Acupuncture and Herbal Medicine

Key issues and essential points in non-clinical pharmacology of traditional Chinese medicine formulas: enhancing the scientization and standardization

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Abstract

As the primary form and means of clinical treatment, traditional Chinese medicine formulas (TCM formulas) embody the core of TCM's syndrome differentiation and treatment approach and serve as a bridge between TCM theory and clinical practice. Exploring the relationship between the chemical constituents of TCM formulas and the body's vital activities, along with their complex interactive mechanisms, represents one of the key scientific challenges in modern TCM research. However, due to the complexity of TCM chemical constituents and the inherent vast systemic nature of the human body, coupled with the fragmented, experiential, and semi-quantitative nature of TCM formulas pharmacology research, bottlenecks such as complex composition, unclear mechanisms, and insufficient standardization and refinement constrain its in-depth development. *Technical guidelines for non-clinical pharmacology research of traditional Chinese medicine formulas* systematically review and summarize the research content and relevant advances in non-clinical pharmacology of TCM formulas, integrate multidisciplinary technical approaches, and establish research standards, providing practical standards for systematically elucidating the integrated mechanisms of action between multi-component drugs and the body. This article interprets the core content of the technical guidelines, thereby initiating the following discussion on TCM formulas pharmacology: analyzing critical points, elucidating the complete evidence chain, and describing research content and application scenarios, which aims to enhance the scientization and reliability of TCM formulas pharmacology and to facilitate the research and development of new TCM drugs.

Keywords: Clinical relevance, Evidence chain, Integrative pharmacology, TCM formulas, Technical guidelines

Introduction

Traditionally, Chinese medicine formulas' pharmacology mainly elucidated the properties, functions, application principles, and mechanisms of action of medicinal substances. Its contemporary conception is defined as a discipline guided by the theory of traditional Chinese medicine (TCM), which employs modern scientific methodologies to clarify the interactions and patterns of action between TCM formulas and vital bodily functions at human, animal, organ, tissue, and cellular levels. This includes the pharmacodynamics and pharmacokinetics of TCM formulas, with a particular focus on analyzing the complex modes of action in TCM (Figure 1). With advances in modern science and technology, particularly the emergence of frontier disciplines such as systems biology, big data, and artificial intelligence, research in TCM formulas pharmacology has entered a new stage. In 1999, Li[1] introduced the concept of network targets, revealing potential links between TCM syndromes and molecular network regulatory mechanisms. In 2004, Liu[2–3] applied systems biology theories and methods to understand drug processes *in vivo*, proposing the establishment of unified pharmaceutical principles and envisioning a grand vision for pharmaceutical science. In 2006, Luo et al.[4] established an integrated systems biology framework including chemical omics to investigate "system-system" interactions between external intervention systems and internal biological response systems. In 2007, Hopkins[5–6] proposed the concept of network pharmacology, inspiring the application of networks to study the pharmacological mechanisms of TCM formulas. In 2009, Hao et al.[7] combined pharmacokinetics, pharmacodynamics, metabolomics, and cheminformatics to establish three key TCM pharmacokinetic technologies: *in vivo*/*in vitro* material association analysis,

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How to cite this article: Li H, Wang P, Li WJ, Wu D, Zhong YT, Xiao XH, Zhang JH, Xu HY. Key issues and essential points in non-clinical pharmacology of traditional Chinese medicine formulas: enhancing the scientization and standardization. Acupunct Herb Med 2025;5(4):379–399. doi: 10.1097/HM9.0000000000000176

Received 11 September 2025 / Accepted 19 November 2025

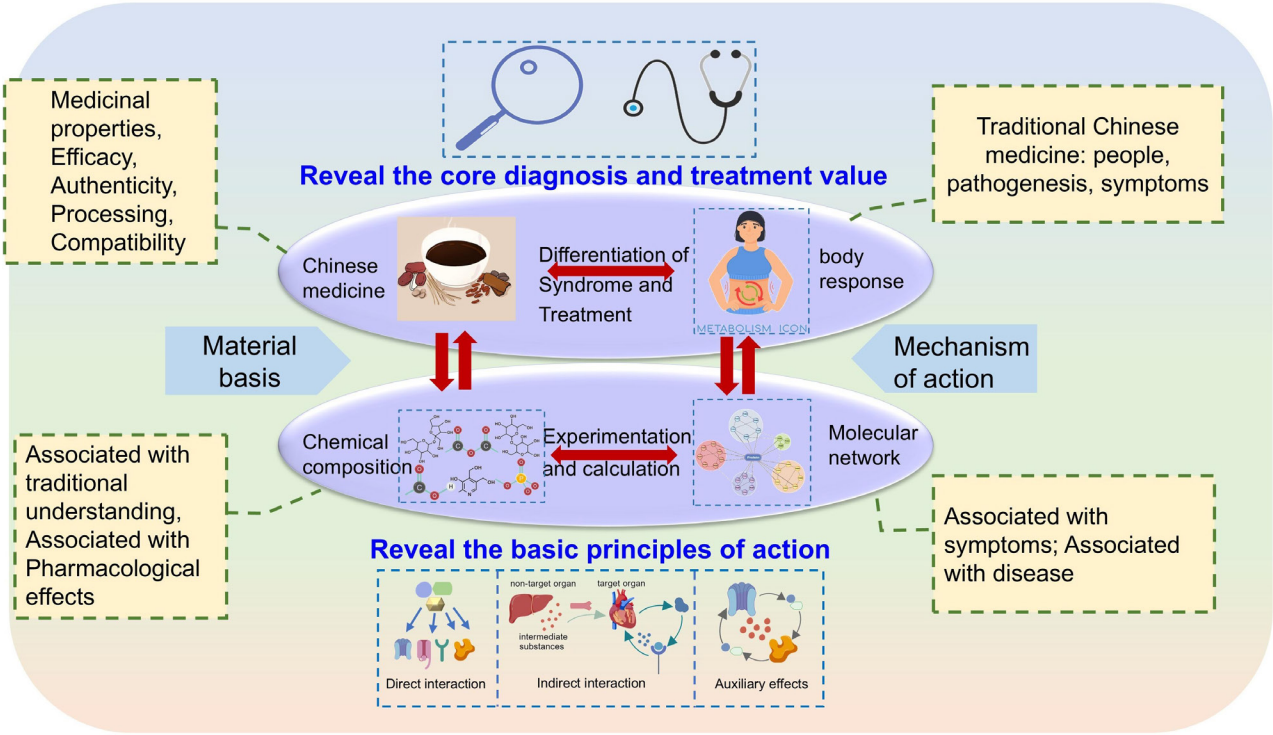

Figure 1. Contemporary understanding of TCM formulas' pharmacology. TCM: Traditional Chinese medicine.

quantitative structure–property/structure–activity relationship research on homologous components, and pharmacokinetic parameter expansion analysis, which formed a novel technical framework for TCM pharmacokinetic studies. In 2010, Wang et al.[8] established a new research paradigm of chinmedomics, employing metabolomics to elucidate the biological essence of syndromes, identifying direct *in vivo* active constituents based on serum pharmaceutical chemistry, and clarifying the material basis and mechanisms of action. In 2013, Wang and Yang[9] developed a systematic model for predictive and validation studies in TCM pharmacology, alongside a TCM systems pharmacology database and analytical platform. In 2014, Xu and Yang[10–11] established a new paradigm for TCM formulas pharmacology research, TCM-integrated pharmacology, emphasizing multi-level integrated studies that combine "the holistic and the particular, the *in vivo* and *in vitro*, computation and experimentation." Yang et al.[12] proposed a novel approach to identifying active component clusters in TCM formulas that can fundamentally represent the efficacy of the entire prescription from its numerous constituents. This cluster can be used as an indicator of quality control and contribute to combination drug discovery. In 2023, Gan et al.[13] elucidated the principles of TCM syndrome differentiation and treatment by analyzing topological proximity relationships between TCM targets and symptom-associated protein modules within the complete human protein network. Combined with systems biology and big data, these studies have developed various research modes and aim to reveal the multi-component, multi-target, and multi-pathway action patterns of TCM formulas, which have significantly advanced TCM formula pharmacology.

From a temporal perspective, the diverse research approaches in TCM formulas pharmacology have broadened our analytical scope. However, a closer look at the industry reveals some inherent constraints: the complex composition of TCM, the intricate nature of the human body, and the empirical and semi-quantitative nature of pharmacological research. Consequently, the complexity of TCM constituents, the unclear mechanisms of its action, and the insufficient standardization and refinement of pharmacology practice of TCM formulas constitute bottlenecks that hinder its in-depth development. The National Medical Products Administration issued the *Technical Requirements for Pharmacological and Toxicological Research of New Traditional Chinese Medicines* in 1999, outlining concise requirements for key pharmacodynamic, general pharmacology, pharmacokinetic, and toxicological studies in new drug development. In 2007, the Centre for Drug Evaluation (CDE) published the *Technical Guidance Principles for General Pharmacological Research of Traditional Chinese Medicines and Natural Medicines*, proposing recommended trial protocols for safety evaluation of TCM. In 2024, CDE released the *Technical Guidance Principles for Pharmacodynamic Research of Traditional Chinese Medicines (Draft for Comment)*, providing reference frameworks for primary and secondary pharmacodynamic studies of innovative TCM drugs, thereby facilitating the accelerated development of new TCM drugs. Although these guidance documents provide a basic framework for various aspects of TCM formulas pharmacology research, they are inadequate at an operational level and do not meet the precise requirements of modern research. Moreover, the internationalization of TCM formula research is hindered by the lack of standardization.

In recent decades, significant advances have been made in the pharmacology of TCM preparations, particularly in the development of animal models, the evaluation of pharmacodynamics, and the exploration of mechanisms of action. Integrating these studies with cutting-edge disciplines such as systems biology, big data, and artificial intelligence has substantially expanded the scope of research. Consequently, it is imperative to conduct a systematic review of the progress of non-clinical pharmacology research on TCM formulas in this context. This will enhance the integration of multidisciplinary techniques and establish technical research standards for TCM formula pharmacology studies. To this end, and to establish a research paradigm for TCM formulas pharmacology, standardize its methodologies, and improve the quality of its research, our team has formulated the *technical guidelines for non-clinical pharmacology research of traditional Chinese medicine formulas*. The guidelines were published on February 17, 2025.

As the first systematic technical guideline to regulate non-clinical pharmacology research on TCM formulas, this document provides specific, categorized guidance and serves as a reference for refined, systematic, and standard pharmacological research on TCM formulas. This article interprets the core content of the technical guidelines, thereby initiating the following discussion on TCM formulas pharmacology: analyzing critical points, elucidating the complete evidence chain, and describing research content and application scenarios, which aims to enhance the scientization and reliability of TCM formulas pharmacology and to facilitate the research and development of new TCM drugs.

