Emerging Applications of Metabolomics in Clinical Pharmacology

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Metabolic disturbances have been associated with many human diseases, including cancer, diabetes, and cardiovascular disease. Metabolomics, a rapidly growing member of the –omics family, investigates cellular metabolism by quantifying metabolites on a large scale and provides a link between metabolic pathways and the upstream genome that governs them. With the advances in analytical technologies, metabolomics is becoming a powerful tool for identifying diagnostic biomarkers of diseases, elucidating the pathological mechanisms, discovering novel drug targets, predicting drug responses, interpreting the mechanisms of drug action, as well as enabling precision treatment of patients. In this review, we highlight the recent advances of technologies and methodologies in metabolomics and their applications to the field of clinical pharmacology. Recent publications from 2013 to 2018 are covered in the review, and current challenges and potential future directions in the field are also discussed.

Metabolism is the foundation of cell physiology involving biomass, energy, and redox balance, all of which are vital for life. Many studies have shown that metabolic reprogramming plays a critical role in various diseases including cancer, diabetes, and cardiovascular and neuropsychiatric diseases. Thus, the study of metabolism is essential for providing insights into the diseased physiology as well as novel therapeutic strategies.

Metabolomics is a relatively new member of the –omics family and an increasingly powerful tool for understanding cellular and systemic metabolism. It is generally defined as the qualitative and quantitative analysis of small-molecule metabolites (<1,000 Da) involved in various biochemical reactions in biological systems.[1,2] It aims to characterize the ultimate status of physiology or pathophysiology by measuring the time-dependent metabolite alterations in a global metabolic network.[2–4] It can profile a large amount of the small-molecule metabolites in a variety of biological samples including serum, plasma, urine, cerebrospinal fluid, cells, and tissues.[5,6] The advent of the metabolomics approach allows researchers to identify metabolites of interest and novel markers and pathways associated with pathophysiology at unprecedentedly low concentration levels, and as a result, the field of metabolism is rekindled after several decades.[4,7] To date, metabolomics has been widely used in basic and translational research areas including biomarker discovery, disease mechanistic study, nutrition, drug development, and pharmacology.[8–10]

It is well established that individuals respond differently to drug treatment, owing to both genetic and environmental influences, such as xenobiotics, gut microbiota, and drug–drug interactions. Individual variability in drug response may lead to treatment failure arising from severe toxicity and/or lack of efficacy, necessitating patient stratification for personalized treatment in clinical trials and bedside treatment. The application of –omics technologies such as genomics[11] and metabolomics[5] makes it possible to maximize therapeutic drug effects and/or minimize toxicity. Pharmacogenomics reflects the inherited difference among patients of various genotypes and shows great promise for the development of individualized drug therapy.[11] However, it has not considered the environmental factors, such as age, gender, lifestyle, and the gut microbiome, and thus has achieved limited success in clinical applications.[12] Pharmacometabolomics, on the other hand, reflects the comprehensive effects of genetic, environmental, and physiological impacts and can therefore be used as a powerful complementary tool in clinical pharmacology and personalized therapy.[5] The past decade has witnessed the increasing applications of metabolomics in clinical pharmacology.[5,13–15] In this review, we summarize the recent advances in the methodologies of metabolomics and then highlight the applications of metabolomics in clinical pharmacology over the past 5 years.

METABOLOMICS ANALYTICAL TECHNOLOGIES

Metabolomics can be categorized into "untargeted" and "targeted" approaches. Untargeted metabolomics mainly provides global profiles of a large number of metabolites in biological samples and contains much information on known and unknown metabolites.[2,16] Targeted metabolomics usually focuses on the analysis of a specific subset of known metabolites, such as tricarboxylic acid cycle intermediates or amino acids.[17,18] The metabolomic workflow for both targeted and untargeted metabolomics analysis usually comprises the sequential steps of experimental design, sample collection and preparation, data acquisition, data analysis, metabolite identification (for untargeted metabolomics), and biological interpretation (**Figure 1**).[19,20] Many reviews have previously summarized the advances in metabolomics technologies, including sample collection[21] and preparation[22,23] and data acquisition[2,5] and analysis.[24] Herein, we aim to brief the key development of the mass spectrometry (MS)-based metabolomics technologies that were frequently used in clinical pharmacology studies in the past 5 years.

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Received March 26, 2019; accepted May 18, 2019. doi:10.1002/cpt.1538

Mass spectrometry and nuclear magnetic resonance (NMR) are the most popular platforms for data acquisition in metabolomics and metabolic flux studies. The advantages and disadvantages of MS and NMR have been reviewed previously.[2,5] Although NMR is more quantitative and can provide more information about molecular structure, its application has been limited by its relatively lower sensitivity and throughput than MS.[25] In addition, MS can be coupled with chromatographic separation technologies, including liquid chromatography (LC), gas chromatography (GC), and capillary electrophoresis, thereby allowing improved separation, quantification, and characterization of analytes from complex biological samples.[2] Among these, high sensitivity, ease of sample preparation, high resolution power, and reproducible chromatographic retention times make LC-MS the most widely used platform in metabolomics.

The choice of MS instruments usually depends on the type of metabolomics. Triple quadrupole or quadrupole linear ion trap mass analyzers are usually used in targeted metabolomics studies because of their high sensitivity and selectivity.[25] Based on an LC-based triple quadrupole mass spectrometer, Gu *et al.*[26] detected 595 precursor ions and 1,890 multiple reaction monitoring (MRM) transitions under positive and negative ion modes. Hu *et al.* developed a targeted metabolomics method monitoring ~200 metabolites using a triple quadrupole mass spectrometer coupled to a high-performance liquid chromatograph. This method has been widely adopted for mechanistic studies of cancer and infectious diseases targeting cell metabolism.[27–30] In particular, the method is ultrasensitive and can detect ~60 metabolites from only 10,000 hematopoietic stem cells, which enables metabolomics profiling of rare cell populations.[28] Recent studies have also utilized high resolution MS instruments such as Orbitrap and time-of-flight (TOF) for targeted metabolite quantification.[31] For example, by using a quadrupole-Orbitrap LC-MS (Q-Exactive, Thermo Scientific, Bremen, Germany), Zhou *et al.*[32] developed an efficient large-scale quantitative method targeting 237 metabolites by using the scheduled parallel reaction monitoring mode. In untargeted

