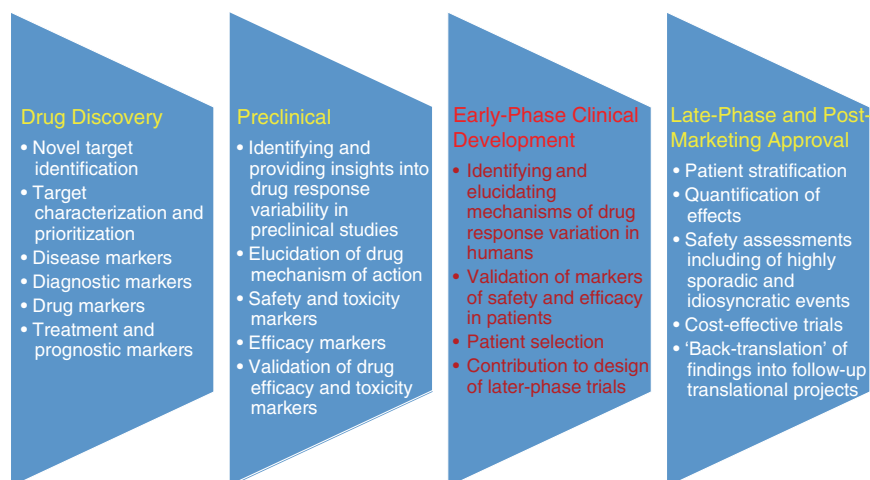
The vision of the “personalized medicine” initiative is the prospect of selecting treatments according to individual patient's unique characteristics, and, in particular, those characteristics that are relevant to treatment safety and efficacy.<sup>28–30</sup> Metabolomics is potentially a useful prognostic indicator to complement other personalized biomarker domains (genomics, transcriptomics, and proteomics) because endogenous biochemical are ontologically closer and interact directly with the elements affecting the organism (e.g., pathological factors, environmental modifiers, treatment interventions, and the genome as well), thus, a more complete and authentic representation of disease effects and intervention outcomes. In contrast, genomic, transcriptomic, and proteomic information is controlling in nature and has yet to be “translated” and “actualized” downstream before exerting its effects and does not take into account the dynamic status of the entire organism or external effects.<sup>8,25</sup>

Indeed, variation in response to pharmacotherapy is determined by both genes and the environment. Pharmacometabolomics identify characteristics of response to pharmacological interventions based on individuals' metabotype.<sup>1,4,27,31,32</sup> The “metabotype” is the totality of person's characteristics associated with metabolic health

**Table 2** Key pharmacometabolomic clinical trials

	Drug	Therapeutic area	Findings	Implications	Reference
1.	Acetaminophen	Healthy volunteers	High predose urinary levels of p-cresol sulfate had low postdose urinary ratios of acetaminophen sulfate to acetaminophen glucuronide	First published human pharmacometabolomics study; host microbiome affects drug metabolism	Clayton <i>et al.</i> <sup>10</sup>
2.	Tacrolimus	Healthy volunteers	Predose urine metabolites and modeling predict tacrolimus PK parameters	Baseline metabolomic phenotypes can be used to characterize PK parameters and provide insight into mechanisms responsible for PK variation	Phapale <i>et al.</i> <sup>36</sup>
3.	Acetaminophen	Healthy volunteers	Postdose (but not predose) urine metabolites were predictive of ALT elevation after acetaminophen dose	Pharmacometabolomics may be used to predict DILI	Winnike <i>et al.</i> <sup>9</sup>
4.	Simvastatin	Cardiovascular disease	Baseline cholesterol ester and phospholipid metabolites correlated with LDL-C response to simvastatin treatment	Metabolic profiles could elucidate mechanisms of action of drugs and explain response variability	Kaddurah-Daouk <i>et al.</i> <sup>37</sup>
5.	Sertraline	Neuropsychiatric diseases	Metabolic profiles (including phenylalanine, tryptophan, purine and tocopherol) partially identified responders to sertraline and placebo	Metabolic profiles could help differentiate true drug responders from placebo responders	Kaddurah-Daouk <i>et al.</i> <sup>38</sup>
6.	Capecitabine	Oncology	Baseline metabolic profiles identify subpopulations susceptible to capecitabine toxicity in inoperable colorectal patients	Pretreatment serum samples could help identify subpopulations susceptible to treatment-limiting adverse events	Backshall <i>et al.</i> <sup>39</sup>
7.	Taxane or FEC	Oncology	Impaired glucose tolerance and elevated plasma glucose levels most significantly associated with poor response in patients with breast cancer and metabolic syndrome	Single metabolite may identify patients at risk of reduced response to chemotherapy. Metabolomic profiles can provide insights into the role of metabolism in cancer pathogenesis and clinical evaluation.	Stebbing <i>et al.</i> <sup>40</sup>
8.	Simvastatin	Cardiovascular disease	Baseline amino acid metabolic profiles may be correlated with good or poor responders to simvastatin treatment	Untargeted metabolomics approach may identify metabolites relevant to variation in treatment response and help elucidate response mechanisms	Trupp <i>et al.</i> <sup>41</sup>
9.	Sertraline	Neuropsychiatric diseases	Tryptophan pathway metabolites differentiate sertraline from placebo responders in treatment of depression	Metabolomic profiles can separate drug from placebo response	Zhu <i>et al.</i> <sup>42</sup>
10.	Atenolol	Cardiovascular disease	Whites and African Americans have different changes in fatty acid metabolites in response to atenolol treatment of hypertension	Racial and genetic variability expressed in metabolomic profiles may provide useful marker of drug response	Wikoff <i>et al.</i> <sup>43</sup>
11.	Aspirin	Hematology; healthy volunteers	Serotonin levels correlated with platelet reactivity parameters (e.g., collagen-induced platelet aggregation) in response to aspirin treatment in healthy volunteers	Single metabolite levels can explain variability in known intermediate physiological markers (e.g., platelet aggregation) implicated in drug response	Ellero-Simatos <i>et al.</i> <sup>44</sup>
Pharmacometabolomic-informed-pharmacogenomic studies					
12.	Citalopram / escitalopram	Neuropsychiatric diseases	Glycine was negatively associated with escitalopram response in MDD patients. This helped identify GLDC SNP as potential SSRI response biomarker in depression.	Metabolomic studies may provide clues into mechanisms of treatment response and may help identify genomic correlates of drug response	Ji <i>et al.</i> <sup>45</sup>
13.	Aspirin	Cardiovascular disease	Aspirin nonresponders had higher adenosine and inosine levels. Genetic variants in adenosine kinase were identified as associated with aspirin response. Resistance to aspirin therapy may be mediated through the purine pathway.	Metabolomic studies may provide insights into mechanisms of treatment response and resistance. Metabolomic approach may guide identification of genomic correlates of drug response.	Yerges-Armstrong <i>et al.</i> <sup>35</sup>