Key issues in non-clinical pharmacological research of TCM formulas

Strengthening the relevance of TCM formulas, pharmacology, and clinical practice

TCM has accumulated extensive clinical experience in disease prevention and treatment and formed a unique theoretical framework through millennia of practice. Non-clinical pharmacology research on TCM formulas emphasized guidance by TCM theory and grounding in clinical practice. When constructing and selecting animal models, relevance to clinical settings must be considered across both disease and syndrome dimensions. Evaluations of similarity, scientific validity, reproducibility, reliability, controllability, and cost-effectiveness should be conducted, as shown in Table 1. In the process of establishing a pharmacodynamic indicator system, it is imperative that it is aligned with clinical efficacy evaluation frameworks. This process involves the integration of various efficacy indicators at multiple levels, including macro- and micro-levels, subjective and objective levels, primary and secondary levels, short-term and long-term levels, and qualitative and quantitative levels, as well as syndrome-related and disease-related indicators. Specifically, physiological parameter indicators should incorporate clinically recognized parameters such as body temperature, electrocardiograms, and neurological function; biochemical indicators should select highly specific markers or disease-related proteins, enzymes, RNA, and metabolites; and pathological indicators should employ widely accepted histological staining and examination methods, including immunohistochemistry, hematoxylin-eosin (HE) staining, and oil red O staining. Moreover, in the context of fundamental pharmacology research and the development of new Chinese medicine drugs supported by human experience, there is a necessity for trial samples to align with actual clinical applications. The route of administration, dosage, and frequency of administration should be relevant to, and correspond with, clinical practice.

Categorized guidance facilitates fundamental research and development of new TCM drugs

The non-clinical pharmacology of TCM formulas should be driven by clinical value, ensuring that fundamental research findings are translated into clinical applications or disease prevention/treatment strategies in a timely and effective manner. From an industry perspective, non-clinical pharmacology research on TCM formulas can be divided into two categories: fundamental research and new drug development. Fundamental research focused on elucidating the active constituents and mechanisms of action of TCM prescriptions that are supported by robust TCM theoretical foundations and substantial human application evidence, including classical prescriptions, clinically validated empirical formulas, folk remedies, and marketed proprietary Chinese medicines. New Chinese medicine drug development encompasses three scenarios: development based on TCM formulas with robust theoretical and clinical evidence, development based on experimental research or lacking clinical experience, and priority review for TCM formula-based new drug development. The middle, originating from experimental research or lacking clinical experience, further comprises two stages: drug suitability evaluation and registration approval.

During fundamental research, test samples, administration routes, dosages, and frequencies must align with clinical practice; the formulation composition, botanical origin, medicinal parts, and processing methods of TCM formulas must be clearly defined, and the test samples can be laboratory-scale. For new traditional Chinese formula drug development originating from experimental research or lacking human experience, the drug suitability evaluation stage requires comparative studies on the prescription's fundamental information alongside administration routes, dosage, and frequency to provide a scientific basis for key information selection. The samples should be laboratory-scale preparations. However, during the registration approval stage, samples must correspond to an actual clinical application. Information such as the botanical origin, medicinal parts, place of origin, harvesting period, cultivation or farming methods, fixed drug composition and ratios, preparation processes, herbal materials, formula, and manufacturer must be clearly defined and uniformized. The route, dosage, and frequency of administration should be maintained consistent or relevant with the proposed clinical trial. For registration approvals of new Chinese medicine formulas supported by robust TCM theory and human

Table 1

Key points for evaluating the relevance of animal models and clinical practice

Evaluation content	Disease animal model	Syndrome/symptom animal model
Similarity evaluation	Disease animal models should replicate human pathologies as closely as possible in terms of their anatomy, pathology, and physiology, while making full use of the specific reactions inherent to different breeds and strains of experimental animals.	Animal models of syndromes must correspond to the primary and secondary symptoms and tongue and pulse patterns of clinical Chinese medicine syndromes. They must also exhibit similarities in specific pathological, physiological, functional, metabolic, and structural aspects to clinical conditions.
Scientific evaluation	-	A scientific evaluation must be conducted between animal models of syndromes and clinical syndromes, based on symptoms (primary syndromes), pathogenesis (forward syndromes), treatment (opposite syndromes), associated factors (supporting syndromes), and objective indicators (supporting syndromes).
Repeatability evaluation	Consistency must be maintained and controlled across all aspects, including animal species, strain, age, sex, body weight, health status, feeding management, experimental conditions/steps, pharmaceutical manufacturer, drug administration, and experimental techniques.	See also "disease animal model."
Reliability evaluation	The model is required to reliably reflect the characteristics of human disease, including the disease itself, as well as specific functional, metabolic, or structural changes. It is also necessary for the model to exhibit the primary symptoms and signs of the disease. The model must be validated through a series of tests.	Establishing a conversion model for the four diagnostic methods between humans and animals by combining macro-level characterization with micro-level indicators. Developing in-depth research into TCM clinical syndromes and clarifying their pathological, physiological, functional, metabolic, and structural characteristics.
Controllability and cost-effectiveness	Animals that are highly sensitive to pathogenic factors, prone to mortality, or difficult to control are unsuitable for use as animal models in scientific research. In the context of animal models, the disease progression should be readily manipulable, the methodology straightforward to implement, and the approach economically viable.	See also "disease animal model."

experience, such as category 1.1 innovative TCM prescriptions and category 3.1 classical TCM formulas, test samples must clearly be defined and standardized with the aforementioned information. Test samples, administration routes, dosages, and frequency must also align with clinical studies. Moreover, the technical guidelines categorize and recommend suggestions for other content, as outlined in Table 2. Through comprehensive categorization of different aspects, this guideline provides a reference for the priority review and approval of new TCM drugs with well-defined medicinal substance bases and clear mechanisms of action.

Improving research precision through cutting-edge technologies and interdisciplinary approaches

This technical guideline encourages the adoption of state-of-the-art technologies in non-clinical pharmacology research for TCM formulas to further enhance precision. For example, in constructing multidimensional "pharmacokinetic–pharmacodynamic (PK–PD)" correlation models for TCM prescriptions, we can employ nonlinear algorithms such as multi-objective optimization techniques based on entropy values and neural networks; in the context of comprehensive molecular mechanism prediction for TCM formulas, the integration of omics technologies, namely single-cell omics and spatial omics, has been demonstrated to enhance the precision of mechanism research. The employment of techniques such as organoid and microfluidic chip simulation of *in vivo* PK–PD dynamics has facilitated the transition of TCM pharmacology from experience-driven to data-driven approaches and from qualitative descriptions to quantitative analysis, thereby improving its accuracy, scientization, and standardization.

Organoids are defined as miniature cellular structures cultured in defined three-dimensional (3D) environments *ex vivo*, generated from tissue-resident stem/progenitor cells, adult stem cells, embryonic stem cells, or induced pluripotent stem cells (iPSCs). Yan et al.[14] employed intestinal organoids to corroborate the hypothesis that the traditional Chinese formula Liangxue Guyuan Yishen tang promotes intestinal stem cell recovery by restoring beneficial gut microbiota, particularly *Akkermansia muciniphila* (AKK), and elevating isobutyric acid levels. This approach shows great potential in mitigating the side effects of intestinal damage following cancer radiotherapy.

Organ-on-a-chip represents a micro-physiological system, serving as a simplified functional model at the organ or even organism level. Numerous mature organ-on-a-chip platforms have been developed, encompassing the brain/blood-brain barrier, lung, heart, liver, kidney, intestine, vasculature, skin, and tumors[15]. By coupling multiple organ-on-a-chip platforms *via* fluidic perfusion or shared culture media, researchers can investigate

Table 2

Categorized recommendations for non-clinical pharmacology of TCM formulas in fundamental research and new Chinese medicine drug development

				Development of new TCM drugs			
					TCM formulas derived from experimental research or lacking clinical experience		
Main content	Relevant requirements		Fundamental research	Human application experience	Drug suitability evaluation stage	Registration approval stage	Priority review
Sample information	Basic information	Clarify formulation composition, botanical origin of medicinal materials, processing methods, etc.	+	+	+	+	+
	Preparation process/scale	Specify extraction, drying, and process information/small-scale trials and above.	+	—	+	—	+
		Specify extraction, drying, and process information/pilot-scale and above.	+	+	+	+	+
	Material basis	Clarify the material basis through qualitative and quantitative analysis.	+	—	—	—	+
Administration method	Consistency or correlation with clinical administration in terms of route, dosage, frequency, etc.		+	+	—	+	+
	The route of administration, dosage, frequency, etc, provides scientific justification for the proposed clinical application.		+	+	+	+	+
Selection based on an animal model	Similarity assessment		+	+	+	+	+
	Repeatability assessment		+	+	+	+	+
	Reliability assessment		+	+	+	+	+
	Economic evaluation		+	+	+	+	+
Pharmacodynamic indicator system construction and evaluation	Primary pharmacodynamic indicators	General phenotypic indicators	+	+	+	+	+
		Physiological and biochemical indicators	+	+	+	+	+
		Pathological and morphological indicators	+	+	+	+	+
		Traditional Chinese medicine syndrome-related indicators	+	+	+	+	+
		Disease-symptom-syndrome–related molecular markers	+	+	+	+	+
		Omics technologies and biomarkers	+	+	+	+	+

TCM: Traditional Chinese medicine.

"+" denotes studies recommended for implementation; "—" denotes studies that may be omitted.

organ-organ interactions and systemic diseases such as off-target drug toxicity, cancer metastasis, and inflammation[16].

Single-cell omics refers to the collective term for technologies that enable systematic, high-throughput analysis of specific types of biomolecules (such as genomes, transcriptomes, epigenomes, proteomes, etc) at single-cell resolution. Its core objective is to elucidate the cellular heterogeneity that exists within populations, thereby unveiling the individual variations in cells that have been obscured by conventional bulk-level analysis, which enables a more precise understanding of cellular states, types, functions, developmental trajectories, intercellular interactions, and their roles in physiological and pathological processes. Single-cell transcriptome sequencing is currently the most widely applied technique. Tao et al.[17] collected tissue samples from mice with colorectal adenomas and humans before and after administration of the Shenbai Jiedu decoction performing single-cell transcriptome sequencing to analyze cellular type shifts within the tumor microenvironment, CCL22 accumulation, dendritic cell (DC) differentiation trajectories, and DC-T cell communication intensity. The present study has established single-cell atlases for both mouse models and clinical patients, thus confirming that Shenbai Jiedu decoction reverses the significant accumulation of CCL22 and DCs observed in model mice and patients. These cell types exhibit higher abundance in colorectal cancer tissues.

Spatial omics preserves the original spatial positioning of biological tissues to reveal spatial heterogeneity in molecular functions, gene expression, protein distribution, and metabolic activities across different regions within tissue structures. Yin et al.[18] integrated spatial transcriptomics (ST-seq), spatial metabolomics, and mass spectrometry imaging (AFADESI-MSI) to systematically elucidate the spatiotemporal dynamic reprogramming and regulatory mechanisms of Shenhua tablet through modulation of glutathione metabolism, glycolysis, and L-proline metabolism. This approach localized the spatial distribution of active components within the renal cortex, revealing the multi-target synergistic mechanisms of TCM formulas at the anatomical micro-region level.