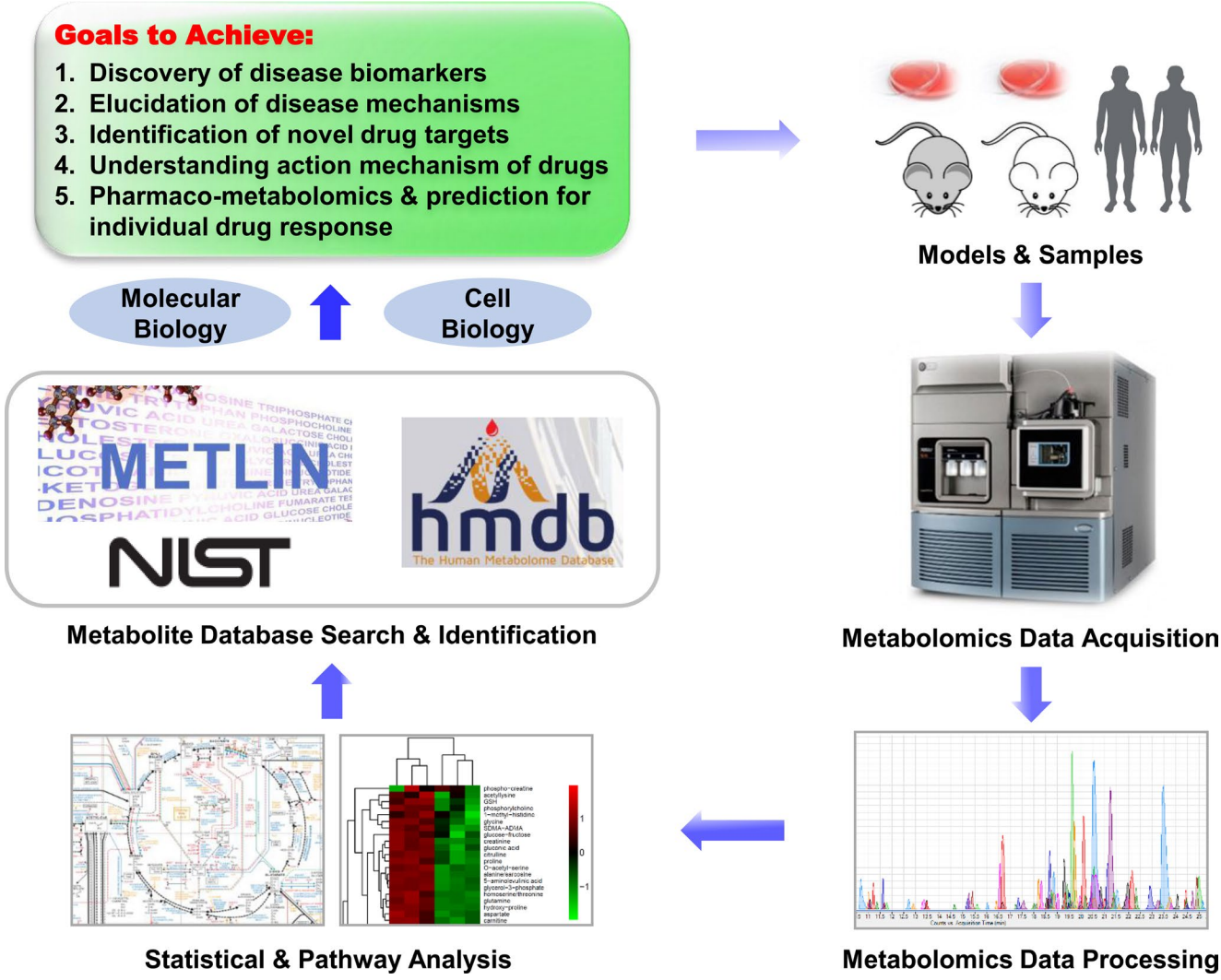

Figure 1 A typical workflow for metabolomics study usually comprises experimental design, sample collection, data acquisition, data processing and statistical analysis, metabolite identification, and biological validation. NIST, National Institute of Standards and Technology.

metabolomics, high resolution MS instruments such as quadrupole TOF and Orbitrap mass analyzers are extensively used because of their high mass resolution, high mass accuracy, and high scan speed. The obtained high-resolution spectral data facilitates a rapid library search for metabolite identification. Raro *et al*.[33] evaluated the performances of hybird quadrupole TOF (Waters Micromass, Manchester, UK) and hybrid quadrupole Orbitrap (Thermo Scientific, Bremen, Germany) for untargeted metabolomics and concluded that both instruments were equally efficient for metabolic profiling studies. Recently, a new strategy of combining untargeted and targeted metabolomics has been proposed. Xu and colleagues developed comprehensive pseudotargeted methods on Triple TOF (AB SCIEX, Framingham, MA), as a practical approach with high repeatability and throughput, that is able to generate high-quality and information-rich metabolomics data.[34,35] Using a systematic and automated approach and software (MRM-Ion Pair Finder, Guowang Xu lab, Dalian, China), they were able to define 854 MRM metabolite ion pairs in serum samples. This method generated data sets that are close to those from a targeted metabolomics approach with improved metabolite coverage and reliable biomarkers identification.

(Q-Exactive, Thermo Scientific) Additionally, new metabolomics technologies are emerging, such as matrix-assisted laser desorption/ionization mass spectrometry,[36] desorption electrospray ionization mass spectrometry,[37] and iKnife (Waters).[38] Moreover, the combination of two or more above-mentioned platforms can achieve a broader coverage of different types of metabolites from complex biological samples.[39,40]

APPLICATIONS OF METABOLOMICS IN CLINICAL PHARMACOLOGY

Pharmacogenomics focuses on the identification of genome variants that influence drug response, while metabolomics investigates the biochemical variations associated with varied drug responses and disease heterogeneity. As a pathophysiological state often involves metabolic perturbation, metabolomics is increasingly recognized as a powerful tool for its ability to profile and delineate metabolic perturbations and, thus, linking metabolic phenotypes to genotypic variations. Clinical applications of metabolomics involve identification of diagnostic biomarkers, elucidation of the disease mechanisms, discovery of novel drug targets, and prediction of the drug responses (**Table 1**). Patients often show great heterogeneity at the molecular level due to their diverse genotypes, gut microbiota, nutrition status, and lifestyles. All these factors contribute to interindividual variability in drug response and treatment outcome. Therefore, it is vital to investigate these differences from a systematic perspective using multiplex analytical and computational/data mining tools (e.g., genomics and metabolomics).