ALT, alanine aminotransferase; DILI, drug-induced liver injury; FEC, fluorouracil, epirubicin, and cyclophosphamide; GLDC, glycine dehydrogenase (decarboxylating); LDL-C, low density lipoprotein cholesterol; MDD, major depressive disorder; PK, pharmacokinetics; SNP, single nucleotide polymorphism; SSRI, selective serotonin reuptake inhibitors.



**Figure 1** Pharmacometabolomic in drug development. Pharmacometabolomics can be a resourceful drug development tool capable of contributing to every step in the process. This schematic illustrates the various potential uses of pharmacometabolomics in the different drug development phases (role in early-phase development is highlighted).

and that which dictate disease heterogeneity and drug response.<sup>7</sup> The “metabotype” reflects not only the constitution of the individual (e.g., the genetic makeup, gender, age, and ethnicity), and the impact of the disease (including any genetic components), but also the product of environmental exposure (e.g., diet, climate, environmental xenobiotics, gut microbiota, and circadian rhythms) as well as any effects of past and concomitant treatments (e.g., polypharmacy) that have impacted the organism during its lifetime and therefore provide a unique and comprehensive profiling of its constitution.<sup>2</sup>

Beyond the general conceptual argument it is evident that some metabolic products are more sensitive indicators of health states than others.<sup>8,25,33</sup> In addition, drugs may affect gene expression and protein synthesis and may also have direct pharmacological interactions with metabolic products not directly affected by the genome or proteome that lead to therapeutic and toxicological effects.<sup>8</sup> Finally, heterogeneous populations that appear phenotypically similar could display variability in molecular, metabolic, and other biological factors, which are important in determining drug response, allowing the use of metabolomics to decipher “behind the scenes” heterogeneity. In all these cases, choice of the optimal metabolomic biomarker out of a complex interconnected biological environment is of essence to the accomplishment of healthcare objectives, including successful drug development programs and pharmacotherapy. Several studies have demonstrated the use of pharmacometabolomics to guide the selection of the right drug for the right metabotype<sup>9,34,35</sup> (Table 2).<sup>9,10,35–45</sup>