The application of cutting-edge technologies has been significantly advanced by innovative data analysis platforms that leverage big data and artificial intelligence algorithms. Examples include algorithms for identifying key cellular populations responsive to drugs within single-cell transcriptomic databases of disease states[19], intelligent and quantitative analysis technologies and systems for Chinese and Western medicine based on network targets (UNIQ system)[20], and the mitochondrial phenotype dynamic recognition algorithm for facilitating mechanism identification[21]. These advancements are propelling pharmacological research on TCM formulas into more microscopic domains and precision-oriented research paradigms. However, the challenges posed by new technologies, such as insufficient reproducibility and standardization, also warrant consideration.

Systematic research to enhance the integrity, scientization, and standardization of non-clinical pharmacological evidence for TCM formulas

The specific operational framework for integrated pharmacology comprises four modules. First, systematically characterizing the chemical fingerprint and *in vivo* metabolic fingerprint of TCM formulations to identify three categories of bioactive constituents. Second, investigating three distinct action pathways to delineate primary effect mechanisms. Third, establishing qualitative and quantitative PK–PD relationships to identify key active components, core molecular targets, and principal pharmacological actions. Fourth, conducting in-depth experimental validation through techniques such as component or gene knock-out/knock-in targeting key active components and core molecular targets.

Guided by TCM-integrated pharmacology, this technical guideline systematically outlines *in vivo* and *in vitro* pharmacological research models, multidimensional efficacy evaluation systems, *in vivo* component migration patterns by qualitative and quantitative PK–PD relationships, direct/indirect/auxiliary pathways, and network computing combined with multi-omics analysis for mechanism elucidation. This approach embodies the characteristics of integrated research, covering clinical and fundamental pharmacology aspects, holistic and localized perspectives, *in vivo* and *in vitro* studies, macro- and micro-levels, pharmacokinetics and pharmacodynamics, and computational and experimental methodologies. It establishes a comprehensive system for the evidence-based evaluation of TCM formulations, enabling the transition from general characterization to precise mechanism research. This fully reflects the unity between systems theory and reductionism, forming the fundamental research mode for non-clinical pharmacology research on TCM formulas, specifically as follows.

Integrity of non-clinical pharmacological evidence chains for TCM formulas

Clinical and fundamental research

Clinical research addresses questions of efficacy at a relatively macro-level, whereas basic research focuses on the micro-level, seeking to understand why treatments are effective. When conducting evaluations of the advantages and mechanisms of action in TCM, emphasis should be placed on establishing a chain of evidence for TCM efficacy[22–23]. Research should be systematically planned around clinical positioning and value, with evidence bodies refined at multiple levels. From an evidence-based medicine perspective, Zhang et al.[24] proposed the concept of evidence chain: guided by clinical value, these chains link TCM clinical research with fundamental research through outcome indicators, forming an evidence set that encompasses clinical, pharmacological efficacy, and mechanism levels. This addresses the questions of both "whether it is effective" and "why it is effective." Liu et al.[25–26] constructed a "multidisciplinary evidence chain for treating *bi* syndrome from the spleen" based on syndrome-pattern integration studies, clinical practice research, clinical efficacy studies, multi-omics

experimental techniques, and multi-target mechanism investigations. Gao et al.[27] established a clinically integrated evidence chain by drawing upon clinical and fundamental research evidence regarding Guizhi Fuling capsules for treating various conditions. This provided a systematic summary of the therapeutic advantages of the capsules for specific conditions, while also exploring their clinical efficacy and mechanisms of action. Based on a clinical meta-analysis, Yupingfeng san combined with conventional therapy was found to enhance treatment outcomes for chronic obstructive pulmonary disease (COPD) by improving pulmonary function and quality of life.[28] Network pharmacology identified potential therapeutic mechanisms involving calcium and cyclic adenosine monophosphate (cAMP) signaling pathways. Isoflavone and isorhamnetin emerged as active components with a strong binding affinity for COPD treatment. This provides comprehensive clinical evidence for the use of Yupingfeng san in COPD management.

Holism and partialism

The holistic perspective is one of the fundamental theories of TCM, with the unity of heaven and humanity embodying its innate natural philosophy. Chinese herbal formulas are harmoniously composed by integrating seven emotions to form organic entities that interact with the coordinated, orderly dynamic environment of the human body, including its viscera, meridians, and channels. Within this complex system, they exert a collective, holistic effect of balancing *yin* and *yang* and harmonizing *qi* and blood. At the same time, the pharmacological actions of TCM formulas represent the organic integration of multiple therapeutic effects. Various efficacy indicators are interrelated and combined to form a dynamically stable, indivisible functional whole (Figure 2). Furthermore, non-clinical pharmacology research on holistic TCM formulas covers systematic studies of drug metabolism, including serum and tissue pharmaceutical chemistry and multi-component pharmacokinetic investigations, which are conducted to obtain the metabolic fingerprints and pathways of herbal formulations; system pharmacology studies of TCM formulas through systems biology techniques, including genomics, transcriptomics, proteomics, metabolomics and network pharmacology, with enhanced multi-omics data integration, are used to elucidate the molecular biological networks through which TCM formulas regulate diseased imbalances; establishing correlations between the metabolic fingerprint of TCM formulas and the molecular biological networks of diseases and constructing multidimensional interactions between TCM formulas and the body based on a "point-line-surface" framework; and revealing associations between pharmacokinetic markers and biomarkers, composition-effect relationships, and constructing multi-component "PK–PD" models through data mining.

In vivo and in vitro

In vitro studies eliminate interference from complex *in vivo* environments. They often offer advantages such as straightforward operation, high sensitivity and specificity, and easily controlled conditions. In the context of drug absorption, distribution, metabolism, and excretion pathways, mature *in vitro* models exist for every stage, from "drug dissolution-gut microbiota conversion-intestinal absorption" to "hepatic enzyme

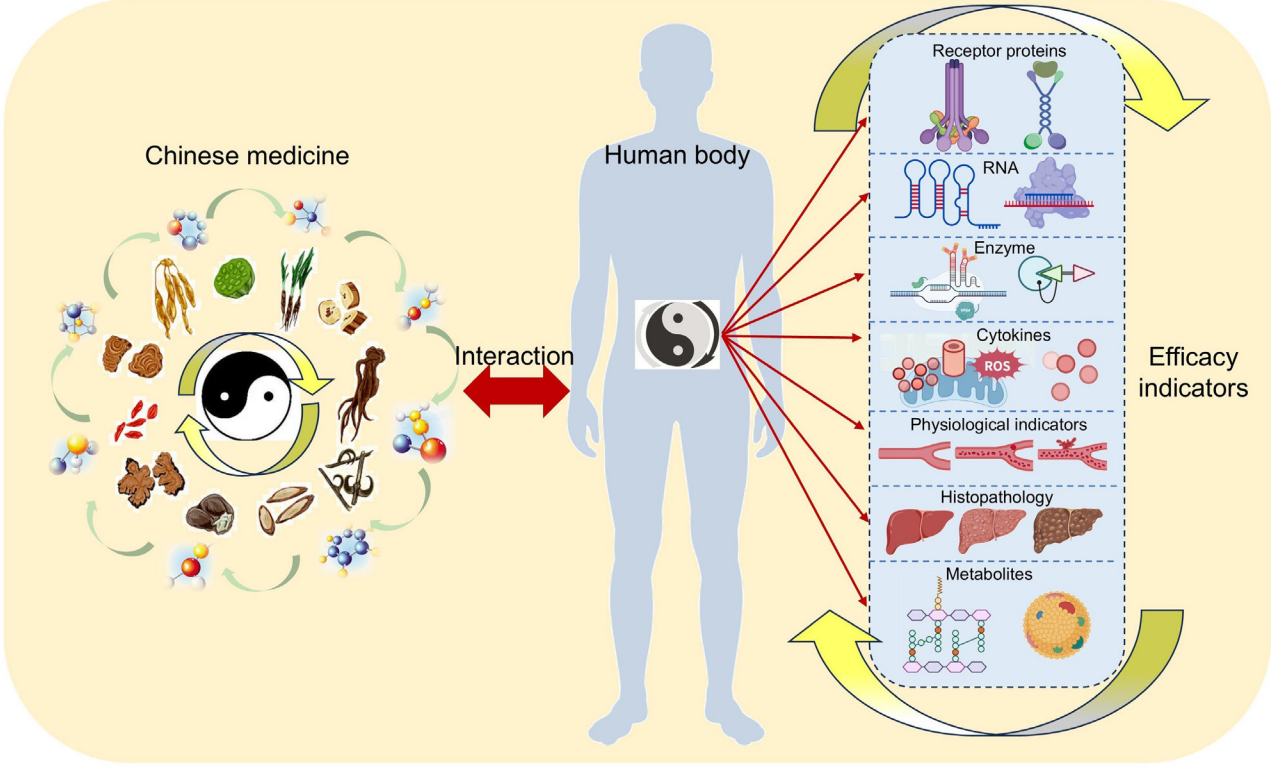

Figure 2. Holistic perspective on the mechanism of TCM formulas. ROS: Reactive oxygen species; TCM: Traditional Chinese medicine.

metabolism-blood-brain barrier." Examples include the bio-mimetic extraction method simulating gastrointestinal digestion, the Caco-2 cell model and everted gut sac method simulating intestinal absorption, and the liver microsome *in vitro* incubation method simulating hepatic metabolism. It should be noted that multiple factors must be considered when employing these models. For instance, *in vitro* trachea/tissue evaluation models require consideration of organ/species, anatomical location (eg, colonic or ileal smooth muscle in *ex vivo* intestinal preparations), modeling methodology, drug-related factors (drug-containing serum/plasma, drug-containing intestinal absorption solutions, drug-containing liver incubation media, extract or formulation solutions), and *ex vivo* culture conditions. For organoid model evaluations, factors such as organoid tissue morphology, pathological characteristics, heterogeneity and stability, molecular biological properties, and drug-related factors must be considered regarding their impact on the pharmacological effects and potency of TCM prescriptions. Cell/organelle evaluation models should undergo short tandem repeat (STR) identification of cells, considering the influence of cell species origin, cell line/primary status, cellular genetic background (gene expression levels), co-culture status and method, modeling approach, drug factors, culture medium, and culture environment on the pharmacological effects and potency of TCM prescriptions. Models for evaluating enzyme activity should consider the impact of enzyme concentration, reaction environment (pH, ionic strength, cofactors, detergents, etc), substrate concentration (natural *vs*. synthetic), the mechanism of the inhibitor (competitive, non-competitive, or uncompetitive), and reaction time on the pharmacological effects and potency of TCM formulas.