Discovery of metabolite-based diagnostic and prognostic biomarkers

Diagnostic and prognostic biomarkers of disease could play an essential role in personalized treatment and precision medicine. Metabolic phenotyping provides a new dimension of patient information that will enable a patient stratification based on their metabolic profiles. The applications of MS in biomarker discovery have been reviewed previously.[41–43] There have been numerous examples of metabolomics applications to identifying biomarkers in various cancers, including gastric cancer,[44] bladder cancer,[45] epithelial ovarian cancer,[46] cancer cachexia,[47] and colorectal cancer (CRC).[48] Mutations in isocitrate dehydrogenase (IDH)1 and IDH2, present in most gliomas in adults, are associated with the accumulation of 2-hydroxyglutarate (2-HG) in the tumor. Choi *et al*.[49] developed and optimized a noninvasive detection method of 2-HG by proton magnetic resonance spectroscopy and then estimated 2-HG levels in the tumors of 30 subjects. The results showed that 2-HG levels were correlated with mutations in IDH1 or IDH2, indicating that the noninvasive detection of 2-HG might be feasible. With the US Food and Drug Administration (FDA) approval of inhibitors for mutant IDH1 and IDH2 for acute myeloid leukemia (AML), this marker could serve as a complementary diagnostic biomarker. Xu and his colleagues[50] performed a biomarker study on hepatocellular carcinoma (HCC). They analyzed serum metabolic profiles from 1,448 subjects, including healthy controls and patients with chronic hepatitis B virus infection, liver cirrhosis, and HCC, using LC-MS-based metabolomics methods. The data revealed a serum metabolite biomarker panel including phenylalanyl-tryptophan and glycocholate. This panel performed better than alpha-fetoprotein for diagnosis and provided a new method in early detection and differentiating HCC from a high-risk population of cirrhosis. In a recent metabolomics study, Jia *et al*.[48] were able to isolate a panel of 15 metabolites that discriminated between CRC tissue and normal tissue adjacent to the tumor. The metabolites were identified as follows: elevated (relative to normal tissue) lactate, glycerol, and glutamate, which are associated with metabolic pathways for energy production due to the Warburg effect; elevated β-alanine, aspartate, palmitoleate, kynurenine, and uracil, which are associated with pathways leading to synthesis of macromolecules; elevated putrescine, cysteine, hypoxanthine, 5-oxoproline, 2-aminobutyrate, and downregulated myo-inositol, which are associated with pathways that promote adaptation to oxidative stress. This panel was hypothesized to represent a core metabolome capable of identifying CRC for patients that had varied genetic backgrounds, mutations, pathologic stages, and geographic locations as four different CRC cohorts originating from Hangzhou, Shanghai, Beijing, and the United States were tested. In addition, this panel of metabolites was shown to distinguish the treatment outcomes of CRC patients with better outcomes with a longer time-to-recurrence (52.9 vs. 25.9 months) and better 5-year survival rate. The conclusion from this work was that there was a possibility for a distinct metabolic signature of human CRC with prognostic potential.

Metabolic biomarkers were also used to differentiate pancreatic ductal adenocarcinoma from chronic pancreatitis.[51] In a case-control metabolomics study performed using both GC-MS and LC-MS, a biomarker signature comprised of nine metabolites and additionally carbohydrate antigen 19-9 was identified to differentiate pancreatic ductal adenocarcinoma from chronic pancreatitis with a sensitivity of 89.9% and a specificity of 91.3%, respectively. The clinical application of this biomarker panel improved the

Table 1 Emerging applications of metabolomics in clinical pharmacology

Application	Disease	Sample	Metabolomic platform	Major findings	Reference
Discovery of diagnostic and prognostic biomarkers	Gastric cancer	Human plasma	LC-MS	Tryptophan, kynurenine, and phenylacetylglutamine might serve as potential biomarkers for diagnosis of gastric cancer.	44
	Bladder cancer	Human urine	LC-MS	Ten metabolites showed the potential to be biomarkers for the diagnosis of bladder cancer.	45
	Epithelial ovarian cancer (EOC)	Human plasma	LC-MS	Three groups of metabolites were identified as potential diagnostic biomarkers for different stages of EOC.	46
	Cancer cachexia	Human serum and urine	NMR	45 metabolites were found to be cancer cachexiarelated in serum and urine.	47
	Colorectal cancer	Human tumors	GC-MS	Fifteen metabolites were used to predict the recurrence and survival of colorectal cancer patients.	48
	Acute myeloid leukemia (AML); glioma	Human tumors, serum, urine, and marrow	NMR; LC-MS; GC-MS	2-Hydroxyglutarate might be a valuable diagnostic and prognostic biomarker of AML and gliomas.	49,68–71
	Hepatocellular carcinoma (HCC)	Human serum	LC-MS	Phenylalanyl-tryptophan and glycocholate in serum had the potential for diagnosis of HCC.	50
	Pancreatic ductal adenocarcinoma (PDAC); chronic pancreatitis (CP)	Human serum and plasma	GC-MS; LC-MS/MS	Nine metabolites coupled with carbohydrate antigen 19-9 were identified for the differential diagnosis between PDAC and CP.	51
	Alzheimer's disease (AD)	Human serum and brain tissue	FIA-MS/MS; LC-MS/MS	Sphingolipids were associated with AD pathology and progression, which could serve as biomarkers for AD diagnosis.	52
	Cardiovascular disease	Human serum	NMR; LC-MS; GC-MS	Phenylalanine, monounsaturated fatty acids, and polyunsaturated fatty acids could predict cardiovascular risk.	53
	Type 2 diabetes and cardiovascular disease (e.g., coronary heart disease, myocardial infarction, and heart failure)	Human plasma	LC-MS/MS; GC-MS	Mannose might serve as a biomarker of type 2 diabetes and cardiovascular disease.	54
Understanding mechanisms of diseases	Bipolar disorder (BD)	Human cerebrospinal fluid	CE-MS	IDH3A-mediated abnormal isocitrate metabolism in the mitochondria was involved in the pathogenesis of BD.	14
	Prostate cancer	Human prostate specimens	LC-MS	mTORC1 regulated polyamine metabolism through regulation of S-adenosylmethionine decarboxylase 1 stability.	15
	Pulmonary hypertension	Cells isolated from patients	LC-MS	Relationship between microRNA-124–PTBP1–PKM and overall metabolic, proliferative, and inflammatory state of hypertensive adventitial fibroblasts was clarified.	55
	Acute myeloid leukemia (AML)	Human serum; mouse serum; cells	GC-MS	AML was prone to *SLC2A5*-mediated fructose utilization to offset glucose insufficiency.	57

(Continues)

Table 1 (Continued)

Application	Disease	Sample	Metabolomic platform	Major findings	Reference
	Non–small cell lung cancer (NSCLC)	Human plasma and tumor; mouse plasma and tumor; cells	GC-MS; NMR	Glucose metabolism was heterogeneous within and between human tumors; lactate was a fuel in human NSCLC.	58,59
	Severe fever with thrombocytopenia syndrome virus (SFTSV)	Human serum	LC-MS/MS	Arginine metabolism was involved in SFTSV infection.	30
	Non-alcoholic fatty liver disease (NAFLD)	Human plasma	LC-MS/MS; GC-MS	GSH and NAD+ metabolism were potential intervention targets in NAFLD.	60
	Zostavax vaccination	Human plasma	LC-MS	Inositol phosphate, glycerophospholipids, and sterol metabolism affected immune outcome.	61
Identification of new drug targets	AML; gliomas	Human gliomas; cells; human peripheral blood, bone marrow, and pheresis samples	LC-MS; GC-MS	2-hydroxyglutarate was found to be a biomarker of AML.	62,63
	AML	–	–	Enasidenib and ivosidenib, inhibitors of mutant enzymes IDH1 and IDH2, respectively, were developed and approved.	65–67
	Gliomas	Primary patient-derived glioma cells; xenografts	LC-MS; NMR	2-HG produced by IDH1/2 mutations could enhance the sensitivity to PARP inhibitor in tumor cells.	72
	AML	Human plasma	GC-MS; LC-MS/MS	Drug resistance of mutant IDH1/2 inhibitors and relevant mechanisms were uncovered.	73,74
Pharmacometabolomics for predicting the drug efficacy	Resistant hypertension	Human urine	NMR; LC-MS/MS	Citrate, oxaloacetate, α-ketoglutarate, and malate might predict future response to spironolactone before treatment.	80
	Acute lymphoblastic leukaemia (ALL)	Human whole blood	LC-MS	A metabolite panel could be used to monitoring the disease progress of ALL.	81
	Prostate cancer (PCa)	Human serum	LC-MS	Seven metabolites might serve as predictive biomarkers for assessing the therapeutic response to endocrine therapy in PCa patients.	82
	Breast cancer	Human serum	LC-MS	Spermidine and tryptophan were correlated to the response of patients who received trastuzumab-paclitaxel neoadjuvant therapy.	83
	Renal cell carcinoma (RCC); melanoma	Human serum	LC-MS	Kynurenine level was associated with drug response to nivolumab in melanoma patients; higher baseline level of adenosine predicted worse progression-free survival and poor response to nivolumab in RCC patients.	84
	–	Human serum	LC-MS	Serum oxylipids derived from linoleic acid were associated with the response to low dose aspirin.	85