### Pharmacometabolomics use in early-phase drug development

Early-phase development is defined as the first-in-human safety, and proof of concept efficacy clinical trials, typically conducted in healthy volunteers and patients, respectively. They are usually small (10–80 research participants) and short in duration (days to weeks) and aimed at obtain-

ing initial information about drug effects in humans before the definitive large, long, late-phase approval clinical trials. Pharmacometabolomics can contribute to drug discovery and development at multiple points along the translational and clinical process (Figure 1, Table 3<sup>46</sup>). The specific benefits are outlined in Table 2. The contribution is particularly relevant and valuable in early-phase human clinical development where little is known about drug toxicity and efficacy, and where reliably identifying “true positives” and “true negatives” can spare the expensive late-phase studies or loss of effective therapeutics, respectively, and reduce the costs and delays of developing innovative treatments. About half of all new chemical entities fail at phase III stage of clinical development, meaning they are the “false positives” of earlier trials; the “false negatives” may never be known as they do not get a “second chance” at retesting in large, adequately powered trials.<sup>47,48</sup> Because early-phase clinical development studies (i.e., phase 0, I, and II) are usually small, short, and underpowered, the value of traditional outcomes is limited. Any improvement in this expensive and lengthy outcome of early-phase inefficiency, such as the availability of reliable and powerful surrogate pharmacometabolomic biomarkers, can increase the predictive validity of early-phase trials and overall effectiveness of clinical development.<sup>49</sup>

Pharmacometabolomics, as other “omics” platforms, holds the promise of reliably predicting pharmacotherapy outcomes in a quicker and more efficient way than traditional approaches. This can be accomplished by identifying and utilizing metabolomic components as “surrogate” or “intermediate” biomarkers of longer-term clinical outcomes of interest to drug developers (e.g., toxicity, remission, mortality, and wellbeing). In addition, pharmacometabolomics can help account for the “non-genetic” components of human heterogeneity (e.g., lifestyle, diet, and environmental exposures). Such heterogeneity could account for important efficacy and toxicity variability in humans.<sup>50,51</sup> Pharmacometabolomic studies can then be done with limited exposure (dose and duration) to the novel drug, allowing fewer risks of

adverse effects, minimal delays in delivery of standard treatment to research participants, quicker arrival at “go-no-go” developmental decisions, and reduced developmental timelines.

Several unique features of pharmacometabolomic approaches need to be considered when incorporating into clinical development programs. The ethical aspects (e.g., confidentiality and inclusiveness) of sample collection and use should be taken into account in study design, storage, processing, and dissemination of results. Samples collected for the pharmacometabolomic evaluation are generally minimally invasive and could be easily collected over study time points or therapeutic time course. However, to maximize their “informatics” potential, sophisticated banking infrastructure has to be established and maintained so that the complex and large amount of information could be analyzed, processed, and compared with intra- and interindividual samples over long periods of time.

## ANALYSIS OF CLINICALTRIALS.GOV DATABASE

### Purpose

The purpose of this study was to assess the type and scope of metabolomic applications in clinical trials as reflected in trials registered in clinicaltrials.gov.

### Methodology

Clinicaltrials.gov database was accessed on 4 July 2015 using the key word “metabolomics.” Each study entry was independently reviewed and categorized by the two authors (T.B. and S.D.) by phase, sponsor, therapeutic area, objectives, study start date, and outcome data (see **Supplementary Material**). Studies were categorized as “discovery” if the clinical trial was used to identify, study, or validate metabolomic biomarkers, and were identified as “clinical development” (phase 0 through phase IV) if the biomarkers were used as study outcome of pharmacotherapy interventions in clinical trials. We defined “early-phase development” as phase 0, I, or II studies, of developmental programs of new molecular entities, or new indications of known drugs. Studies evaluating only drug metabolite profile (e.g., mass balance studies) were not included. Any discrepancies between the authors’ assessments were reconciled in a consensus discussion.

### Results

Over the 18 years (1997–2015) available in the clinicaltrials.gov database, a total of 469 studies were identified in which metabolomic biomarkers were used as primary (51.8%) or secondary (48.2%) outcomes. One hundred sixty-six (35.4%) were drug development studies, 270 (57.6%) discovery studies, and 72 (15.4%) other (e.g., diet, exercise, and acupuncture) studies, with some overlap (see **Figure 2**). Study objectives were efficacy (57.4%), pathophysiology/pathogenesis (20.3%), diagnosis (19.2%), safety (16.0%), and prognosis (15.4%), with some overlap. Of the drug development

studies, 92 (19.6% of the total) were “early-phase development” studies, however, only 7 (1.5%) of all metabolomic studies were used in development of new molecular entities. There has been a gradual increase over the past 14 years in trials utilizing metabolomic outcomes as one of the end points, especially after 2006. The trend appears to plateau after 2011 with another increase in 2014 (see **Figure 3**). Nevertheless, even the highest utilization frequency (92 studies) in 2014 constituted <0.5% of reported clinical trials (0.39% of 23,286 trials). The majority of the studies (438; 93.4%) were conducted by or in collaboration with academic institutes, 66 (14.1%) were conducted by industry, and 35 (7.5%) were industry/academia collaborations. Endocrinology, oncology, central nervous system, cardiovascular, and gastroenterology were the most represented therapeutic areas with endocrinology, at 215 studies, comprising almost half (45.8%) of all studies followed by oncology at 12.4% (see **Supplementary Figure S1**). A search using the near-synonym term “metabonomics” yielded 42 studies, of which 19 included drug intervention (**Table 4**,<sup>52</sup> **Supplementary Material**). Of these, two were early-phase and two were late-phase clinical development studies. Sixteen (84.2%) were done by academia and four (21.1%) by industry (one study was done in collaboration). Results are similar to those from the “metabolomics” search.