To ensure the accuracy and repeatability of experimental results, *in vitro* pharmacological studies should incorporate repeated sample numbers and trial runs. Blank, negative, and solvent controls must be included in experimental groups. Positive control drug groups should be established where necessary. The design of the dose for test drug formulations should take into account the intended clinical human dose, the results of preliminary trials, and other experimental data. At least three doses should be used in testing the drug to explore its dose–response relationships. The observation and detection criteria should be established alongside *in vivo* animal studies. These criteria should adhere to the fundamental principles of sensitivity and specificity while also supplementing and enriching the findings of *in vivo* research.

Macro and micro

In the pharmacodynamic evaluation of TCM formulations, both macro-level efficacy indicators (eg, phenotypic markers such as the mental state, activity levels, respiration rate, feeding behavior, water intake, defecation frequency, and body weight of animals) and micro-level molecular indicators (eg, biochemical markers including proteins, enzymes, RNA, and metabolites associated with disease pathogenesis) should be employed. Further exploration of the "dose-efficacy" and "time-efficacy" relationships may be conducted, with the objective of identifying chemically detectable biomarkers associated with therapeutic efficacy. This provides a foundation for the quality assessment of TCM formulations.

Furthermore, previous standards have generally regulated pharmacological research techniques at the macro-level of therapeutic efficacy, with minimal attention paid to normalizing the investigation of action pathways in TCM formulas. Guided by a TCM-integrated pharmacology research model, this guideline provides a micro-level summary of the three action pathways of Chinese herbal formulas, along with a detailed description of the research content and requirements for each pathway. Based on the association patterns between TCM multi-constituents and biological effects, alongside ADME (absorption, distribution, metabolism, and excretion) characteristics, active substances in TCM can be categorized into three types: absorbed prototypes, absorbed metabolites, and unabsorbed constituents. The therapeutic effects of these substances in the human body are achieved through three distinct pathways[11] (Figure 3). The first mechanism involves the direct action of absorbed prototypes and absorbed metabolites on disease targets. The second mechanism represents the indirect action where all three active components regulate non-target organs to produce intermediary mediators that subsequently act on disease target organs. It also encompasses the regulatory role of the gut microbiota and the way in which human extracellular vesicles mediate intercellular communication. For instance, research indicated that during myocardial infarction, the liver secretes a crucial intermediary substance, fibroblast growth factor 21 (FGF21), which does not act locally in the liver. Instead, the substance circulates *via* the bloodstream to the heart, where it further modulates the interleukin (IL)-1β/IL-6 signaling pathway to ameliorate myocardial infarction[29]. The third mechanism involves non-covalent interactions between components arising from hydrogen bonding, van der Waals forces, π–π stacking, molecular complexation, and electrostatic effects, or auxiliary biological processes involving altered ADME behavior through metabolic enzyme/transporter activity, or the effects of TCM active ingredients or miRNA carried by plant-derived vesicles on cells or the microenvironment. For instance, the interaction between berberine and emodin results in the formation of supramolecular nanoparticles, thereby significantly enhancing their antibacterial activity[30]; quercetin and kaempferol enhance the intestinal absorption of isorhamnetin by inhibiting its efflux *via* the intestinal cell efflux transporter MRP2[31]. These may all be understood as auxiliary effects. By focusing on these three pathways, a chain of evidence system for the efficacy of Chinese herbal formulas comprising "chemical substances-action targets-network targets-disease effects" has been established. This development represents a significant advancement in the field of non-clinical pharmacology research concerning TCM formulations, thereby further enhancing the scientization and standardization of the research. Requirements for research content on the three pathways are outlined in Table 3.

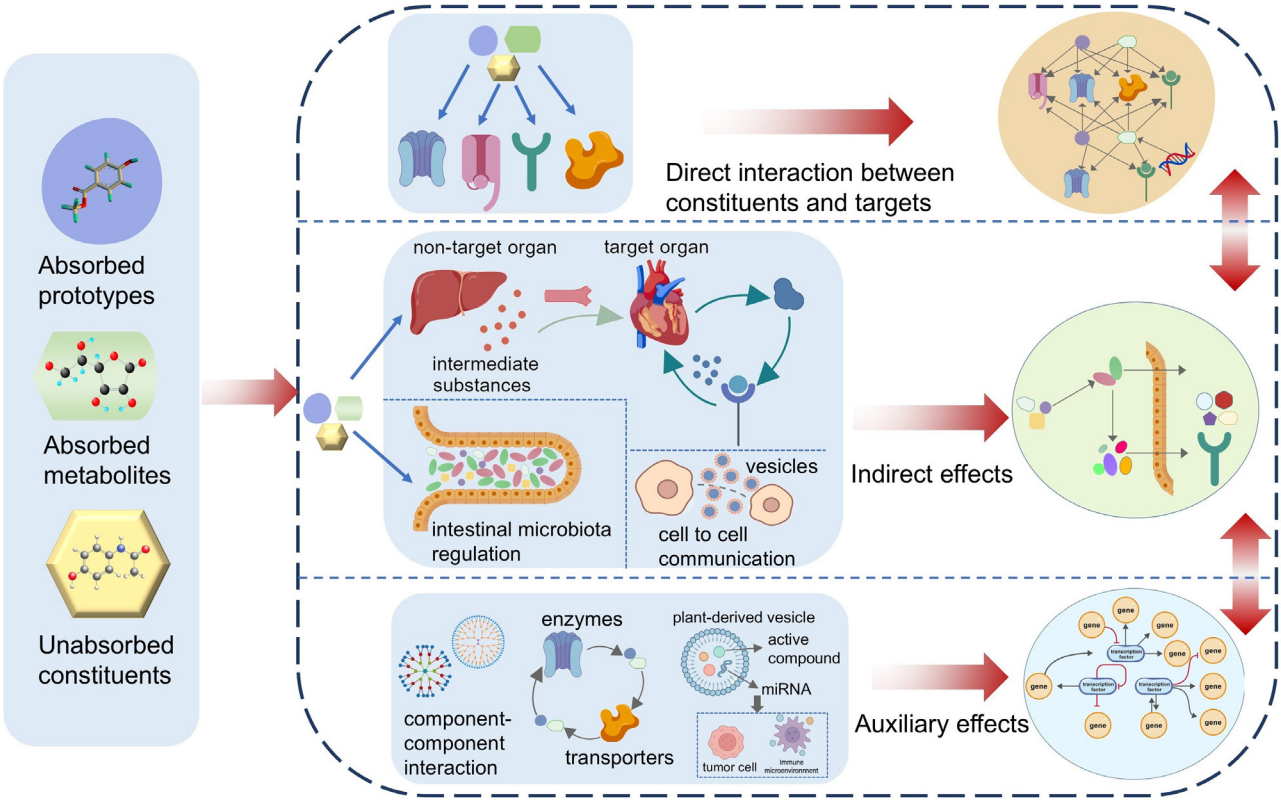

Figure 3. Three pathways of action in TCM formulas. TCM: Traditional Chinese medicine.

Pharmacokinetics and pharmacodynamics

Pharmacokinetic-pharmacodynamic (PK–PD) correlation studies are an effective method of dynamically characterizing and quantitatively describing the relationship between active components and their pharmacological effects. By integrating time, drug concentration, and effect based on constituent content, distribution patterns within the body, the potency of the pharmacological action, and the characteristics of the network topology, the pharmacodynamic basis and the pharmacokinetic markers of TCM formulations can be elucidated. PK–PD models can be categorized into five types: linear models, log-linear models, maximum effect (E_{max}) models, sigmoid E_{max} models, and β-function models. The characteristics and applications of each model are detailed in Table 4.

The connection modes of PK–PD models can be categorized into four types: direct *versus* indirect connections, direct *versus* indirect effects, soft *versus* hard connections, and time-dependent *versus* time-independent models. Direct linkage denotes an equilibrium relationship between the effect of the drug and the concentration at the site, where the peak effect coincides with the maximum plasma concentration with no time lag. Therefore, plasma concentration can be used as an input function to describe the effect of the drug. Indirect linkage indicates that equilibrium between site and plasma concentrations cannot be immediately attained. The peak effect lags behind the peak plasma concentration, and there is no direct correlation, with a significant time delay. The direct and indirect effects correspond to the direct and indirect regulatory actions in Table 3, respectively. Consequently, indirect effects also exhibit notable time lags. Soft coupling links PK and PD using concentration-effect data, whereas hard coupling connects them based on drug mechanisms by utilizing PK data alongside *in vitro* affinity experiments involving enzymes and receptors. This enables the prediction of new compound activities *in vivo*. Time-independent models describe drug effects that vary solely with changes in the concentration of the effect compartment, where the PD parameters remain constant over time. Most drugs conform to this paradigm. Conversely, time-dependent models indicate that drug effects are unaffected by target-site concentration, with PD parameters evolving over time. Here, changes in effect intensity occur even when the concentration of the effect compartment remains stable. Tan et al.[33] utilized a PC12 cell death model induced by $Na_2S_2O_4$ and Glu to establish a PK–PD model *via* a support vector machine algorithm, revealing the active components of Nao Mai Tong that inhibit neuronal cell death. Zhang et al[34] employed Q-marker theory and the activation of the renin-angiotensin-aldosterone system to establish an E_{max} model that relates the pharmacokinetics of 17 plasma components and the angiotensin II efficacy levels to identify quality markers for Qili Qiangxin capsules when used to treat chronic heart failure. Based on TCM combination theory and a rat osteoarthritis model, Wu et al.[35] employed WinNonlin software to establish an effect-chamber-atrio-ventricular sigmoid E_{max} model. This model was used to investigate the pharmacological basis and mechanism of action of the synergistic effect of the *Epimedium brevicornu-Ligusticum chuanxiong* drug combination.