(Continues)

Table 1 (Continued)

Application	Disease	Sample	Metabolomic platform	Major findings	Reference
Pharmacometabolomics for predicting the drug toxicity	–	Human urine	GC-MS	A group of metabolites was found to help to understand the relationship between *SLCO1B1* SNPs and methotrexate toxicity.	89
	Esophageal squamous cell carcinoma (ESCC)	Human serum	GC-MS; LC-MS	Glutaric acid, glucuronic acid, and cystine were correlated with hematological toxicity, pyruvic acid was correlated with nephrotoxicity after neoadjuvant chemoradiotherapy for ESCC.	90
	Atopic dermatitis	Human urine	LC-MS	The long-term side effects of pimecrolimus and desonide were predicted by metabolomics.	91
Drug metabolism and pharmacokinetics	–	Human plasma	GC-MS; LC-MS	A poly-PK strategy was developed that can simultaneously characterize the PK of Chinese herbal medicine as well as the endogenous metabolic response.	92
	–	Human plasma	GC-MS	Predose metabolites were significantly correlated with the PK behavior of atorvastatin.	93
	–	Human urine	LC-MS	Deferoxamine metabolites, C9:1 carnitine, C12:1-OH carnitine, and phenylacetylglutamine were significantly associated with high exposure of busulfan.	94
	Lymphoid malignancies	Human urine	GC-MS	A set of 28 metabolites in the baseline urine samples of the patients were predictive of individual MTX clearance.	95

–, without specific disease; CE, capillary electrophoresis; FIA, flow injection analysis; GC, gas chromatography; GSH, glutathione; NAD+, nicotinamide adenine dinucleotide; IDH, isocitrate dehydrogenase; LC, liquid chromatography; MS, mass spectrometry; mTORC1, mammalian target of rapamycin complex 1; MTX, methotrexate; NMR, nuclear magnetic resonance; PK, pharmacokinetics; SNPs, single nucleotide polymorphisms.

diagnosis and treatment stratification when compared with carbohydrate antigen 19-9 alone.

Recently, a quantitative and targeted metabolomics method revealed potential biomarkers for Alzheimer's disease (AD) and cardiovascular diseases. Varma, *et al.*[52] discriminated AD patients from healthy controls using a panel of 26 metabolites from sphingolipids and glycerophospholipids metabolism. Some sphingolipids were associated with the pathology and progression of AD and were proposed to be biomarkers for early AD diagnosis. In another biomarker discovery study involving 7,256 patients, quantitative NMR metabolomics identified 33 lipids and metabolites significantly associated with incident cardiovascular events.[53] With further meta-analyses, four metabolites were revealed to be highly associated with future cardiovascular events: higher serum phenylalanine and monounsaturated fatty acid levels were associated with increased cardiovascular risk, while higher omega-6 fatty acids and docosahexaenoic acid levels were associated with lower risk. Another study showed that plasma mannose levels were elevated in subjects with higher risk to develop type 2 diabetes and cardiovascular disease.[54]

Understanding mechanisms of diseases

Improved understanding of disease mechanisms would facilitate new drug discovery and enable precision clinical pharmacology. Recently, metabolomics has demonstrated its usage in understanding fundamental insights into molecular mechanisms of neuropsychiatric diseases, cardiovascular diseases, and cancer.[14,15,55]

Particularly, a complementary approach integrating both discovery metabolomics and metabolic flux analysis involving stable-isotope labeling was shown to provide significant biological insights into cancer metabolism.[56] Using both metabolomics and metabolic flux analysis, Jia *et al.*[57] reported that AML was prone to *SLC2A5*-mediated fructose utilization to offset glucose insufficiency. They also revealed that high expression of the fructose transporter, *SLC2A5*, was correlated with inferior survival of AML patients. Meanwhile, serum metabolomics study revealed that fructose utilization was negatively associated with the therapeutic outcomes in AML patients. Pharmacological blockage of fructose uptake ameliorated leukemic phenotypes and potentiated the cytotoxicity of the antileukemic agents. This study highlighted enhanced fructose utilization as a metabolic feature of AML and its potential as a therapeutic target. Deberardinis *et al.* used metabolic flux analysis to analyze the *in vivo* metabolic heterogeneity as well as lactate utilization in non–small cell lung cancer patients.[58,59] Their data indicated that tumors, including *bona fide* human non–small cell lung cancer, can use lactate as a fuel *in vivo*. Strikingly, these *in vivo* metabolism studies also indicated that when directly comparing lactate and glucose, lactate's contribution to the tricarboxylic acid cycle predominates.

In a recent work by Hu and DeBerardinis *et al.* on small cell lung cancer (SCLC),[29] cell lines were profiled using metabolomics, and two groups of metabolites were identified correlating with high or low expression of the Achaete-scute homolog-1 (ASCL1) transcription factor ($ASCL1^{High}$ and $ASCL1^{Low}$). Metabolic flux analysis further proved that purine *de novo* synthesis pathway was upregulated in $ASCL1^{Low}$ cell lines and tumors, which abundantly express the guanosine biosynthetic enzymes inosine monophosphate dehydrogenase-1 and -2 (IMPDH1 and IMPDH2). An

IMPDH inhibitor potently suppressed ASCL1^{Low} cell growth in culture, selectively reduced growth of ASCL1^{Low} xenografts, and when combined with chemotherapy it improved survival in genetic mouse models of ASCL1^{Low}/MYC^{High} SCLC. The data defined an SCLC subtype-selective vulnerability related to *de novo* guanosine nucleotide synthesis, and therefore, this key metabolic enzyme could be a potential therapeutic target for ASCL1^{Low}/MYC^{High} SCLC. This work provided a potentially new strategy for targeted treatment of a subpopulation of SCLC.