### Limitations

Studies before phase II (i.e., phase I and phase 0, or exploratory clinical trials) are not required to be registered in the public domain and may have not been included in the clinicaltrials.gov database. This may have exposed our analysis to reporting bias. Our search strategy was dependent on the use of the term “metabolomics.” It is possible that studies used metabolomic biomarkers but have not identified them as such.

### Conclusions

Over the 18-year period of the clinicaltrials.gov database, a total of 469 studies included metabolomics applications in clinical trials, most (57.6%) in discovery phase (i.e., clinical trials used to discover/validate biomarkers), 19.6% in early phase drug development but only seven studies (1.5%) used metabolomics in development of new molecular entities. Almost half (45.8%) of the applications were in endocrinology, followed by oncology (12.4%). The large majority of metabolomic trials (93.4%) are conducted by academia rather than by drug developers and even with recent growth in utilization metabolomics are used in <0.5% of all reported clinical trials. The limited application may be due to the complex nature of metabolomics, the limited availability of qualified metabolomic biomarkers, and with sophisticated combinations of statistical, analytical, and scientific capabilities necessary for interpretation of results yet to be developed.<sup>12</sup>

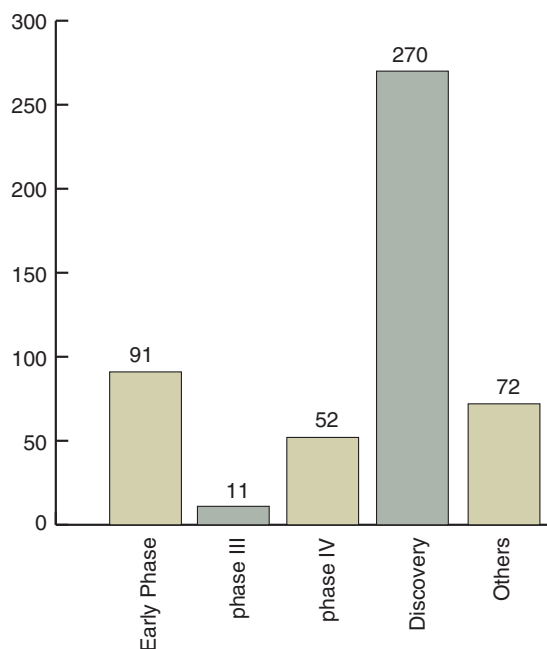
**Table 3** Benefits of pharmacometabolomics applications in drug development

- A. Identifying new drug targets relevant to the drug's efficacy, safety, PKs
- B. Mechanistic insight into disease pathophysiology
- C. Insight into the impact of genotype and phenotype variability on pharmacotherapy outcomes
- D. Study design:
  1. Outcomes:
    - Functioning as "surrogate biomarkers" allowing early detection of safety and efficacy signals. This is particularly valuable in the typically underpowered early-phase trials
    - PKs – metabolomic correlates of PK parameters (area under the curve,  $C_{max}$ ,  $T_{max}$ , clearance, volume of distribution, half-life, trough drug concentrations)
    - Pharmacodynamics – identifying metabolomic markers predictive efficacy and/or toxicity
    - DDIs
    - Therapeutic window: identifying drug plasma concentrations that are between toxic levels (upper limit) and noneffective levels (lower limit)
  2. Participant selection – by establishing more meaningful inclusion/exclusion criteria
  3. Dose selection – influenced by existing population and subpopulation information on dose-response and concentration-response relationships relevant to the drug or disease under study
  4. Validation of biomarkers identified in preclinical work and thus:
  5. Increasing the efficiency of later-phase trials
  6. Pharmacometabolomics used to inform the design of pharmacogenomic studies
- E. Sample collection: can be collected noninvasively, in most cases, with multiple samples easily collected over any required time course
- F. Ethics: adhering to pharmacometabolomics principles would enable more ethical study designs by limiting the testing of new medications to those most likely to benefit and least likely to experience adverse outcomes:
  1. Identifying at-risk population
  2. Identifying those most likely to experience beneficial response to the drug
  3. Limiting duration of exposure to ineffective drugs
  4. Early identification of toxic potential
  5. Increasing the efficiency of drug development with quicker delivery of new therapeutics
- G. Drug "rescue" and "repurposing": using newly validated metabolomic biomarkers to identify new value in existing drugs or previously unseen value in drugs that had their development terminated (Collins<sup>51</sup>)
- H. Vulnerable populations, disease subpopulations, and rare disease drug development: pharmacometabolomics could increase the efficiency of identifying subpopulations, and reduce the duration of exposure, leading to accelerated development for these conditions
- I. Increasing translational effectiveness: by lowering risk, duration, and, ultimately, cost of drug development

$C_{max}$ , peak plasma concentration; DDI, drug-drug interaction; PK, pharmacokinetics.