To ensure model robustness and parameter stability, internal cross-validation methods, such as leave-one-out,

Table 3

Research content and requirements for the three action pathways of TCM formulas

Action mode	Research content	Requirements
Direct regulatory action	Analysis of target-reaching components in traditional Chinese medicine.	Based on target organ information for the disease, utilize ADME/PK predictive models to identify potential TCM components (prototypes and metabolites) that are absorbed into the body and reach the target tissue, ie, TCM target-reaching components. These components can be identified in blood, target organs, target tissues, and target cells.
	In vitro activity evaluation of target-reaching components.	*In vitro* pharmacological models are employed to evaluate the activity of target-reaching components for key disease-specific effects.
	Research on direct targets of the targeted components.	Potential direct targets of TCM components may be identified based on virtual predictions or based on literature reports. Target identification is conducted using experimental methods employing either chemical biology (active component probes covering biotin, alkyne, or azide groups) or label-free approaches (DARTS, CETSA, and TPP). Additionally, studies investigating the mode of action and binding sites between target-reaching components and direct targets may utilize amino acid mutation experiments or crystal structures of complexes formed between TCM components and target sites.
	Research in regulatory relationships of target-reaching components.	Research into the regulatory relationships between the components of TCM that reach the target, their direct targets, molecular pathways, and the effects on disease, using techniques such as gene knock-out and overexpression.
	Research into coordinated actions and mechanisms of multi-component Chinese medicine formulas.	To investigate the coordinated actions of multiple components within TCM formulations and the molecular mechanisms underlying synergistic interactions, animal models and cellular models may be employed to study key disease-specific effects.
Indirect regulatory action	Determination of indirect regulatory effects.	The presence of the following phenomena suggests indirect regulatory effects: (1) The exposure concentration of the migrating components of the Chinese herbal compound in the disease target organs/tissue is significantly lower than the concentration at which these components can exert their effects in the disease target organs/tissue; (2) Altering the route of administration (eg switching from gastrointestinal administration to injection) increases the exposure concentration of the migrating components of the Chinese herbal compound in the disease target organs/tissue, yet the therapeutic efficacy of the compound either fails to improve or even diminishes; (3) In non-target organs/tissue with high exposure levels of migrating components, surgical removal or inhibitory procedures (eg, adrenalectomy to study the relationship between kidney-tonifying TCM and adrenal hormones; administration of composite antibiotics to clear intestinal flora to investigate the relationship between spleen-strengthening TCM and gut microbiota regulation) result in a marked reduction or complete loss of the TCM compound's therapeutic effect.
	Screening for intermediate mediator substances.	Intermediary substances primarily encompass bioactive endogenous substances within the human body, such as hormones, cytokines, extracellular vesicles, and gaseous signaling molecules, as well as metabolites derived from gut microbiota. These can be identified using transcriptomics, proteomics, metabolomics, or metagenomics approaches. Once candidate mediators have been identified, the most appropriate experimental approaches for manipulating these molecules can be selected. These include constructing plasmids, viral vectors, or gene-edited animal models to perform knock-out or overexpression experiments on genes that encode the mediator. The resulting changes to the pharmacological effects of the TCM formulas can then be examined to elucidate the cross-organ regulatory mechanisms mediated by the intermediary substance. It should be noted that, in certain circumstances, the indirect regulatory effects of TCM formulations may involve multiple intermediary substances, constituting multi-level indirect regulation.
	Elucidating the mechanisms by which TCM prescriptions regulate intermediate mediators.	The direct regulation of intermediate mediators by TCM formula components can be investigated using the research approach for direct regulation. Specifically, to target the primary expression organs (tissues) of the intermediate mediator, the cell types regulated by the components of the TCM formulas must first be identified. Through *in vitro* and *in vivo* studies, the pathways and molecular mechanisms by which TCM formula components regulate the expression of the intermediate mediator should then be elucidated.
	Elucidating the mechanism by which mediators regulate disease target organs.	The regulation of disease target organs by mediators is a direct regulatory effect that can be investigated using a direct regulation research approach. First, the cell types within the disease target organ (tissue) that are regulated by mediators must be identified. Through *in vitro* and *in vivo* studies, the pathways and molecular mechanisms by which mediators regulate disease can then be elucidated.

(Continued)

Table3

(Continued)

Action mode	Research content	Requirements
Auxiliary regulatory action	Confirmation of auxiliary regulation	The alteration of metabolic enzyme/transporter activity by one component of a compound formulation may influence the uptake or efflux of another component, thereby affecting pharmacokinetic behavior and therapeutic efficacy. The effects of drug active ingredients or miRNAs carried by plant-derived vesicles on cells or the microenvironment. The components within a compound self-assemble into supramolecular aggregates *via* non-covalent interactions, such as hydrogen bonding, van der Waals forces, π–π stacking, molecular complexation, and electrostatic effects. This enhances the solubility of poorly soluble TCM molecules, improves the *in vivo* stability of TCM constituents, and increases bioavailability and activity.
	Study on PK–PD synergy in multi-component TCM formulas based on auxiliary regulatory mechanisms.	Investigating the pharmacodynamics and mechanisms of TCM formulas through three auxiliary action modes and establishing multi-component PK–PD correlations *via* "drug–drug interactions" to provide scientific evidence for studying the mechanisms of synergistic efficacy and toxicity reduction.
	Molecular mechanisms of multi-component auxiliary regulation in TCM formulas.	Investigate the molecular mechanisms of multi-component PK–PD associations underlying the auxiliary regulatory actions of TCM formulas through multidimensional network analysis linking chemical constituents, intermediary substances, molecular pathways, and disease effects.

CETSA: Cell thermal shift assay; DARTS: Drug affinity responsive target stability; PK–PD: Pharmacokinetic-pharmacodynamic; TCM: Traditional Chinese medicine; TPP: Thermal proteome profiling.

k-fold, and external validation, may be employed. This involves using a completely independent dataset to predict outcomes and comparing the predicted values with the observed values. Metrics such as mean prediction error and root mean square error of prediction (RMSE) are then calculated to quantitatively assess model accuracy. Furthermore, given the multi-component, multi-target nature of TCM formulas, it is equally crucial to identify suitable efficacy indicators and chemical constituents that are sufficiently representative of the entire prescription to ensure model robustness, parameter stability, and cross-system adaptability.

Computations and experiments

The integration of computations and experiments emphasized in this guideline covers three dimensions: First, at the computational level, we can use bioinformatics or network pharmacology analyses to predict potential interaction patterns between TCM formulations and the body using virtual predictions from extensive biological network datasets. Second, at the *in vitro* experimental level, we integrate modules such as drug dissolution, gut microbiota metabolism, intestinal absorption, and hepatic enzyme metabolism, combined with *in vitro* pharmacological evaluations involving cells, tissues, and organs, to study the interactions between TCM formulas and the body from an *in vitro* perspective. Third, at the whole-animal experimental level, integrated *in vivo* pharmacokinetic and pharmacodynamic evaluations are conducted. Particular emphasis is placed on combining TCM formula metabolic fingerprinting with systems biology, linking pharmacokinetic markers to biomarkers to investigate TCM formula interactions within the body. For instance, Li et al.[36] employed bioinformatics to mine biological processes, signaling pathways, and biomarker genes associated with acute gouty arthritis from the Gene Expression Omnibus (GEO) database. Integrating this with chemically identified components of the Shi-Re Bi Capsules, they constructed a regulatory network to identify pathways for further investigation. Experimental validation was conducted in a rat model of damp-heat syndrome induced by intra-articular injection of monosodium urate into the ankle joint. Wu et al.[37] used a chronic constriction injury model to analyze the biological mechanisms underlying the synergistic effects of combining *Corydalis* and *Angelica dahurica*. Using transcriptomic analysis of joint tissue and bioinformatics network computation, alongside experimental validation of key gene expression, they revealed that *A. dahurica* plays an auxiliary role in enhancing the analgesic effect of *Calendula officinalis* by promoting skeletal muscle tissue regeneration and regulating calcium ion transport. Furthermore, our team has independently developed the pharmacology platform, *Encyclopedia of Traditional Chinese Medicine*, which encompasses 402 herbal medicines, 3,959 formulas, and 7,284 chemical constituents[38]. The chemical component data is cross-referenced with the ChEMBL and PubChem databases. The Pipeline Pilot ADMET (absorption, distribution, metabolism, excretion, and toxicity) ensemble model is used to calculate pharmacokinetic parameters and assess the drug potential of each component. This platform is extensively applied in bioinformatics computing and TCM component analysis research[39–40]. Therefore, we built an integrated web platform, SoFDA[41], with a manually curated database of syndrome ontology, a network-based evaluation tool of multi-way associations among diseases, TCM syndromes, and herbal formulas, and a visualization tool for constructing and editing multidimensional networks. This platform establishes functional modules for evaluating the relevance of "disease-syndrome" "formula-syndrome" and "disease-syndrome-formula" relationships, providing robust data foundations and platform support for core TCM theories such as "treating different diseases with the same formula" "treating the same disease with different formulas" and "formula-syndrome correspondence"[42–43]. It bridges information from macroscopic TCM syndromes, diseases, and TCM formulas to microscopic molecular mechanisms.

Table 4

PK–PD model, equation, characteristic, and application[32]

Model	Equation	Characteristic	Application
Linear model	$E = b \times C + E_0$	Simple model with easily derived parameters, but often fails to accurately reflect the PK–PD relationship.	Can predict E_{max} 20%–80% of drug effects.
Log-linear model	$E = a \times \lg C + E_0$	Offers distinct advantages in statistical testing, but the number of variables included in the model must not be excessive.	Model with more statistical tests.
E_{max} model	$E = E_{max} \times C/(EC_{50} + C)$	When drug concentration increases to a maximum level, the effect increases more slowly, reflecting the sensitivity of the effect compartment to the drug.	This is suitable for studies where the efficacy of a drug increases in line with its concentration, such as investigations into drug-receptor interactions.
Sigmoid E_{max} model	$E = E_{max} \times C/(EC_{50} + C) + E_0$	Most widely used.	Widely applied.
β-Function model	$E = X^r(1 - X)^s/g$	This functional equation is often converted into a linear bivariate regression equation to calculate the various parameters of the model.	Used to describe a convex Ce–*E* curve.

PK–PD: Pharmacokinetic–pharmacodynamic.

Take the research on the Yuanhu Zhitong prescription as a case study. We have conducted a systematic qualitative and quantitative chemical analysis of multiple batches of Yuanhu Zhitong tablets[44]. An integrated *in vitro* pharmacology evaluation model that combined intestinal absorption-activity assessment with ADME-bioactivity was established to evaluate its intestinal absorption and vasoactive properties[45]. Serum and cerebrospinal fluid pharmacokinetic studies revealed that multiple constituents in the formula are absorbed into the bloodstream and can effectively cross the blood–brain barrier[46]. Further integrated research combining chemical fingerprinting, metabolite fingerprinting, and network targeting identified several key components, including tetrahydropalmatine, which possesses favorable pharmacokinetic properties[47]. These components can act on core targets to exert significant pharmacological effects and may serve as potential quality markers for the formula[48]. Mechanistic research has demonstrated that pyroptosis, which is mediated by the NLR family pyrin domain containing 3 (NLRP3) inflammasome, exacerbates neuroinflammatory damage in neuropathic pain by disrupting the C/EBPβ-Clec7a axis[49]. The principal herb, corydalis, primarily exerts analgesic effects by inhibiting spinal inflammation and neuropeptide signaling pathways, while the auxiliary herb, Angelica dahurica, promotes skeletal muscle regeneration and regulates calcium ion transport, thereby exerting synergistic effects[37]. This systematic research demonstrates that integrated pharmacology can be successfully applied to the study of TCM formulas.

Major research contents in non-clinical pharmacology of TCM formulas

Due to the diverse composition of TCM formulas and the complexity of their interaction patterns with the human body, as well as the multi-target, multi-pathway regulation inherent in TCM formulas, pharmacology research, and its relative independence within large-scale biological network studies, existing guidelines often fail to provide specific, detailed direction and standardization for experimental investigations. Guided by the TCM-integrated pharmacology research paradigm, this guide provides specific and precise operational guidance for each research phase. It achieves this by identifying connections and patterns across various non-clinical pharmacology research modules for TCM formulas, providing a clear and concise framework. Non-clinical pharmacology studies of TCM formulations should be clinically relevant. Following the population, interventions, comparisons and outcomes (PICO) framework of evidence-based medicine (population: study participants; intervention: intervention measures; comparison: control measures; outcome: key clinical outcomes), each module's research content shall be discussed accordingly.