Using a similar strategy, Hu *et al.*[30] performed metabolic profiling of the serum samples from healthy subjects and patients infected with severe fever with thrombocytopenia syndrome (SFTS) virus. This study aimed to understand the mechanisms underlying the clinical features, including progressive viral replication and severe thrombocytopenia, and identify a potential therapeutic target for a disease with high mortality but no specific therapeutics. Data from this study revealed that the serum arginine declined with SFTS in two independent patient cohorts. Further analysis suggested that arginine metabolism, via nitric oxide synthase and arginase, is a key pathway in severe fever with thrombocytopenia syndrome virus–caused consequential death. Arginine deficiency was found to be associated with decreased intraplatelet nitric oxide concentration, which caused platelet activation and thrombocytopenia. It was also observed that granulocytic myeloid-derived suppressor cells that expresses arginase expanded in severe SFTS patients. This partially explained the arginine depletion that further led to the downregulation of T-cell CD3-ζ chain and disturbance of virus clearance from T cells. In the following randomized controlled trial, Hu *et al.* observed that arginine supplementation was associated with accelerated virus clearance and thrombocytopenia recovery. Together, their findings revealed the arginine catabolism pathway–associated regulation of platelet homeostasis and T-cell dysregulation after severe fever with thrombocytopenia syndrome virus infection, which not only provided a functional mechanism underlying SFTS pathogenesis but also offered an alternative therapy choice for SFTS.

Yoshimi and his colleagues[14] provided some evidence for mitochondrial dysfunction in bipolar disorder using a metabolomics approach. They found that cerebrospinal fluid isocitrate was significantly higher in bipolar disorder patients than in healthy controls, with a relatively lower *IDH3A* and *IDH3B* gene expression level. Zabala-Letona *et al.*[15] discovered that there were alterations in the polyamine pathway both in mouse model and human biopsies of prostate cancer using targeted and untargeted metabolomics. Furthermore, they revealed that mammalian target of rapamycin complex 1–regulated S-adenosylmethionine decarboxylase 1 was upstream of the metabolic reprogramming involved in prostate cancer. Another metabolomics study of hepatic steatosis in patients identified glutathione and nicotinamide adenine dinucleotide metabolism as potential intervention targets in nonalcoholic fatty liver disease.[60]

The emerging role of metabolomics combined with other –omics, such as genomics, in elucidating disease mechanisms has been recognized as well. To comprehensively understand the mechanism underlying the human immune responses to Zostavax vaccination, Li *et al.*[61] established a multiscale, multifactorial response network by integrating metabolomics, transcriptomics, flow cytometry, and plasma cytokine data sets. They found that sterol metabolism and inositol phosphate metabolism were mechanistically associated with the immune response.

In summary, it can be clearly seen that metabolomics plays a pivotal role in understanding the fundamentals of diseases, which, in turn, could lead to the identification of novel drug targets for therapy.

Identification of new drug targets

Applications of metabolomics in drug discovery and precision medicine over the past decade have been emerging and were recently reviewed by Wishart.[5] Metabolomics has been used to investigate the functions of oncogenes and tumor suppressors, as well as metabolic enzymes, leading to the identification of novel drug targets. Many effective drugs being used in clinic are enzyme inhibitors, which can regulate the activity of metabolic enzymes and maintain metabolic balance.

A very good example was the development of ivosidenib (AG-120) and enasidenib (AG-221), two-first-in-class drugs against IDH1 and IDH2-mutated relapsed or refractory AML, respectively. Using metabolomics method, Dang *et al.*[62]and Ward *et al.*[63] revealed that mutant enzymes IDH1 and IDH2 convert α-ketoglutarate into 2-HG in gliomas and AML. The latter metabolite was referred to as an oncometabolite as its increased levels were associated with an increased risk of brain tumors. A later study confirmed that inhibitors of mutant IDH1 prevented 2-HG production and inhibited tumor growth,[64] highlighting the value of the metabolomic approach for the discovery of new therapeutic targets. Subsequently, enasidenib, a new drug targeting the mutant IDH2 enzyme, was developed and approved. It suppressed the mutant IDH2 enzyme and reduced the production of 2-HG *in vitro* and *in vivo*.[65] These findings laid a solid foundation for the following clinical trial. After the clinical trial was completed,[66] enasidenib was approved by the FDA in the United States in 2017 for the treatment of relapsed or refractory AML. In 2018, enasidenib's sister drug ivosidenib, the inhibitor for mutant IDH1 also entered into clinical trial and was subsequently approved by the FDA[67] (**Figure 2**). IDH1 mutations occur in 6–10% of AML patients, while IDH2 occur in 9–13%. The successful development of these two drugs brought new therapeutic options to the patients of relapsed or refractory AML.

Metabolomics has been employed in almost every stage of the development of mutant IDH1/2 inhibitors, including clinical pharmacology (**Figure 3**). DiNardo *et al.*,[68] Jia *et al.*,[69,70] and Fathi *et al.*[71] have proposed oncometabolite 2-HG as a clinical diagnosis and prognostic biomarker in AML patients with or without IDH mutations. Using LC-MS and GC-MS based metabolomics, they found that the levels of 2-HG in serum of AML patients with IDH mutations were higher than those without IDH mutations. Furthermore, 2-HG levels were negatively correlated with the overall survival of IDH-mutant patients. It has also been evaluated as a diagnosis and prognostic biomarker of IDH-mutated gliomas using magnetic resonance spectroscopy.[49] Sulkowski *et al.*[72] revealed that 2-HG produced by mutant IDH1/2 enhanced the sensitivity of poly adenosine diphosphate ribose polymerase inhibitors on patient-derived glioma cells and tumor xenografts. They