### Challenges facing use of pharmacometabolomics in clinical development

The application of pharmacometabolomics introduces multiple potential challenges in terms of study design, bioinformatics infrastructure and skills, and regulatory, ethical and legal requirements (Table 4), and may marginally increase the complexity of clinical trials and associated early developmental costs. A pharmacometabolomic approach in early-phase clinical development may need to contend with the fact that metabolomic markers are not yet fully validated. Novel classes of molecular entities may present particular challenges because of limited familiarity with the test article.<sup>28</sup> The metabolome may have complex and shifting relationships not only with the drug under development

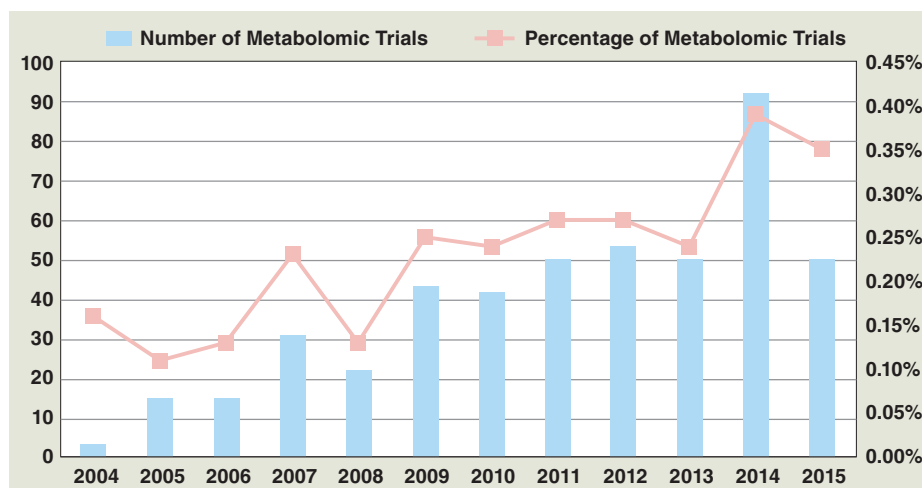


**Figure 2** Inclusion of metabolomic approaches in clinical trials, by phase. 'Early phase' includes phase 0, 1, and 2 clinical development studies of New Chemical Entities (NMEs) or new indications of approved drugs. 'Others' includes non-drug studies (e.g., exercise, diet, acupuncture). 'Discovery' refers to studies that were used to identify, study, or validate metabolomic biomarkers.

but also with evolving disease states and environmental changes. Such variability may be challenging, especially in the context of the typically small-sized and underpowered early-phase clinical development trials. The lack of widespread use and incomplete familiarity with the application of metabolomic principles in clinical developments present additional practical obstacles. Additional details are available in Table 4. These factors may initially be associated with high trial costs, but costs are expected to decrease as economies of scale come into effect.

A recent Institute of Medicine report on biomarkers in drug development recommended that before utilization as a clinical trial end point, a candidate omics-based test should be clearly defined and validated using a two-step developmental process (i) discovery and (ii) evaluation of clinical utility and use.<sup>28</sup> In the discovery stage, the test, methodologies, and computational procedures are being developed and are then tested and validated in a clinical population and locked down to prevent additional changes. Nevertheless, in a recent presentation to a Senate Committee, the US Food and Drug Administration indicated the willingness to work with drug developers to maximize the use of novel biomarkers in drug development, even in cases in which the biomarkers are not yet fully validated or "qualified."<sup>30</sup>

In sites in which the use of biomarkers for screening is not standard practice, preidentification of potential patients for the trial may be challenging. Ethical implications concern confidentiality of information stored in bioinformatics systems and the risk of delaying delivery of optimal health-care because of using metabolomic markers that are not fully



**Figure 3** Number and percentage of trials using metabolomics approaches in study design. Percentage is of all trials reported for that year. Data from clinicaltrials.gov 2004–2015.

validated or not fully correlated against the gold standard of care.<sup>28</sup> The lengthy turnaround of nonstandard screening assays could also delay patient management. Sample collection and processing should be standardized to minimize variability among the sites.