Animal models and in vitro *pharmacological models*

Animal models refer to populations that are specifically affected by a particular disease. The selection of animal models must be based on a comprehensive determination of the clinical positioning of the TCM formula, its prescription characteristics, and the research objectives. Animal models based on syndromes may be created using TCM etiological modeling, drug-based modeling, direct pathological modeling, or a combined etiological-pathological approach. Disease-syndrome integrated animal models can be developed in the following ways: multifactorial composite modeling, combining Chinese and Western medical approaches, identifying TCM syndromes based on Western disease models, and exploring Western diseases based on TCM syndrome models. It is recommended that evaluations be conducted of the correspondence between syndromes, tongue, pulse findings, and clinical TCM syndromes; evaluations of the scientific validity and similarity between pathophysiology, functional metabolism, and clinical manifestations; and evaluations of the reproducibility, reliability, and cost-effectiveness of animal models when used.

Furthermore, the technical guidelines provide detailed recommendations for pharmacological models in *in vitro* experiments, such as *ex vivo* organs, *ex vivo* tissues, organoids, cells, organelles, enzyme activity, and molecular interaction models for virtual screening. It is necessary to examine the influence of the *ex vivo* model's own environment and characteristics on the intensity of the pharmacological effects of TCM formulations. The guidelines outline the advantages, disadvantages, and applicable scenarios for different administration methods: direct drug administration, drug-containing serum, and drug-containing intestinal absorption fluid. It is worth noting that the drug-containing serum method more closely approximates the direct *in vivo* action of TCM compound formulations. However, it presents challenges, including significant serum matrix interference, low drug concentrations, and the potential for false positives or false negatives. This method is generally suitable for evaluating the direct regulatory effects of oral formulations *in vitro*. The drug-containing intestinal absorption fluid method is primarily employed when the prototypes serve as the primary active ingredient in TCM formulations and is unsuitable when metabolites constitute the active substance.

Administration samples, routes, doses, and frequency

This guideline provides categorized, systematic, and specific guidance on administration samples, routes, doses, frequencies, and positive drugs for both TCM fundamental research and new drug development. It clarifies the intervention and control measures within the PICO framework. For example, with regard to positive drug selection, the technical guideline recommends first-line Western drugs for animal models of disease and recognized effective TCM formulas for animal models of syndrome.

Pharmacodynamic evaluation indicator system

The technical guidelines propose establishing a multidimensional pharmacodynamic evaluation indicator system to describe clinical outcomes within the PICO framework. This encompasses phenotypic indicators, physiological parameters, biochemical markers, pathological indices, imaging metrics, omics evaluation indicators, and TCM syndrome/symptom-related indicators. Fuzzy mathematics and mathematical statistics methods should be applied to analyze the weighting and correlations of associated indicators, thereby forming an organically integrated, interrelated, and dynamically stable efficacy evaluation system. For key efficacy indicators, comprehensive evaluations should also be conducted regarding specificity, sensitivity, accuracy, repeatability, and cost-effectiveness. The significance, scope of application, grading, and thresholds for selected indicators should be elaborated, with the aim of achieving broad recognition and adoption.

Pharmacokinetic study of TCM formulas

Pharmacokinetic study of TCM formulas involves the systematic analysis of the components *in vivo*, as well as multi-component pharmacokinetic studies and the relationships between the pharmacokinetics (PK) and pharmacodynamics (PD) of multiple components and their biological effects. Experimental design should maintain consistency with pharmacodynamic studies regarding samples, administration routes, dosages, frequency, and animal models.

The systematic analysis of the *in vivo* movable components of TCM formulations encompasses qualitative metabolization and quantitative metabolization. The sampling timepoints after administration should be designed according to the formulation's pharmacodynamic characteristics, particularly its time-effect relationship. Target tissues include the contents of the gastrointestinal tract, serum, dejecta, urine, and various organs, such as the heart, liver, spleen, lungs, and kidneys. High-resolution, high-sensitivity chromatography-mass spectrometry (MS/MS) is generally employed for systematic identification and analysis of bioavailable components, encompassing both prototypes and metabolites through qualitative and quantitative assessment. At the same time, methodological considerations must be emphasized, including control experiments (blank solvent, negative biological samples, drug-containing biological samples, etc), repeatability of retention time deviation, the relative abundance of standard spectra, and the signal-to-noise ratio of detected ions.

Chromatography remains the primary analytical method for multi-component pharmacokinetic studies of TCM formulations. Quantitative analysis methodologies encompass accuracy, precision, specificity, sensitivity, repeatability, stability, standard curves, extraction recovery rates, quantitative ranges, biological media, and medium effects. Commonly used pharmacokinetic parameter calculation software includes the 3P87/3P97 practical pharmacokinetic calculation program, DAS statistical software, WinNonlin software, and Kinetica software. These can be used to calculate statistical moment parameters of drug concentration–time curves to describe the pharmacokinetic characteristics of multi-component TCM formulations.

Based on a systematic analysis of active components' migration patterns, the guidelines recommend investigating the complexity and nonlinear characteristics of PK/PD. If the active components migrating within the body (both prototypes and metabolites) are primarily distributed in plasma and target tissues, it is hypothesized that they exert regulatory effects through direct action, thereby establishing a multidimensional network of qualitative associations linking migrating active components, action targets, regulatory pathways, and disease effects. Should the systemic components predominantly distribute within non-target tissues, they may exert cross-organ (or tissue) regulation through indirect mechanisms. If migratory components are predominantly distributed in non-target tissues, they may exert regulatory effects across organs or tissues through indirect mechanisms. Therefore, identifying the intermediary substances and regulatory actions becomes a research priority, enabling the construction of a multidimensional network of qualitative associations linking migrating active components, intermediary mediator substances, cross-organ

regulation, regulatory pathways, and pathological effects. Furthermore, pharmacokinetic markers can be systematically identified based on chemical content, *in vivo* exposure levels, bioactivity intensity, and biological network topology characteristics. Moreover, biomarkers can be identified by integrating multi-objective optimization techniques to enable the establishment of a quantitative PK–PD correlation model for the multi-component network effects of TCM. This statistical model describes the dose-time-effect mechanisms that underlie the action patterns of TCM formulations.

Molecular mechanism

Regarding molecular mechanism research, we propose adopting the TCM-integrated pharmacology strategy as guidance to investigate the molecular mechanisms of TCM formulas' actions from a holistic perspective. This encompasses panoramic molecular mechanism analysis based on omics databases and bioinformatics, determination of primary effects in TCM formulas, and validation of their molecular mechanisms *via* bio-network computational analysis, disease-syndrome-formula correlation analysis for TCM prescriptions, and regulatory mechanism analysis of direct, indirect, and auxiliary actions based on PK–PD correlations.

Application

Based on an integrated pharmacology strategy, the research model discussed in this guideline can be applied to fundamental studies of Chinese herbal compound pharmacology. These include investigations into the pharmacological basis of efficacy, the biological implications of syndromes, the safety and efficacy of Chinese herbal medicines, the mechanisms of action, novel approaches to quality control, and the development of new TCM drugs (Figure 4).

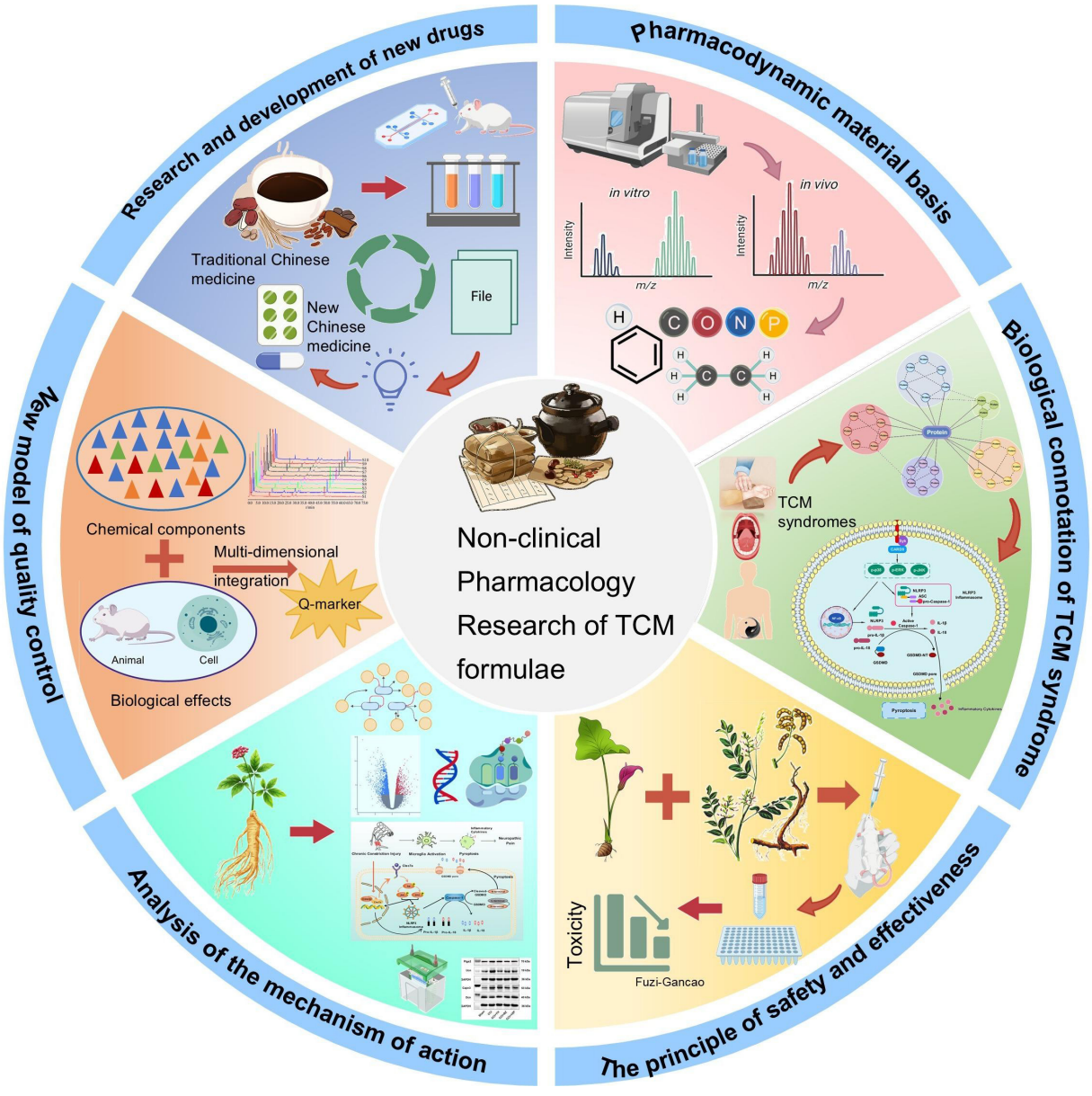

Figure 4. Application of non-clinical pharmacology for TCM formulations. TCM: Traditional Chinese medicine.