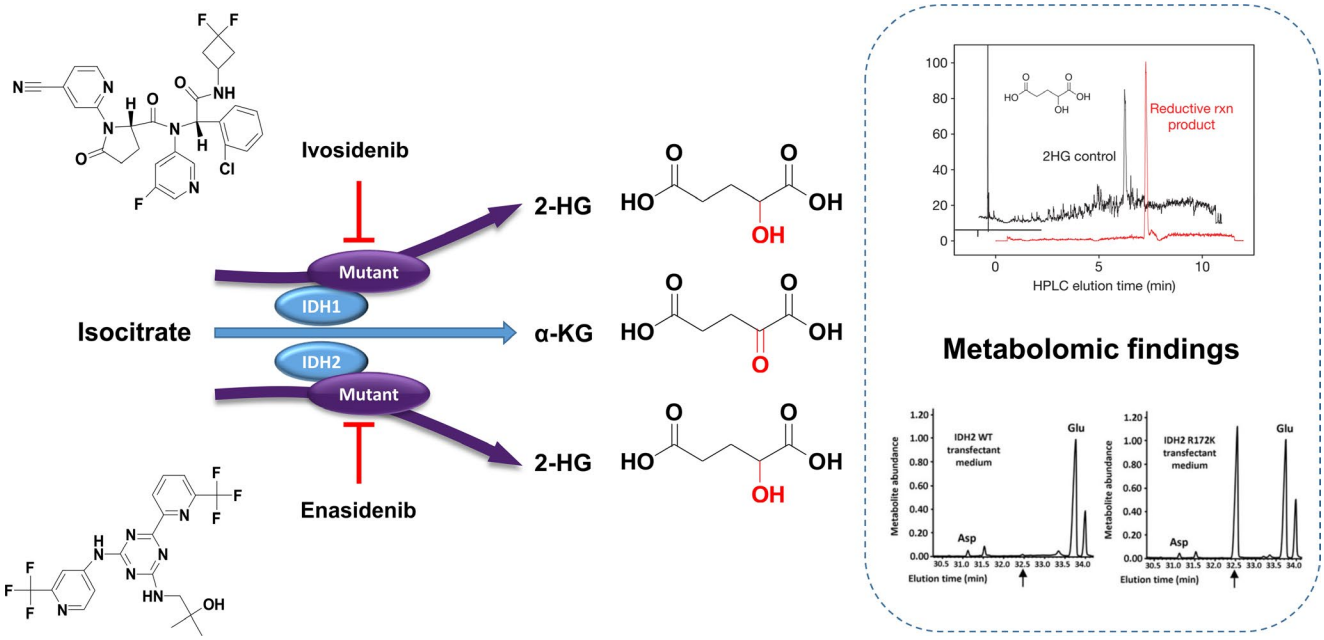

Figure 2 Metabolomics played a critical role in the identification of 2-HG, the oncometabolite generated by mutant IDH1 and IDH2, the targets of the corresponding inhibitors, ivosidenib and enasidenib. 2-HG, 2-hydroxyglutarate; α-KG, α-ketoglutarate; Asp, aspartate; Glu, glutamate; HPLC, high-performance liquid chromatograph; IDH, isocitrate dehydrogenase; WT, IDH2 wild-type. Figures in the right dotted box were adapted with permission from Springer Nature, *Nature* (Cancer-associated IDH1 mutations produce 2-hydroxyglutarate; Lenny Dang, David W. White, Stefan Gross, Bryson D. Bennett, Mark A. Bittinger *et al.*; 2009)[62] and adapted from *Cancer Cell*, vol. 17, Patrick S. Ward *et al.*, The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting α-ketoglutarate to 2-hydroxyglutarate, p. 225–234, 2010, with permission from Elsevier.[63]

demonstrated that IDH1 mutant tumor cells were more sensitive to poly adenosine diphosphate ribose polymerase inhibitors, which provided new clues to a personalized treatment strategy. Harding *et al.*[73] revealed isoform switching between mutant IDH1 and IDH2 as a mechanism involved in the acquired drug resistance to mutant IDH1/2 inhibitors by analyzing four patients. By gene sequencing and 2-HG production monitoring, Intlekofer *et al.*[74] showed the appearance of second-site IDH2 mutations *in trans* or formation of IDH dimer-interface mutations *in cis* might be another mechanism for the acquired drug resistance to mutant IDH inhibitors.

Precision medicine and pharmacometabolomics

As patients respond differently to therapies, it is important to take a more personalized approach to maximize drug efficacy and minimize drug toxicity. Prediction of therapeutic effects and adverse events is a crucial mandate to guide patient selection for personalized treatments. Pharmacogenomics focuses on the identification of genome variants that influence drug response and has been practiced in clinical settings. However, it only reflects information associated with genetics but does not encompass variations in drug-response phenotypes induced by other effects, such as the use of other medications, different diets and nutrition, and the gut microbiota. Pharmacometabolomics, however, could determine the metabolic phenotypes resulting from the interplay of genetics, gut microbiota, and environmental influences at baseline and after drug treatment. Therefore, it could potentially be used to better define mechanisms related to variations in drug response of the patients.

Metabolomics has also been used for the prediction of disease susceptibility in a patient population as well as drug metabolism and pharmacokinetics. The pharmacometabolomics approach identifies individual metabolomic characteristics that allow for the prediction of drug effectiveness and toxicity in a given individual patient population.[12,75–79] A representative schematic chart for the pharmacometabolomics-enabled personalized treatment is shown in **Figure 4**.

Pharmacometabolomics has also been integrated with pharmacogenetics to better predict and understand an individual's drug response. Kaddurah-Daouk *et al.* initially proposed a "pharmacometabolomics-informed pharmacogenomics" strategy to investigate the efficacy of citalopram/escitalopram in major depressive disorder patients utilizing a GC-MS platform. Their results showed that glycine levels were negatively associated with treatment outcome and thus may be useful as a biomarker for predicting drug response.[76]

Pharmacometabolomics for predicting the drug efficacy. Martin-Lorenzo *et al.*[80] investigated the treatment response of spironolactone in resistant hypertension (RH) patients. By combining both NMR and LC-MS–based metabolomics, they discovered that citrate and oxaloacetate could discriminate between RH and pseudo-RH, while coupled with α-ketoglutarate and malate, they could discriminate between responders and nonresponders to spironolactone treatment. This group of metabolite markers could be used to predict the drug response of spironolactone prior to the treatment and thus help to optimize