#### Recommendations for the application of pharmacometabolomics principles in clinical trials

Drug development programs should include a plan for the identification and development of pharmacometabolomic biomarkers that may be useful in drug testing (**Figure 4**). Such plans should be prepared as early as possible, preferably before candidate selection. This will allow sufficient time to develop and validate the biomarkers allowing application during the clinical development process. Developers should arrive at a decision whether to proceed with non-targeted metabolomics approach (i.e., the “agnostic,” semi-quantified exploration of a wide-range systemic response to drug treatment with no prespecified hypothesis, aimed at identifying biomarker/s, and generating hypotheses), or a targeted approach (i.e., the quantified characterization of a subset of metabolites, based on validated assays and predefined hypotheses, aimed at developing or utilizing an existing biomarker). If validated biomarkers exist, they should be incorporated as outcomes in the design of early-phase clinical trials. Whether they are primary or secondary outcomes may depend on existing experience with the biomarker and its (estimated) predictive validity with respect to desired clinical outcomes. If novel metabolomic biomarkers are identified as valuable and feasible, exploration and characterization should be initiated as early as possible. If sufficiently robust signals and potential benefits are identified, then development and clinical validation of the analytic test should follow.<sup>28</sup> Early-phase programs would then play a critical role in biomarker validation allowing utilization of the biomarkers in later phases of development.

Regulatory authorities should be involved in feasibility assessment, and the details of the validation process and clinical trials applications, from the very early

stages. Regulators encourage such early involvement (e.g., pre-investigational new drug meetings) and data suggest more efficient clinical development ensues.<sup>30</sup> Regulators can assist with the choice and qualification (see below) of putative biomarkers and provide guidance on the regulatory approval process and the role the biomarkers can play in it. Voluntary submission of study results and discussion of the implications of metabolomic data to the development process should be routinely considered and encouraged.

In the clinical validation and qualification process, every effort should be made to choose biomarkers with the greatest specificity and sensitivity, and, hence, predictive value. It is also critical in the validation and qualification process to use a study that is independent from the analytical and clinical studies in which the diagnostic test was initially developed. That is, the analytical characterization (e.g., accuracy, sensitivity, cut-points, etc.) of a diagnostic test should be based on a dataset that is independent from the samples with which it is to be clinically validated.<sup>28</sup> In parallel, and to maximize assay utility, it is important to build up capabilities in terms of understanding related biology and complementary “omics” (e.g., genomics) markers of disease and drug effects, handling of the bioinformatics component, identifying suitable technology platforms, and managing intellectual property issues related to the use of the specimens, biomarkers, assays, and computer software used. The validation of metabolomic biomarkers with no immediate drug development applications but potentially with important long-term applications in translational science may require collaboration and resource-sharing among industry, academic, and regulatory stakeholders.

Informed consent documents should include statements about future use of biospecimens and potential risks because of delayed or misinformed clinical management. The methods and procedures for sample collection, amount of sample required, processing, storage, screening out poor quality specimens, and related logistics should be established and incorporated in laboratory manuals and protocols well in advance of the clinical trials. The turnaround time for

**Table 4** Challenges of pharmacometabolomics applications in early-phase drug development

## Methodological

- Need to validate biomarkers before their use in patient selection. Validation of biomarkers may be done in parallel to clinical development but may delay the application of the biomarker to the drug being developed.
- “Complexity of a moving target” – the metabolome responds to other effects besides those of the drug, including environmental conditions, diet, host microbiome, immune response, drug interactions, the effect of the disease being treated, and changes because of improvement or worsening of the condition (Bai<sup>52</sup>, Trupp *et al.*,<sup>41</sup> Zhu *et al.*<sup>42</sup>). These may confound controlled clinical trials and may require long-term effectiveness trials to assess the validity of a pharmacometabolomic biomarkers in reflecting drug response.
- Pharmacometabolomics signals may be too weak for the limited power of early-phase studies
- Statistical and bioinformatics challenges: there is still limited knowledge on handling of the large amount of information generated by metabolomic data and the value of novel statistical and informatics approaches

## Operational

- Metabolomics-related expertise is still not widely available
- Pre-identification of patients for enrollment may be challenging as metabolomic information is not collected as part of standard of care
- Limited availability of technology and expertise to design and interpret pharmacometabolomics studies
- Studies may be limited to sites which can handle the complexity of “omics” studies
- Multiple sites may have to be opened for the enrollment as the patient selection is based on metabolomics data
- Sample collection, processing, and storage requires standardization across sites and studies to minimize variability
- Turnaround time of specialized labs may introduce delays