Analysis of the pharmacological basis of TCM formulas

TCM is a complex chemical system of ambiguous nature. Since its modernization, the fundamental question of which of the substances in these preparations have therapeutic effects has remained unanswered. The efficacy varies considerably during the transition from drug to formulation, yet when focused on the disease itself, the active substances and targets are limited. The mechanisms of action and efficacy of TCM formulas must be elucidated through modern biological techniques and approaches within clinical application scenarios, guided by the principles of TCM theory. Research into the pharmacological basis of TCM formulas has progressed through four phases. The initial stage employed phytochemical methods centered on extraction, separation, purification, structural characterization, and bioactivity testing. This is exemplified by the isolation and purification of ephedrine[50] and camptothecin[51]. The subsequent phase focused on bioactivity-guided isolation, leading to the discovery of artemisinin[52] and paclitaxel[53]. The third phase involved serum pharmaceutical chemistry in TCM and was exemplified by the identification of blood-penetrating components in Dachengqi decoction, as well as the analysis of their colon cancer regulation mechanisms[54]. The fourth phase involved formula-syndrome metabolomics in TCM and included the material basis and action mechanisms of Keluoxin capsules for treating diabetic retinopathy[55], as well as the active constituents and quality markers of Danggui Jianzhong decoction for treating primary algomenorrhea[56].

The integrated pharmacology research mode employs UPLC-QTOF-MS/MS for systematic characterization of chemical constituents in TCM formulas. Building upon its demonstrated efficacy in *in vivo* and *in vitro* models, the Traditional Chinese Medicine Integrated Pharmacology Research Platform (TCMIP) v2.0, or omics analysis, is utilized to conduct "disease-syndrome-formula" correlation analyses or "disease-target-pathway" network calculations based on TCM clinical syndromes or diseases, with the result of prediction of action pathways and efficacy modules. Finally, by combining molecular biology experiments with virtual validation *via* component-gene molecular docking, we offer a comprehensive interpretation of the underlying mechanisms and material basis of drug-mediated disease prevention and treatment. For instance, based on mass spectrometry-identified components and transcriptome analysis, Mao et al.[57] found that the combination of mangiferin and glycyrrhizic acid in Baihu Guizhi decoction alleviates the severity of rheumatoid arthritis by reversing dysregulation in thermogenesis and energy metabolism. Hou et al.[58] analyzed potential active components of purslane seeds for diabetes treatment through integrated application of UHPLC-LTQ-Orbitrap and TCMIP v2.0, combining high-throughput chemical analysis, target prediction, and network computing. Moreover, novel material forms such as supramolecular structures of Chinese herbal medicine[59], inorganic elements[60], and carbon dots[61] have recently been proposed for use in studying the pharmacology of Chinese herbal medicines. They are expected to play a role in elucidating the mechanisms of action within these formulations.

Interpretation of the biological connotations of TCM syndromes

TCM syndromes, as the core of its foundational theory, serve as the key basis for syndrome differentiation and treatment for the distinct individuals under the guidance of TCM theory. The individualized philosophy they embody aligns perfectly with precision medicine. The personalized approach they represent is perfectly aligned with precision medicine. The disease-syndrome integration research model is one of the main approaches used to improve the effectiveness of TCM treatment. By combining disease diagnosis with syndrome differentiation, it considers the dynamic interplay and interactions of complex factors, providing a comprehensive understanding of the fundamental nature of disease. The research paradigm of this technical guideline integrates pharmacokinetic studies and systems biology approaches of TCM formulas, elucidating the interactions between the metabolic fingerprint of the formulas and the molecular biological networks of syndromes or diseases. On the one hand, in pharmacological efficacy studies, utilizing animal models and selecting appropriate physicochemical indicators allows for a relatively intuitive perception of disease states. Diagnostic criteria for various TCM diseases or syndromes have been incorporated into these indicators, which serve as diagnostic tools. However, these indicators often fail to reflect the onset and progression of disease accurately. Consequently, when no changes in these indicators are apparent, integrating disease and syndrome enables flexible diagnosis based on TCM symptoms. Interventions guided by this diagnosis can prevent disease before it manifests. Conversely, when TCM symptoms show marked improvement, but physical and chemical indicators clearly indicate the persistence of pathogenic factors, appropriate consolidation therapy can facilitate a favorable prognosis. On the other hand, in fundamental molecular research, the integrated application of multi-omics analysis techniques alongside biomolecular network analysis, combined with information on diseases, symptoms, syndromes, genes, and targets, enables the screening of syndrome-specific candidate molecular markers. This approach comprehensively uncovers the distinct pathological mechanisms of different syndromes within the same disease. Employing a multi-omics approach centered on the gut microbiota, Guo et al.[62] elucidates the biological foundations of intestinal damp-heat syndrome and gastrointestinal solid-heat syndrome in type 2 diabetes mellitus (T2DM). The study explores the systemic characteristics of the multi-omics in relation to diverse dejecta profiles in T2DM and screens and validates the clinical significance of the key bacterial genus *Blautia* for precision interventions in T2DM. This demonstrates the scientific value of TCM syndromes as indicators of the body's holistic state, which can be used for disease classification and treatment.

Elucidation of the principles of safety and efficacy of TCM formulas

Toxicity is one of the fundamental principles of TCM. The chemical constituents of herbal medicines act as

initiating factors, with positive effects manifesting as efficacy and adverse effects as toxicity. Controlling toxicity while ensuring therapeutic efficacy is important for the modernization of TCM. The toxicity of Chinese herbal medicines presents challenges, including concealment, uncertainty in material basis, ambiguity in safe dosage, complexity in toxicity-efficacy mechanisms, and idiosyncrasy in individual variations[63]. Based on this technical guideline and guided by TCM-integrated pharmacology strategies, clinical multi-omics integration research can elucidate the toxicity-efficacy patterns of toxic herbals, along with their toxicity targets and biological pathways, with a focus on the interrelationships between TCM components, their effects, and their toxicity mechanisms.

Tripterygium glycoside tablets demonstrate significant efficacy in treating rheumatoid arthritis, yet they exhibit pronounced hepatotoxicity, with the underlying mechanism remaining unclear. Wang et al.[64] employed an integrated research strategy combining clinical phenomics, transcriptomics, and proteomics to construct an efficacy-toxicity association network involving core targets for both therapeutic efficacy and toxicity of Tripterygium glycoside tablets. Through SoFDA enrichment analysis of its TCM clinical symptoms and modern disease phenotypes, the association characteristics and molecular mechanisms of the efficacy-toxicity relationship in Tripterygium glycoside tablets were systematically elucidated. This enriches the scientific understanding of the toxic properties of the Chinese medicinal herb *Tripterygium Wilfordii* and provides evidence to guide the rational clinical use of Tripterygium preparations. Further research may also be conducted into the analysis of pharmacologically active and toxicological component groups, the relationship between dose, efficacy, and toxicity, identifying and controlling toxicity markers, and the correlation between acute and chronic toxicity. These studies could be integrated with the combined pharmacology strategy set out in this technical guideline to achieve more precise control over the safety and efficacy of TCMs.

Analysis of the mechanisms of action in TCM formulas

Analyzing the mechanisms of action of TCM formulas is crucial for clarifying and explaining their therapeutic efficacy. The approach to analyzing the mechanisms of action of the TCM formula in this technical guideline is guided by the TCM-integrated pharmacology strategy. It focuses on the primary effects of the formula, describing the active components, key targets, pathways, and their associated biological functions, and conducting in-depth, multi-level research into the regulatory interactions between the formula and its primary effects. This analytical model is reflected in the following aspects.

The first analytical approach is to integrate multi-omics, high-throughput detection data with bioinformatics analysis to identify the molecular basis that determines the efficacy of TCM formulas. As an alternative, existing databases such as the Human Phenotype Ontology or the SoFDA can be used. However, the relevance, rationality, and scientific reliability of data from prior animal studies must be considered. Combining the primary effects of the formula with network parameter calculations can uncover and validate molecular mechanisms at a molecular level. Chen et al.[65] employed UPLC-Q-TOF/MS combined with network pharmacology to identify the active components and mechanisms of Gegen Qinlian decoction in treating irinotecan-induced diarrhea. *In vitro* experiments using SN-38-activated NCM460 cells and *in vivo* irinotecan-induced diarrhea studies validated the anti-inflammatory activity of Gegen Qinlian decoction in relation to intestinal barrier function, alongside its effects on inflammation-associated protein expression and mRNA levels within the phosphoinositide 3-kinase and AKT serine/threonine kinase (PI3K-AKT) signaling pathway, thereby establishing a foundation for elucidating the drug's molecular mechanisms.

The second analytical approach involves constructing and examining a multidimensional interaction network that links disease genes, syndrome genes, and candidate formula targets from the perspective of disease-syndrome-formula associations. The aim is to elucidate the connections between drug candidate targets, disease phenotypes, and TCM syndromes. Using computational methods, functionally significant network modules of topological importance are identified, revealing the molecular basis and mechanisms by which formulas intervene in TCM syndromes and treat diseases[66].

The third interpretative approach focuses on explaining TCM theories, such as treating different diseases with the same method and treating the same disease with different methods. *Scutellaria baicalensis* and *Paeonia lactiflora* constitute a clinically effective drug pair for treating depression and tumors, though their dual-targeted mechanism remains unexplained. Sun et al.[67] employed UPLC-MS to detect the components of the drug pair, integrating network pharmacology to investigate the multi-pathway regulation of their active constituents and co-targeted mechanisms when treating depression and colorectal cancer. Using chronic unpredictable mild stress and orthotopic tumor transplantation mouse models, the study validated the therapeutic efficacy of *Scutellaria baicalensis* and *Paeonia lactiflora* in achieving the concept of "different diseases treated by the same method." Tawulie et al.[68] used 16S rRNA gene sequencing and UPLC-MS/MS to assess alterations in the distal ileal microbiota and bile acid metabolism profile of obese T2DM rats. Bioinformatics analysis and molecular mechanism validation confirmed that the hypoglycemic formula Sanhuang wan exerts therapeutic effects by modulating the metabolism alterations above and upregulating the intestinal FXR/FGF15 and TGR5/GLP-1 signaling pathways. Furthermore, Huanglian decoction can treat T2DM by interfering with acetaldehyde and dicarboxylate metabolism by enhancing the expression levels of the genes and proteins responsible for glucose transporter 4 (GLUT4), insulin receptor (INSR), and mitogen-activated protein kinase 1 (MAPK1)[69].