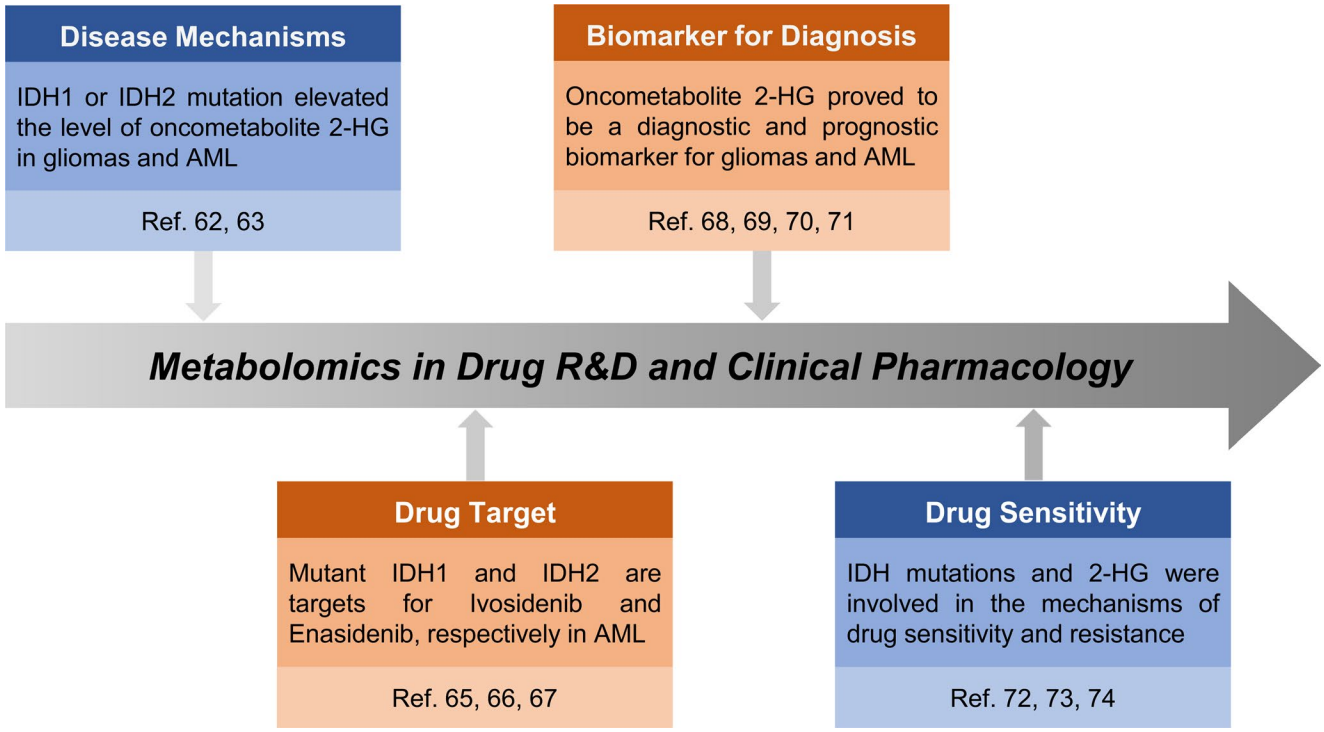

Figure 3 The applications of metabolomics in the drug research and development (R&D) and clinical pharmacology of mutant IDH1/2 inhibitors. 2-HG, 2-hydroxyglutarate; AML, acute myeloid leukemia; IDH, isocitrate dehydrogenase.

the treatment of RH patients with spironolactone.

Many similar studies have used metabolomics to predict the outcome of drug treatment in various cancers, including acute lymphoblastic leukemia and prostate and breast cancer.[81–83] In a study investigating the efficacy of nivolumab in 79 patients with advanced melanoma and 82 patients with metastatic renal cell carcinoma, the results revealed that the change in kynurenine levels in melanoma patients correlated well with the response to nivolumab. In addition, higher baseline levels of adenosine in renal cell carcinoma patients were associated with worse progression-free survival and lack of response to nivolumab.[84]

Pharmacometabolomics has also been used to explore the mechanisms underlying the interindividual variation in response to aspirin treatment: Global decreases in serum oxylipids in response to aspirin intervention (14 days, 81 mg/day) were observed in 156 healthy subjects.[85] The results indicated that the non-COX1-mediated variability in response to aspirin could be partially induced by linoleic acid–derived oxylipids and may help explain the mechanism of action of low dose aspirin.

Pharmacometabolomics for predicting the drug toxicity. Metabolomics has been first applied in evaluating drug toxicity in preclinical models in the Consortium for Metabonomic Toxicology (COMET) project.[86] Recently, it has been employed to understand mechanisms associated with drug-induced nephrotoxicity.[87,88] Martinez and his colleagues investigated the relationship between polymorphisms in gene *SLCO1B1*, coding for organic anion transporting polypeptide 1B1 (OATP1B1), and the toxicity of methotrexate (MTX) in adult patients with hematological malignancies using targeted urinary metabolomics.[89] According to the results of this study, 38 and 34 altered metabolites were found between wildtype and carrying the variant allele, *c.388A>G* or *c.521T>C*, respectively. Moreover, half of these metabolites were related to MTX toxicity, and most were shown to be fatty acid derivatives and microbiota catabolites. These results suggest that the combination of pharmacometabolomics and pharmacogenomics would be a better choice to achieve precision medicine. To identify serum metabolite biomarkers that could predict the side effects of neoadjuvant chemoradiotherapy for esophageal squamous cell carcinoma (ESCC), Nishiumi *et al.*[90] performed a metabolomics study of serum samples from 26 patients with ESCC before neoadjuvant chemoradiotherapy. The results showed that significantly higher serum levels of glutaric acid, glucuronic acid, and cystine were associated with hematological toxicity, while the serum level of pyruvic acid was significantly lower in nephrotoxicity. These metabolites, therefore, could be used to predict drug-induced hematological toxicity and nephrotoxicity after neoadjuvant chemoradiotherapy for ESCC. In another study, Lee *et al.*[91] showed that urinary metabolomics effectively predicted long-term side effects of topical calcineurin inhibitors (TCI) in young children (<2 years) with primary atopic dermatitis. Previously, these drugs were banned for use in young children, based on the known carcinogenic potential of immunosuppressants and the results of animal studies where the drugs were orally administered and toxicity was observed only when blood levels reached 100 ng/mL. However, when TCIs are topically applied, blood levels remain low or