## Ethical, legal, and regulatory

- Divergence of (yet not fully validated) metabolomic results from the therapeutic “gold standard” – can lead to delay of or substandard clinical management
- Ensuring proper inclusion in informed consent process
- Limited regulatory guidance on the design and acceptability of “OMIC” data for drug development decisions. Generally done on case-to-case basis.
- Limited guidance on standardization of pharmacometabolomic study methodologies and validation of biomarkers
- Delay in delivery of patient care due to laboratory turnaround times
- Intellectual property issues due to use of the specimens, biomarkers, assays, and computer software used for calculation of the predictor

## Economic

- Pharmacometabolomic is an emerging field and yet with few success stories to demonstrate value in drug development
- The cost for early-phase development increases with inclusion of the metabolomics profiling and analysis, and the potential need for validation. Any benefits need to offset the investment.
- Although healthcare payers are enthusiastic about pharmacometabolomics, there is little evidence on translation of study findings into effective healthcare policies

biomarker tests, especially novel and uncommonly used in clinical practice, should be taken into consideration during the design of clinical trials. Certified laboratories and preferably certified central laboratories should be used. Statistical and modeling methods, bioinformatics and data management, and related quality assurance plans and standards should be established in advance, preferably in consultation with regulatory authorities.

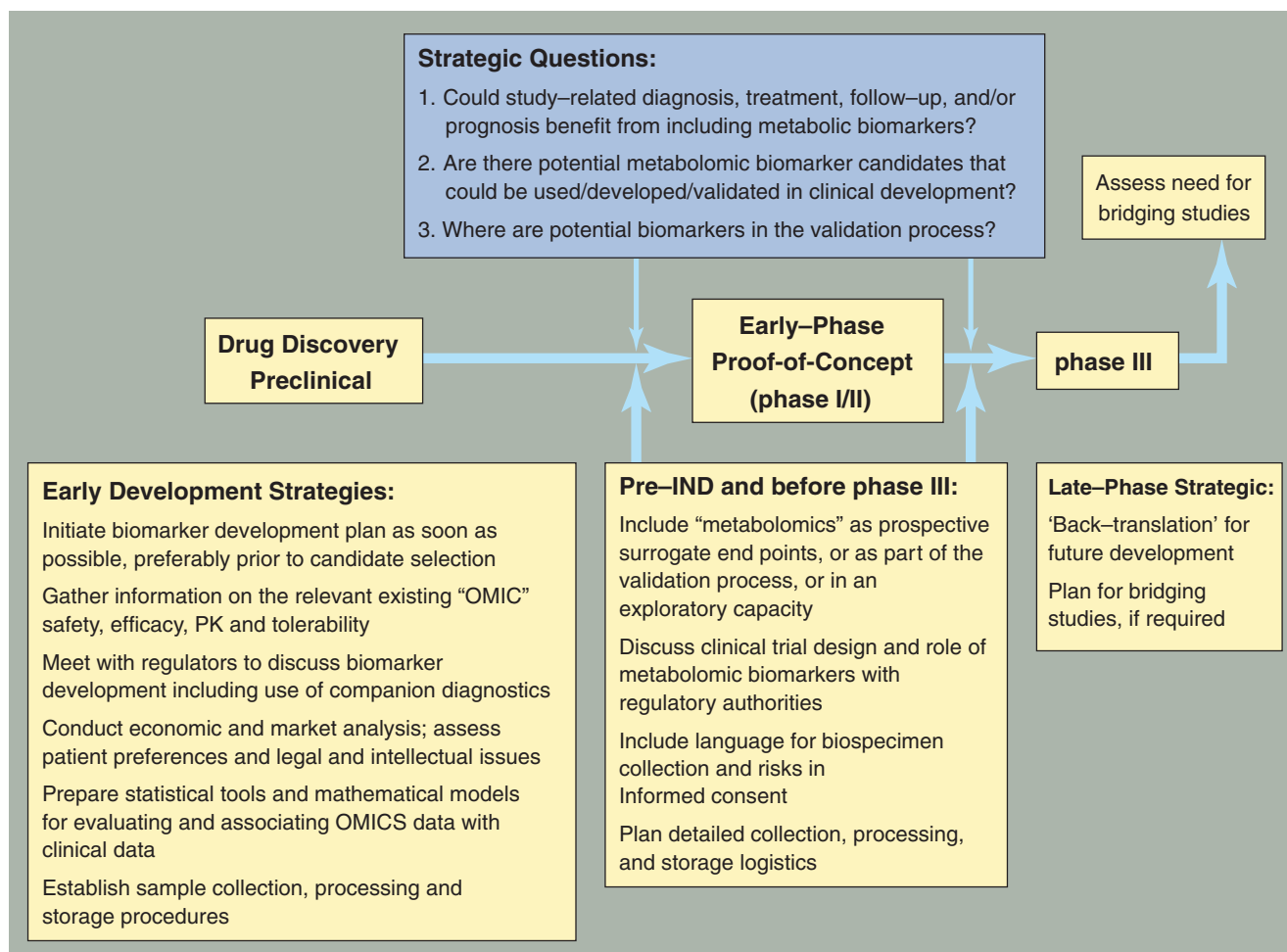
**Considerations to the design of clinical trials**