The fourth analytical model is based on the PK–PD relationship between the multiple components of TCM and their biological effects, from which three pathways of action for TCM formula have been deduced: direct regulatory action, indirect regulatory action, and auxiliary regulatory action. Traditional pharmacological research theories and

paradigms are primarily based on isolated subsystems and typically focus on the direct antagonistic effects of drugs on molecular targets in diseased organs. Although this regulatory mechanism is straightforward, obvious, and easily verifiable, it still presents challenges when it comes to complex diseases such as metabolic disorders, autoimmune diseases, tumors, and aging. In recent years, the significance of organ dialogue in disease progression and treatment has become increasingly recognized. The systemic therapeutic principles and corresponding treatment methods of TCM, such as the mutual generation and restraint among the five *zang* organs (five elements), the internal–external relationships between *zang-fu* organs and meridians, treating *zang* organ disorders by addressing the *fu* organ, mother passing illness to child, suggest cross-organ effects and the mechanisms through which medicinal substances intervene *via* mediating substances[70]. Moreover, the auxiliary effects exert their influence through interactions between absorbed prototypes and metabolites, as well as between enzymes and transporters, thereby providing crucial insights into the complex mechanisms of TCM.

Insights into a new model for quality control in TCM

The quality evaluation system for TCMs incorporates traditional assessment, chemical evaluation, and biological evaluation. The prevailing approach typically involves controlling the quantity of one or more indicators or active constituents in the medicine as a quality benchmark. However, TCMs contain numerous constituents, and the basis for efficacy varies among different formulations. Furthermore, the correlation between these constituents and therapeutic effects is often weak, meaning this approach is unable to accurately reflect the true quality of TCMs. This technical guideline's research paradigm suggests that studies on quality markers for TCMs can be conducted. Specifically, this involves conducting a preliminary screening of quality markers based on their specificity to a given substance, their relevance, and their medicinal potential, while simultaneously identifying candidate quality markers associated with the pharmacological effects of TCMs and validating such markers through component knock-in/knock-out approaches[71–72]. The determination of chemical constituents in samples, as proposed by the technical guidelines, can be used to establish substance-specific correlations. The confirmation of target constituents and similar pharmacokinetic characteristics helps to identify constituent-constituent relationships and the pharmaceutical potential of active ingredients. The establishment of multi-level efficacy indicator systems and PK–PD correlations can be used to validate efficacy. Consequently, the basic concept of TCM-integrated pharmacology research strategies and the "Five Principles" for quality biomarkers are similar. Gao et al.[73] identified wilforine as the quality marker for Tripterygium glycoside tablets based on their chemical composition characterization and PD-PD studies. LI[74] has identified 23 potential quality markers that can be used to distinguish between 15-year-old wild-grown and cultivated ginseng for the first time. Dong et al.[75] utilized network pharmacology and pharmacokinetics combined with experimental validation to preliminarily predict potential quality biomarkers for Huangqin Qingre Chubi capsules in treating rheumatoid arthritis. However, to establish scientifically sound, high-quality standards, we must explore the precise quantitative relationship between index constituents and biological activity further. Based on this, we must validate our findings through knock-in/knock-out experiments and investigate how quality markers in TCM contribute to its overall efficacy, which will enhance the quality evaluation system for TCM.

Guidance for the development of new TCM drugs

The Special Provisions on the Registration Management of Traditional Chinese Medicines encourage the development of new TCM drugs and simplify the approval process. The research and development of TCM's new formula drugs represent a crucial measure for the inheritance and innovation of TCM, serving as a powerful tool for its modernization and internationalization. This technical guideline is closely integrated with the "three-combination" review system, providing a comprehensive collation and summary of pharmaceutical research for the development of TCM's new formula. It outlines the recommended and non-recommended content of research and the associated requirements, which are adapted to different stages and scenarios. This enhances the scientization and standardization of the development of new TCM drugs, and it offers technical references for priority review scenarios where the material basis and mechanism of action are clearly established.

Outlook

Holistic analysis of active compounds in TCM

Identifying and characterizing the active constituents of TCM *in vitro* and *in vivo* is the primary challenge in elucidating the mode of action within its complex systems. The rapid screening of novel active constituents in TCM can be achieved by utilizing novel techniques such as receptor-ligand-specific binding, bio-mimetic affinity for cell membranes, and cell membrane chromatography. This effectively supplements the discovery of *in vitro* bioactive substances[76]. However, the *in vivo* characterization of bioactive compounds remains complex. To systematically analyze the dynamic changes of drugs within the body and their correlation with therapeutic efficacy, it is necessary to construct *in vitro* composite models based on the pathways of component migration. Examples of such models include intestinal absorption-activity evaluation, intestinal absorption-hepatic drug metabolism enzymes-activity evaluation, and gut microbiota transformation-intestinal absorption-bioactivity evaluation, which simulate the entire metabolic process. By dynamically tracking the transformation of substances within the body, these models elucidate the relationship between the three component categories in TCM—absorbed prototypes, metabolites, and unabsorbed constituents—and their pharmacological activity. This approach identifies active pharmaceutical substances and their mechanisms of action, enabling a comprehensive analysis of bioactive components in TCM formulations.

Discovery of direct-action targets and refined analysis of regulatory networks

The discovery and validation of direct targets for TCM, the elucidation of regulatory mechanisms in signal pathways, and the development of techniques for the refined analysis of target-regulatory networks are critical challenges in understanding the complex modes of action of TCM. There are presently two approaches to direct target identification. One approach is reverse mode, which involves screening target proteins starting from TCM components and employing methods such as chemical proteomics and chemical genomics. Chemical proteomics encompasses techniques such as chemically active probe labeling and non-labeling methods that detect alterations in the thermal stability of target proteins. It also includes novel approaches for identifying common targets in complex systems based on fragments of the target protein[77–78]. Another approach involves inferring the phenotypic alterations and the associated known pathways and functional networks induced by TCM active components acting in the human body. This involves identifying the direct targets of these medicines by analyzing changes in the expression levels of key proteins at the upstream and downstream stages of pathways. The methods employed include genomics, proteomics, metabolomics, and cellular morphology analysis.

Based on intracellular effect regulatory networks, novel technologies for multi-omics data integration and network regulation analysis could be developed to elucidate the mechanisms of drug efficacy. These technologies enable the precise evaluation of the therapeutic effects and potential risks of active molecules through changes in molecular nodes within the network. Focusing on key gene functional modules and their associated therapeutic pathways enables the identification of critical node genes and the mechanisms of synergistic interactions between drug constituents, thereby revealing collaborative action patterns among multiple active components. At the same time, an artificial intelligence-driven engine could be developed to perform GPU-accelerated dynamic metabolic simulations of the entire range of Chinese herbal medicine constituents. This would establish a cloud map of human physiological parameters, including hemodynamics, lipid/protein microenvironments, and enzyme/transporter expression profiles. At subcellular resolution, the engine would locate constituents, their metabolites, and action targets within Chinese herbal formulas inside cells. Integrating multi-omics data, biochemical models, and artificial intelligence algorithms would enable the creation of a multi-scale virtual metabolic model covering molecules, organelles, cells, tissues, organs, and systems. This would enable the real-time simulation of the spatiotemporal dynamics of the absorption, distribution, metabolism, and excretion of TCM compound constituents, revealing their metabolic patterns *in vivo*. At the molecular level, this approach would systematically reveal the complex mechanisms of TCM action and provide an iterative, interpretable, and expandable digital platform for delivering personalized care with precise drug dosing and TCM modernization.

Integration of multidimensional effect system

Multidimensional data, including chemical composition, target networks, disease-pathology effects, and PK–PD profiles, are to be integrated to establish dynamic target networks reflecting the spatiotemporal evolution of disease pathogenesis in TCM. A multi-layered, dynamic, microscopic molecular interaction network map could be constructed through the integrated analysis of multi-omics molecular characteristic profiles, which encompass genomics, proteomics, metabolomics, and epigenomics. Following a systematic research model combining micro-level molecular networks, meso-level pharmacological activity, and macro-level TCM clinical disease-syndrome-effect, and integrating refined analysis of target networks within TCM formulas, we can map the local sub-networks of herbal actions onto the holistic disease-syndrome-effect network. This enables analysis of how TCM formulas harmonize global network imbalances, organically linking TCM holistic thinking with the molecular network evolution of Western medical diseases, thereby elucidating the mode of action and characteristic features of TCM formulas.

Original innovation based on the interpretation of TCM theories

TCM and modern medicine are two distinct systems for studying life and health. Exploring TCM theories in-depth can inspire us to examine scientific problems from different perspectives, thereby promoting original innovation in the life sciences. Theories such as “the lungs and large intestine are paired organs” “the heart governs the spirit and consciousness” and “the lungs are the upper source of fluids” may provide an understanding of the mechanisms underlying respiratory disorders, intestinal diseases, psychiatric conditions, and disorders of fluid metabolism. For example, is respiratory disease associated with the gut microbiota? Are cardiac extracellular vesicles linked to human mental health? Can respiratory disease affect the function of renal tubules or collecting ducts? Traditional Chinese medical theory is a vital source of original innovation and deserves the attention of researchers.

Conclusion

Non-clinical pharmacology studies of TCM compound formulations should be more closely connected to clinical TCM practice. Distinctions must be made between sample information, administration methods, and other parameters in fundamental research and new TCM drug development contexts. Systems biology research should integrate cutting-edge technologies and interdisciplinary approaches to elucidate the holistic and localized effects of TCM compounds on the human body alongside macro-phenotypic manifestations and micro-level mechanisms. *In vivo* and *in vitro* studies should be combined to validate drug effects, and PK–PD studies should be integrated to elucidate dynamic regulatory networks. Computational network analysis should be employed alongside experimental validation to clarify mechanisms of action. The integrity, scientific rigor, and standardization of the non-clinical

pharmacology evidence chain for TCM formulas should be enhanced through multidimensional, comprehensive approaches. Based on the technical guidelines published by our team, the integrated pharmacology strategy for TCM can be applied to elucidate the pharmacological basis of TCM formulations, interpret the biological significance of TCM syndromes, explain the principles of safety and efficacy of TCM formulations, clarify the mechanisms of action of TCM, inspire novel approaches to quality control of Chinese herbals, and guide the research and development of TCM new drugs. However, further research is required to comprehensively analyze pharmacologically active substances, accurately identify direct targets and regulatory networks, integrate multidimensional effect systems, and enhance original innovations based on TCM theories.

Conflict of interest statement

Junhua Zhang is an editorial board member of this journal. None of the other authors declare any conflicts of interest.

Funding

This study was supported by the National Key R&D Program of China (2023YFC2308200 and 2023YFC3502900).

Author contributions

Hai-Yu Xu and Junhua Zhang conceived and designed this study. Han Li wrote the original draft. Xiao-He Xiao participated in the definition of the relevant concepts. Ping Wang, Wei-Jie Li, Dan Wu, and Yu-Te Zhong participated in the discussion and edited the manuscript. All of the authors have read and approved the published version of the manuscript.

Ethical approval of studies and informed consent

Not applicable.

Acknowledgments

None.

Data availability

All data generated or analyzed during this study are included in this published article.

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