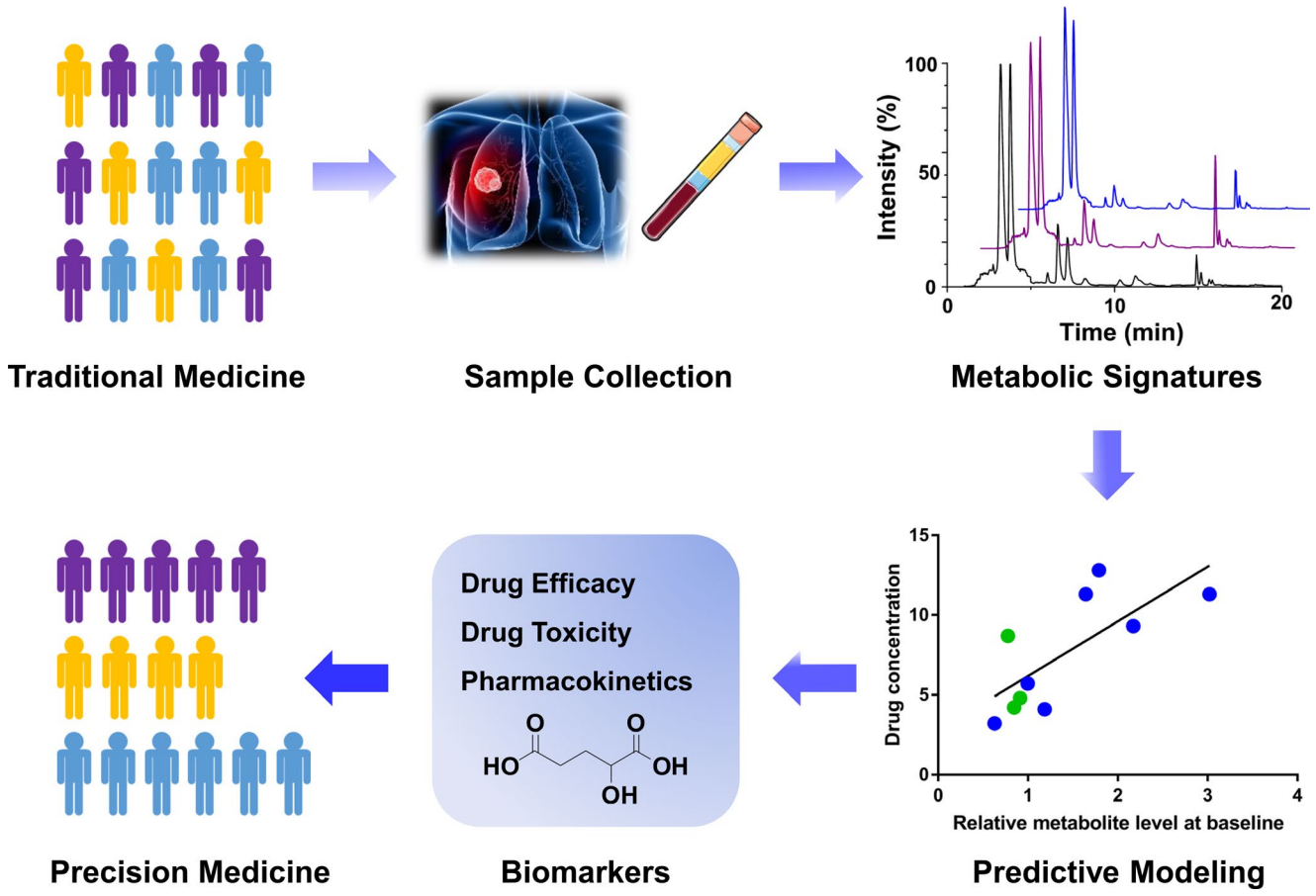

Figure 4 A representative schematic chart for the pharmacometabolomics-enabled precision treatment.

undetectable. Therefore, a generalized, 6-month clinical trial was done to compare the effects of the TCI, pimecrolimus, and the corticosteroid, desonide, using urinary metabolomics. Although topical steroids were found to alter metabolites associated with cancer, TCIs did not have this effect in the long-term side effects prediction model. Moreover, tryptophan and phenylalanine metabolism were altered in the steroid-treated group, and phenylalanine metabolism was altered in the pimecrolimus-treated group.

Metabolomics for predicting drug metabolism and pharmacokinetics. Metabolomics has also been used as a means of studying multicomponent herbal medicines. Jia *et al*.[92] used this approach to investigate the pharmacokinetics of the Huangqi decoction (HQD, consisting of *Radix astragali* and *Radix glycyrrhizae*) in healthy Chinese.

HQD was extracted along with serum samples taken from subjects before and after ingestion of HQD so that comparisons could be made among parent drug components, absorbed parent components, and metabolites of HQD derived from the absorbed components. A panel of 115 phytochemical compounds was detected in HQD, 370 metabolites in predose plasma and 1,013 metabolites in post–HQD intervention plasma. The detection and identification of the metabolites and HQD compounds in the plasma provided initial information about the pharmacologically active substances in HQD. Ultimately, a total of 56 absorbed HQD compounds and 292 secondary HQD metabolites were identified in post–HQD plasma samples. Measurements were made at 4, 8, and 12 hours post-HQD ingestion to analyze the kinetics of the formation of secondary metabolites as well as the time required for absorption of parent HQD compounds. In addition, 166 metabolites measured at the three timepoints were identified as altered endogenous metabolites formed in response to HQD. Diet, gender, and fasting before drug ingestion were all found to influence the metabolomic results, which in turn may affect drug efficacy.

Drugs with narrow therapeutic windows and high pharmacokinetic variability require the precise prediction of the effects of drug exposure to avoid potential toxicities. Metabolomic profiling of predose plasma samples from 48 healthy volunteers was performed using a GC-MS platform to explore the individual differences in pharmacokinetics of atorvastatin.[93] The results suggested that endogenous metabolites such as tryptophan, alanine, arachidonic acid, 2-hydroxybutyric acid, cholesterol, and isoleucine could serve as markers for predicting individual differences in pharmacokinetics and facilitate individualized drug therapy of atorvastatin. Busulfan is a frequently used chemotherapeutic agent with a narrow therapeutic window and high pharmacokinetic variability. Kim and his colleagues investigated the biomarkers in the urine of pediatric patients

who received hematopoietic stem cell transplantation after busulfan administration to predict its pharmacokinetics using global metabolomics.[94] As a result, ferritin, acylcarnitine, and phenylacetylglutamine were associated with the area under the concentration-time curve of busulfan and could be potential biomarkers for the prediction of busulfan exposure. The identification of metabolites associated with drug exposure could also serve as predictive biomarkers that would help optimize the therapy.

Likewise, MTX showed variable pharmacokinetics at high dose. A metabolomics study was performed with urine samples collected from a cohort of adult patients with lymphoid malignancies before MTX administration.[95] The results showed that a set of 28 metabolites in the baseline urine samples of the patients were predictive of individual MTX clearance.

CONCLUDING REMARKS AND FUTURE PERSPECTIVE

Over the past few years, significant progress has been made in the development of novel technology and methodology used in metabolomics and their applications in clinical pharmacology. Metabolomics has shown tremendous potential to provide new insights into the disease mechanisms. In addition, it has a great potential to make a powerful impact on pharmaceutical and clinical research. Recent advances in methodology and applications of the metabolomics approach in clinical pharmacology studies, as highlighted in this review, provide exciting new opportunities to enhance the treatment response and therapeutic efficacy.

Although a great deal of effort has been made in this area to date, we continue to face multifaceted challenges. Technically, when compared with other –omics, especially genomics and transcriptomics, that have achieved great standardization, the application of clinical metabolomics is hindered by its interlaboratory variations among different experiments. Studies in clinical pharmacology involve large sample sizes, which require highly reproducible and reliable metabolomics analyses. Further advancements in global metabolite profiling are needed especially in methodological standardization enabling more consistent and reproducible data across various metabolomics laboratories and centers. Determining metabolites with improved accuracy and precision will allow investigators to detect subtler differences in metabolic phenotypes. In addition, the detection and annotation of more low-concentration metabolites to achieve a broader coverage of the entire metabolome is another technical challenge for metabolomics.

Application wise, the standardized protocols should be developed in clinical pharmacology studies, starting from sample collection, preparation, and processing through the data analysis and interpretation. Metabolomics studies of large populations and patient cohorts may help to achieve more unbiased clinical data for better understanding of the drug response and offer better predictive power for outcome evaluation. In addition, novel bioinformatics tools that enable integrating metabolomics with other –omics (genomics, transcriptomics, and proteomics) and predictive modeling of –omics data and drug response (pharmacokinetics, pharmacodynamics, and toxicity) need to be developed to accelerate research in clinical pharmacology and precision medicine. We anticipate significant advancements to be made in metabolomics with increased consistency and better integration ability with other –omics data sets in the clinical pharmacology application.

ACKNOWLEDGMENTS

We appreciate many other excellent publications that have contributed to the metabolomics field and its applications in clinical pharmacology studies. Due to the space limitation, we regret that we were unable to include all the relevant work in this review.

FUNDING

Z.H. is supported by grants from Tsinghua-Peking Joint Center for Life Sciences, Beijing Frontier Research Center for Biological Structure, Tsinghua University (53332200517), and National Science and Technology Major Project for "Significant New Drugs Development" (2017ZX09304015). Z.H. is the recipient of Bayer Investigator Award.

CONFLICTS OF INTEREST

The authors declared no competing interests for this work.

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