Biomarker-driven research participant selection in clinical trials should only take place with biomarkers already validated and qualified in respective populations. However, the manner of qualification and amount of data required should be determined in discussions with the regulatory authorities on a case-by-case basis and may well be influenced by the expected healthcare benefit of the drug under development (e.g., breakthrough therapy designation).<sup>28,30</sup> A checklist for criteria used to determine the readiness of omics-based data to guide patient selection has been developed by the National Cancer Institute.<sup>53</sup> Nevertheless, clinical development programs may be used, and could play an important role in the validation of metabolomic biomarkers. In these cases, however, the drug development programs should use other means for patient selection and characterization of primary outcomes. The informed consent should include the relevant sections describing not only the experimental nature of the drug under study but also the experimental nature of the biomarker used to assess drug response. It should also include a description of confidentiality implications, including those of long-term storage and use. Even if no immediate plans for biomarker development exist, collection of specimens for future prospective-retrospective studies should be contemplated.

Specific applications in study design include use of metabolomic biomarkers to:

1. Elucidate pathophysiological mechanisms and monitor disease progression with and without drug response, thus providing practical and powerful short-term surrogate end points. In fact, metabolomic correlations with lack of response or lack of correlation with traditional biomarkers could provide important insights into disease mechanisms. For example, the lipidomic profile correlated with response to statin treatment in which traditional low-density lipoprotein-cholesterol levels did not, providing an opportunity to elucidate previously unrecognized disease and treatment mechanisms.<sup>42</sup>
2. Define and screen heterogeneous disease populations for inclusion in clinical trials. Disease subtypes, including disease severity, response to various treatments, and prognosis, may be characterized by their metabolite, including participants with a particular metabolite may reduce study variability and thereby increase its power and ability to detect meaningful treatment effects. Metabolomic biomarkers could also help screen out prospective research participants at risk for experiencing adverse events.





**Figure 4** Recommendations for the application of pharmacometabolomics principles in clinical trials. The figure illustrates the points along the translational research continuum where pharmacometabolomic biomarkers development and application might be considered. IND – Investigational New Drug Application – the regulatory process governing the first testing of new drugs in humans.

3. Characterize and stratify clinical trial populations by identifying variation in drug response. Subsets of healthy volunteers or patients with the illness under study may respond differently to the drug, even after the targeted (and “biased”) screening. This allows each clinical trial to pursue agnostic exploration of disease and treatment heterogeneity. For example, a metabolite or metabolomic profile may be involved in the assessment of:
  - a. Pharmacokinetics – help identify the subpopulations with absorption, distribution, metabolism, and elimination properties, including considerations of drug-drug interactions, consistent with drug disposition within desired parameters.<sup>36</sup>
  - b. Safety – help characterize the subset of research participants likely to experience and those likely to not experience adverse events.<sup>9,39</sup>
  - c. Efficacy – help characterize the subset of patients likely to respond to therapeutic intervention or likely to not respond.<sup>45,54</sup>

- d. Pharmacometabolomic-informed pharmacogenomic trial results – help corroborate genomic findings, provide detail characterization (i.e., phenotyping) of genomic variants in disease manifestation and treatment response, and identify genomic-metabolomic biomarker combinations that are more powerful as surrogate end points than either biomarker class alone.<sup>13</sup>

## CONCLUSIONS

Pharmacometabolomics is an emerging “omics” biomarker field that has potential to accelerate drug development by identifying, early in the clinical development process, patients most likely to experience beneficial treatment effects and least likely to experience adverse outcomes. Metabolomic information represents the integration of genomic, proteomic, and environmental influences on the organism and can provide information on drug response not captured by the other “omics.” The potential value

is greatest in early-phase clinical development, in which studies are small, short, and often underpowered, and where pharmacometabolomics can help reduce variability of study populations and act as a powerful surrogate of drug response. Nevertheless, analysis of clinicaltrials.gov in 2015 identified only limited application of pharmacometabolomics in drug development clinical trials. We propose strategies for adoption and incorporation of pharmacometabolomics principles in clinical development. These include early planning and identification of potential biomarker candidates, attention to ethics considerations, education, and sample processing. The most critical recommendation is to start early in the discovery phase, preferably with regulatory endorsement, by validating and qualifying clinically relevant pharmacometabolomic biomarkers so that they can be used at the earliest stages of human testing. Notwithstanding the required investment in novel tools and skills, pharmacometabolomics has the potential to shorten clinical development timelines, bring down overall developmental costs, and lead to considerable improvements in overall translational effectiveness and delivery of healthcare benefits.

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